IAEAC

BBMEC

12th Workshop on Biosensor & Bioanalytical Microtecniques in Environmental, Food & Clinical Analysis

INTERNATIONAL BIOSENSOR CONFERENCE

25. – 29. September 2017 | Rome, Italy

Edited by

Katia Buonasera

Consiglio Nazionale delle Ricerche

BBMEC 12th Workshop on Biosensor & Bioanalytical Microtechniques in Environmental, Food & Clinical Analysis
INTERNATIONAL BIOSENSOR CONFERENCE
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IAEAC
International Association of Environmental Analytical Chemistry (IAEAC)

Institute of Crystallography (IC)
National Research Council (CNR)

IBAF
Institute of agro-envinronmental and forest biology (IBAF)
National Research Council (CNR)

CNR-IIA
Institute of Atmospheric Pollution (IIA)
National Research Council (CNR)

Sponsors

ABC Analytical & Bioanalytical Chemistry

Biosensing Technologies
Innovative start-up

Biosensors-MDPI an open acces journal by MDPI

IJEAC Journal on Environmental Analytical

Sensors-MDPI an open acces journal by MDPI

PalmSens_Compact Electrochemical Interfaces

Sensor100
Sensor 100 The International Bio-Sensor and Chemo-Sensor Network

Topics

- Novel Concepts in Biorecognition, Transduction, Signal Amplification & Sample Preparation
- Lab-on-a-chip, microTAS, Biosensors
- Nanomaterials
- Microtechniques
- Surfaces & Interfaces
- Screening
- Array Technology
- Imaging
- On-site Analysis
- Label-free, Electrochemical, Optical, Mass-based, Magneto Transducers
- Application to clinical, food, environmental and processing challenge

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Message: The 12th International Workshop on Biosensors and BioAnalytical μ-techniques in Environmental, Food and Clinical Chemistry (BBMEC12), held in Rome from September 25th to 29th, was the result of a trustful collaboration between CNR and IAEAC.

The International Association of Environmental Analytical Chemistry (IAEAC) is a world-wide operating organization that aims at promoting and maintaining scientific excellence in analytical science as applied to different environmentally relevant research areas. Not only the traditional compartments soil, water, and air are included in its activities, but likewise fields such as process analysis, human health, or food quality. In these efforts, the IAEAC strongly interacts with relevant disciplines other than Chemistry. I enthusiastically approached the leading spirit of the association when I participated to several IAEAC workshops and finally when elected vice-president of IAEAC in 2015. In these last years, due to my increasing involvement in the association, I greatly appreciated its idealistic aims thanks to our President, Prof. Josè Broekaert (Hamburg University).

CNR is the Italian National Research Council and its activities are targeted to fundamental and applied research, innovation technology and advanced services in several fields of sciences. CNR performs research activities and provides services in support to public administrations, public and private enterprises and citizens. Environmental aspects of these activities are studied in several departments involving engineers, biologists and analytical chemists.

The event held this year, has been the 12th of a series of successful IAEAC workshops. They started in 1994 in Paris and had their last edition in 2015 in Regensburg, Germany, chaired by Prof. Antje Baeumner. The symposium held in Rome last Semptember, once again underlined the IAEAC's idealistic goals, but also added something new. Traditional conferences we are accustomed to follow, indeed, are based on the synchronization of our scientific interests with the timing of oral and poster sessions; this is why precise time schedules and theme sessions are identified. In this way, however, the multidisciplinarity of our topic is mortified, and participation focused only to presentations that affect individuals. The format of our workshop, therefore, wanted to detach itself from this traditional vision based on synchronicity, to embrace sequentiality. We knew only the start time, every day, but that did not mean “anarchy”. Together with the Chairmen and their supporters, we created a workshop focusing on the scientific content and discussion, not on schedule. To do this, the program did not include timetables and theme sessions, but a mix of presentations divided by sessions. In each session, an invited speaker, a researcher and a student gave their oral presentations with the appropriate time for questions and insights. Poster sessions did not take place during the coffee-break or lunch, because we thought the most advanced and interesting results, still not enough mature, are the salt of future science and deserve the right attention.

Following this approach, students and young researchers had the opportunity to check their activity in front of an international scientific community by both oral and poster presentations but also intervening in the session management as supporters, alongside the Chairman. These arrangements made it possible for a totalizing participation, a stress free program and a better networking.

It is now up to all of us, organizers and participants, to fill in this program with a breath of life, to exchange know-how and brand-new results, and by these means building bridges between our respective research institutions or countries, contributing for a little to pull down the existing walls as we did during our workshop and we wil do in our future research activities.On behalf of the BBMEC12 scientific and organizing committees and the IAEAC executive committee, I wished all of you a successful and scientifically stimulating workshop in our city and now, after the workshop, an intense scientific collaboration.

I am extremely thankful to the CNR staff, especially to Dr. Katia Buonasera, editor of this book, and Laura Passatore for accompanying me in the organization of the workshop together to Mrs Clara Grossi and Mirta Varvesi (OmniaCongressi). Thanks to Dr. Angelo Massacci, Director of CNR-IBAF (Institute of Agro-Environmental and Forest Biology) for his friendly help. Through their engagement I hope a good frame has been established for an international event.

Roberto Pilloton
Chair of BBMEC12
IAEAC Vice President

Program

Final program

Monday September 25, 2:00 p.m.

Registration

Welcome

Afternoon Session, Chair Lisa Hall *(A.Stroia, supporting)*

IL01 - Novel nanomaterials for the detection of pathogens in microanalytical systems
A.J. Baeumner

O01 - Electrospun functional nanofibers for electrochemical microfluidic biosensors
N.Wongkaew, M.Simsek, L.von Kruechten, M.Buchner, A.Duerkop, A.J. Baeumner

S01 - Rapid and simple surface functionalization strategy for lipid-based membranes
C.Figalist, T.Hirsch, A.J. Baeumner

Tuesday September 26, 8:30 a.m.

1st Morning Session, Chair Antje J. Baeumner *(E.Sepe,supporting)*

IL02 - Engineering silk-like proteins for biosensors
E.A.H.(Lisa) Hall, Z.Zhao, J.Chun

O02 - Monitoring of sewage biomarkers with community sewage sensors for public health
Z.Yang, M.Tanyeri, B.Kasprzyk-Hordern, C.G. Frost, J.Cooper, P.Estrela

S02 - Microfluidic spheroid-generation glass platform for integration in a screening system for endocrine disruptors
K.Gier, P.P.M.F.A. Mulder, J.P.S.H. Mulder, U.Sauer, E.M.J. Verpoorte

2nd Morning Session, Chair Karl-Heinz Feller *(B. Demirbakan, supporting)*

IL03 - Nanoparticles and UV-C induced DNA damage
J.Labuda, J.Blaškovičová, A.Koutsogiannis, J.Sochr

O03 - High-sensitivity screening of soluble ERBB2 in different cell lines using a combined label-free and fluorescence biosensing platform
A.Sinibaldi, M.Allegretti, E.Sepe, N.Danz, P.Munzert, A.Occhicone, E.Tremante, P.Giacomini, F.Michelotti

S03 - Bloch surface wave biosensors for real-time study of fibronectin-phosphorylcholine coatings for biomedical applications
A.Sinibaldi, V.Montaño-Machado, E.Sepe, N.Danz, P.Munzert, F.Sonntag, D.Mantovani, F.Michelotti

1st Poster Session

Lunch

1st Afternoon Session, Chair Karsten Haupt *(B.Ozcan, supporting)*

IL04 - Targeted surfaces for cell imaging and sensing applications
S.Timur

O04 - Driving self-assembly in a microfluidic system for surface enhanced Raman analysis
M.Banchelli, M.de Angelis, C.D'Andrea, M.Cottat, R.Pini, P.Matteini

S04 - Flow injection electrochemical quartz crystal nano-balance based immunosensing platform for detection of 17β-estradiol in water

A.C.Singh, S.Bhand

2nd Afternoon session, Chair Suna Timur *(A.C. Singh, supporting)*

IL05 - Whole cell biosensors for cytotoxicity and chemosensitivity assays
K.H.Feller, B.Büttner, M.Dubiak-Szepietowska, M.Büttner

O05 - Micro and nanofabricated molecularly imprinted polymers as recognition elements for chemical sensors
K.Haupt

S05 - Fluorinated surfaces for matrix-free laser desorption ionization
C.Piotto, G.Guella, P.Bettotti

Wednesday September 27, 8:30 a.m

1st Morning Session, Chair María-Pilar Marco *(S.Şanlı, supporting)*

IL06 - Fully integrated ready-to-use paper-based electrochemical (bio)sensors
D.Moscone, S.Cinti, F.Arduini

O06 - Nanofunctionalized interfaces for Surface Plasmon Resonance based immunosensors
S.Kumbhat

S06 - New quinoline-based chiral ligands and their Europium(III) complexes
C.De Rosa, M.Bettinelli, F.Piccinelli

2nd Morning Session, Chair Jenny Emneus *(T.Lavecchia supporting)*

IL07 - Point-of-care paper based analytical device detecting highly conserved DNA sequence of Dengue virus using ZnO multipods
C.Singhal, Y.K. Mishra, A.Mathur, C.S. Pundir, J.Narang, R.Adelung, D.K. Avasthi

O07 - Molecularly imprinted polymers as bio-mimic sorbents for highly selective solid phase microextraction of triazine herbicides in crops
B.Ebrahimi

S07 - Multiple electrochemiluminescence detection for biosensing
M.Mayer, M.Neumeier, S.Takegami, A.Georgescu, A.Duerkop, T.Hirsch, A.J.von Wangelin, A.J. Baeumner

2nd Poster Session

Lunch

1st Afternoon Session, Chair Danila Moscone *(N.Colozza, supporting)*

IL08 - 2D and 3D Lab-on-a-Chip systems for environmental and life science applications
A.I.Bunea, J.Kajtez, A.Heiskanen., S.S.Keller, M.Alm, P.Thomsen, A.Aspegren, A.Martinez-Serrano, M.Dufva, A.Wolff, J.Emnéus

O08 - A multiplexed and multimodal biobarcode, simultaneously tailored detection of small molecules and proteins
M.Broto, R.McCabe, R.Galve, M.P.Marco

S08 - Enhanced biocide power of Cu/Zn/Co mixed oxides towards *E.coli*
M.Carbone, E.M.Bauer, A.Talone, V.Orlando, S.Biagioni, R.Briancesco, M.Semproni, L.Bonadonna

2nd Afternoon Session, Chair Katia Buonasera *(C.Figalist, supporting)*

IL09 - Challenging the threats of multiplexation through nanobiotechnological approaches
M.P. Marco

O09 - Multiplexed analytical platforms based on the use of antibodies for monitoring pollutants in marine environment
J.P.Salvador, A.Sanchis , K.Kooper, A.Miti, M.P.Marco

S09 - Biotransformation of dinitrotoluene by *Escherichia coli* and its implications for the detection of trace explosives
B.Shemer, C.Hazan and S.Belkin

Thursday September 28, 8:30 a.m. leaving to Porano, Villa Paolina

Networking Session and Votation for the best presentations awards (during transfer, ☺)

Welcome at Villa Paolina (Porano)
Angelo Massacci, Director of CNR-IBAF (Institute of Agro-Environmental and Forest Biology)

Presentation of *TECO Project*: *an opportunity to boost collaboration between Europe and India*
L. Passatore, S. Carloni, M. Zacchini, A. Massacci

Morning Session, Chair Subbiah Alwarappan *(C.De Rosa, supporting)*

IL10 - Nanoelectrodes ensemble: a versatile platform for biosensing
L.M.Moretto, P.Ugo

O10 - Electrospun nanofibers as advanced recognition materials in test stripes for optical quantitation of biogenic amines in food
A.Dürkop, N.Iurova, A.Danchuk, A.J.Bäumner

S10 - Electrochemical bismuth-modified printed sensors as sustainable tools to study the remediation capability of novel carbon-nanotube sponges towards Pb2+ and Cd2+ polluted waters
N.Colozza, D.Florio, F.Arduini, M.Scarselli, D.Moscone

Transfer to Castiglione in Teverina

Lunch @ "Trebotti" Organic Farm

Afternoon at Orvieto

Visit, Tasting and Dinner at MUVIS

Friday September 29th, 8:30 a.m.

POSTER session (P23-P36)

Morning Session, Chair Sunil Bhand *(F.Malvano, supporting)*

IL11 - Electrochemical biosensors based on 2-dimensional nanomaterials
S.Alwarappan, R.Pilloton

O11 - Tricks of the trade: molecular enhancements of microbial bioreporters' performance
S.Belkin

Best Presentations Awards for Young Scientists
Roland Frei Award
Springer Award

O12 - Molecularly imprinted polymer decorated nanoparticles for selective determination of tetracycline in milk
S.Bhand, A.Sharma

S11 - Mechanistic modeling as an effective approach for the understanding and optimization of biosensor performance
D.Semenova, A.Zubov, Y.Silina, L.Micheli, A.C.Fernandes, K.V.Gernaey

Legenda:

IL: Invited Lecture (25')
O: Oral Communication (20')
S: Student Communication (15')

CONFERENCE PICTURES

POSTERS (P01-P38, Y means candidate for awards, V means not present author)

P01-Y - Enzymatic quantification of glyphosate herbicide using a colorimetric PD-CMOS platform
A.Stroia, D.Dheeman, B.C.Cheah, D.R.S. Cumming, M.P.Barrett

P02-Y - Nanofiber architectures for electrochemical biosensing of glucose
I.Yezer, B.Demir, D.O. Demirkol, S.Timur

P03-Y - Detection of bacterial toxins via surface plasmon resonance with fluorescence spectroscopy based biosensor
S. Seherler, A.Bozdogan, T.Arzu, O.Ildeniz, I.Anac, F.Nese Kok

P04-Y - Polypeptide with electroactive endgroups as sensing platform for the abused drug 'methamphetamine' by bioelectrochemical method
B.Demir, T.Y.Sengel, E.Guler, Z.P.Gumus, H.Akbulut, E.Aldemir, H.Coskunol, D.G.Colak, I.Cianga, S.Yamada, S.Timur, T.Endo, Y.Yagci

P05-Y - Development of easy assemble immobilization matrix with magnetic nanofiber layers
S.Şanlı, E.Güler, Z.P.Gümüş, D.O.Demirkol, S.Timur

P06-Y - Detection of Escherichia coli O157:H7 in food products by impedimetric immunosensors
F.Malvano, D.Albanese, M.Di Matteo, R.Pilloton

P07-Y - The azoR gene promoter: a new sensing element for detection of trace explosives
Y.Henshke, B.Shemer, S.Belkin

P08-Y - Detection of contaminants' residues on surfaces by microbial bioreporters
Y.Bykov, D.Shakibai, T.Elad, S.Belkin

P09-Y - Molecularly imprinted polymers as synthetic receptors for cell targeting and imaging
P.X.Medina-Rangel, M.Panagiotopoulou, B.Tse Sum Bui, K.Haupt

P10-Y - Immunosensing based on silver nanoparticles as a model
T.Lavecchia, S.Felici, M.Angjellari, L.Micheli, M.L.Terranova, G.Palleschi

P11-Y - Tracking effects of environmental organic micro-pollutants in the subsurface by coupling HPTLC with genotoxicity test as a tool for effect directed analysis
D.Shakibai, L.Moscovici, C.Riegraf, S.Buchinger, G.Reifferscheid, S.Belkin

P11-V - Paper genosensor based on smart nanocomposites for point-of-care diagnosis of chikungunya virus
C.Singhal, A.Mathur, C.S.Pundir, J.Narang

P12-Y - A novel electrochemical biosensor based on screen printed gold electrode modified with organo-silane for the tumor necrosis factor receptor-associated protein 1 detection
B.Demirbakan, M.K.Sezginturk

P13-Y - Determination of C1 inhibitor human by using screen printed gold electrode modified with silane: higly sensitive and disposable biosensor
B.Özcan, M.K.Sezginturk

P14 - A label-free low cost sensor based on surface-plasmon resonance on plastic optical fiber coupled with a biomimetic receptor for furfural detection in water
M.Pesavento, N.Cennamo, L.Zeni, D.Merli, S.Marchetti

P15-V - Detection of bacteriophages by biosensor based on the microwave resonator
O.I. Guliy, B.D.Zaitsev, A.V.Smirnov, O.A. Karavaeva, I.A.Borodina

P16 - Development of novel biosensor system for fish stress monitoring using self-assembled monolayer
H. Wu, Y. Fujii, H. Ohnuki, M. Murata, H. Endo

P17-Y - New design of label-free human immunoglobulin G impedance biosensor with micro-gap parallel plate electrodes
Y.Kusaka, H.Ohnuki, H.Wu, H.Endo, D.Tsuya

P18-Y - Determination of total antioxidant capacity in plant extracts using electrochemical sensors
A.Serban, M.David, D.Lazurca, C.Radulescu, C.Stihi, M.Florescu

P19-Y - An optical sensor for studying hemocompatibility of biomaterials in a flow system
J.Hutterer, G.Gauglitz

P20-Y - An innovative carbon black modified sensor to detect free chlorine
M.R.Tomei, D.Neagu, F.Arduini, D.Moscone

P21-Y - Potassium sensing with ion selective field-effect transistor
S.Bhandari, U.Singh and S.Joshi

P22-Y - Folat receptor targeted multimodal engineered vesicles for imaging and therapy: a magic of theranostics
B.Demir, F.B. Barlas, Z.P.Gumus, S.Timur

P23 - Sandwich NP-based biobarcode assay for C-reactive protein quantification in plasma samples
M.Broto, R.Galve, M.P.Marco

P24 - High throughput immunoassay for the therapeutic drug monitoring of tegafur
M.Broto, R.McCabe, R.Galve, M.P.Marco

P25 - Development of LED-color-switching type biosensor for the visualization of fish stress response
H.Endo, H.Wu, R.Shinoda, M.Murata, H.Ohnuki

P26 - Recombinant cell biosensors for the detection of Endocrine-Disrupting Compounds
L.Moscovici, D.Shkibai, S.Buchinger, G.Reifferscheid, S.Belkin

P27 - Electrochemical sensor based on ultrathin nanostructural coating for analysis in biological and environmental samples
L.M.Moretto, N.Karimian, A.M.Stortini, P.Ugo

P28 - Multiplexed analytical platforms based on the use of antibodies for monitoring pollutants in marine environment
J.P.Salvador, A.Sanchis, A.Miti, M.P.Marco

P29 - Carbon nanotube modified screen printed electrodes: pyranose oxidase immobilization platform for amperometric enzyme sensors
D.O.Demirkol, C.Ozdemir, R.Pilloton, S.Timur

P30 - Amithiophenol modified montmorillonite as an immobilization matrix for enzymes in biosensor preparation
Y.Y.Yilmaz, E.E.Yalcinkaya, D.O.Demirkol, S.Timur

P31 - Study for developing an electrochemical tongue to differentiate three types of natural waters, using very simple sensors and principal component analysis
M.Tomassetti, M.Castrucci, G.Visco, R.Angeloni, L.Campanella

P32 - Electrochemical detection of hydrogen peroxide by free standing nickel oxide-plastic electrode
S.Susmel, L.Micheli, M.Carbone

P33 - Rapid detection of Escherichia coli in marine water samples by remote optical biosensor system
K.Buonasera, A.Gabrielli, G.Pezzotti Escobar, R.Briancesco, S.Paduano, M.Semproni, L.Bonadonna

P34 - Fluorescent microfluidic device based immunoassay for therapeutic drug monitoring: acenocoumarol case
J.P.Salvador, T.Brettschneider, C.Dorrer, M.P.Marco

P35 - Yeast-based amperometric bioprobe for simazine detection in agricultural water and raw cow's milk samples
G.Grasso, L.Caracciolo, G.Cocco, C.Frazzoli, R.Dragone

P36 - Design of a bioprobe for total estrogenicity levels determination in environmental and food matrices: a biotechnological approach
G.Cocco, R.D'Ovidio, C.Frazzoli, G.Grasso, T.Giardina, R.Dragone

P37 - Technological integrated bioelectronic system and relevant control charting for early intervention on food chain and the environment: the BEST Platform
C.Frazzoli, R.Dragone, A.Mantovani, G.Grasso

P38 - Agreement between Official control and self-monitoring: data report in an Italian dairy chain
F.Martelli, C.Giacomozzi, A.Fadda, R.Dragone, G.Grasso, C.Frazzoli

Legenda:

Pxx(-Y): Poster (Y is for Young and participating to the poster awards; V is for Virtual, not present author)

Scientific session at Porano
Tour and dinner

"Mens sana in corpore sano" - Giovenale (Satire, X, 356)

The fourth day was an unusual meeting in which science, art, history and healthy eating merged into one unforgettable experience. The day started with the transfer of participants from Rome to Porano (Province of Terni, Umbria Region), at Villa Paolina, headquarters of CNR-IBAF. After a brief introduction of Dr. Angelo Massacci, Director of the Institute, and Dr. Laura Passatore, who presented TECO Project, a morning scientific session took place, followed by a tour through the lands that hosted one of the oldest and most fascinating civilization of the past and from which scents and flavors, that make unique Italian food and wines in the world, originate. The tour was divided in three moments: the visit to "Trebotti" winery, located in Castiglione in Teverina (Province of Viterbo), with wine tasting and lunch, the tour of Orvieto and the visit to the MUVIS museum, with aperitif, wine tasting and conference dinner.

VILLA PAOLINA: WHERE SCIENCE AND ART COEXIST IN ARMONY

Villa Paolina represents one of the most relevant park of the Umbria region, due to the avenues and historic gardens that includes, and the variety, the development and the vegetation of the plant species present.
Known in the sixteenth and seventeenth centuries as the Villa of the Seven Cardinals, the complex assumes the connotations of a true homely mansion around 1706 by Giovan Battista Gualtiero, son of Stanislao (1628-79) Marquis of Corniolo, who lives there with his wife, noblewoman Giulia Staccoli of Urbino.
The Gualtiero era ended immediately after the Unity of Italy: in 1874 Villa del Corniolo was alienated and passed into the hands of the marquis Viti Mariani. Since then, the villa will be called Villa Paolina, in honor of the new owner's wife, the marquis Paolina. During the twentieth century, the Villa becomes property of the Province of Terni that for a long time has granted it, under a Convention, to the National Research Council, before as a headquarters of the Institute for Agroforestry and currently, after a restructuring within the CNR, of the Institute of Agro-Food and Forestry Biology (IBAF). The IBAF carries out experiments and research into the branches of Ecophysiology, plant ecology, genetics, forestry and forestry research, some applications in the agro-food industry and food certification.

ORVIETO: THE HERITAGE OF THE ETRUSCAN CIVILIZATION

Located in the Umbria region, the green heart of Italy, Orvieto is a very ancient city situated on the flat summit of a large butte of volcanic tuff.
Inhabited since the Iron Age, Orvieto was a major centre of Etruscan civilization known under the name of "Velzna" (latinized as "Volsinii"). Located in a virtually inaccessible area, equipped with powerful fortifications, the ancient Velzna was a very rich city, which controlled the river communications between Etruria and Rome. Described as "Caput Etruriae" ("Capital of Etruria") by Valerius Maximus (first century AD), and "Oppidum Tuscorum opulentissimum" ("Rich fortified city of the Etruscans") by Pliny the Elder (23-79 AD), Orvieto (whose current name of medieval origin derives from the latin "Urbs Vetus" or "Old Town") offers a lot of itineraries through which visitors can examine the history of this city: from Etruscan constructions (**Etruscan temple of Belvedere**, the **Etruscan Necropolis** of the Crocifisso del Tufo, the **Etruscan wall** and the **Etruscan well**), to the **underground of Orvieto**, full of pits and caves of Etruscan and Medieval periods, the **Saint Patrick's well**, the **Anello della Rupe** path, and the **Cathedral**.

SAINT PATRICK'S WELL

In 1527, during the "Sack of Rome", the then Pope Clement VII took refuge in Orvieto. He commissioned Antonio da Sangallo the Younger in the construction of the well to serve as the water supply in case of siege. The design of Sangallo, who already worked on the fortification of the city, was inspired by the spiral of the Belvedere in the Vatican. He created an ingenious system of two spiral staircases that never meet. They create a way to get in and out of the well (about 53 meters deep) without any traffic problems. Today it is a real pleasure to descend to its depths along the more than two hundred steps.

ORVIETO CATHEDRAL

The ***Orvieto Cathedral*** is widely considered the most glorious example of Italian Gothic. It is a large 14th-century Roman Catholic cathedral dedicated to the Assumption of the Virgin Mary.

The building was constructed under the orders of Pope Urban IV to commemorate and provide a suitable home for the **Corporal of Bolsena**, a miracle which is said to have occurred in 1263 in the nearby town of Bolsena, when a travelling priest who had doubts about the truth of transubstantiation found that his host was bleeding so much that it stained the altar cloth. The cloth is now stored in the Chapel of the Corporal inside the cathedral.

Situated in a position dominating the town of Orvieto the cathedral's façade is a classic piece of religious construction, containing elements of design from the 14th to the 20th century, while inside resides some of the best Italian painters of the period.

VISIT TO THE MUVIS

The MUVIS, Museo del Vino e delle Scienze Agroalimentari (Museum of Wine and Agro-Food Sciences), housed inside the monumental Vaselli's Cellars, is an anthropological museum that aims to return the thousand faces of the territory and offers the visitor a journey, both real and symbolic, in the depths of the earth.

Abstracts

IL01 - Novel nanomaterials for the detection of pathogens in microanalytical systems

A.J. Baeumner

Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Regensburg, Germany

Contact: antje.baeumner@ur.de

Abstract

Microfluidic biosensors, labs-on-a-chip and lateral flow assays for the detection of viable organisms, toxins, and clinically relevant markers have been successfully developed in our research group including analytes such as *B. anthracis, C. parvum*, dengue virus, *E. coli, S. pyogenes*, cholera toxin, CD4+ T-lymphocytes, thrombin and myoglobin. We develop a range of nanomaterials focusing on their analytical function. Liposomes e.g. (Figure 1) are designed as a highly flexible signal amplification strategy for bioanalytical sensors. Taking advantage of their large inner cavity for the entrapment of marker molecules, as well as their phospholipid bilayer for modifications with biorecogniton elements, we can lower limits of detection in comparison to other amplification strategies such as enzymes and nanoparticles. Electrospun nanofibers provide an immense potential to enhance bioassays. The great variety of chemical surfaces available and the large surface-to-volume ratio promise to solve challenges of signal enhancement, non-specific binding and analyte pre-concentration. We have therefore studied nanofibers in paper-based lateral-flow assays (LFA) and in microfluidic systems. In the latter, we focus our studies currently on the use of nanofibers as sample preparation material, as immobilization support matrix and as transducer-element. We have demonstrated that bacterial cells can be pre-concentrated through 3D nanofiber mats in microfluidic channels. Nanofiber materials were chosen to selectively isolate *E. coli* cells from solution while preventing non-specific binding. We were also able to functionalize the nanofibers by bearing biotin on the outer surface without the need of any additional chemical step by adding biotin to the original spinning dope. As the nanofibers function as passive mixers they enhance the bioassay not only through their immense surface area but also by decreasing diffusion limitations. New studies integrate signaling molecules directly into the nanofibers to take advantage of their unique capabilities.

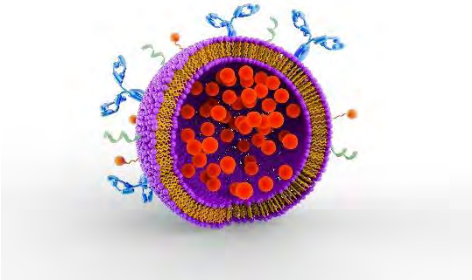

Figure 1 - Schematic of liposomes for use in bioanalytical sensors

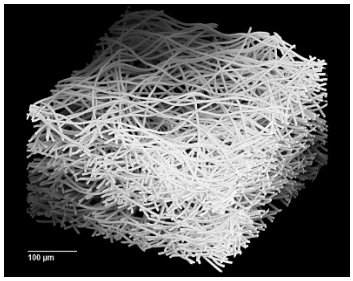

Figure 2 - Model of nanofibers used as separation matrix in microfluidic biosensors

Keywords: Electrospun nanofibers, liposomes, pathogenic organisms, paper-based lateral-flow assays (LFA), microfluidic systems

O01 - Electrospun functional nanofibers for electrochemical microfluidic biosensors

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Abstract

Electrospun conductive nanofibers have become highly relevant in electrochemical sensor development nowadays. In particular, their remarkable features, e.g. high surface-area-to-volume ratio, ease of modification, and simple system integration, make them an excellent candidate for enhancing electrochemical performance as well as promoting miniaturization capability. In the present study, we investigated two different strategies for generating electrospun conductive nanofibers onto indium tin oxide (ITO) electrode from polyaniline (PANI): 1) electrospinning of pre-doped PANI, and 2) electrospinning of non-conductive PANI where a post-doping procedure was performed and designated as pre- and post-doped PANI nanofibers. The latter technique offered a greater nanofiber morphology in a broad range of environmental and electrospinning conditions as well as a novel functionality towards dopamine detection. The conditions for post-doped PANI nanofiber production were thoroughly optimized with respect to the type of acid dopant, acid-dilution solvent, and incubation time. Moreover, additional thermal-treatment of post-doped PANI nanofibers was found to improve their stability. Electrochemical performance and binding stability of acid were mainly characterized by cyclic voltammetry and water contact angle measurements, respectively. Apart from production of electrospun conductive nanofibers we successfully integrated the nanofibers into an electrochemical microfluidic chip by integrating the PANI-ITO electrodes with hot-embossed PMMA microchannel. Further studies will focus on the additional doping with biotin in order to generate facile biological binding moieties on the conductive nanofiber surface. Ultimately, this work will not only provide a robust and powerful tool in advancing analytical applications but also expand towards biomedical research, especially for tissue engineering.

Keywords: electrospinning, conductive nanofibers, polyaniline, electrochemical sensor, dopamine, microfluidic chip

S01 - Rapid and simple surface functionalization strategy for lipid-based membranes

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Abstract

Nanoparticles and nanovesicles such as liposomes are increasingly used for bioanalysis and drug delivery, requiring a simple and reliable surface functionalization strategy to enable specific targeting. The lipid bilayer surface of liposomes renders them highly desirable for such applications, as it promotes biocompatibility and stability in aqueous environments, and allows for a variety of surface modification strategies.

In this study, two different principles for the modification of this lipid bilayer using biotin as model receptor were investigated. Molecules with lipophilic tails bearing the desired surface entity were either added to the lipid composition prior to synthesis, or inserted into the intact lipid bilayer after vesicle formation. In the latter case, three different types of molecules were studied for their ability to efficiently and reproducibly insert into the bilayer of preformed liposomes: a biotinylated lipopeptide, dipalmitoylphosphatidylethanolamine-biotin and cholesterol-biotin.

The functionalized liposomes were characterized and compared with respect to their size, ζ-potential, encapsulation efficiency and binding capability. It was found that in-situ modification of liposomes is a straightforward approach leading to a large number of liposomes, sufficient for thousands of assays, bearing the same characteristics. However, for each surface condition, a new batch of liposomes must be synthesized, which is time-consuming and associated with a certain degree of synthetic unreliability. The insertion strategy provides a solution to these drawbacks as through a simple 1-h incubation any amount of liposomes can be efficiently modified and any variation in the surface tag can be conducted simultaneously and highly reliably. Comparing the three types of insertion molecules, the biotinylated lipopeptide proved to be the most reliable and effective. Lipopeptides with biorecognition elements will pave the road for many analytical applications with liposomes and other lipid-layer approaches.

Keywords: lipid-based membranes, functionalization, liposomes, lipopeptides, biorecognition

IL02 - Engineering silk-like proteins for biosensors

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Abstract

Embracing synthetic biology opens a door to structural and functional materials, inspired by biology but interfacing the biotic/abiotic and with potential to offer new biosensing materials. This presentation looks at emerging bioinspired materials. Silk-like engineered proteins produced in bacteria provide a bulk manufacturing option for new materials, leading people to imagine integrated sensing, therapy, defence or bioremediation and built entirely through assembly of fibrous protein constructs. Protein fibres are natural materials that retain the property of high mechanical strength, but also possess high thermal stability compared to other biological materials. However, silk offers only half the solution since there is no functional element. Synthetic biology provides an alternative pathway for maintaining protein functions while producing the mechanically strong fibrous material.
A ramification of synthetic biology is that nature becomes a starting point for materials, not a definitive source. Honeybee silk from honeybee larvae (*Apis mellifera*) for example, is good in transgenic production. The honeybee silk is encoded by four small genes (AmelF1-4) with low level of repetition in their sequences, which allows them to be expressed in relatively large amounts in recombinant production. Different from many other silk materials, honeybee silk protein adopts an α helical dominant structure with small β sheet domains. It has also been shown that a single recombinant silk protein (AmelF 3) is sufficient to mimic the structural and mechanical property of native protein, so this offers a starting point for introducing functionality to the fibre through protein fusion with an enzyme.
By contrast, the genetic expansions of CAG repeating into different lengths of polyglutamine sequences can also be assembled to form fibres, but in nature appear to derive neurotoxicity. Eight hereditary diseases, including Huntington's disease, have been discovered in relation to this neurodegenerative sequence repeat. Chemically synthesized Q_{15} peptide tends to aggregate into β-sheet-rich short filaments, whereas elongation trends were observed with the assembling from Q_{20} to Q_{42}. Q_{77} creates a readily self-assembled fibre.
The presentation will examine the potential of these materials when fused with enzymes targeting for different analyses. Examples presented include luminescent silk-like fibre biosensors from polyQ or honeybee silk protein that can measure ATP. Other examples look at electrochemical tagging and new ways to use old proteins by engineering the interface between biology and a transduction system. Alternatively, electrochemical functionality can be shown, where the bioelectrode can be used to control enzyme activity and switch the oxidation state or where a silk-like redox protein, pulled in a carbon fibre composite, allows a new kind of enzyme electrode. The opportunity to generate bio-inspired functional materials is opening up new opportunities to extend biosensors in new directions, but this presentation looks at the impact of including a fibrous material as in integral part of an enzyme and considers both the mechanical properties of the materials and the functionality of the enzyme in this novel environment.

Keywords: Silk-like proteins, synthetic biology, honeybee silk, biosensors, luminescence, electrochemical tagging, enzymes.

O02 - Monitoring of sewage biomarkers with community sewage sensors for public health

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Abstract

Wastewater-based epidemiology (WBE) has shown to be an innovative and promising tool for the estimation of community-wide drug consumption[1,2]. WBE focuses on the analysis of drug residues and their metabolites (namely “sewage biomarkers”) excreted by humans in a given area (e.g. a city), which are pooled by urban wastewater systems and transported to a local wastewater treatment plant, from which a wastewater sample is collected for analysis. Compared to the conventional population survey methods[1,2], this approach offers a near real-time and cost-effective evaluation of community-wide drug consumption patterns, with the potential to influence prevention policies for example. To explore the possibility of rapid monitoring of community-wide health, electrochemical sewage sensors have been proposed as novel tools to determine sewage biomarkers, with a potential for a field test[3].

Here we present on electrochemical community sensors for monitoring of sewage biomarkers ranging from small molecules[4] (e.g. illicit drugs such as cocaine, antibiotics), DNA (such as mitochondrial DNA)[5] and protein cancer biomarkers[6], based on the electrochemical (impedimetric and voltammetric) techniques. The immobilization strategy was developed and optimized to achieve a sensitive and selective assay. For examples, a DNA-directed immobilization of aptamer sensors (DDIAS) was proposed for the effective electrochemical impedimetric sensing, and demonstrated to be able to detect both prostate-specific antigen and cocaine at a low level, allowing for quantification of biomarkers in sewage. The assays were validated with classical mass spectrometry. Another voltammetric sensor for mitochondrial DNA in sewage, using a synthesized ferrocene intercalator enables identification of potential population biomarkers associated with cancer in sewage[5].

We demonstrated that electrochemical sensors enable a rapid monitoring of sewage biomarkers at the community level and complement the traditional analytical tools to identify sewage profile for public health.

Keywords: Electrochemical sensors, sewage biomarker, public health, wastewater-based epidemiology

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S02 - Microfluidic spheroid-generation glass platform for integration in a screening system for endocrine disruptors

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Abstract

Endocrine disrupting chemicals (EDCs) are substances which affect the hormone system in organisms through interference with different signalling pathways. They can act as agonists or antagonists on nuclear receptors at concentrations in the picomolar range, and are associated with diseases such as cancer, type 2 diabetes, obesity and developmental disorders. Existing analysis platforms for endocrine activity are generally inadequate, as they either require several sensors to detect this activity, or cannot assess the complex interactions of hormone-active chemicals at all. We are therefore developing a microfluidic screening system to better assess the adverse effects of EDCs on organisms by using hormone-sensitive MCF-7 cells.

The screening platform includes a microfluidic hanging-drop component to generate multi-cellular spheroids, which, once formed, can be automatically transferred to integrated perfusion microchambers for further cultivation. After incubation with medium containing EDCs, the system allows a protein-microarray to be coupled to it, to quantify ten biomarkers which are related to various endpoints of EDC action in organisms. In this way, the effect of potential endocrine disrupting substances on the protein secretion of the cells can be assessed.

The hormone-sensitive epithelial breast cancer cell line, MCF-7, was chosen as the biological model at the heart of the sensing platform. We decided to use 3D-cell culture to better mimic *in vivo* tissue conditions and thus improve the *in vivo* predictability of our assay. The cells are cultured in serum-free, phenol red-free medium to ensure that no hormones or hormone-active substances are present, thus circumventing possible interferences by these compounds on experimental results. In order to avoid leaching of potential hormone-active components or additives from the plastics conventionally used in cell culture, we have developed the microfluidic spheroid-generation platform in glass.

The hanging-drop system (Figure 1) consists of two thermally bonded BOROFLOAT ® glass slides (75.6 x 25 mm). Six hanging drops are formed in the row of 6 holes (wells) in the lower chip. Each pair of holes is connected by parallel channels to a single inlet and outlet for medium and cell introduction, which guarantees a more stable and even drop formation than if six holes were connected to one in- and outlet via one channel. The 6 hanging-drop wells have a diameter of 3 mm for the generation of 3D spheroids in droplets having volumes up to 45 µL drops. For stable drop formation in a hydrophilic glass device, a hydrophobic rim or ring is required around the lower edge of each well, to avoid spreading of the liquid. We have introduced a microfabricated ring made of the inert material, perfluoropolyether (PFPE), to the system. Once cured, using UV light, each ring is attached to the well chip using the epoxy, NANO™ SU-8.

During static operation in a simplified device with drop wells and without channels, we observed up to 40% evaporation of the hanging drops after 24 hours, especially for the outer wells. An incubation chamber with an integrated glass window was thus designed and constructed by 3D printing for operation of the hanging-drop system. The hanging-drop chip system can be inserted and positioned in this chamber to minimize evaporation and detect the spheroids under the microscope. Polystyrene beads (ø 15 µm) were used to mimic cells in the droplets and look at direct effects of the flow on the beads at different flow rates. No effect could be detected at flow rates from 1 to 10 µL/min. Ongoing

experiments focus on the generation and cultivation of MCF-7 spheroids in the glass device and effects on the cells through exposure by EDCs.
This contribution will describe the developed glass hanging drop device and its integration in the screening system as well as the latest results gained for generation and testing of MCF-7 spheroids with EDC.

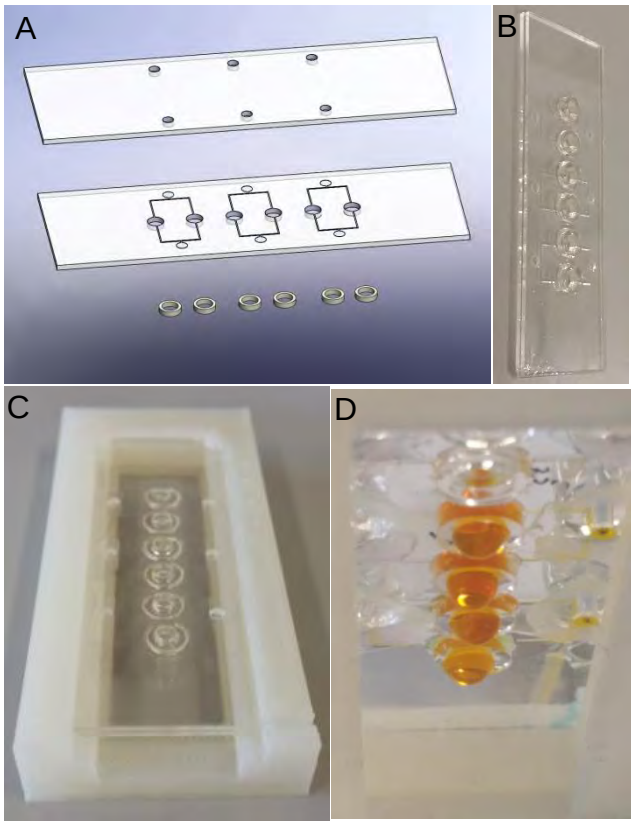

Figure 1 - Microfluidic hanging drop system made of glass with integrated PFPE rings for a better stability of the droplet. A) Single sections of the hanging drop device. B) Prototype of the microfluidic spheroid-generation glass platform. C) Hanging drop incubation chamber with glass device. D) Droplet generation in the glass hanging drop device using stained water.

Keywords: glass hanging drop system, microfluidics, screening system, endocrine disruptor

Acknowledgements: This project is part of the MARIE CURIE ITN (FP7-PEOPLE-2013-ITN) SAMOSS. Additional, we like to thank M.D. Skolimowski for sharing his pre-work for a cell perfusion system.

IL03 - Nanoparticles and UV-C induced DNA damage

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Abstract

The aim of this study was (i) to characterize the ability of selected nanoparticles such as CdTe and CdS quantum dots and gold and silver nanoparticles to transfer energy to proximate oxygen molecules, under the generation of reactive oxygen species (ROS), and (ii) to propose procedures based on biosensor and biosensing for the assessment of a potential risk of the UV irradiation, towards the structure of isolated dsDNA.

A time dependent formation of ROS in solution of individual nanoparticles under the 253.6 nm wavelength (UV-C) treatment was evaluated spectrophotometrically using the appearance of new absorption wave of the indicator 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) at 470 nm and decrease in the absorption waves of α-diphenyl-β-picrylhydrazyl (DPPH) at 350 nm and 550 nm. On the other side, trapping of generated $O_2^{\bullet-}$ by radical scavengers such as quercetin and dsDNA in solution was observed depending on their concentration. The generation of ROS becomes limited under the formation of protein corona at an incubation of metallic nanoparticles with fetal bovine serum.

Electrochemical DNA based biosensors were constructed by a surface modification of conventional glassy carbon electrode by the layer of calf thymus, plasmid or salmon sperm dsDNA and the layer of corresponding nanoparticles. After the UV-C treatment of the biosensors for a given time, cyclic voltammetry and electrochemical impedance spectroscopy in the presence of hexacyanoferrate(III/II), as well as square-wave voltammetry of the guanine moiety, were utilized for the quantification of structural damage to immobilized DNA. The biosensing procedure consisted of UV-C treatment of DNA solution in the presence of nanoparticles, followed by an immobilization of oxidatively damaged DNA onto the glassy carbon electrode and electrochemical measurements. The UV-C enhanced degradation of in the presence of nanoparticles under study has been found depending on their size and fluorescence ability. The electrochemical DNA-based biosensors are shown as effective tools for the estimation of unwanted nanosize effects of nanomaterials towards the nucleic acid biomacromolecule.

Keywords: quantum dots, metallic nanoparticles, protein corona, UV radiation, detection of ROS, DNA damage

This work was supported by the Scientific Grant Agency VEGA of the Slovak Republic (Project No. 1/0489/16).

O03 - High-sensitivity screening of soluble ERBB2 in different cell lines using a combined label-free and fluorescence biosensing platform

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Abstract

ERBB2 is a tyrosine kinase receptor that acts as master integrator of human epidermal growth factor receptor signalling, regulating a variety of cell activities as proliferation, growth and differentiation. In particular, ERBB2 overexpression occurs in approximately 20–30% of breast cancers and it is generally associated with a dismal prognosis, collocating breast cancer as the most common, potentially fatal cancer of women[1].

According to international guidelines, the target therapy based on Trastuzumab is administered when IHC staining either reaches a 3+ intensity, or is at least 2+ and the gene is amplified, as assessed by FISH. This method, although accurate and highly standardized, relies on semi-quantitative, subjective IHC scores, and yields a discontinuous scale. In the present work, we propose a new analytical tool that is able to quantify very low concentrations of ERBB2 cancer biomarkers in biological complex matrices.

In particular, we report on the use of one-dimensional photonic crystal (1DPC) biochips to detect clinically relevant concentrations of ERBB2 expressed in different cell lines. To do that, we have developed an optical platform, combining both label-free and fluorescence detection, which makes use of 1DPC biochips tailored with monoclonal antibodies for highly specific biological recognition. The excitation of a Bloch surface wave (BSW) is obtained by a prism coupling system leading to a dip in the angular reflectance spectrum[2]. Similar to surface plasmon resonance, the measurement of the dip's position shift, due to refractive index perturbations as well as biomolecular interactions at the surface, is exploited for biosensing. Moreover, in presence of fluorescent labels at the 1DPC surface, the platform can interrogate the BSW biochip also in the enhanced fluorescence mode, thus obtaining further information on the cancer biomarker assay and making bio-recognition more robust and sensitive[3]. For the fluorescence operation mode, a limit of detection below 1 ng/mL, about 10 times lower than for the label-free approach, has been reached, thus enabling an ultimate resolution for ERBB2. Such an approach permits us to quantify ERBB2 content in complex matrices and it is used to successfully discriminate cell lysates over-expressing different amounts of ERBB2. The presented method definitely meets international recommendations (15 ng/mL) for diagnostic ERBB2 assays that in the future may help to more precisely assign therapies counteracting cancer cell proliferation and metastatic spread.

Keywords: Optical biosensors, Bloch surface waves, Breast cancer, ERBB2

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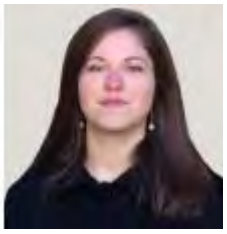
S03 - Bloch surface wave biosensors for real-time study of fibronectin-phosphorylcholine coatings for biomedical applications



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Abstract

In the last two decades, bio-inert based materials for biomedical coatings have been widely studied. The emphasis has only recently moved towards a new generation of biomaterials[1] aimed at improving the interactions with the biological environment. For this reason, the characterization of the interaction of proteins with surfaces is of high relevance to understand the performance of biomaterials. An interesting case is described by molecules with high difference in molecular weight as fibronectin (FN, 450 000 g/mol) and phosphorylcholine (PRC, 181 g/mol). Such biomolecules were largely studied for cardiovascular applications to improve endothelialization and hemocompatibility[2,3]. In the present work, a label-free optical technique was used to quantify adsorbed/grafted FN and PRC. To study such biomedical coatings, one dimensional photonic crystals (1DPCs) supporting a Bloch surface wave (BSW) were used[4]. To excite a BSW, a prism coupler (Kretschmann-Raether configuration) has been used producing a dip in the reflectance; by tracking the position shift of such minimum, it is possible to detect changes in the refractive index as molecular interactions at the interface. FN and PRC were added and changes on the angular position of the dip while in contact with the molecules were related to the mass adsorbed/grafted to the surface. Similar mass densities (in the range of $163 \div 173$ ng/cm^2) were recorded at the 1DPC surface for both FN adsorbed (FNa) and grafted (FNg). Nevertheless, a significant decrease of the total mass deposited was observed after the addition of PRC to FNa, implying desorption of the protein. On the other hand, for FNg, an increase of $7 \div 17$ ng/cm^2 was detected after either adsorption or grafting of PRC. Hence, FNg showed higher stability and better interaction with PRC than FNa. Such results are in accordance with previous characterizations[5]. In addition, BSW biosensors can also work in a combined label-free/fluorescence configuration, exploiting fluorescence features to further confirm label-free results[6]. In conclusion, this approach could give an experimental evidence of the growth of bioactive films in real-time providing a new tool for the further understanding of the biological performance of such a coating for biomedical applications.

Keywords: biomedical coatings, Bloch surface waves biosensors, photonic crystals

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IL04 - Targeted surfaces for cell imaging and sensing applications

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Abstract

The generation and fabrication of nanoscopic structures are of critical technological importance for future implementations in areas such as nanodevices and nanotechnology, bio-sensing, bio-imaging, cancer targeting, and drug delivery. The final aim of any sensing technology is design and fabrication of fully integrated, cheap, portable and reliable single platforms, able to detect and identify simultaneously different molecules in real time with high sensitivity, even at the single cell and single molecule level. Especially, novel materials including nano-structures are sought by chemists, biologists and engineers for a variety of applications. Biological sensing platforms have the ability to interact with the extremely low concentrations of the analyte and this sensitivity makes the usage of biosensors for a variety of applications such as bio-molecular recognition and process monitoring. Here, an overview of our works on the development of novel probes and sensing platforms as well as their use as targeted diagnosis applications will be given in detail.

Keywords: Biofunctional surfaces, Biosensor, Cell imaging

O04 - Driving self-assembly in a microfluidic system for surface enhanced Raman analysis

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Abstract

An advanced optofluidic system for protein analysis based on Raman signal amplification via dewetting and self-assembly of molecules on the surface of a microreservoir is herein presented. A large droplet of micromolar protein solution is allowed to evaporate on the surface of a circular aluminum microwell reservoir at constant temperature, which results in an outward-receding contact line geometry motion through typical stick-and-slip dynamics. The radial convective flows during evaporation carry molecules from drop center to drop edge, where they concentrate and self assembly and these aggregates are eventually deposited after complete withdrawal of the droplet solution, leaving periodic ring-shaped stains of the solute. The enrichment of the protein molecules at the drop-edge can be finely addressed through proper control of the evaporation-rate and kinetics of the process, confining the protein in a local very highly concentrated state, suitable for enhanced Raman detection. If the surface of the microreservoir is coated with a SERS-active substrate of assembled silver nanocubes, the Raman signal of the protein detected at the edge of evaporating drop is further enhanced due to the accumulation and self-assembly of the protein molecules nearby the hot spot regions of the nanocube surface.
The Raman signals of cytochrome C, taken as a model for small proteins with high self-assemblying properties, are increased by a factor of 100 at the edge of the evaporating droplet with respect to the bulk solution on the SERS-active nanocube layer. Analysis of the SERS spectrum of the protein unveils localized reorientation of the heme group of cytochrome C due to a tight packing of the protein molecules at the contact line of the drop between the nanocube surface and the water-air interface.
The evaporation-induced SERS technique proposed herein allows detection and analysis of proteins in their native state with high accuracy and sensitivity and represents a promising method to study self-assembly protein processes. Thereby the SERS microfluidic system presented can give real perspective of applications in several biochemical and biological studies.

Keywords: Self-assembly, Raman spectroscopy, microfluidic device

S04 - Flow injection electrochemical quartz crystal nano-balance based immunosensing platform for detection of 17*β*-estradiol in water

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Abstract

Steroidal hormone 17*β*-estradiol (E2) is a commonly found endocrine disrupting chemical present in the environment and is believed to enter water systems via human and animal waste i.e. urine and faeces. When the concentration of E2 in water bodies or aquatic environment reaches to 1 ng L^{-1} (10-12 mol L^{-1}), it may lead to alter reproductive system, immunological diseases and male fish can be feminized (gender imbalance). The continuation of E2 contamination in aquatic life would affect the human life through food chain even present at trace level. Low levels of E2 in the aquatic environment causes the same endocrine disruption effects in animals as EDCs. In the present work, a label-free piezoelectric immunosensor integrated with flow injection analysis (FIA) has been developed for detection of E2 in water. For construction of immunosensor, the monoclonal antibodies highly specific to E2 (mAb-E2) was immobilized over the surface of gold-coated quartz crystal. Different self-assembled monolayers have tested to get better sensitivity such as 11-Mercaptoundecanoic acid, *L*-Cysteine, 1,4-Dithiothreitol and Cysteaminium chloride. Surface modified with SAMs of 11-mercaptoundecanoic acid (11-MUA) was more stable so further measurements have been carried out using 11-MUA. Immobilization of mAb-E2 was confirmed by fourier transform infra-red spectroscopy (FT-IR), scanning electron microscopy (SEM) and atomic force microscopy (AFM). The recognition of E2 by immunosensor was based on the resonance frequency change (ΔF) due to binding of E2 on mAb-E2 immobilized gold quartz crystal surface compare to the signal of blank recorded (F_o). E2 detection was carried out in the range from 1-200 pg mL^{-1} in binding buffers. A limit of detection (LOD) was found to be 1 pg mL^{-1} (S/N=3) with correlation coefficient (R^2) 0.9902 (n=3) in E2 spiked water samples. The developed immunosensor showed no significant cross reactivity with other structural analogues and existence of high precision and reproducibility strongly recommend its application in other matrices.

Keywords: Endocrine Disrupting Chemicals, 17*β*-estradiol, Piezoelectric, Immunosensor, Water

IL05 - Whole cell biosensors for cytotoxicity and chemosensitivity assays

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Abstract

This lecture will deal with a new method for the complex description of cellular effects to investigate the toxic potential of substances on different cell model lines and biopsies, in a real time, sensitive and high throughput manner[1-4]. In case of the cell lines, the cells were transfected with a reporter/promoter plasmid construct as an early biomarker of stress induction (leading to GFP expression under stress). In this manner, the measurements can be made under incubator-free conditions, without any limitations. In 2D cell cultivation, it was possible to show the cytotoxicity effect at various cell lines and in real time with plant extracts, chemicals, as well as nanoparticles.
The development of 3D cell cultures with Matrigel scaffold and a hepatocyte cell line even more increases the relevance of the sensor towards the human skin, or the behavior of the human organ (see Fig. 1). This is clearly shown in experiments comparing the toxic behavior of nanoparticles in 2D- and 3D- environment.

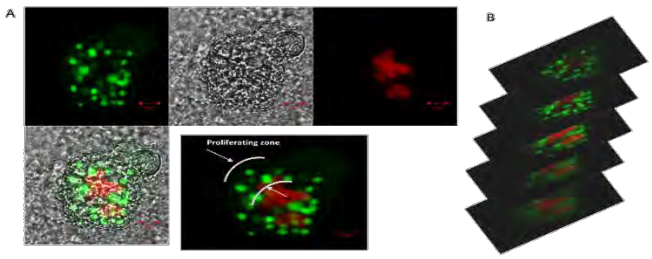

Figure 1 - Cultivation of hepatocytes in 3D matrix (scaffold Matrigel, green living cells, red dead cells)

Keywords: LoC, whole cell-based biosensors, cytotoxicity, 3D cell structure

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O05 - Micro and nanofabricated molecularly imprinted polymers as recognition elements for chemical sensors

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Abstract

Biomimicry is the general term covering any approach aimed at reproducing artificially essential properties of one or more biological systems. This is done in order to exploit natural mechanisms or materials for direct applications in different technological domains, in particular in materials science. At molecular level, one example of biomimetic materials is molecularly imprinted polymers (MIPs), also known as 'plastic antibodies'.MIPs are synthetic receptors that specifically recognize molecular targets[1]. They are highly cross-linked polymers that are synthesized through the polymerization of monomers bearing suitable functional groups, in the presence of the target molecule acting as a molecular template. This templating induces three-dimensional binding sites in the cross-linked polymer network that are complementary to the template in terms of size, shape and chemical functionality. The plastic antibody can then recognize and bind its target with an affinity and selectivity similar to a biological antibody.
We present new approaches allowing for the synthesis of MIP by controlled/living radical polymerization and spatially controlled localized photopolymerization. This allows for example to obtain protein-size, soluble MIP nanogels with a homogeneous size distribution[2]. They show specific binding of their targets, small organic molecules or proteins[3], with a nanomolar affinity and a good selectivity. Since MIPs are compatible with standard micro and nanofabrication techniques, they can also be obtained in any other physical form, and at the same time interfaced with other materials including transducers. The use of these functional nanomaterials for chemical and biosensing[4-8], and for cell and tissue imaging[9] will be discussed.

Keywords: Molecular imprinting, synthetic antibody, biomimicry, nanofabrication

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S05 - Fluorinated surfaces for matrix-free laser desorption ionization

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Abstract

Matrix Assisted Laser Desorption Ionization (MALDI) is a widely used ionization technique with very low detection limit. It is often exploited in peptides and proteins high-throughput mass spectrometric analyses, thanks to its soft ionization nature that induces small fragmentation onto delicate biomolecules. One of MALDI main drawback is that it requires the analyte to be mixed with a proper low molecular weight molecule (called matrix) that promotes desorption/ionization (D/I) processes but it also generates strong interfering signals in the low mass spectral region that might prevents the detection and structural characterization of low molecular weight analytes. Moreover the molar ratio [analyte]/[matrix] is still found using empirical approaches and the inhomogeneous analyte-matrix co-crystallization produces poor shot-to-shot and sample-to-sample signal reproducibility.
The possibility to avoid matrix use will ease both sample preparation and mass spectrum interpretation and it has been investigated for long time.
In recent years nanostructured substrates have been proposed as suitable substrates for matrix-free laser desorption ionization (LDI) measurements.
Here we propose a very simple, fast and highly reproducible method to obtain substrates for matrix-free LDI: the only step needed to detect analytes is the fluoro-silanization of bulk conductive and UV absorbing surfaces. With our bulk substrates the analytes down to femtomolar concentration can be easily detected. This approach has two main advantages compared to the so-far proposed nanostructured chips: (1) nanostructures are no longer required; (2) several materials, once fluorinated, can be used as substrates (e.g. the routine metallic sample holder).

Keywords: laser desorption ionization, bulk surfaces, silanization

IL06 - Fully integrated ready-to-use paper-based electrochemical (bio)sensors

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Abstract

The use of disposable electroanalytical platforms is nowadays widely used for many applications, ranging from the clinical to the environmental surveillance. Although the use of these portable strips (e.g. glucosensors) represents a keystone in the self-monitoring assays, drawbacks related to their production cost and waste removal need to be carefully evaluated. Low-cost and eco-friendly materials are strongly required to address these requirements. Paper-based substrates, due to their properties, are effectively involved in the development of such strips, both as electrochemical or as colorimetric tests[1,2]. The real advantage related to paper-based devices is the possibility to realize reagent/equipment free tools. By pre-loading all the reagents onto the cellulose network, the end-user is capable to perform the analysis without particular expertise. Moreover, paper is of low-cost and lightweight, and this makes the devices highly deliverables to the all those setting-limited countries.

Herein simple approaches to fabricate screen-printed electrodes (SPEs) using paper substrate are described. Depending on the analytical requirement, different types of paper can be adopted in SPE manufacturing: filter paper, office paper, and nitrocellulose; all of them offer a wide range of solutions. Coupled to the well-established wax printing technology[3], screen-printing on paper allows to create diverse sensing devices, just by tailoring the printed electrodes depending on the needs. Paper-based SPEs have been manufactured by using different nanomodifiers such as carbon black (CB) and prussian blue (PB) in order to obtain superior electrochemical platforms, and morphologically and electrochemically characterized.

The analytical suitability of the proposed approach has been evaluated over the quantification of alcohol in beer, phosphate ions in river water, and organophosphorus pesticides in both river and waste waters. Results showed that these innovative, disposable, ready to use device are perfectly suitable for the application in the selected areas, both for sensitivity and handiness. Applications in clinical field such as the measurement of chloride ions in sweat, glutathione in whole blood and butyrylcholinesterase in serum are in progress.

The herein proposed manufacturing approach offers an affordable and sustainable tool in order to realize highly variable electrochemical sensors and biosensors.

Keywords: Paper-based (bio)sensors, ready-to-use, nanomaterials, electrochemistry

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O06 - Nanofunctionalized interfaces for Surface Plasmon Resonance based immunosensors

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Abstract

Surface Plasmon Resonance (SPR) based immunosensors is an ever growing area of research in the development of high-performance diagnostic devices for point-of-care analysis. Immunosensor based on antibody-antigen binding is a promising method for the detection of disease biomarkers, food pathogens, explosives and environmental contaminants due to its high sensitivity, selectivity and feasibility for miniaturization.

Despite the active research on biosensors around the world, most of them have limitations with respect to portability and real-time applications. One of the major challenges in biosensors development is the fabrication of immunosensor by the immobilization of ligands/biomolecules on the transducer surface. Self-assembly method is one of the most promising route for simple and highly effective immobilization of biomolecules. Self-assembled monolayers offer several attractive features such as bare minimum resources, easy miniaturisation, high degree of order and control, ability of mimicking the cellular microenvironment and chemical stability for high-performance applications. The functionalization of gold surfaces by organic molecules containing free anchor groups such as thiols, disulphides, amines and silanes allows tremendous flexibility for functionalization with respect to their terminal groups such as hydrophilicity, hydrophobicity and chain length.

Here, we have demonstrated the fabrication of immunosensors based on self-assembly method. The SAM functionalized surfaces have been explored for the detection of clinically relevant molecules such as dengue, dopamine and metal ions with suitable functionalization. Optimization of the self-assembled surfaces, biomolecule immobilization and blocking of the free active groups have been performed by using SPR and electrochemical techniques. The results are compared with physically immobilized bio-molecules with respect to sensitivity and stability. The results showed good performance of the SAM interfaces for highly sensitive and selective detection of analytes down to ppb level with good reproducibility and storage stability. The results suggest that the SAM based immunosensor interfaces have promising applications in point of care analysis.

Keywords: Surface Plasmon Resonance, immunosensor, Self-assembled monolayer

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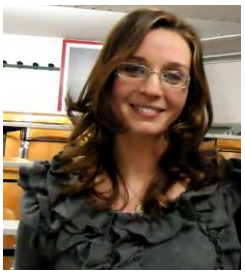
S06 - New quinoline-based chiral ligands and their Europium(III) complexes



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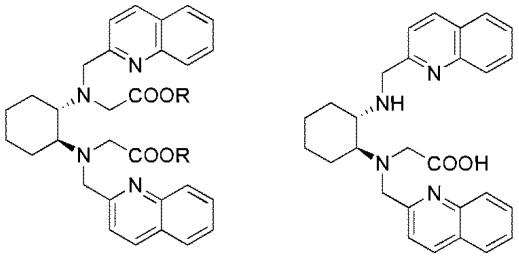
Abstract

Luminescent complexes of Eu(III) and Tb(III) soluble in alcohol and water have been extensively exploited in biomedical field, as their excited states (in particular the ones of Tb(III) ion) are less sensitive to non-radiative vibrational quenching caused by high energy oscillators (such as OH)[1]. This behaviour gives rise to reasonable values of the luminescence quantum yield. In addition, when a lanthanide complex is designed for biomedical applications, a strong overall luminosity or Brightness (B) is required, $B = \varepsilon \cdot \phi$, where ε is the molar absorption coefficient and ϕ the luminescence quantum yield. B can be increased if the ligand is capable to strongly absorb the exciting light and efficiently transfer the excitation energy to the lanthanide ion (antenna effect). This may be ensured by the presence in the ligand backbone of a suitable heteroaromatic fragment able to bind the metal ion. The ligand should be also capable to hamper the intrusion in the inner coordination sphere of solvent molecules, which would increase non-radiative quenching phenomena of the Ln(III) excited state.

In the presence of a chiral Ln(III) environment, the metal may also display circularly polarized luminescence (CPL), a chiroptical phenomenon, which has found interesting applications such as chirality sensing[2,3] and medical imaging techniques[4].

In this contribution, we focus our attention on the synthesis of new chiral ligands containing the quinoline fragment (Fig. 1) and their Eu(III) complexes, following by characterization with the luminescence spectroscopy in the solid state and in solution of alcohol and water.



R = alkyl group or H

Fig. 1. Molecular structure of the ligand discussed in the present contribution

The low symmetry of the ligand (C1 point symmetry) guarantees a strongly distorted Eu(III) geometric environment and the predominance of the $^5D_0 \rightarrow {}^7F_2$ hypersensitive band, both in the solid state and in alcohol solution, as documented by the inspection of the Eu(III) luminescence emission spectra. Further, the decay time of the $^5D_0$ Eu(III) luminescence for all the complexes falls in the ms range and the heteroaromatic quinoline antenna efficiently sensitizes (around 320 nm) the red luminescence of this lanthanide ion.

All these peculiarities candidate this complex for applications in the biomedical field where stability and solubility in protic polar solvent is strongly required.

The presence of several easy displaceable solvent molecules in the inner coordination sphere of the metal ion, candidates these complexes for applications in the field of sensing as potential luminescent probes of important analytes.

Keywords: Eu(III) complexes, chiral ligands, quinoline fragment, biomedical appliacation

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IL07 - Point-of-care paper based analytical device detecting highly conserved DNA sequence of Dengue virus using ZnO multipods

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Abstract

An electrochemical paper analytical device (EPAD) based genosensor consisting of complex shaped ZnO nanostructures and microstructures (ZnO multipods) was fabricated for detection of consensus DNA sequence of Dengue virus (DENV) using methylene blue (MB) as an intercalating agent for sensing DENV DNA. To achieve it, probe DNA (PDNA) was immobilized on the surface of ZnO multipods at EPAD. The synthesized multipods were fully characterized. This PDNA modified electrode (PDNA/ZnO/EPAD) served as a signal amplification platform for the detection of the target DNA (TDNA) of dengue. The hybridization between PDNA and TDNA was detected by reduction in current, generated by interaction of MB with free guanine (3'G) of ssDNA. The detection range was wide from 0.1 nM to 10^5 nM. The present sensor shows a high degree of specificity towards DENV. Furthermore, this genosensor is able to detect all types of dengue. The developed paper based sensor showed many advantageous features such as facile preparation, low sample volume, homogenous distribution of nanoparticles onto the surface and economic.

Keywords: Conserved sequence, genosensor, Dengue virus, ZnO multipods, DPV, CV

O07 - Molecularly imprinted polymers as bio-mimic sorbents for highly selective solid phase microextraction of triazine herbicides in crops

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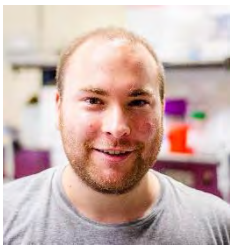
Abstract

Molecularly imprinted polymers (MIPs) are bio-mimic materials which can selectively bind to analytes. MIPs based extractor phases have demonstrated superior sensitivity and selectivity. In our past studies these polymers have shown excellent potential in solid phase micro-extraction (SPME) of atrazine (chloro-triazine) and ametryn (methylthiotriazine) as two hazardous triazine herbicides. The aim of this work is further study of previously published works by use the mixture of two templates (ametryn and atrazine) in structure of fabricated imprinted polymer with dual templates to clarify the effects of this change on selectivity of fabricated fiber. A simple polymerization method was used to produce intended polymer which can be coupled with GC and GC/MS. Methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA) and mixture of ametryn and atrazine have used as functional monomer, cross-linker and dual templates, respectively. The overall results in optimum conditions showed an array molecular recognition for selective micro-extraction methylthiotriazine as well as chloro-triazine herbicides. These results clearly showed the effect of used dual templates in MIP structure. The obtained detection limits were shown good array recognition for group of triazine herbicides as 9, 13, 16, 8, 17, 19 and 16 ng mL^{-1} for ametryn, prometryn, terbutryn, atrazine, simazine, propazine, and cyanazine respectively by GC-FID detection. The reliability of the prepared fiber for extraction of analytes in real samples has been investigated by using spiked crop samples such as cucumber, onion, rice and maize.

Keywords: Molecularly imprinted polymers, Methacrylic acid, ethylene glycol dimethacrylate, ametryn, atrazine, GC-FID, crop samples

S07 - Multiple electrochemiluminescence detection for biosensing



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Abstract

Research is presented focusing on a novel microfluidic biosensor for the detection of pathogenic organisms. Electrochemiluminescence (ECL) is a highly sensitive analytical technique that combines advantages from electrochemical and luminescence principles, i.e. requiring simple hardware and offering lowest limits of detection as no background signal occurs. Here, we studied the possibility of dual detection based on commercially available ECL reagents and realizing it in a simple polymer-based microfluidic system. Luminol and Tris-(2, 2'-bipyridyl)ruthenium(II) chloride ($Ru(bpy)_3^{2+}$) were used as ECL compounds, ITO and gold as electrode material. It was found that luminol-based ECL provided the same low limits of detection on gold and ITO electrodes around 20 ± 1 nmol/L. For $Ru(bpy)_3^{2+}$ ECL, the same limit of detection was obtained for ITO electrodes, but only 40 nmol/L on gold electrodes. Different surface modifications were tested for enhancement of ECL signals and long-time electrode stability on ITO electrodes. In order to further lower the possible limits of detection when analyzing DNA molecules, liposomes were synthesized to entrap both ECL markers. Ruthenium liposomes were shown to be highly stable over more than a year at 4 °C. However, luminol could not be entrapped within the inner cavity of the liposomes. Therefore, a more hydrophilic derivative was synthesized, meta-carboxy-modified luminol (*m*-COOH luminol) which exhibits outstanding ECL performance with a 4-fold overall signal increase compared to standard luminol. Furthermore, it has a high solubility (approximately 20 mM) in water at pH 7.0 in contrast to luminol, which is not soluble under these conditions. Hereupon, stable, luminescent liposomes entrapping *m*-COOH luminol were obtained. These were used in a microtiter plate-based sandwich hybridization assay for a highly sensitive detection of the model analyte *Cryptosporidium parvum* with an excellent detection limit of 3.2 pM. Currently, both ECL-liposome systems are under investigation for the detection of pathogen DNA sequences within the microfluidic device.

Keywords: ECL, Biosensor, Microfluidic, Multiple Detection

IL08 - 2D and 3D Lab-on-a-Chip systems for environmental and life science applications

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Abstract

2D and 3D Lab-on-a-Chip (LOC) and microfluidic systems will be presented that can be applied in different biomedical and environmental biosensing- and organ-on-a-chip applications. Microfluidic systems are designed with two basic modules: (1) a motherboard equipped with fully integrated pumping, valving and sample/waste reservoirs, (2) an exchangeable microfluidic chip designed to fit the desired application. 2D chips are equipped with a planar electrode array explored for intra and extracellular events using voltammetric and/or impedimetric detection. 3D systems are equipped with different scaffolds for support and culturing of cells, having: (a) structured perfusable channel network, enabling delivery of necessary nutrients and oxygen to the interior of the scaffolds, (b) secondary more arbitrary random porous network that can enclose a hydrogel phase with a “nearby” source of important cell factors, supporting the growth and differentiation of cells, (c) ability to conduct, or sense, electrical currents. Examples of 2D and 3D systems applied in relation to drug delivery, Parkinson´s disease and environmental application, e.g. biofuel cells or biophotovoltaics, will be presented.
The applicability of the presented systems spans from enzyme/antibody based biosensor array systems for environmental and diagnostic biomedical bioanalysis to cell- and organ on chip system for better *in vivo* mimicking toxicity or drug screening.

Keywords: Lab-on-a-Chip, microfluidic systems, voltammetry, impedance, biosensors, biomedical and environmental applications.

O08 - A multiplexed and multimodal biobarcode, simultaneously tailored detection of small molecules and proteins

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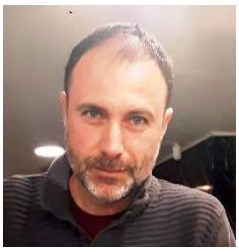
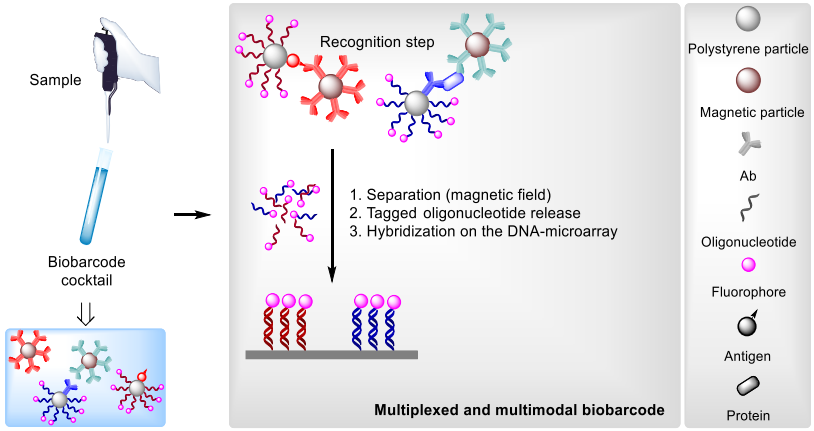


Abstract

For the first time, we report a multiplexed and multimodal NP-based biobarcode assay. The proposed strategy is based on: (1) encoded polystyrene particles (ePSP) functionalized either with an antigen, for a competitive assay format, or an antibody, for a sandwich assay format, (2) magnetic particles (MP) functionalized with specific antibodies and (3) DNA-microarray for the selective quantification of each coding oligonucleotide.



Thus, within this assay design we could tailor two calibration curves for acenocoumarol (ACL) and C-reactive protein (CRP), cardiovascular disease (CVDs) related compounds. Each of them presented a different type of assay format, indirect competitive (LOD 3.6 nM) and sandwich (LOD 78.4 ng/mL) respectively. In addition, the platform capability for quantifying spiked buffer has been demonstrated reproducible, obtaining recoveries around 110 and 96%. Samples were spiked within human sample concentration range and analyzed within a sole dilution for both compounds. Thus, we provide a tool to quantify compounds of different chemical nature within human sample requirements in one single run.

Keywords: Biobarcode, multiplexed, multimodal, cardiovascular disease

S08 - Enhanced biocide power of Cu/Zn/Co mixed oxides towards *E. coli*

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Abstract

E. coli are a large and diverse group of bacteria. Most species are harmless and usually inhabit human and animal gut of healthy people. Some serotypes are instead pathogenic and are able to cause bloody diarrhea, kidney failure and even death. Common conveying pathways of E. coli are undercooked ground beef, raw milk, juice, fruit, vegetables as well as contaminated water. Single oxides nanoparticles, expecially CuO and ZnO, proved to be an efficient tool to reduce E. coli concentration in water[1]. Here we present the enhanced effects of synthesized nanosized mixed Cu/Zn and Cu/Zn/Co oxides against E. coli in water. The mixed oxides were synthesized from hydroxycarbonates precursors and fully characterized by XRD, IR and SEM, hence their structure and size were determined.

Afterwards, the nanoparticles were dispersed thoroughly in a culture medium and E. coli bacteria were cultivated on Tryptic Soy Agar plated containing different concentrations of metal oxides nanoparticles (50-100-200-400 mg/ml). Bacteria were counted in terms of colony forming units (CFU). E. coli counts decreased in the culture medium containing metal oxide nanoparticles and the dose-response relationship was calculated. In general, all mixed oxides display better performances as compared to the corresponding single oxides. Furthermore, the ternary mixed oxide Cu/Zn/Co oxides nanoparticles display a higher biocidal power than the binary Cu/Zn ones. Tests were performed also in comparison with single oxides mechanically mixed in the same proportion of the solid solutions of mixed oxides, with systematic lower performances. The bacterial inhibition rate increases linearly with the concentration of nanoparticles, the highest viability reduction values (> 99%) being obtained at the highest doses.

Cytotoxicity tests were performed on the single, binary and ternary oxide which displayed the highest biocidal power and revealed a level of toxicity comparable for all the nanoparticles, regardless of their composition.

Keywords: Cu/Zn/Co, Mixed oxides, Escherichia coli

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IL09 - Challenging the threats of multiplexation through nanobiotechnological approaches

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Abstract

The so-called “omic” technologies point to a future in which the health status of an individual will be better defined by molecular signatures or footprints than for the analysis of single biomarkers. Hence, there is a need to develop new diagnostic technologies that respond to this multiplexation demand while providing results in a rapid, reliable and efficient manner. The microarray technology permits the simultaneous detection of different substances, mainly through spatial codification of the target analytes. Technological advances in micro (nano) biotechnology have allowed implementation of novel transducing schemes and miniaturization increasing even more the efficiency and the number of substances to be analyzed in a single chip. However, besides genomics, microarrays for peptide, protein or small molecule analysis represent a big challenge. The analyses of these substances is not as straightforward as DNA technology due to the molecular variability and complex nature of proteins (different hydrophobicities, acidic or basic characters, functionality, etc.). Unlike nucleic acids, which are relatively homogeneous in terms of structural and electrostatic properties, proteins can be extremely diverse regarding chemical structure and biological properties. Preventing protein denaturation and maintaining structural conformations are key issues in microarray technology. This is the reason because DNA microarrays are much more standardized. Moreover, simultaneous measurement of different chemical entities is often difficult to accomplish, because of their particular features, the need to use different immobilization chemistries or even distinct assay protocols. On top of this, there is the fact that often, target analytes are present at different concentration ranges, being impossible to analyze them in the same run, being necessary either to dilute the sample or to concentrate it depending on the detectability that needs to be accomplished. In this communication, several nanobiotechnological approaches to face these challenges will be presented. Thus, DNA-Directed Immobilization (DDI) may allow circumventing the limitations associated to immobilization of distinct biomolecules in a single chip. On the other hand, biobarcode assays based on the use of a DNA transduction scheme may provide the possibility to modulate signal and to analyze different chemical entities simultaneously.

Keywords: biomarkers, multiplexation, DNA microarrays, nanobiotechnological approaches, biobarcode assays

O09 - Multiplexed analytical platforms based on the use of antibodies for monitoring pollutants in marine environment

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Abstract

The development of novel methodologies for environmental monitoring sea water contaminants has gained prominence for quality assurance in aquaculture and protect marine biodiversity. The aquaculture industry represents nowadays the 20% of the total fish production with an expected increasing of this percentage in the following years. To ensure food safety and quality it is essential to guarantee environmental health. In this way, the Marine Strategy Framework Directive (MSFD) and the Water Framework Directive (WFD) support the development of new technologies for monitoring pollution and management of good practices in environmental vigilance and food safety. Immunochemical techniques are based on the use of antibodies as a biorecognition element for the sensitive and specific recognition of the targeted pollutant. These kind of techniques can be use as alternative or complementary tools in analytical chemistry, to lighten the great amount of analysis that have to carry out with less cost. Moreover, allow configurations for on-site monitoring not requiring highly qualified personnel and high-cost equipment.
In this communication we will present the work performed to develop distinct multiplexed immunochemical analytical platforms will be presented. Thus, ELISA, fluorescent microarray and electrochemical sensors have been developed to simultaneously detect the presence of pesticides, antibiotics, hormones, persistent organic pollutants or marine toxins in aquaculture facilities.

Keywords: antibody, amperometric immunosensor, microarray

S09 - Biotransformation of dinitrotoluene by *Escherichia coli* and its implications for the detection of trace explosives

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Abstract

DNT (2,4-dinitrotoluene), a volatile impurity in military grade TNT-based explosives, is a potential tracer for the detection of buried landmines and other explosive devices. An *Escherichia coli*-based bioreporter strain engineered to detect traces of DNT was recently designed in our laboratory, but the pathway by which this bacterium utilizes DNT has not been deciphered. The present study aims to characterize the biotransformation of DNT by *E. coli* and identify the DNT metabolite which induces the *yqjF* gene promoter, which is the sensing element of the above mentioned bioreporter.
To pursue these objectives, tools from the fields of molecular biology and analytical chemistry were employed in tandem. Several DNT metabolites originating from reductive biotransformation of DNT were identified by liquid chromatography coupled with mass spectrometry (LC/MS), and the response of the *yqjF*-based bioreporter to these products was investigated. 4-hydroxylamino-2-nitrotoluene (4HA2NT) was shown to be a direct but weak inducer of *yqjF*, while trihydroxytoluene, a downstream metabolite of 4HA2NT, appears to be the main inducer of this gene.
In order to identify the enzymes involved in the biotransformation process, the influence of single and double gene deletions on both the response of the *yqjF*-based bioreporter to DNT and the accumulation of DNT biotransformation products was investigated. Several enzymes involved in this process were identified, including the nitroreductases NfsA and NfsB, and their role in the biotransformation of DNT was characterized.
To further investigate the bacterial response mechanism to DNT we performed a transcriptome analysis (RNA-seq). Several genes were shown to be significantly upregulated in the presence of DNT, and their involvement in the process is being investigated. One of these genes, the azo-reductase *azoR*, was shown to be upregulated in the presence of DNT significantly more than *yqjF*. Therefore, this gene was selected as a candidate for a new generation of bioreporters for explosive detection. The response of this gene to a spectrum of compounds was tested and its role in the cell's response to DNT is investigated. Finally, we employed random mutagenesis on the promoter region of this gene in order to obtain clones with enhanced response to DNT.

Keywords: Bioreporter, dinitrotoluene, landmine, explosive

IL10 - Nanoelectrodes ensemble: a versatile platform for biosensing

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Abstract

Since the first reports dating back to the mid-1990s[1-3], ensembles and arrays of nanoelectrodes (NEEs and NEAs, respectively) have gained an important role as advanced electroanalytical tools. Their unique characteristics which include, beyond the typical advantages of electroanalytical devices, a strongly improved signal/noise ratio, enhanced mass transport and suitability for extreme miniaturization. From the year 2000 onward[4,5], these properties have been exploited to develop electrochemical biosensors in which the surfaces of NEEs/NEAs have been functionalized with biorecognition layers using immobilization modes able to take the maximum advantage from the special morphology and composite nature of their surface.
In this communication we present an overview of this field, beginning from the different nanofabrication methods and the operating principles of NEEs/NEAs, focusing on the more relevant features for the development of highly sensitive and miniaturized biosensors. Successively, an overview of the work done by our group dealing with bioanalytical and biosensing applications of sensors based on biofunctionalized arrays/ensembles of nanoelectrodes is presented.
With this communication we would like to provide information on fundamentals of the utilization of this versatile platform and stimulate new ideas for future developments.

Keywords: Nanoelectrodes, ensembles, arrays, electrochemical biosensors

O10 - Electrospun nanofibers as advanced recognition materials in test stripes for optical quantitation of biogenic amines in food

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Abstract

Biogenic amines (BAs) have long been identified as an ideal marker for the determination of quality and safety of food, i.e. as freshness indicator. Especially in protein-rich or fermented foods, microorganisms produce BAs by decarboxylation of amino acids or amination of carbonyls. As BAs can cause food poisoning, even at concentrations well below the odour threshold of the human nose, rapid and inexpensive methods for their overall quantitation are highly desirable. Therefore, test stripes are well regarded as a rapid pre-screening alternative before more time-consuming and expensive separation methods are chosen for suspicious samples. We introduce electrospun nanofibers doped with a chromogenic and fluorescent amine-reactive dye (Py-1) as a new sensor matrix in test stripes for quantitation of biogenic amines in foods with most inexpensive instrumentation.

For this purpose, we embedded 2 mg/mL of Py-1 in a 18% cellulose acetate matrix that was electrospun for 30 min under optimized conditions on ITO as a transparent substrate. CA forms a uniform anionic fiber mat with high surface-to-volume ratio. The fiber matrix not only extracts cationic BAs from real samples but also provides reactive sites of Py-1 to transduce the concentration of BAs into a fluorescence signal. Test stripes are cut and an extract of a real food sample is dropped onto the stripe. The BAs contained convert the weakly blue fluorescent pyrylium dye Py-1 into a strongly red emitting pyridinium dye. After development in an ethanol chamber a digital image is taken upon fluorescence excitation with a simple UV lamp. A linear calibration plot for the BA-content is obtained against the fluorescence ratio extracted from the red channel divided by the one from the blue channel of the digital image, respectively. The quantitation range of BAs is from 10-500 µM and matches the permitted limits for BAs in foods. The stripes show very similar sensitivity towards various BAs like Histamine, Putrescine, Tyramine, Cadaverine or Spermidine. Most importantly, there is no response towards secondary and tertiary amines. Therefore, the stripes are well suitable for determination of the total BA content in foods which was demonstrated with several seafood samples.

This shows that electrospun nanofibers can provide a favourable matrix for test stripes for foods due to their ease of production, analyte extraction and signalling properties. Their in-field applicability to real samples with simple instrumentation suggests further applications in packaging sensor technology.

Keywords: Electrospun nanofibers, Test stripes, Biogenic Amines, Food

S10 - Electrochemical bismuth-modified printed sensors as sustainable tools to study the remediation capability of novel carbon-nanotube sponges towards Pb^{2+} and Cd^{2+} polluted waters

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Abstract

The intensive global industrialization and urbanization have progressively polluted the environment, degrading water quality. The parallel growth of clean water demand highlights the importance to study synergic solutions to tackle this pervasive worldwide problem. Among pollutants, heavy metals such as lead (Pb) and cadmium (Cd) have been included in the list of 33 Priority Substances of Directive 2008/105/EC, because of their highly toxic effects on living beings. Monitoring and remediation plans are needed to face the pollution issue. Besides the official spectroscopic methods, square wave anodic stripping voltammetry (SWASV) has been widely used for heavy metal detection at trace levels. SWASV combined with miniaturized electrochemical sensors pave the way to the development of sustainable tools for in field analysis. Carbon-nanotubes (CNTs) are suitable candidates for water purification, as they have shown notable capability to adsorb several chemicals, including heavy metals[1,2]. Hence, a novel material, based on CNTs 3D network with a sponge-like structure (3D-CNTs), has raised great interest because of its high surface-to-volume ratio. It has been shown that these sponges can remove oils and organic compounds from water, and then be regenerated by squeezing or incineration[3].

Herein, our goal was to develop an electrochemical sensor to evaluate the capability of 3D-CNTs to remediate Cd^{2+} and Pb^{2+} polluted water. Heavy metals were measured by SWASV analysis with Nafion®-coated screen-printed electrodes, modified *in situ* with a bismuth film, which have demonstrated their suitability for Cd^{2+} and Pb^{2+} monitoring in previous studies[4,5]. Firstly, the formation of the bismuth film was deepened to improve the sensor working stability, while the storage stability was examined by storing the Nafion-coated sensors at different temperatures and humidity. Thus, the sensor was applied for the measure of Cd^{2+} and Pb^{2+} in water samples filtered using 3D-CNTs. The filtering system was composed by a siringe for the withdrawal of the sample and a tailor-made cell fabricated with 3D printing to allocate the 3D-CNTs. About 500µL of Cd^{2+} and Pb^{2+} standard solutions were flowed through the cell containing 16 mg of 3D-CNTs. A percentage of decrease of 40±4% and 46±5% was obtained for 60 ppb of Cd^{2+} and Pb^{2+}, respectively. According to these preliminary results, great progress can be expected by using printed sensors combined with 3D-CNTs in water monitoring and treatment.

Keywords: Heavy metals, Water remediation, Carbon-nanotubes, Bismuth-based electrodes

IL11 - Electrochemical biosensors based on 2-dimensional nanomaterials

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Abstract

Recent advancements in nanotechnology, availability of variety of nanomaterials ranging from “quantum dots to graphene” with unique electronic, catalytic and optical properties opened up a route for constructing novel transducing platforms for a variety of biosensing applications. Herein, we discuss the biosensor systems that employ graphene as the transducer. Graphene is a one atom thick 2D allotrope of carbon with excellent mechanical strength, high elasticity and thermal conductivity. Further, at room temperature graphene exhibits the highest electron mobility, tunable band-gap and tunable optical properties. Moreover, the large surface area of graphene provides large number of defects and thereby it has a large number of electroactive sites. For all these reasons, graphene is the material of interest for several electrochemical biosensing applications. For example, graphene electrochemical biosensors find potential application in security, environmental analysis and in point of care analysis of diseases. Herein, we discuss the method employed for the design of graphene based biosensors that finds potential biomedical application such as neurotransmitters detection, biomarkers detection and DNA mutations analysis to identify chronic diseases such as cancer, HIV etc.

Keywords: electrochemical biosensor, nanomaterials, graphene, biomedical application

O11 - Tricks of the trade: molecular enhancements of microbial bioreporters' performance

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Abstract

The relative ease by which molecular sensing and reporting elements can be fused together in microbial whole-cell biosensors to generate dose-dependent quantifiable physical (luminescent, fluorescent, colorimetric, electrochemical) responses to pre-determined conditions allows the construction of diverse classes of sensors. Over the last two decades we and others have employed this principle to design and construct microbial bioreporter strains for the sensitive detection of either (a) specific chemicals of environmental concern (e.g. trinitrotoluene), or groups of compounds sharing either (b) chemical characteristics (e.g. heavy metals, halogenated organics etc.) or (c) global biological effects on living systems (e.g. toxicity or genotoxicity). In many of these cases, additional molecular manipulations beyond the initial sensor-reporter fusion may be highly beneficial for enhancing the performance of the engineered sensor systems. In the present communication we highlight several of the approaches we have adopted over the years to achieve this aim. These include random mutations induced in a "directed evolution" process, splitting of the lux reporter cassette, introduction of viral "amplifiers", manipulation of host permeability and the integration of quorum sensing elements.

Keywords: Microbial bioreporter, sensor-reporter fusion, whole-cell biosensors, environmental pollutants, molecular manipulations

O12 - Molecularly imprinted polymer decorated nanoparticles for selective determination of tetracycline in milk

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Abstract

The antimicrobial residual contamination has gained significant attention due to their indispensable uses in dairy, food and agriculture field. Their residual presence in food cause apparent toxic effect and allergic reactions. Based on their characteristics features, molecularly imprinted polymeric nanoparticles (MIPNPs) have greatly advanced the field of food and environmental analysis. MIPNPs, which allow the specific recognition coupled with suitable transducer, are now widely used for development of sensors in food, environmental and diagnostics. Herein, a novel technique for the preparation of MIPNPs based on radical polymerization method has been demonstrated for tetracycline (TET) analysis. Effect of monomers, photo initiator and porogen were quantified to study the binding interactions. The MIPs were subsequently decorated on magnetic nanoparticles for ease of separation. The characterization and performance of prepared MIPNPs were evaluated utilizing particle size analysis (PSA), scanning electron micrograph (SEM), UV-Vis and Fourier transform infra-red spectroscopy (FT-IR). MIPNPs showed better performance in terms of significantly reduced reaction time, enhanced binding efficiency and low solvent requirement over other reported thermal polymerization methods. Post optimization of experimental conditions, the MIPNPs showed rebinding capacity of 85.40 ± 3.90 % (n=4) against TET. Obtained recoveries were in the range 92.00- 95.60% with maximum percent relative standard deviation (% R.S.D.) 5.44% (n=3) from TET spiked milk samples.

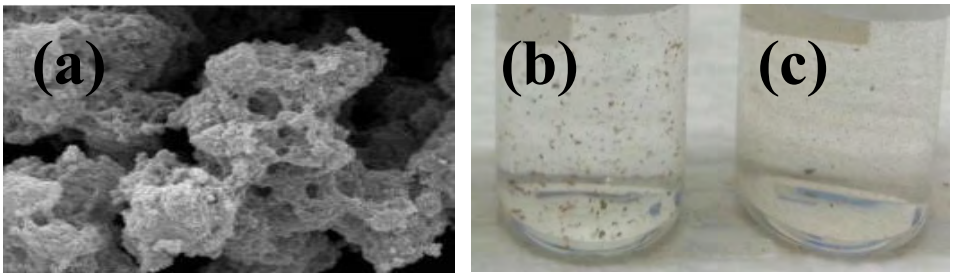
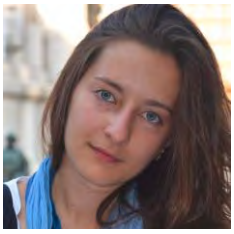


Figure 1. **(a)** Scanning elelctron micrograph of MIPNPs after extraction; **(b)** MIPNPs before separation; **(c)** MIPNPs after separation

Keywords: Molecularly imprinted polymers (MIP), Tetracycline, Radical Polymerization, Milk

S11 - Mechanistic modeling as an effective approach for the understanding and optimization of biosensor performance



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Abstract

The design, optimization and further integration of biosensors in small scale reactors, such as microbioreactors (MBRs), is an adequate solution addressing the current analytical needs in biotechnology. It holds a great potential for the development of rapid, high-throughput, and cost-effective screening technologies, as well as synthesis of valuable chemicals. Therefore, providing novel data interpretation methods and supporting software is a crucial step towards a significant progress in this field. In this work, we present the mechanistic model constructed for interpretation of the multi-layer glucose biosensor response under cyclic voltammetry conditions. The mechanistic modeling approach was applied to the first generation glucose biosensor, presented here as a spatially one-dimensional dynamic system composed of three layers (mediator, enzyme/membrane, diffusion) deposited on the surface of the working electrode (Figure 1). The derived non-linear second order partial differential equations describe the diffusion and reaction of the involved (bio)chemical species inside the enzyme/membrane layer, as well as electrochemical reaction on the electrode surface. All the governing equations were processed by the finite difference method and solved numerically, using the implicit Euler method.

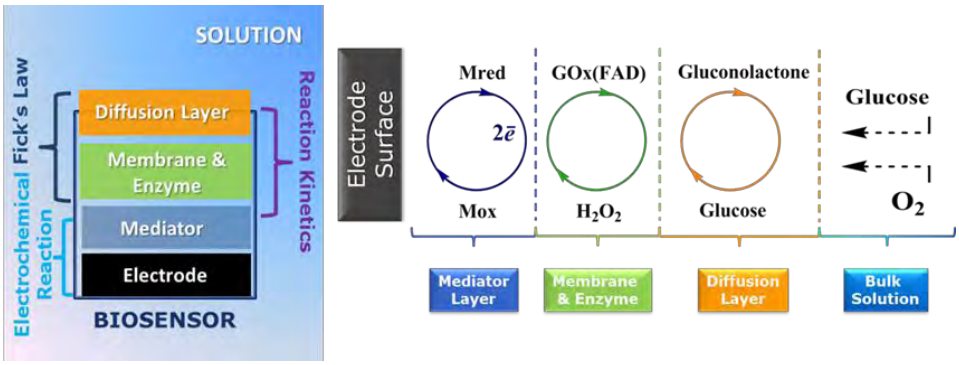


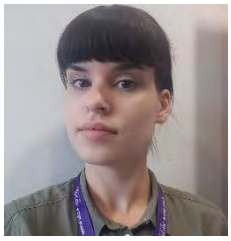
Figure 1 – Schematic representation of the biosensor design and operation: $GO_x(FAD)/GO_x(FADH)_2$ – oxidized/reduced forms of glucose oxidase, M_{ox}/M_{red} - oxidized/reduced forms of Prussian Blue.

The developed model shows a good qualitative and quantitative response towards the obtained experimental data. It allows studying the evolution of the reacting species concentrations as well as the electric response (current) of the sensor for amperometric measurement and cyclic voltammetry. Using an existing Matlab™ toolbox developed at DTU, the model was subjected to uncertainty and sensitivity analysis with the aim of mapping the relative importance of the model parameters. The presented mechanistic model approach enables to get better insights in the bioprocess and electrode performance. It can be used to estimate the mechanism of the enzymatic kinetics together with the

electrochemical reaction. Comparison of the simulation results and experimental data with subsequent uncertainty and sensitivity analysis leads to development of a powerful tool. Such tool estimates the correlations between the system parameters and their influence on the biosensor response, which can be further used to optimize the biosensor performance.

Keywords: Mechanistic modeling, cyclic voltammograms, glucose biosensors, amperometric biosensors

P01-Y - Enzymatic quantification of glyphosate herbicide using a colorimetric PD-CMOS platform



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Abstract

Glyphosate is currently the most intensively used herbicide worldwide for weed control. Due to contrasting claims with respect to the safety of glyphosate, the European Union has initiated an investigation into the carcinogenic potential of this agrochemical. To this end, our work focused on developing an enzymatic biosensor for glyphosate quantification using a photodiode complementary metal oxide semiconductor (PD-CMOS) platform. We employed the use of an optimised glyphosate N-acetyltransferase enzyme which developed a colorimetric reaction in the presence of an acyl-donor. A good linear correlation was obtained in the concentration range of 10-600 nM (r^2= 0.98), with a response time of less than five minutes. In Europe, the tolerated limit for herbicides in drinkable water is 0.59 µM. As a result, recent reported glyphosate biosensors have detection limits from 25 pM to 47 uM, making our proposed biosensing model fit for development. Therefore, with a simple and specific enzymatic glyphosate sensing procedure coupled with an economically scalable CMOS platform, the proposed biosensor represents an ideal prototype for glyphosate monitoring in the environment.

Keywords: Glyphosate, CMOS, environmental, herbicides

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P02-Y - Nanofiber architectures for electrochemical biosensing of glucose

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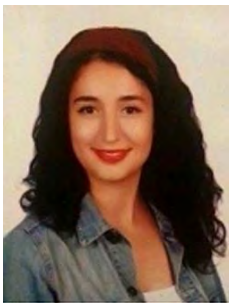

Abstract

Owing to the requirements for the detection of various compounds from metals to mammalian cells in the fields of medical diagnosis, the food industry, bioprocess monitoring, and environmental screening, highly sensitive and selective detection systems in low detection limits have attracted great attention[1]. Numerous nanomaterials have been used to prepare a novel analysis system. Among them, nanofibers have been applied in a wide range because of their large surface area, availability of controlling diameter and thickness, flexibility, and porous structure[2]. Electrospinning is a technique by which a polymer solution is charged to high voltage to produce diverse forms of nanofibers[3]. After the electrospinning of polymers, biomolecules are immobilized on the surface of produced nanofibers. The designed biomolecule-modified electrospun nanofibers can be used to prepare biosensors systems, and the electrospun nanofibers play a critical role as an immobilization matrix of biological materials. Herein, we constructed a cellulose acetate/chitosan (CA-CHIT) nanofiber surface as a novel platform for the sensing of Glucose oxidase (GOx). Initially, GOx were covalently bound to the nanofiber surface by cross-linking of glutaraldehyde. CA-CHIT/GOx biosurface was tested for the detection of glucose by amperometric biosensing. Afterwards, the investigation of analytical and surface characteristics of CA-CHIT/GOx platform were accomplished by electrochemical techniques as the final step. This developed biosurface may be an alternative platform for glucose sensing with the support of modifiable nanofiber structures and also can be used as an novel platform for biosensing.

Keywords: Electrospun nanofibers, electrospinning, biosensor, glucose oxidase

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P03-Y - Detection of bacterial toxins via surface plasmon resonance with fluorescence spectroscopy based biosensor

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Abstract

Water contamination and access to the clean water is one of the important problems concerning both developed and underdeveloped countries. Fast detection of waterborne pathogens would prevent the spread of the related diseases and help to take immediate action against them. This study aims to construct fast-response biosensor that recognizes toxins of waterborne pathogens. For this purpose, surface plasmon resonance with florescence spectroscopy (SPFS) method was used. Recognition layer was prepared on gold-coated LASFN9 slides using three different modifications: Poly(N-isopropylacrylamide) (PNIPAAm)-based terpolymer (containing methacrylic acid and benzophenone methacrylate comonomers), a carboxy-methylated dextran (CMD), and 11-mercaptoundecanoic acid (11-MUA). The PNIPAAm and CMD based films were prepared by spin coating from polymer/ethanol solution and crosslinked and attached to the modified gold substrates via UV irradiation. For the detection, sandwich assay format was chosen and truncated form of cholera toxin (Cholera toxin beta subunit; Ch-B) was used as model analyte. 11-MUA modified gold substrates were first activated using EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride)/NHS(N-Hydroxysuccinimide) chemistry. Streptavidin (0.01 mg/mL) solution was passed through the system and the binding curve was obtained real-time by kinetic SPR measurement till the signal was stabilized. Subsequently, system was washed with PBS to remove unbound streptavidin. Afterwards, biotinylated cholera toxin antibody was diluted in 1:1000 ratio and passed through the system until binding was completed. This first recognition surface was ready for the detection of Ch-B. Cy5 conjugated cholera toxin antibody will be used as the secondary antibody and the fluorescence signal induced by surface plasmons will be monitored. The same procedure is being optimized for both the PNIPAAm and CMD based films coated gold substrates. Preliminary data showed that the preparation of the recognition layer is easy and fast and the real-time monitoring of the all steps by SPR offers an advantage to follow the critical steps of both preparation and detection process.

Keywords: surface plasmon resonance, immunosensor, cholera toxin, surface engineering

The grant from The Scientific and Technological Research Council of Turkey (TÜBİTAK-115E015) is gratefully acknowledged.

P04-Y - Polypeptide with electroactive endgroups as sensing platform for the abused drug 'methamphetamine' by bioelectrochemical method

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Abstract

Affinity-type sensors have emerged as outstanding platforms in the detection of diagnostic protein markers, nucleic acids and drugs. Thus, these novel platforms containing antibodies could be integrated into the monitoring systems for abused drugs[1]. Herein, we established a novel detection platform for the analysis of a common illicit drug: methamphetamine (METH). Initially, a fluorescent-labeled polypeptide (EDOT-BTDAPala), derived from L-alanine N-carboxyanhydride (L-Ala-NCA) via ring-opening polymerization using 4,7-bis(2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)benzo[c][1,2,5]thiadiazole-5,6-diamine (EDOT-NH_2-BTDA) as initiator, was employed as a glassy carbon electrode (GCE) covering host, in order to immobilize the METH selective antibody. Prior to the examination of analytical features, GCE/EDOT-BTDA-Pala/Antibody surface was successfully characterized in the way of electrochemical (cyclic voltammetry and electrochemical impedance spectroscopy) and microscopic techniques (scanning electron microscopy and fluorescence microscopy). As for the analytical characterization, linearity and limit of detection (LOD) were found as 10–100 µg/mL with an equation of y=0.0429x−0.2347, (R^2=0.996) and 13.07 µg/mL, respectively. Moreover, sample application using artificial urine, saliva and serum samples spiked with METH (10, 25, 50 µg/mL) were performed and LC-MS/ MS system was used for further confirmation. The described platform can be adapted to monitor the other types of abused drugs by using suitably selected biorecognition elements.

Keywords: Surface modification, Abused drug analysis, Immunosensor, Methamphetamine

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This work is supported by Republic of Turkey, Ministry of Development (Project Grant No: 2016K121190).

P05-Y - Development of easy assemble immobilization matrix with magnetic nanofiber layers

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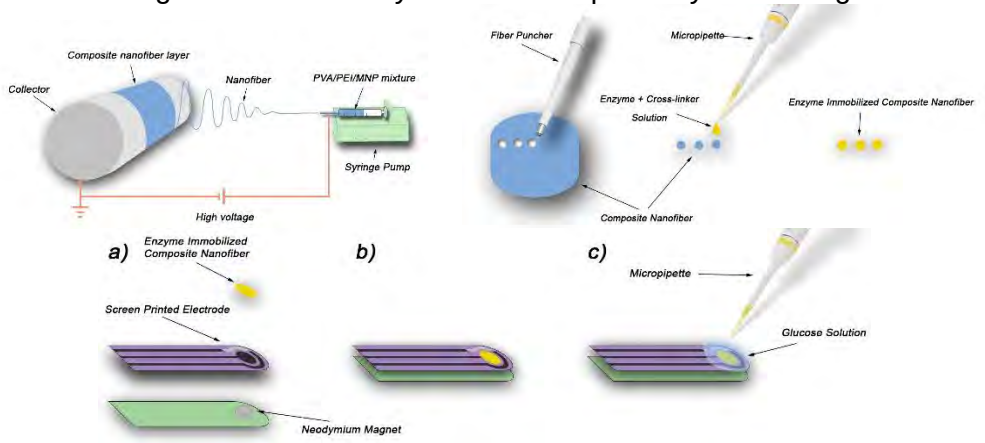
Abstract

Stabilization of biomolecules on biosensor immobilization matrices is critically important. Many polymers and nanomaterials are used as immobilization matrix with different methods. Direct electro-spinning the fibers on electrode surface could be one of the examples from literature. Here in this work it is aimed to form composite nanofibers with superior morphological and magnetic properties by electro-spinning of magnetic nanoparticles in appropriate polymer matrix on collector surface for multiple usages. Fe_3O_4 (magnetite) is used as magnetic nanoparticle (MNP) with a mixture of polyvinyl alcohol (PVA), polyethylene imine (PEI), to produce a homogenous composite nanofiber layer with electrospinning process. As a model enzyme, glucose oxidase (GOx, glucose 1-oxidase, β-D-glucose: oxygen 1-oxidoreductase, EC 1.1.3.4) is immobilized on a piece of produced composite nanofiber layer which cut same size of the working electrode surface, with the usage of glutaraldehyde as crosslinker. Magnetic properties of nanofibers are provided to assemble the fiber layer on screen printed electrode by the help of neodymium magnet for electrochemical measurements. This method is prevented handicaps such as reproducibility, excessive chemical and time usage and also allowed to reuse screen printed electrode by cleaning quickly and effortlessly with removing the used MNF layer within the split away of the magnet.



Keywords: magnetic nanofiber biosensor

This work is supported by TUBITAK (Project No:215Z194)

P06-Y - Detection of Escherichia coli O157:H7 in food products by impedimetric immunosensors

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Abstract

In the last 20 years, *Escherichia coli* O157:H7 has been considered as one of the most virulent foodborne pathogenic bacteria worldwide. This strain of *E.coli* is classified as an enterohemorrhagic bacterium with the ability to cause hemorrhagic diarrhea, renal failure, anaemia and other health problems. Traditional methods employed to detect *E.coli*, such as plate culture, enzyme-linked immunosorbent assays and polymerase chain reaction, are generally tedious and time-consuming, too expensive and require a laboratory and a highly trained technician. Therefore, a rapid, sensitive and low-cost method for the detection of *E.coli* is highly necessary.

Among biosensors, electrochemical immunosensors are broadly investigated for bacteria detection due to their specific affinity reaction, sensitive recognition and simple fabrication. In particular, Electrochemical Impedance Spectroscopy (EIS) is an efficient electrochemical technique for the direct transduction of biosensing events on electrode surface, including the monitoring of the antigen-antibody interaction, by probing the interfacial properties of electrodes. Because the immobilization of antibodies on the electrode surface readily affects the immunosensor analytical performances, different label-free immunosensors designs were reported and compared in this work, with the aim to detect very low *E.coli* O157:H7 cells.

EIS and CV were used to probe the features of the surface-modified electrodes: analysis of the system response provided information concerning the electrical behaviour of the interface and the interaction antibody-antigen occurring on it. An equivalent electrical circuit model was proposed to fit the impedimetric results.

Due to the specific immuno-interaction on the electrode surface, the impedance changes: in particular, for all immunosensor configuration, it was observed that with increasing bacteria concentration, the Electron Transfer Resistance (R_{ct}) value increases continuously, indicating that it has been captured at the site of the antibody and the electron transfer process is hindered. The analytical performances of the immunosensors developed were compared and the immunosensor with a limit of detection of 3 cfu/mL was used to analyse meat and milk samples spiked with known concentration of *E.coli*, showing a good recovery.

This technique has great potential for direct, rapid, sensitive and specific detection of *E.coli* O157:H7 cells.

Keywords: E.coli, electrochemical immunosensors*,* Electrochemical Impedance Spectroscopy (EIS)

P07-Y - The *azoR* gene promoter: a new sensing element for detection of trace explosives

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Abstract

Around 110 million landmines are buried throughout the world, and their detection is hazardous and challenging. The explosive content of landmines is mostly of 2,4,6-trinitrotoluene (TNT), along with its manufacturing impurities 2,4-dinitrotoluene (DNT) and 1,3-dinitrobenzene (1,3 DNB). Vapors of these compounds permeate through the casing of the landmine, migrate to the soil surface, and can be used to determine the location of buried landmines.

A bioreporter for DNT and TNT has previously been developed in our laboratory, based on a genetically engineered *E. coli* strain harboring a fusion of a sensing element, the *yqjF* gene promoter, to a reporter element, either *gfp* or *luxCDABE*. Transcriptome analysis revealed that the most highly up-regulated gene in the presence of DNT was *azoR*. This gene encodes a FMN-dependent NADH-azoreductase. A bioreporter based on the fusion of the *azoR* promoter region to *luxCDABE* was designed, and its activity in response to DNT and TNT was investigated.

Preliminary results show that *azoR* is not directly activated by DNT, but rather by a downstream metabolite. The role of certain enzymes in the biotransformation process was also investigated; gene deletions of the nitroreductases *nfsA* and *nfsB* resulted in a significant decrease of the response to DNT. This suggests that the inducing molecule is the product of DNT reduction. A deletion of *hcr*, an oxidoreductase that catalyzes the reduction of the *hcp* hydroxylamine reductase, showed significant increase in the response to DNT, hinting at its role in the further biotransformation of the inducing molecule.

Keywords: landmines, trace explosives, TNT, DNT, bioreporter, transcriptome analysis, *azoR* gene promoter, *hcr* oxidoreductase

P08-Y - Detection of contaminants' residues on surfaces by microbial bioreporters

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Abstract

We present preliminary results of an innovative approach for the detection of pollutant residues on everyday surfaces, by spraying live bioluminescent bacterial sensor strains and monitoring the ensuing light emission. Sensor spraying may be carried out manually or semi-automatically, and is shown to be low cost, rapid and sensitive. We demonstrate the method using 2,4,6-trinitrotoluene (TNT) and 2,4-dinitrotoluene (DNT) as model targets, and a luminescent *E. coli* bioreporter, genetically engineered to detect these chemicals, as the model sensor. Different types of surfaces exposed to these two compounds were sprayed with a thin layer of the bioreporter, and following a short incubation, luminescence was imaged by bioluminescence Imaging Systems. We have detected DNT and TNT spots containing down to 40ng per spot. In the future we will continue to improve the performance of the spraying method, increase detection efficiency, and expand the spectrum of detectable compounds.

Keywords: Bioluminescence, Spraying method, Surfaces, TNT/DNT, *E.coli*, Imaging

P09-Y - Molecularly imprinted polymers as synthetic receptors for cell targeting and imaging

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Abstract

Advanced tools for cell imaging are of particular interest as they can detect, localize and quantify molecular targets like abnormal glycosylation sites that are biomarkers of cancer and infection. Targeting these biomarkers is often challenging due to a lack of receptor materials. Molecularly imprinted polymers (MIPs) are tailor-made synthetic receptors that are able to specifically recognize target molecules. They are synthesized by co-polymerizing functional and cross-linking monomers in the presence of a molecular template, thus resulting in the formation of binding sites with affinities and specificities comparable to those of natural antibodies[1].

We applied fluorescently-labeled MIP particles for molecular imaging of fixed and living human keratinocytes in order to localize and quantify hyaluronan and sialylation sites[2-4]. Hyaluronan is composed of alternating units of D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine while sialic acid refers to N and O-derivatives of neuraminic acid, of which the most common member is N-acetylneuraminic acid (NANA). Thus, MIPs were prepared with the monosaccharides GlcA and NANA as templates. The organic dye rhodamine and two InP/ZnS quantum dots (QDs), one emitting in the green and the other in the red region were used as fluorescent probes. Rhodamine-MIPGlcA and rhodamine-MIPNANA were synthesized as monodispersed 400 nm sized particles and were found to bind selectively their target located in the extracellular region, as imaged by epifluorescence and confocal microscopy. In contrast, when MIP-GlcA and MIP-NANA with a smaller size (125 nm), prepared in the form of thin shells around green-QDs and red-QDs respectively, were used, staining of the intracellular and pericellular regions were rendered possible as well. The simultaneous multiplexed labeling and imaging of human keratinocytes with green QDs conjugated with MIP-GlcA and red QDs conjugated with MIP-NANA was also demonstrated. The specificity of binding was verified with a non-imprinted control polymer and by enzymatically cleaving off the terminal GlcA and NANA moieties.

Keywords: Molecularly imprinted polymers, keratinocytes, hyaluronan, rhodamine, quantum dots

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P10-Y - Immunosensing based on silver nanoparticles as a model

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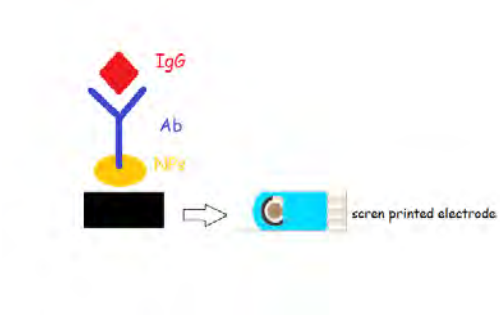
Abstract

Properties of nanoparticles such as light absorption and electroactivity are bringing interesting immunosensing alternatives. The interaction between nanoparticles (NPs) and biomolecules may reveal new material functions, where the NPs introduce new properties into the molecules, or alternatively, the biomolecules affect the structure or properties of the NPs. The aggregation of nanomaterials, especially silver, on the surface of working electrode of screen printed electrodes (SPEs) induced by application of specific oxidation potential, propose a new approach for immunoassay, using electrochemical detection to obtain high sensitivity. In this work, using the electroactive properties of silver NPs, an electrochemical enzymeless system is reported using the NPs functionalized with anti-IgG antibody and IgG as target molecule. Silver NPs are used as marker for electrochemical measurements and support of an immunological chain for generic analyte. The capabilities of this system, using in optical methods and electrochemical sensor (based on screen printed electrodes and voltammetric techniques) are highlighted. The Ag-NPs are synthesized modifying the Brust method in terms of time and amount of reagents. The conjugation between the antibody (Ab) and silver NPs is monitored through the UV-VIS spectroscopy following the the plasmonic shift of metal particles, and cyclic voltammetry (CV). The initial plasmon resonance of silver nanoparticles occurs at 394 nm, but the immobilization of Ab causes a shift of about 15 nm. The study of NP-Ab interaction is carried out with CV technique, using silver nanoparticles functionalized with growing amounts of antibody. The stable interaction between Ab and NPs shows that the anodic peak current downturn when the antibody concentration increases. The anodic stripping of the silver NPs, reveals that the peak area of the oxidative signal corresponding to the stripping of silver to silver ions decreases with increasing of IgG incomplete stripping of the silver NPs.This result stimulates our interest to review a major research work related to the use of NPs as biochemical sensors, and to highlight the advantages of using this strategy over the common analytical methods.



Keywords: electrochemical, silver nanoparticles, enzymeless immunosensor, anodic stripping

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P11-Y - Tracking effects of environmental organic micro-pollutants in the subsurface by coupling HPTLC with genotoxicity test as a tool for effect directed analysis

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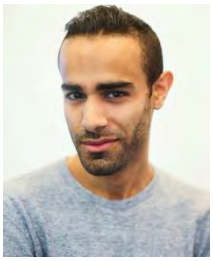

Abstract

Tracking the occurrence, distribution and fate of micro-pollutants compounds in the subsurface can be achieved by precise analytical methods. However, these methods are costly and require specialized laboratories and instrumentation. In addition, analytical methods do not provide data on agonist or antagonist properties, bioavailability of pollutants, and their effects on living systems. Thus, we aim to develop an innovative technological platform for monitoring organic micro-pollutants based on the assessment of their biological effects using our collection of *E. coli*-based bioluminescent toxicity and genotoxicity sensors, applied directly onto the surface of high-performance thin layer chromatography (HPTLC) plates. The sensor strains express the bioluminescent *luxABCDE* genes in the presence of toxic chemicals, thus allowing the detection of toxic compounds separated on the TLC plate. The advantages of using HPTLC allow us to do so in a cost-effective manner, with better analytical precision and dealing simultaneously with multiple samples and standards, combining two complementary approaches for the detection of organic micro-pollutants. Preliminary results have demonstrated a dose-dependent response to the model genotoxicant Nalidixic acid using a *recA*-based bioreporter immobilized in alginate directly on the surface of an HPTLC plate. We think that this innovative approach will prove to be an effective tool for the effect-detection of organic micro-pollutants in the environment.

Keywords: micro-pollutants, subsurface, genotoxicity, sensors, HPTLC, *luxABCDE,* nalidixic acid

P11-V - Paper genosensor based on smart nanocomposites for point-of-care diagnosis of chikungunya virus

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Abstract

Detection of *Chikungunya virus* (CHIKV) in a sensitive, specific and rapid way is the requirement in developing countries. The present work develops a paper based biosensor for ultrasensitive and specific detection of CHIKV DNA. The nanocomposites were used to serve as the signal enhancing platform due to the high surface area-to-volume ratio. The proposed biosensor showed very high sensitivity and selectivity for target DNA with a wide linear range. Use of paper makes this biosensor economic, portable and moreover ready to be used for point-of-care devices if constructed on a commercial scale.

Keywords: Chikungunya, DNA, paper analytical device, point-of-care

P12-Y - A novel electrochemical biosensor based on screen printed gold electrode modified with organo-silane for the tumor necrosis factor receptor-associated protein 1 detection

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Abstract

The tumor necrosis factor (TNF) receptor-associated protein 1 (TRAP1) was initially identified as a protein interacting with the intracellular domain of the type 1 TNF receptor (TNFR-1). Later, sequence analysis revealed that TRAP1 was identical to heat shock protein 75 (HSP75), highly homologous to HSP90. It is well described that the level of heat shock proteins (HSPs) increases in tumors in response to stresses of various kinds in order to restore the normal protein-folding environment and that they are important in maintaining oncogenes in an active conformation. Silane coupling agents are compounds whose molecules contain functional groups that bond with both organic and inorganic materials. It is this characteristic that makes silane coupling agents useful for improving the mechanical strength of composite materials, for improving adhesion, and for resin modification and surface modification.

In this study, we designed a novel biosensor to detect TRAP1 biomarker constructed on modified screen printed gold electrode (SPE-Au). Firstly, the bare electrode was incubated with 6-mercaptohexanol overnight. Later, the electrode was incubated with 3-cyanopropyltrimethoxysilane. After these processes, Anti-TRAP1 was covalently immobilized onto the modified electrode surface. To obtain a stable, sensitive and linear biosensor, the parameters such as 3-cyanopropyltrimethoxysilane solution concentration, anti-TRAP1 concentration and incubation time were optimized. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) methods were applied to characterize the immobilization of anti- TRAP1 and to determine TRAP1. For analytical characterization of the biosensor; linear range, reproducibility, repeatability and shelf life of the biosensor studies were tried. Finally, the designed biosensor was applied in real human serum.

Keywords: biosensor, electrochemical impedance spectroscopy, screen printed electrode

We are thankful for financial support from the *TÜBA* Üstün Başarılı Genç Bilim İnsanı Ödüllendirme Programı (TÜBA-GEBİP, Project number: 58765284-205.02/1489).

P13-Y - Determination of C1 inhibitor human by using screen-printed gold electrode modified with silane: highly sensitive and disposable biosensor

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Abstract

C1 inhibitor (C1-INH) is a serine protease inhibitor (serpin), also known as SERPING1. C1-INH is important in controlling a range of processes involved in vascular homeostasis, including inflammation, blood pressure and coagulation. A silane compound is a monomeric silicon-based molecule containing four constituents. One of the useful silane modification agents is 3-glycidoxytriethoxysilane (3-GOPE) compound containing reactive epoxy groups. Surface covalently coated with this silane coupling agent can be used to conjugate thiol-, amine-, or hydroxyl-containing ligands, depending on the pH of the reaction. Thus, 3-glycidoxypropyltriethoxysilane can be used to link inorganic silica or other metallic surfaces containing OH groups with biological molecules containing any three of these major functional groups.

In this study, a biosensor based on screen-printed gold electrode (SPE-Au) was designed to determine C1-INH. Firstly, bare electrode was incubated with 6-mercaptohexanol overnight. Later, the hydroxylated surface of the electrode was treated with 3-GOPE solution overnight. After these steps, Anti-C1-INH was covalently immobilized on modified SPE-Au electrodes. Optimization steps are very important and necessary to obtain a good, stable, repeatable and reproducible biosensor. For this purpose, all parameters such as 3-GOPE concentration, anti-C1-INH concentration and incubation time were optimized. To determine the immobilization steps and optimization of the biosensor, electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were used. For detection analytical characterization of the proposed biosensor, linear range, repeatability and reproducibility studies were applied. Shelf-life of the designed biosensor was also investigated. Finally, the designed biosensor was applied to real human serum and hopeful results were obtained.
We are thankful for financial support from the *TÜBA* Üstün Başarılı Genç Bilim İnsanı Ödüllendirme Programı (TÜBA-GEBİP, Project number: 58765284-205.02/1489).

Keywords: C1 inhibitor (C1-INH), 3-glycidoxytriethoxysilane (3-GOPE), screen-printed gold electrode (SPE-Au), biosensor, electrochemical impedance spectroscopy, human serum

P14 - A label-free low cost sensor based on surface-plasmon resonance on plastic optical fiber coupled with a biomimetic receptor for furfural detection in water

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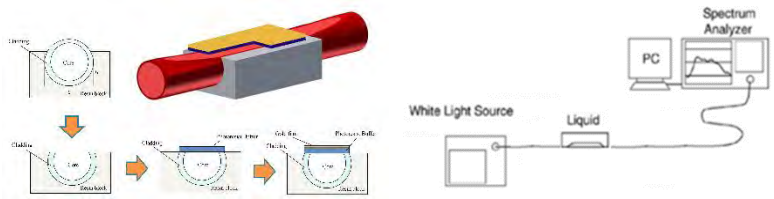
Abstract

Surface-plasmon resonance (SPR) is a label-free technique for direct monitoring of specific receptor/substrate interactions. The popularity of this technology has grown rapidly since the 1980s and resulted in a vast array of platforms. The versatility of SPR-biosensor technology has lent itself to many disciplines from life sciences to pharmaceutical, food and environmental monitoring. Nevertheless, the commercialization of biosensor based on SPR technology has lagged significantly behind research output, due primarily to both technical and cost issues[1]. Evidently in fields like industrial, food and environmental monitoring, costs and quickness are of overwhelming relevance, together with the possibility of performing in-situ measurements.

Recently, it has been shown that optical fibers can be useful tools for the development of optical sensing devices[2,3], potentially allowing much cheaper and smaller experimental set-ups. In particular plastic optical fibers (POF) are especially advantageous due to their excellent flexibility, simple manufacturing and handling procedures, great numerical aperture, large diameter, and the fact that plastic is able to withstand smaller bend radii than silica and allows an easy miniaturization, which is a very favorable characteristic for in situ determination[4]. Another point which must be considered in sensors development and practical application is the nature of the receptor. Bio-receptors, as for example antibodies, present some drawbacks in terms of costs, possibility of utilization in harsh environments and time required for development. To improve this point, biomimetic receptors, i.e. molecularly imprinted polymers have been proposed[5]. MIPs are porous solids obtained by molecular imprinting methods, which are relatively easily deposited over the gold layer at the surface of which the surface plasmons are excited. Having in mind these points, a sensor based on surface plasmon resonance and plastic optical fiber (SPR-POF) for furfural (furan-2-carbaldehyde, 2-FAL) in environmental waters has been here developed. 2-FAL derives from the de-hydration of pentose and from the Maillard reaction, so it is widely present in the environment, also because of contamination deriving from its many industrial applications, for example as selective solvent in the refining of lubricating oils, and as chemical feedstock for the production of 5-membered oxygen-containing heterocycles. 2-FAL is certainly toxic to some microorganisms, while its toxicity to the human beings is still under investigation. Its determination in environmental and food monitoring is of large interest.

A schematic view of the SPR platform for 2-FAL here developed is reported in the figure, together with a sketch of the platform preparation and of the measurement set-up.



POF (PMMA) has a core diameter of 960 µm diameter, and cladding of 20 µm.
The thickness of the gold film is 60nm.

The molecularly imprinted polymer layer was formed directly over the platform, according to a previously reported procedure [3], i.e. a typical molecular imprinting technique. The composition of

the prepolymeric mixture was similar to the previously reported one [3], excepted the template, that in this case was 2-FAL. The polymer layer contains some recognition sites included in a three-dimensional matrix. The measurements are performed by dropping the sample, (about 20 µL of water solution containing 2-FAL or blank), directly over the platform. The adsorption spectra are registered after 3 minutes' incubation, from 400 to 950 nm. A low cost apparatus is required. The spectra are normalized against the transmission spectrum of the same platform in air, a dielectric in which not any surface plasmon resonance takes place, due to its low refractive index. Between successive measurements the platform is washed by flushing with ethanol and hexane. The combination of the template molecule (2-FAL) with the specific sites in the MIP layer produces a variation of the refractive index of the polymer, which is measured as a shift of the surface plasmon resonance wavelength (Δλ). Due to the use of the optical fiber, the resonance wavelength is measured instead of the classical resonance angle. The resonance wavelength was shifted to higher values upon adsorption of 2-FAL on MIP, indicating that the refractive index of the polymer increases. The dependence of the wavelength variation (Δλ) on 2-FAL concentration is not linear, as usual when a limited number of receptor sites are present. Thus the experimental data were fitted by the Langmuir isotherm. Typical results from the standardization curve are: affinity constant K_{aff}=1.09 10^6 M^{-1}; $\Delta\lambda_{max}$=2.73 nm; sensitivity al low concentration= 1.8 10^7 nm M^{-1}; LOD=3.5 10^{-7} M. Notice the high value of the affinity constant from a solvent, water, which could strongly compete with the interaction of 2-FAL with the imprinted sites of the polymer. These platforms can be re-utilized at least 5 times.

The concentration of 2-FAL in water at levels from about 0.1 to 10 µM, which is of interest in environmental water, as well as for food monitoring can be determined by the proposed SPR-POF sensor. The determination is rapid, and requires unexpensive and small instrumentation. The sample volume required for the determination is small too. The optical platforms were prepared manually in a very simple way, but probably for this reason the reproducibility from platform to platform is not very good. Nevertheless, these sensors are promising for other aspects, in particular since they can be re-utilized for at least one month.

Keywords: Molecularly Imprinted Polymers; Furanic Compunds; Electrochemical Sensors; SPR sensors

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P15-V - Detection of bacteriophages by a biosensor based on the microwave resonator

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Abstract

As long as viral infections remain one of the global problems, the studies aimed at the development of new express methods for their determination are very urgent. For the first time, the possibility of recording the interaction between immobilized microbial cells and bacteriophages, using the detection system based on a microwave resonator (5 – 8.5 GHz), was shown. It has been demonstrated that the biosensor can differentiate the case of the interaction of bacterial cells with specific bacteriophages from the control experiments, when such interaction is absent. The possibility of immobilization of microbial cells on the surface of thin polystyrene films modified by plasma radio frequency argon discharge (13.56 MHz) was also shown. The optimal conditions of the immobilization of the cells *Azospirillum lipoferum* Sp59b, which retained the activity of the cells in relation to specific bacteriophages, were selected. By using the microwave sensor one can determine the content of bacteriophages ΦAl-Sp59b in the analyzed suspension. The sensitivity of the sensor is ~10^6 phage/mL, while analysis time is about 10 min. An additional option of the specified sensor is the determination of the viability of microbial cells after immobilization. The obtained results are novel and promising from the point of view of the development of methods able to determine the presence of viral particles and the viability of microbial cells by use of the sensor based on microwave resonator.

Keywords: bacteriophages, microwave resonator, thin polystyrene films, plasma radio frequency argon discharge, microbial cells viability, viral particles detection

This work was supported in part by the Russian Foundation for Basic Research (projects nos. 16-07-00818 and 16-07-00821).

P16 - Development of novel biosensor system for fish stress monitoring using self-assembled monolayer

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Abstract

We developed a biosensor system to measure the glucose concentration in fish as an indicator of fish stress. Uniform immobilization of the enzyme on the electrode surface was difficult, however, and thus the sensor response was often reduced to the high glucose concentration range observed during stress monitoring. Therefore, we attempted to enhance the sensor response by immobilizing the enzymes using a self-assembled monolayer (SAM) in which molecules can spontaneously assemble to form a regular array.

The oxidation current is measured by applying a voltage to the hydrogen peroxide produced by the enzymatic reaction of glucose oxidase (GOx) and glucose. GOx was immobilized on the working electrode modified with a SAM. After immersing the sensor in a phosphate buffer solution (0.1 M, pH 7.8), standard glucose solution was added. In order to test the practical application of the sensor, we also used biological sample taken from the test fish. We also monitored stress response of the fish in real-time with different stressors by using our proposed wireless biosensor system.

Optimization of the conditions revealed that the optimal pH for the sensor was 7.4-7.8. The proposed biosensor showed a good correlation between the output current and a glucose concentration range of 10-3500 mg dl^{-1}. Compared with the dynamic measurement range of the most responsive conventional biosensor (10-100 mg dl^{-1}), the dynamic measurement range of this newly developed sensor was significantly improved in the high concentration range which can help the sensor to get better performance in fish stress monitoring. The uniformity of the enzyme array on the electrode by the immobilization of GOx using a SAM led to an efficient enzymatic reaction. In addition, the output current and glucose concentration were well correlated in an actual fish sample. We also succeeded in monitoring stress response of test fish when fish swims freely with different stressor.

Keywords: glucose biosensor, fish stress, self-assembled monolayer, real-time monitoring

P17-Y - New design of label-free human immunoglobulin G impedance biosensor with micro-gap parallel plate electrodes

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Abstract

In recent years, electrochemical impedance spectroscopy (EIS) attracts considerable attention. Since this technique can detect the affinity binding event which produce no electroactive species such as H_2O_2 for the oxidase reaction, thus the detection is difficult by traditional electrochemical measurement. To improve sensitivity of EIS, the interdigitated micro-electrodes (IDE), that have a series of parallel microelectrodes with alternating microbands connected together, have been widely employed. Because of the short distance between the anodic and cathodic electrodes, the speed of oxidation and reduction cycle is extremely enhanced, which results in a high efficiency of data collection. According to our current density analysis on IDE, it was found that the current was highly concentrated on the edge of electrodes, whereas little current was observed on the central surface area (Fig. 1). The surface condition of the edge part is not identical for biosensing, because the roughness or disordering of surface structure can be easy introduced by the lithography and etching processes, which can cause instability or inaccurate measurement of biosensing performance.

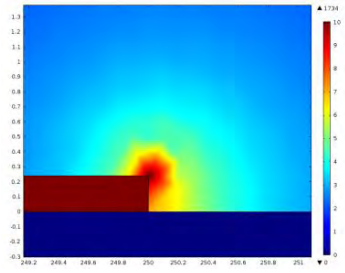

Fig. 1 - Simulated distribution of electric current density of IDE

To overcome this problem, we develop a new electrode structure of parallel plate electrodes with a micrometer scale gap (Fig. 2). The electric current is considered to be distributed homogeneously on the top flat surface with a smooth and well-ordered structure from the estimation of the parallel plate capacitor model. The enhancement of redox cycle is also expected for the parallel plate electrodes because of the microscale gap distance. As the first step, we performed cyclic voltammetry (CV) measurements using the parallel plate electrodes under the presence of $[Fe(CN)_6]^{3-/4-}$. The CV profiles were recorded as the function of gap distance of the electrode (Fig. 3). It was observed that the faradic current gradually increased with decreasing the gap distance. In addition, when the gap was decreased, CV curves show more like sigmoid having less hysteresis character between the forward and backward scans. The observed behavior indicates that the enhancement of redox cycle happens for the parallel electrodes with the shorter gap distance.

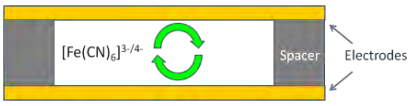

Fig. 2 - The structure of parallel plate electrodes

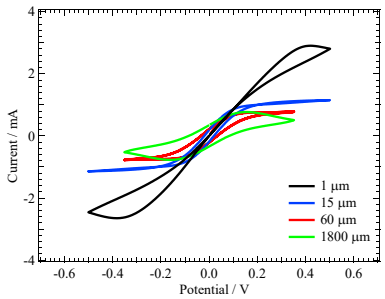

Fig. 3 - CV profiles with various gap distances

We also prepared human immunoglobulin G (IgG) biosensor with the parallel electrodes. Protein G (PrG) was employed to detect IgG, for the selective PrG-IgG binding formation. We immobilized PrG on the one surface of the parallel electrodes and measured EIS spectra as the function of IgG concentration. It was observed an improved IgG sensor performance for the parallel electrodes. The detail results will be discussed in the presentation.

Keywords: Electrochemical impedance spectroscopy, Parallel plate electrodes, Immunoglobulin G, Biosensor

P18-Y - Determination of total antioxidant capacity in plant extracts using electrochemical sensors

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Abstract

Various plants contain high concentrations of several antioxidants, which can inhibit the oxidation of biomolecules by the free radicals that are formed in living organisms. In this work lavender and sea buckthorn extracts have been analysed by using a label-free sensor based on gold nanoparticles to determine their antioxidant capacity. The used extracts were obtained by two different methods and solubilised in different mixtures as solvents. As pro-oxidant, hydrogen peroxide in variable concentrations was used. Optimization studies by means of pH, working potentials, and extracts were carried out. The results were validated and correlated using the classical methods of spectroscopy (UV-VIS, FTIR and Raman). Highest antioxidant capacity and optimized sensors configuration together with and a correlation between the extracts antioxidant capacity and the consumption of hydrogen peroxide have been outlined.

Keywords: electrochemical sensors, gold nanoparticle, plant extracts, antioxidant capacity

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, PN-II-RU-TE-2014-4-2801.

P19-Y - An optical sensor for studying hemocompatibility of biomaterials in a flow system

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Abstract

When implants get into contact with body fluids such as blood, lymph or saliva, proteins start to adhere to their surface within milliseconds. The amount, type and possible conformational changes of the adhering proteins initiate various processes, such as platelet accumulation, activation of immunological cascades, formation of foreign body giant cells or apoptosis of adherent cells[1]. These unintended interactions of a foreign material with the human body can - inter alia - result in implant failure. To study the adsorption of proteins to the implant surface, numerous methods including surface plasmon resonance, ellipsometry, infrared spectroscopy, atomic force microscopy and optical microscopy are available. However, a technique to investigate single steps in protein adsorption and subsequent processes is needed.

The here presented sensor has been developed to be particularly suitable for testing hemocompatibility. The formation of a protein film that develops on the sensor surface is recorded using reflectometric interference spectroscopy[2]. This temperature independent technique monitors the change in optical thickness – the product of physical thickness and the reflective index – caused by association or disassociation processes. By using a semi-steady flow of the protein solution across the sensor surface, the fluid dynamic properties of blood vessels are mimicked. The influence on the formation of protein films of different commonly used materials in cardio-vascular implants, such as polyethylene glycol and polyurethane, and furthermore a biomimicking phospholipid membrane surface was evaluated.

In the future, the here presented method can be extended to adherence analysis of further blood components, such as platelets, erythrocytes and lymphocytes. By testing the adherence of these cellular blood components individually or in combination with other blood components, a more complete picture of the body response to cardio-vascular implant surfaces could be achieved.

Keywords: biosensor, hemocompatibility, foreign body reaction, RIfS

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P20-Y - An innovative carbon black modified sensor to detect free chlorine

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Abstract

Chlorine is the most widespread chemical compound for the disinfection of drinking water and swimming pools, due to its high oxidizing power. The most known disinfectant is sodium hypochlorite, but other chlorine derivatives are also used. In aqueous solution, there is an equilibrium between hypochlorite ion and hypochlorous acid, and the sum of these two species is defined as "free chlorine". The official method for its detection is the N,N-diethyl-p-phenylenediamine spectrophotometric method (DPD)[1]. In agreement with WHO's directives, the level of free chlorine in swimming pools should be between 3 and 5 ppm, while the Italian legislation establishes a range between 0.7 and 1.5 ppm[2,3]. Thus, analytical tools are required to control the disinfectant level, and the development of cost-effective and easy to use sensors could be really helpful. The goal of the Tecnosens Spin-off is to develop and fabricate miniaturized and sustainable electrochemical probes for the monitoring of disinfectant compounds. Herein, we report a sensitive and cost-effective sensor for the determination of free chlorine in water. The sensor was realized using screen-printed electrodes modified with carbon black (CB-SPE), a raw cost-effective nanomaterial chosen for its excellent electrocatalytic properties[4,5]. The working conditions were optimized in order to obtain the best sensitivity and repeatability of the probe. In particular, the working graphite electrode was modified through drop casting with 10 µl of CB dispersion 1 mg/ml in N,N-Dimethylformamide/H2O 1:1 (v/v). The amperometric study, conducted by applying a potential of -0.1 V vs Ag/AgCl, showed that the sensor possesses a linearity range between 0.05 and 200 ppm (R^2= 0.995), a sensitivity of 0.32 ± 0.02 µA/ppm, and a LOD of 0.01 ppm, calculated as three times the S/N. The selectivity and the sensitivity of developed sensor were evaluated up to 200 ppm of trichloroisocyanuric acid. The results underline that the sensitivity (0.36 µA/ppm) of CB-SPE toward this compound is similar to the sensitivity for sodium hypochlorite, in the same working conditions. After studying the matrix effect of the swimming pool water, the accuracy of the sensor was estimated through a recovery test, which percentage resulted (97 ± 10)% in swimming pool water. These results were satisfactory and confirmed the possibility to use the developed sensor in real samples.

Keywords: sensor, free chlorine, carbon black

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P21-Y - Potassium sensing with ion selective field-effect transistor

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Abstract

Extension of ion selective field effect transistors (ISFETs) for measuring species other than hydrogen ions has significant research potential in the field of sensing. ISFETs with ion-sensitivity and selectivity to different ionic species can be fabricated by depositing polymeric membranes containing specific receptor molecules on the gate surface. We modify gate oxide with nano thin gold films followed by surface functionalization with specific ligand for detection of specific analytes. Here we present potassium ion (K+) sensing by a self-assembled monolayer (SAM) of thiol-modified crown ethers in a differential measurement setup. SAM functionalized nanogold surface is characterized by cyclic voltammetry and specific host-guest complexation of potassium ion and chosen crown ethers is characterized by conductance, UV-Vis and FTIR Spectroscopic measurements. The sensor response to potassium ions is linear in the concentration range 0.001 mM to 1mM. The performance of sensor is validated as it obeys Nemst law and gives a slope value ~ 52 mV/decade in a KCl solution. Sensor surface is specifically selective to potassium ions even in the presence of much higher sodium concentration. This simple device is expected to have potential application in biomedical as well as agricultural field for selective determination of potassium concentration in blood serum samples well as soil extracts.

Keywords: Ion selective field effect transistors (ISFETs), Potassium Sensor, Crown ether

P22-Y - Folat receptor targeted multimodal engineered vesicles for imaging and therapy: a magic of theranostics

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Abstract

Theranostic therapy is one of the most promising technology in cancer research, which simultaneously allows treating and real-time monitoring of cancer[1]. In the present study, a novel material has been developed for theranostic approach. Gold nanoparticles (AuNPs) and Protoporphyrin IX (PpIX) both have encapsulated in niosomes (PpIX-AuNP). Here niosomes have been used for the encapsulation of PpIX-AuNP due to the fact that niosomes possess high biocompatibility, physical and chemical stability. AuNP and PpIX are good sensitizer for radio therapy (RT) and PpIX is one of the more studied agent for photodynamic therapy (PDT). This developed formulation was also targeted with folic acid (FA). Treatment was performed by the combination of PDT and RT, by using human alveolar type-II (ATII)-like cell lines (A549) (folat receptor negative) and human cervical cancer cell line (HeLa) (folat receptor positive). Cell lines were monitored by fluorescence microscopy. Characterization of sizes and zeta potential of vesicles were carried out by dynamic light scattering (DLS) and atomic force microscopy (AFM). MTT method was used to measure cell. Our results showed that PpIX-AuNP-FA are homogeneous and consistent less than 100 nm. In conclusion, PpIX-AuNP-FA can be considered as good candidates for PDT and RT treatment in theranostic approaches.

Keywords: Theranostic therapy, gold nanoparticles, niosomes, folic acid, photodynamic therapy, radio therapy, fluorescence microscopy

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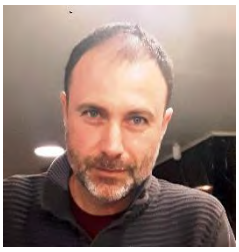
This work is supported by Ege University Research Project (Project Grant No:2017 FEN 007 and 2012 FEN 025)

P23 - Sandwich NP-based biobarcode assay for C-reactive protein quantification in plasma samples



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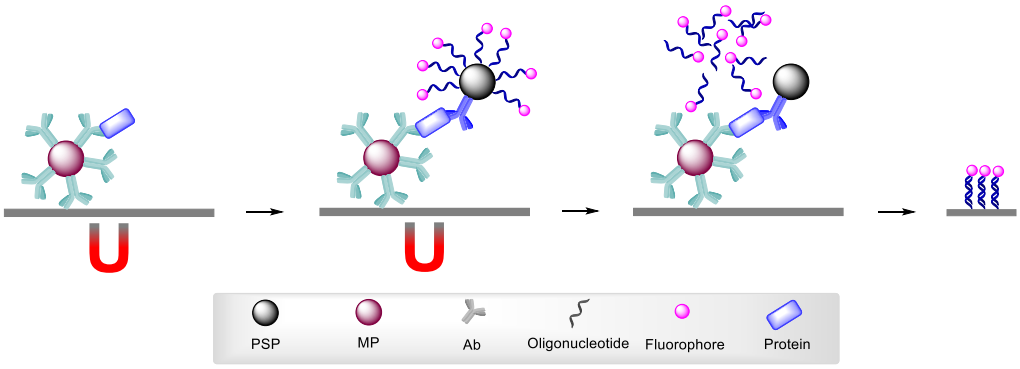
Abstract

A *NP-based biobarcode* for C-reactive protein (CRP) quantification in plasma samples is reported for the first time. The assay uses capture antibody functionalized magnetic beads (pAbCRP2-MP), multifunctional oligonucleotide encoded probes modified with a detection antibody (pAbCRP1-ePSP), and a fluorescent DNA microarray. Thus, magnetic beads are added to the sample to form immunocomplexes that will be isolated, to then add the codified particles to form a sandwich complex with both particles and the target protein, subsequently the complexes are treated to release the oligonucleotide codes, which are finally hybridized in a fluorescent DNA microarray.



The assay has been implemented to the analysis of plasma samples being able to quantify CRP within 900 ng mL^{-1} to 12500 ng mL^{-1} with an excellent accuracy (mean of recovery of 99.5 ± 4.2%, *N = 3*). The *CRP biobarcode* has been used on a small pilot clinical study in which plasma samples from patients suffering different pathologies, most of them related to cardiovascular diseases (CVDs), have been analyzed and the results compared to a reference method demonstrating that the assay developed can be useful for monitoring this biomarker on patients being suspicious to be under risk to suffer CVDs or other diseases involving inflammatory processes.

Keywords: nanoparticles, biobarcode, C-reactive protein, antibody, magnetic beads, plasma samples

P24 - High throughput immunoassay for the therapeutic drug monitoring of tegafur

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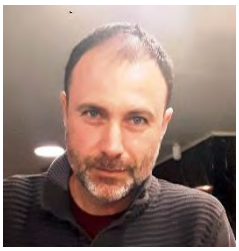
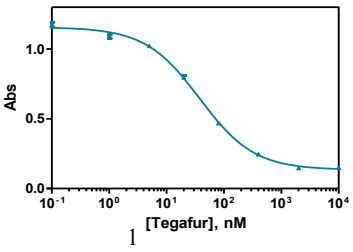


Abstract

Cancer is a term for diseases in which abnormal cells growth and divide without control, and with the potential to invade other parts of the body. Chemotherapy is one of the cancer treatments that uses chemical agents to treat the disease. Because of these drugs are toxic and produce undesirable adverse drug reactions, they need to be monitored in order to establish a personalized treatment to achieve the maximum efficiency and reduce the drug toxicity. Nowadays, therapeutic drug monitoring (TDM) is not routinely used for chemotherapy agents. However, TDM has the potential to improve the clinical benefit of chemotherapy drugs due to their narrow therapeutic window and highly variable pharmacokinetics. Tegafur, prodrug of 5-fluorouracil (5FU), is one of the main anti-cancer drugs used worldwide. Herein, a reproducible and sensitive indirect competitive ELISA has been developed and validated in plasma samples.



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Parameters of the assay (As337/5FU6-CONA)

As dilution	1/7000	$Signal_{min}$	0.134 ± 0.010
[Competitor]	0.25 µg mL^{-1}	$Signal_{max}$	1.157 ± 0.010
Competition time	10'	Slope (m)	-0.915 ± 0.043
Agitation	600 rpm	R^2	0.992 ± 0.001
pH	7.5	IC_{50}, nM	35.6 ± 2.6
Ionic strength	15.0 mS/cm	DR^a, nM	from 7.5 ± 1.3 to 157.5 ± 5.1
Tween 20	0.05 %	LOD, nM	2.7 ± 0.7

The assay shows an IC_{50} of 35.6 nM, reaching a limit of detection of 2.7 nM. It is highly reproducible and it does not show cross-reactivity with any related compound. Summarizing, this assay provides a sensitive, accurate and high throughput analytical method for tegafur quantification in plasma, which fits TDM requirements.

Keywords: Therapeutic drug monitoring, high throughput, immunoassay, tegafur

P25 - Development of LED-colour-switching type biosensor for the visualization of fish stress response

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Abstract

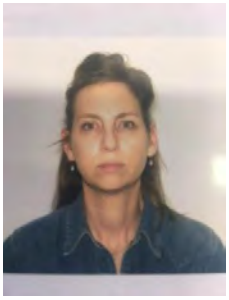
A biosensor system was developed in our lab to measure blood glucose concentration, which is a stress indicator of fish, in real-time while fish swimming freely. This system digitizes obtained data and monitors the stress response by sending the data to the personal computer using optical communication route. However, it is hard to make an instant judgment of fish stress level unless the data treatment which contains the lag. In this research, we aimed to make a new visualization system which can easily judge 3 different levels for fish stress response by visual observation based on LED colour change.

The present system is based on the principle of converting the output current value measured by glucose biosensor corresponding to the stress response, into a voltage value on the electronic circuit and changing the colour of the LED (Red, Yellow, Green) based on the voltage value. First, fish eye interstitial fluid (EISF) in the vicinity of the adventitia of the eye was sampled from the test fish (*Oreochromis niloticus*) and adjusted to concentrations used to define the yellow and red LED by adding glucose. By using these samples, the colour switching values of the LED corresponding to the glucose concentration were set. In addition, the measurable communication range of the sensor was confirmed in air, fresh water, and seawater. Furthermore, we attempted to insert a sensor which connected to the colour-switching system with a lead wire, into the EISF of fish and to monitor the colour change of the LED in real time, *in vivo*, while providing stressor to the fish.

When the glucose concentration in the collected EISF was changed to the pre-set concentration, it was successful to switch the colour to green, yellow and red, according to the value. Next, we confirmed the measurable communication range; it showed that it is possible to communicate in the lab scale under ever the condition of air, fresh water or sea water. Furthermore, when monitoring the stress responses of the fish *in vivo*, a colour change corresponding to the sensor output current value was observed successfully. Therefore, fish stress responses can be visually grasped by using our proposed system.

Keywords: biosensor, fish, stress

P26 - Recombinant cell biosensors for the detection of Endocrine-Disrupting Compounds



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Abstract

The presence of Endocrine-Disrupting Chemicals (EDC's) in wastewater, surface water, ground water and even drinking water is a major concern worldwide as it affects human health by disrupting normal endocrine function. The steroid receptors, such as estrogen receptor α (ERα) and β (ERβ), the androgen receptor (AR) and the progesterone receptor are part of the nuclear receptor superfamily which is the largest family of transcription factors in eukaryotes. The yeast *Saccharomyces cerevisiae* provides a relatively simple and well defined eukaryotic system for the expression of genes from other organisms. Yeast cells do not express estrogen, androgen or progesterone receptors and thus can be used as a powerful tool for detecting EDC's. Yeast based endocrine disruption bioassays consist of engineered yeast cells harboring two foreign genetic elements: one expressing a vertebrate nuclear receptor and the other is the hormone receptor element (HRE) fused to a reporter gene. The reporter gene will only be expressed in the presence of the receptor ligand complex. A wide variety of yeast-based bioreporter assays for EDC's detection is available. These assays, however, are compound-specific and are limited to the detection of one EDC group per assay. The aim of our study is to design yeast strains, which could be used in a single assay for the parallel detection of different groups of EDC's. This will be achieved by using diverse fluorescent reporters coupled to several endocrine receptors. By coupling this biological assay with high-performance thin layer chromatography (HPTLC), a standard method for EDC separation, a wide variety of compounds could be simultaneously detected and quantified.

Keywords: Endocrine-Disrupting Chemicals, steroid receptors, engineered *Saccharomyces cerevisiae,* bioreporter

P27 - Electrochemical sensor based on ultrathin nanostructural coating for analysis in biological and environmental samples

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Abstract

Perfluoroalkyl substances (PFAS) is a wide group of man-made chemicals largely employed in industry and consumer products. Its presence in aqueous environment is a problem of global concern, since they bioaccumulate in the blood and organs of exposed humans and animals and remain there for extended periods of time[1]. Smart recognition systems using synthetic analogues offer improved stability, cost effectiveness and a means of rapid fabrication. Molecularly-imprinted electrodes can provide a promising alternative and direct approach to produce a template and to prepare artificial antibodies to be used in the field of (bio)sensors. Molecular imprinting of polymers (MIPs) is a technique used to create mimetic receptors by the formation of a polymer network around a template. The electrosynthetic approach simply and rapidly creates an adherent and compact polymeric film with controllable thickness, which could be very helpful both in improving the molecular imprinting polymerisation procedure and in extending the application of MIPs[2,3]. In this study, the fabrication and characterization of novel sensor for perfluorooctane sulfonate (PFOS) based on a molecularly-imprinted electrosynthesised polymer is reported. A PFOS sensitive layer was prepared by electropolymerization of o-phenylenediamine (o-PD) on a gold electrode in the presence of PFOS as template so producing an ultrathin film with molecular recognition capability. To develop the MIP the template molecules were removed from the modified electrode surface by a suitable procedure. Electrochemical methods were used to monitor the processes of electropolymerization, template removal and binding in the presence of a redox probe. The imprinted layer was characterized by cyclic voltammetry (CV), differential pulse voltammetry (DPV), quartz crystal microbalance (QCM), profilometry and scanning electron microscopy (SEM). The incubation of the MIP-modified electrode with respect to PFOS concentration resulted in a suppression of the probe signal. The sensor was successfully tested for analysing PFOS in water at 0.1 nM - 1.5 µM concentration levels, giving results comparable with those obtained by HPLC/MS/MS analysis.

Keywords: Perfluoroalkyl substances, MIPs, cyclic voltammetry

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P28 - Multiplexed analytical platforms based on the use of antibodies for monitoring pollutants in marine environment

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Abstract

The development of novel methodologies for environmental monitoring sea water contaminants has gained prominence for quality assurance in aquaculture and protect marine biodiversity. The aquaculture industry represents nowadays the 20% of the total fish production with an expected increasing of this percentage in the following years. To ensure food safety and quality it is essential to guarantee environmental health. In this way, the Marine Strategy Framework Directive (MSFD) and the Water Framework Directive (WFD) support the development of new technologies for monitoring pollution and management of good practices in environmental vigilance and food safety. Immunochemical techniques are based on the use of antibodies as a biorecognition element for the sensitive and specific recognition of the targeted pollutant. These kind of techniques can be use as alternative or complementary tools in analytical chemistry, to lighten the great amount of analysis that have to carry out with less cost. Moreover, allow configurations for on-site monitoring not requiring highly qualified personnel and high-cost equipment.
In this communication we will present the work performed to develop distinct multiplexed immunochemical analytical platforms will be presented. Thus, ELISA, fluorescent microarray and electrochemical sensors have been developed to simultaneously detect the presence of pesticides, antibiotics, hormones, persistent organic pollutants or marine toxins in aquaculture facilities.

Keywords: antibody, amperometric immunosensor, microarray

P29 - Carbon nanotube modified screen printed electrodes: pyranose oxidase immobilization platform for amperometric enzyme sensors

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Abstract

Screen-printing technique has emerging area because of enabling to simple, rapid and inexpensive biosensor preparation in large scale production. Biosensors, which were prepared using screen-printed electrodes (SPEs), have been extensively used for detections of biomolecules, phenolic compounds, pesticides, antigens and anions. Modification of SPEs with various nanomaterials such as graphene oxide, carbon nanotubes (CNTs), palladium nanoparticles, fullerenes etc. to improve analytical performance of electrochemical sensors has been reported nowadays. Carbon nanotubes (CNTs) are members of the carbon-based nanomaterials offering unique mechanical, electronic and chemical stability properties. When CNTs have been used as an electrode, they have a characteristic to mediate electron-transfer reactions with electroactive species. Because of some advantages such as low cost, versatility, and miniaturization in screen-printing technology, the disposable amperometric biosensors based on SPEs has increasing potential. The alteration of SPEs with CNTs has enabled the production of sensitive and stabile sensors[1].

Here, a novel enzymatic biosensor was developed using multiwalled carbon nanotube including screen printed electrodes (MWCNT-SPE). Pyranose oxidase (PyOx) was immobilized on the electrode surface by way of gelatin membrane and then cross-linked using glutaraldehyde. Glucose was detected at -0.7 V (vs. Ag/AgCl) by watching consumed oxygen in enzymatic reaction after addition substrate. After optimization of pH and enzyme loading, the linearity was found in the range of 0.1–1.0 mM of glucose. After that, the effect of MCNT on the current was tested. Also the enzymatic biosensor including glucose oxidase instead of pyranose oxidase was prepared and the biosensor response followed for glucose. Furthermore, this system was tested for glucose analysis in soft drinks.

Keywords: Carbon nanotube, screen-printed electrodes, Pyranose oxidase, glucose, amperometry

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P30 - Amithiophenol modified montmorillonite as an immobilization matrix for enzymes in biosensor preparation

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Abstract

Biomolecule-based diagnostic techniques has become a dynamic area of research because of its remarkable potential for a variety of applications, such as in food/beverage industries, diagnosis of disease, bioprocess monitoring and screening of environmental pollutants. However, the relevant literature often cites that immobilization of biomolecules generally causes a decline in enzyme activity or impaired biological characteristics. To improve the stability of biomolecules which are used in these systems for different requirements, modification of transducer surface for immobilization of biomolecule is a key point. To improve the sensitivity and selectivity of these systems, the interaction between sensor surface and analyzed solution, in other words surface topology, must be thought in nanoscale. Clays are one of the advantageous materials to design different immobilization matrices for biomolecule immobilization, due to their high stability, good adsorptive capacity, large specific surface area, stick out adhesiveness and low costs. Nowadays, clays have a great potential of use as a matrix for biomolecule immobilization[1].

A novel support material for enzyme immobilization based on 4-aminothiophenol modified Montmorillonite (ATP-Mt) was successfully synthesized and used to manufacture pyranose oxidase (PyOx) biosensors (ATP-Mt/PyOx). The intercalation of ATP into Mt was confirmed by FTIR, XRD and TGA techniques. PyOx was immobilized onto the glassy carbon electrode via glutaraldehyde crosslinking, by using ATP-Mt as a support. In order to test the analytical performance of CTAB-Del/PyOx biosensors, chronoamperometric measurements were carried out. After optimization studies, analytical characterization was performed. Also ATP-Mt was applied to detect glucose in real samples.

Keywords: Immobilization, Montmorillonite, 4-aminothiophenol, pyranose oxidase, biosensors, cronoamperometry, glucose

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This project was supported by Ege University Research Funds (Project number:16FBE023). Dr. D.O. Demirkol thanks The Turkish Academy of Sciences-Outstanding Young Scientists Award Program (TUBA-GEBIP2015) for partial financial support.

P31 - Study for developing an electrochemical tongue to differentiate three types of natural waters, using very simple sensors and principal component analysis

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Abstract

The study and the analysis of natural waters is a very current topic, especially related to the problems of pollution, to which natural waters are very exposed. The collection of many and different samples of natural waters, for analytical purposes, made sometimes without a careful discrimination of the numerous sample types, often creates additional difficulties, also for the purposes of programming further, more in-depth and targeted analysis of various natural water samples. In the present research, it was therefore addressed the problem of recognizing, in a very simple, rapid, and safe way, three different types of natural waters (rain, river and groundwater) by means of sensors of low cost and a most popular chemometric elaboration, such as principal components analysis (PCA). The research was conducted using simple, but proved effective methodologies; in particular, they have been employed: a glass potentiometric electrode for the pH measurement, an amperometric gaseous diffusion electrode (Clark type) for measuring the dissolved oxygen content, a temperature sensor and, finally, a sensor recently developed for measuring the concentration of alcoholic traces (ethanol + methanol) eventually present, which consists of a small enzyme catalytic fuel cell[1,2]. This latter type of sensor has been chosen because the pollution from ethanol of natural waters, especially groundwater, is now an increasingly sensitive problem, since they are used biofuels for gasoline and diesel engines, containing relevant percentages of ethanol, even higher than the allowed limit (i.e. 10%, but up to 80% in some cases, despite now completely prohibited) in countries such as Brazil, or also Australia[3] and USA[4]. In this research, it is shown as the simultaneous use of only four sensors, applied on a certain number of rain, river and groundwater samples, and the subsequent processing of the data using a simple PCA program easily available on the market, makes it possible to distinguish and correctly classify the three different types of natural waters considered in this research, so that this study can represent a basic investigation for the development of a simple, but effective and inexpensive "electrochemical tongue".

Keywords: electrochemical tongue, natural waters, principal components analysis

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P32 - Electrochemical detection of hydrogen peroxide by free standing nickel oxide-plastic electrode

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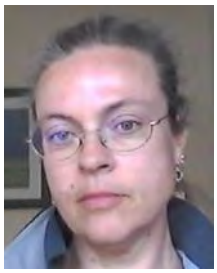

Abstract

The transition metal oxides have attracted the attention of research to prepare new materials, with applications ranging from the development of capacitors, or electrochromic coatings or organic photovoltaics. Oxides of Mn, Cu, Sn, or Ni are promising candidates in electrochemical sensors development as consequence of their catalytic properties associate to a relative simplicity in preparation[1]. Catalytic properties of NiO were exploited to optimize nonenzymatic sensors i.e. for H_2O_2 determination or for the non-enzymatic determination of glucose. These sensors aim to overcome some limitations arising from the use of enzymes i.e. immobilization procedures, risk of run-off from the electrode surface of the enzyme or enzyme instability with regard to specific application conditions. The present work describes the preparation of a freestanding graphite-based plastic electrode (PE)[2], whose formulation is bulk-modified by the addition of NiO powders with the aim of optimizing a catalytic surface for H_2O_2 determination. The plastic electrically-conductive material, which is obtained with a simple and low cost procedure, can be modified in both thickness and shape. The versatility of the applications expands because we demonstrated the ability to bulk-modify the basic composition of PEs.
Figure 1 shows the plastic NiO (NiO-PE) bulk-modified electrodes. A typical cyclovoltamogram recorded with either bare-PE or NiO-PE in 0.1M of NaOH solution and in presence of added H_2O_2 is reported in Figure 2.

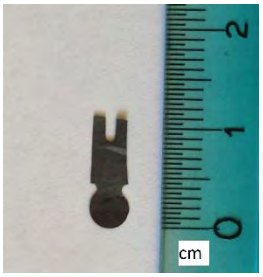

Figure 1 - Plastic electrode (bare-PE or NiO-PE)

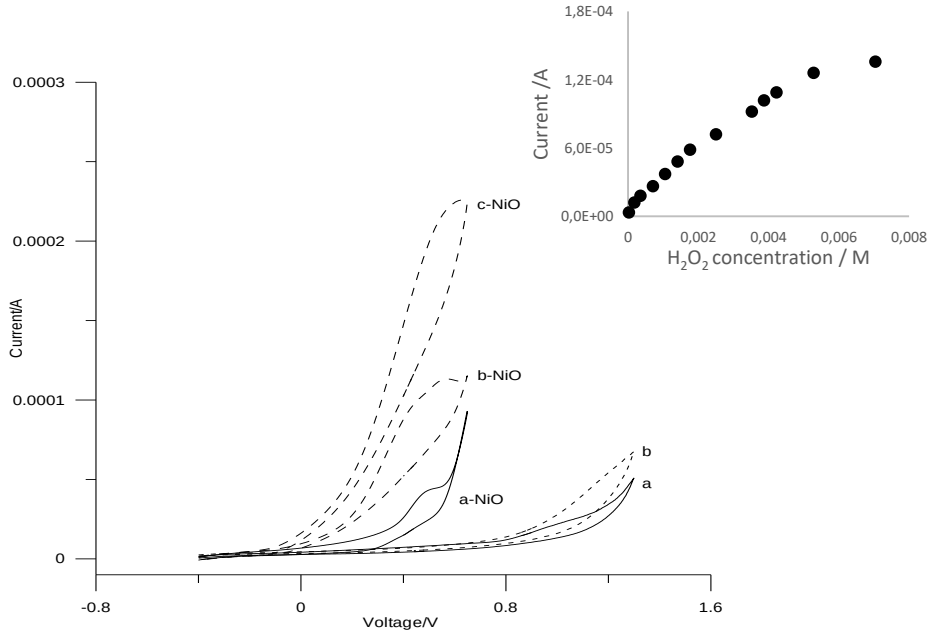

Figure 2 - Cyclic voltammogram of bare-PE in NaOH 0.1 mM (a) and in NaOH 0.1 mM added with H_2O_2 1 mM (b); Cyclic voltammogram of NiO-PE in NaOH 0.1 mM (a-NiO), in NaOH 0.1 mM added with H_2O_2 1 mM (b-NiO) and with H_2O_2 2 mM (c-NiO). Scan rate 50 mVs^{-1} *Insert:* calibration curve obtained from amperometric responses of NiO-PE polarized at + 300 mV due to successive addition of H_2O_2 into 0.1 M NaOH solution.

The calibration curve, performed by amperometry, using a single NiO-PE polarized at + 300 mV and adding H_2O_2 in a concentration from 20 μM to 5 mM (Figure 2 insert), is linear up to 4 mM with $y = 2 \times 10^{-8} + 9 \times 10^{-6}$ (A/μM)($R^2 = 0.990$) and LOD ($3\sigma_{blank}$) of 5 μM.

Keywords: free-standing plastic electrode, nickel oxide, electrochemical, hydrogen peroxide

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P33 - Rapid detection of *Escherichia coli* in marine water samples by remote optical biosensor system

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Abstract

Conventional cultural methods defined by the European Directives require 18-24 hours for the statutory assessment of the bacteriological water quality, and 1-2 days more, for results confirmation. Since delayed results can threaten public health, cutting-edge technologies able to speed up analyses are needed. Biosensors can represent devices which allow easy, rapid and on site analyses suitable for environmental applications. The advantage is their highly specific target's identification reducing the analytical steps. On this basis, a novel, completely automatic optical biosensor system (patent pending) able to rapidly detect *Escherichia coli* (*E. coli*) in marine water was developed. It can perform the entire analytical procedure and its autonomy is guaranteed by the provision of a stand-alone supply box, including substrate stock and a communication system that constantly interacts, by UMTS network, with a web server where results are shown for remote control.

The principle of the analysis is based on the hydrolysis of the substrate beta-D-glucuronide contained in the culture medium added to the sample. The hydrolysis, operated by the microbial enzyme beta-D-glucuronidase, leads to 4-methylumbelliferone, whose fluorescence is measured, giving evidence of the presence of *E. coli*. In order to validate the system, results were compared with those gained with a standard method (ISO 9308-1:2012) able to quantify *E. coli* growth. The developed biosensor system reliably detected *E. coli* as low as 1 cfu/mL in marine water in a maximum of 10-12 hours. This enabled a minimum of two analyses per day, therefore a tighter control of water conditions and near real-time results.

The study paves the way for the development of an automatic monitoring platform, remotely controllable, intended for the management of water resources and acting as an early warning system launching alarm signals when fixed threshold values are exceeded.

Keywords: Biosensor, Escherichia coli, Microbiology, Remote control

P34 - Fluorescent microfluidic device based immunoassay for therapeutic drug monitoring: acenocoumarol case

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Abstract

A high-throughput screening (HTS) immunochemical method for the measurement of plasmatic levels of oral anticoagulants (OAC) is presented. Oral anticoagulant therapy (OAT), such as acenocoumarol (ACL), warfarin (W) and phenprocoumon (PPC), is prescribed to prevent deep vein thrombosis, pulmonary embolism, myocardial infarction and stroke. About 2% of the population is estimated to be under OAT, which expenditures was about 144 million dollars in 2011. The main problem associated to OAT is related to the narrow therapeutic window of these drugs and to the unpredictable dose-response relationship, thus, it is one of the causes for visiting the emergency room at the hospitals.

We present the production of specific antibodies for W, ACL and PPC, and their use to establish an ELISA. The developed immunochemical method is able to accurately quantify these OACs in plasma samples at concentration in the nanomolar range. The ELISA has been used to measure the plasmatic levels of patients under OAT efficiently, accurately in short period of time. Moreover, the high-quality of the antibodies produced allows envisaging the possibility to develop a point-of-care (PoC) device to assist on the patient compliance assessment programs. Therefore, a microfluidic system has been developed and merged with the bioreagents to achieve proof-of-principle for a disposable device that could help clinicians monitor patients under OAT. The read-out of the device is based on fluorescent labels that can be easily read with a scanner. The system was tested in order to have a robust and reproducible signal and subsequently an accurate result.

Keywords: high-throughput screening, anticoagulants, anticoagulant therapy, antibodies, immunochemical method, point-of-care device, microfluidic system, fluorescence

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Patent WO2014083226 A1

P35 - Yeast-based amperometric bioprobe for simazine detection in agricultural water and raw cow's milk samples

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Abstract

Weed management practices in farming systems associated with chloro-s-triazine herbicides use or misuse can result in chemical contamination of environmental compartments and foodstuffs. Simazine is chloro-s-triazine of great agronomical importance worldwide in particular in the United States, Canada, Brazil and China (the world's largest producer and consumer of simazine). Due to its moderate environmental persistence, simazine can contaminate surface waters and groundwaters and thus it can be transferred into the food chain (e.g. in milk chain) through cow consumption of contaminated forage crops and/or contaminated water. Such environmental and food simazine contamination events are rising public health concerns, in particular for reported both short- and long-term toxic effects on non-target organisms' health (e.g. endocrine disrupting). Against this background, simplified, sensitive and rapid screening methods and portable sensoristic devices for detection of chemical contaminants (including simazine) in environmental and food matrices are increasingly required.

In this work, a *Saccharomyces cerevisiae*-based amperometric biosensoristic device (hereafter referred as yeast-based bioprobe) was utilized to detect the presence of simazine in fortified agricultural water and raw cow's milk samples. Percentage interference (%ρ) with aerobic cellular catabolism (as biomarker of exposure) was assessed by measurements of oxygen consumption of exposed yeast cell suspensions compared with control yeast cell suspensions. More specifically, positive %ρ values indicate cellular respiratory inhibition and negative %ρ values indicate cellular respiratory hyper stimulation (i.e. over the maximum physiological rate) of exposed yeast cells. Considering the European legal limits for simazine residues in the matrices considered (0.1 ppb drinking water and 10 ppb in cow's milk), various concentrations (0.1, 0.2 and 0.02 ppb for agricultural water and 200 and 2 ppb for cow's milk) were tested.The results obtained from short-term (2h) exposures showed that the yeast-based bioprobe is able to detect the presence of simazine in agricultural water (14.00 %, 36.80% and 20.29% for 0.02, 0.1 and 0.2 ppb respectively) and in raw cow's milk (20.29% and -24.45% for 2 and 200 ppb respectively) without any necessary sample pretreatments. In particular, for both tested matrices yeast-based bioprobe was able to detect simazine residues up to concentrations 5 times below the legal limits. The proposed yeast-based probe for simazine proved to be very sensitive and the absence of samples pretreatments make it potentially employable as field screening method. Thanks to simplicity of execution of tests, this bioprobe lend itself to automation and integration in the patented technological platform BEST (PCT WO/2010/001432): (Bio)Sensors' system in Food Safety as at-line monitoring system for environmental and food surveillance at critical control points.

P36 - Design of a bioprobe for total estrogenicity levels determination in environmental and food matrices: a biotechnological approach

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Abstract

Endocrine active substances (EASs) include natural hormones and phytoestrogens but also endocrine disrupting compounds (EDCs), a large class of structurally diverse compounds (both of natural and anthropic origin) whose interaction or interference with normal hormonal functions resulting in adverse effects. In particular, estrogenic EDCs can negatively influence endogenous hormone balance of exposed organisms, and such unbalance has been associate with onset of adverse health effects like testis, prostate and breast cancers.

Both animals and humans are widely exposed to those compounds both through interaction with environmental compartments and through consumption of foodstuffs contaminated e.g. by residues of chemicals like pesticides (e.g. chloro-s-triazines and organochlorines) polychlorinated biphenyls(PCBs) and various chemicals (e.g. monomers and additives from plastics like phthalates and bisphenol A) migrating from food contact materials.

Several bioassays and screening methods for estrogenic endocrine active substances detection described in literature. In particular, yeast screening assayes exploit genetically modified yeast with ER-α and ER-β estrogenic receptors, linked to genomic signaling events and a two plasmids cloning strategy (one containing the gene for the responsive element and the other for the reporter element) is frequently used.

In this work, developing a genetically modified yeast (*Pichia pastoris*) yeast-based bioprobe for the determination of the total estrogenicity levels (linked to the presence of EAS and/or estrogenic EDCs) in food matrices (e.g. raw milk) and environmental matrices (e.g. zootechnical sewages) is described.

In order to reduce response time of genetically modified *P. pastoris* cells in presence of estrogenic target compounds, a one plasmid-based genetic engineering strategy was adopted. More specifically, the plasmid was designed and constructed with a membrane estrogenic-receptor (GPER-1) gene tagged with a gene encoding fluorophore mCherry. Thus, genetically modified *P. pastoris* cells harbor a fluorescently-tagged GPER-1 (that involve non-genomic signaling events) whose interaction with target EASs could result in fluorescence signal variations.

Performances of the bioprobe will be pre-validated with fluorescence analyses on genetically engineered *P. pastoris* cells exposed to EASs (both as single chemicals and as mixtures) first in simulated milk solutions and simulated wastewater solutions and then in fortified real samples. Following laboratory validation, the possibility to integrate the designed bioprobe in the patented technological platform BEST (PCT WO/2010/001432): *(Bio)Sensors' system in Food Safety* will be evaluated.

P37 - Technological integrated bioelectronic system and relevant control charting for early intervention on food chain and the environment: the BEST Platform

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Abstract

The Hazard Analysis and Critical Control Points (HACCP) approach is the widely used on-enterprise strategy to control and manage the safety of food production process as well as to support traceability and liability. The milk chain is particularly challenging and challenged by practices (e.g. water dilution of milk or mixing with milk from different species) possibly hindering safety issues, or unintended/unexpected contaminations. Indeed, the milk chain would benefit of early identification of anomalies to shield from commercial frauds and guarantee both authenticity and traceability of the food product.

Innovative technologies for monitoring such factors as farm animal health, productivity, food wholesomeness and traceability can make a substantial difference. The BEST Platform (PCT WO/2010/001432) is a system for environmental diagnostics and monitoring and toxicological self-monitoring and traceability in the food chain, including primary production. BEST comprises: i) a detecting unit, having a plurality *n* of independently selectable probes; ii) a data processing unit for acquiring data from said probes, that is connectable to a data collecting module provided with user interface means. The detecting unit is provided with a plurality *m* of biological media ($0 \leq m \leq n$), and each one of said n probes is suitable to detect a biological, chemical, or physical parameter relevant to quality and/or safety thereby generating a suitable signal. The data-collecting module is suitable to store data, and the data processing unit simultaneously control chart the signal of the said probes, thus identifying and determining concurrent anomalies of said parameters in control charts and/or other diagrams. A warning indication is produced via the said interface means based on at least one alert threshold or at least one intervention threshold through the comparison of data with external or self-educated thresholds, in one or more Critical Control Points (CCP) and/or points of particular attention of a food chain and/or a environmental compartments.

The system comprises a plurality n of electronic boards, each one connected to one respective probe and to the data collecting module, and suitable for carrying out a pre-processing of the signal of the respective probe. The probes and the relevant electronic boards are connected in parallel, so that the system is suitable to carry out diagnostic, environmental monitoring and toxicological self-monitoring and traceability in the food chain, including primary production, by early identification, control, monitoring and managing of anomalous variations of an integrated grid of indexes, with respect to a variation range which is considered normal. Finally, the data processing unit is suitable to carry out a statistic analysis, such as a multivariate analysis, to assess integrated indexes.

The BEST Platform was applied to the milk chain monitoring in the ALERT project - www.alert2015.it - (Consortium integrates 3 public scientific bodies: Istituto Superiore di Sanità, Consiglio Nazionale delle Ricerche, Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, and 7 qualified Italian small medium-sized enterprises encompassing the fields of dairy production, sensor technologies and marketing). Through these applications, BEST patent aims for a fundamental step forward: from invention to innovation through the encounter between Technology Industry and Public Health.

P38 - Agreement between Official control and self-monitoring: data report in an Italian dairy chain

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Abstract

Cow milk is a relevant component in the Western Consumer's diet. The nutritional and economic value of raw milk, as well as its physicochemical properties are directly dependent on milk composition, which, in turn, give valuable information on herd nutritional status and general health. Daily measurements of milk components, both at the individual and herd level, is becoming a common tool to assess the safety and economic value of milk production. Following EU Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002, Italian milk processing industries follow a strict self monitoring process per HACCP, and are subject to Official control by the Italian Competent Authority (Ministry of Health, Moh). A network of 10 Experimental Zooprophylaxis Institutes (IZS) provide accredited laboratory services for analyses for raw milk samples.

However, limited scientific evidence is known about measurements agreement between the milk analyses carried on by official control bodies and the internal ones done by milk processing industries. A number of factors could affect this agreement. Different methods in milk component quantifications (different techniques, with different LOD and LOQ, as well as measurement uncertainty) could be a primary source of disagreement. A second difference between the two measurement chains is that, while the internal industrial monitoring routines are carried on immediately after the raw milk arrival at the processing premises, official monitoring procedures are carried on by sampling the milk, and delivering the sample to the accredited laboratory. While this procedure is validated and refrigerated transportation is routinely used, the effect on raw milk samples biological parameters, especially during the hot season, may be still partly understood.

The main aim of the present study is to verify the agreement between Official Control and Self-control measurements in raw milk processing industry, identify any possible discordances or biases, and to possibly point out procedures to overcome these discordances.

In the framework of the Alert 2015 (http://www.alert2015.it/) project funded by the "Industria 2015" program of the Italian Ministry of Economic Development, a chance was given to collect simultaneous data coming from the official control IZS- LT (Lazio and Tuscany Section) and the industrial Partner Centrale del Latte di Roma Spa (CLR).

Milk production and component measurement data were obtained during a purposely developed monitoring program involving CLR, a FSSC 22000:2010 – certified milk processing plant with an average 350000l/day throughput. The monitoring program started in March 9, 2014 and ended in June 24, 2014. Data collection regarded the daily raw milk collection carried on by CLR on a regional basis, consisting in a daily average 137000 Kg of raw milk collected from local farmers via a fleet of 15-metric tons refrigerated trucks. Traceability of bulk milk was limited to groups of individual farmers.

All samples were taken following rules matching both CLR internal procedures and IZS procedures. The first sample was immediately sent to CLR internal labs for analyses, while the second sample was kept refrigerated and sent to the IZS labs.

A total of 114 twin samples were collected and analyzed for 7 twinned variables (Freezing Point, Aflatoxin, Total Bacterial Count, Somatic Cell Count, fat, lactose and proteins percentages). Descriptive statistics, correlation analysis, paired sample Wilcoxon Signed Rank test and a Bland-Altman analysis was performed in order to assess measurement agreement between CLR and IZS determinations. A General Linear model was used to investigate the effect of CLR Total Bacterial

count, time between sampling and IZS analysis, truck tank temperature and farmer's group on the difference between CLR and IZS Total Bacterial Count.
No outlier removal procedure was used. For all statistical analyses, the significance threshold was set at .05.
A Shapiro Wilk normality test performed on each variable in the data set showed that almost all variables could not be modeled by a normal distribution, except for CLR Lactose, CLR Proteins, and IZS Proteins. A Wilcoxon signed ranks test on the twinned variables assessed that Freezing Point, Aflatoxin, Total Bacterial Count, Somatic Cell Count, Lactose and Fat data showed a significant difference in the median of the paired samples.
Correlation and Bland-Altman analysis concluded for a sound agreement between CLR and IZS Proteins measurement. A moderate to good agreement was found for Somatic Cell Count, Fat, Lactose and, to a lesser extent, for Freezing Point determinations. Low agreement was found between CLR and IZS Total Bacterial Count determinations. The General Linear Model highlighted that the difference between CLR and IZS Total Bacterial Count was not influenced by time between sampling and analysis and truck tank temperature. A significant influence on the difference was CLR Total Bacterial Count. Farmer's group (i.e. the group of farmers whose bulk milk was collected together in the truck tank) had a lower contribution on the difference between CLR and IZS Total Bacterial Count.
This study confirmed a good/ moderate to good agreement between Official Control and Self-control measurements in raw milk processing, at least for Proteins, Somatic Cell Count, Fat, Lactose, and for Freezing Point determinations. A remarkable discordance was found for Total Bacterial Count, which, in line of principle, could not be attributed to differences in the analytical methodologies. Truck tank temperature, and the number of hours between sampling and IZS analysis did not affect this discordance. In turn, the absolute value of CLR Total Bacterial Count did affect the difference, as well as the milk origin (traced up to groups of farmers). Further research is needed to identify how these factors can influence the observed discordance.

Enzymatic quantification of glyphosate herbicide using a colorimetric PD-CMOS platform

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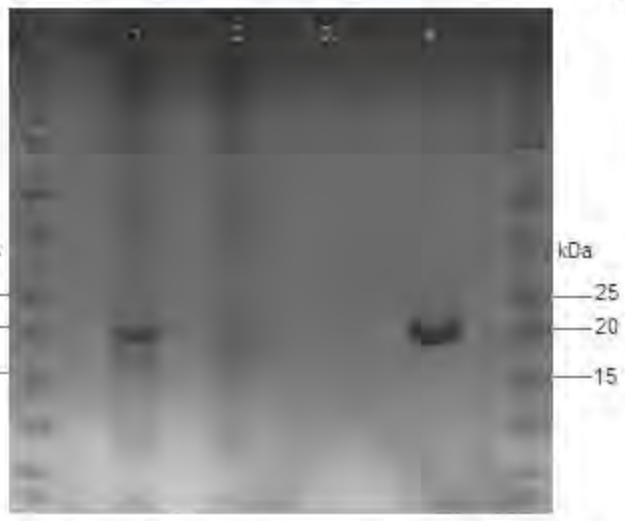
Introduction

Glyphosate is currently the most intensively used herbicide worldwide for weed control. Due to contrasting claims with respect to the safety of glyphosate, the European Union has initiated an investigation into the carcinogenic potential of this agrochemical. To this end, our work focused on developing an enzymatic biosensor for glyphosate quantification using a photodiode complementary metal oxide semiconductor (PD-CMOS) platform. We employed the use of an optimised glyphosate *N*-acetyltransferase enzyme which developed a colorimetric reaction in the presence of an acyl-donor. A good linear correlation was obtained in the concentration range of 0.01-100 µM (R^2= 0.98), with a response time of less than five minutes. In Europe, the tolerated limit for herbicides in drinkable water is 0.59 µM. As a result, recent reported glyphosate biosensors have detection limits from 25 pM to 47 uM, making our proposed biosensing model fit for development. Therefore, with a simple and specific enzymatic glyphosate sensing procedure coupled with an economically scalable CMOS platform, the proposed biosensor represents an ideal prototype for glyphosate monitoring in the environment.

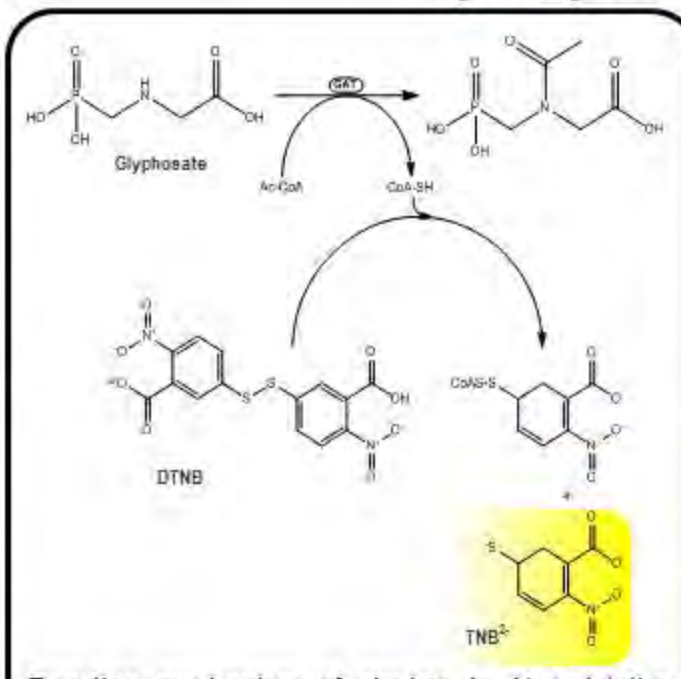
Biological component



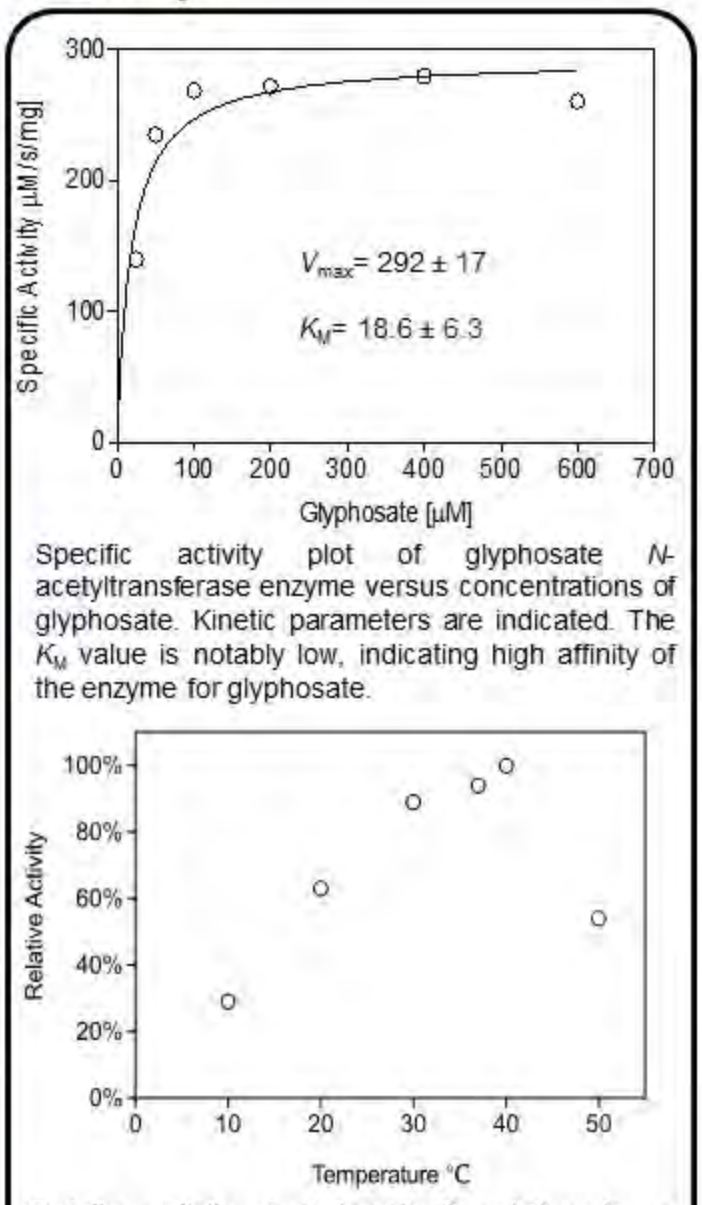
SDS-polyacrylamide 12% gel of purification stages for glyphosate *N*-acetyltrasferase enzyme. Lane 1 crude protein; lane 2 flow through; lane 3 wash; lane 4 purified protein (~18 kDa).

Recombinant protein yield
N-acetyl transferase: 20-38 mg/mL

Spectrophotometric analysis



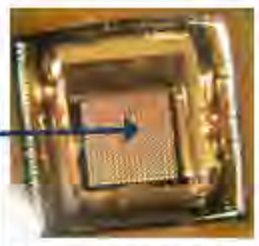
Reaction mechanism of glyphosate *N*-acetylation coupled with TNB^{2-} formation for colorimetric detection and quantification. The glyphosate *N*-acetyltransferase enzyme (GAT), transfers an acyl group from acetyl coenzyme A (Ac-CoA) to glyphosate concomitantly releasing CoA-SH. Then, Ellman's reagent (DTNB) reacts with the released CoA-SH forming a yellow dianion TNB^{2-}(λ_{max}= 412nm).



Specific activity plot of glyphosate *N*-acetyltransferase enzyme versus concentrations of glyphosate. Kinetic parameters are indicated. The K_M value is notably low, indicating high affinity of the enzyme for glyphosate.

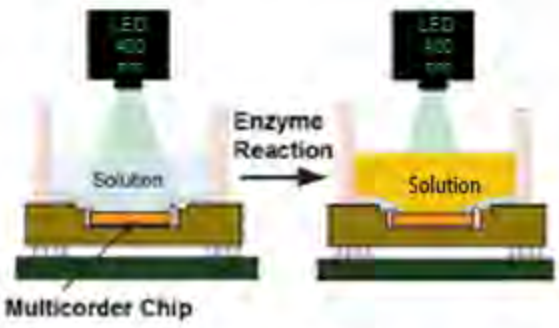
Relative activity of glyphosate *N*-acetyltransferase versus varying temperatures. The optimal thermal conditions are crucial for activity. It can observed that the enzyme has the highest activity when the temperature is set at 40 °C. Our standard assays are carried out at room temperature (~20 °C), where nearly 70% of activity is retained.

Biosensor modality and calibration

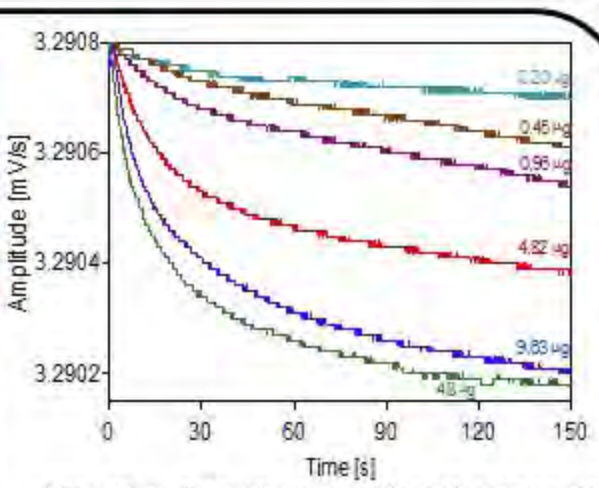


The photodiode sensor comprises of 256 active pixels, each individually collecting data. An average read-out can be generated through LabVIEW Software.

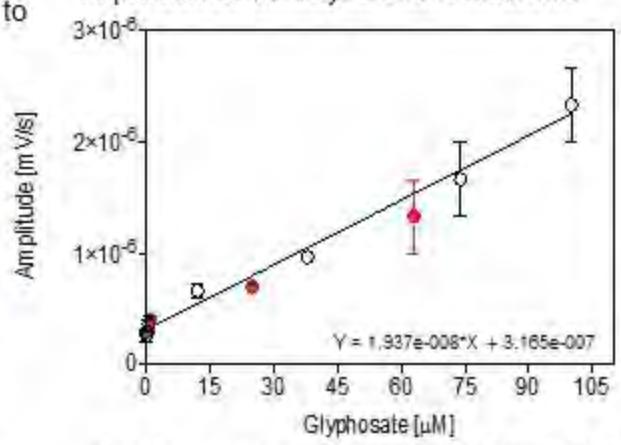
Handheld PD-CMOS platform. The microchip is detachable and easy to manufacture. The main body of the platform converts the electrical signals recorded into digital data. Transmission of recorded data is through Bluetooth via a connected device. The programmed software is able to access the received input and process the data.



Schematic of function for the glyphosate PD-CMOS platform. The reaction mix is placed on top of the photodiode sensing area. Each pixel records the absorbance at λmax= 400 nm versus time. The enzymatic reaction is initiated only after a satisfactory baseline is established. Averaged response time of the sensor for glyphosate detection is under 1 minute.



Microchip data showing different ranges of enzyme concentrations added to a fixed concentration of glyphosate (74 µM). An appropriate addition of enzyme concentration is crucial for determining the precise concentration of enzyme in the assay. The PD sensor depicts rate dependence on enzyme concentrations.



Linear range of detection established for glyphosate on the PD-CMOS sensor. The concentrations tested varied from 0.01-100 µM. The R^2 value is 0.98, indicating a good linearity between the measured rates and the concentrations of glyphosate tested. Initial validation tests were carried out in water samples and rates were determined for unknown samples (red circles).

Conclusions and future work

- Kinetic parameters were determined for glyphosate *N*-acetyltransferase enzyme followed by a temperature stability study.
- We have managed to successfully reach low detection limits for glyphosate quantification (0.01 µM) using the PD-CMOS sensor.
- Key measurements were established by using the PD sensor by incorporating the reaction mechanism proposed.
- Future proposed studies would include optimising the calibration curve further and adding more validation samples. A variety of spiked and unspiked samples would be tested for glyphosate detection and quantification (soil ,water, beer, honey or orange juice).
- Enzyme immobilisation on microchip surface is a crucial step that is currently under testing and development.

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NANOFIBER ARCHITECTURES FOR ELECTROCHEMICAL BIOSENSING OF GLUCOSE

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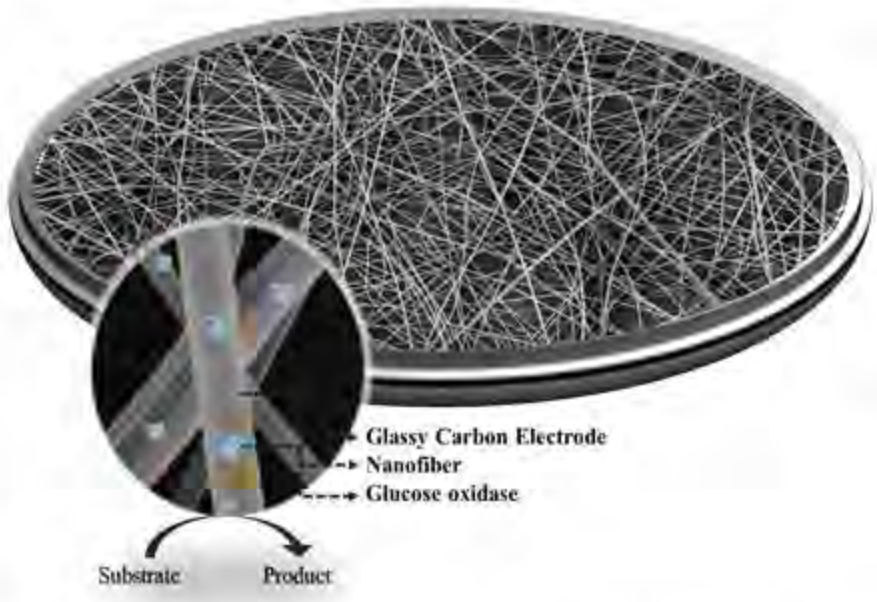
BBMEC 12th Workshop on Biosensor & Bioanalytical Microtechniques in Environmental, Food & Clinical Analysis
INTERNATIONAL BIOSENSOR CONFERENCE
25. - 29. September 2017 | Rome, Italy

Abstract

We constructed a cellulose acetate/chitosan (CA-CHIT) nanofiber surface as a novel platform for the biosensing of glucose. Initially, Glucose oxidase (GOx) were covalently bound to the nanofiber surface by cross-linking of glutaraldehyde. CA-CHIT/GOx biosurface was tested for the detection of glucose by amperometric detection. Afterwards, the investigation of analytical and surface characteristics of CA-CHIT/GOx platform were accomplished by electrochemical techniques as the final step. The developed biosurface may be an alternative platform for glucose sensing with the support of modifiable nanofiber structures and also can be used as an novel platform for biosensing.



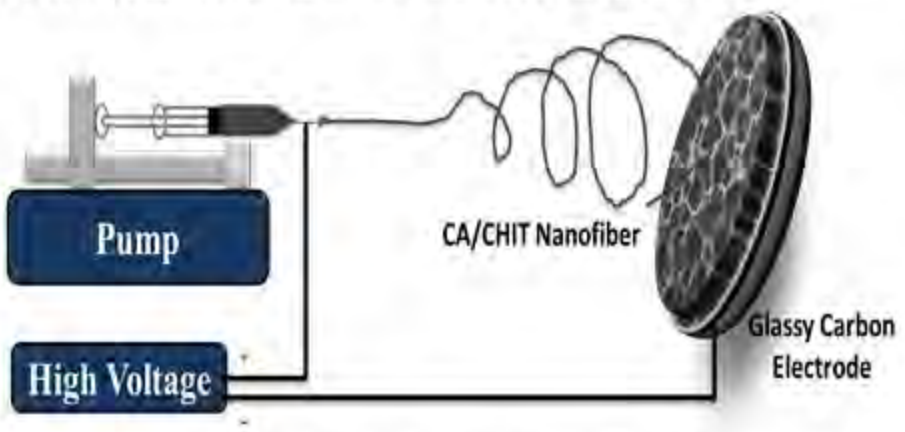
Introduction

Development of highly selective and sensivite glucose sensors is highly active area of sensor research on account of its wide range of application in the fields of diabetes management, food industry, environmental screening,bioprocess monitoring and development of fuel cells. Compared to non-enzymatic systems, enzymatic glucose detection, based on the oxidation of glucose in the presence of Glucose oxidase (GOx) enzyme, is widely used to construct glucose sensors. In recent years, researchers have focused on nanomaterials to enhance the stability, sensitivity and selectivity of glucose sensors [1]. For the immobilization of enzymes on the electrode surface, conducting polymers, functionalized polymers, clays, calixarenes, composite materials, sol-gel, and natural polymers such as gelatin, chitosan and nanomaterials can be used. Among these, electrospun nanofibers can be a useful alternative material as an immobilization matrix due to its high surface area, controlable thickness and diameter, porous structure and chemical inertness which can increase the performance of the sensor. Compared with other methods, electrospinning is the most common and cost-effective technique to obtain long continues fibers. In electrospinning process, the polymer solution is charged to high voltage to produce diverse forms of nanofibers [2].

Results

Parameters that considered to construct smooth CA/CHIT nanofibers

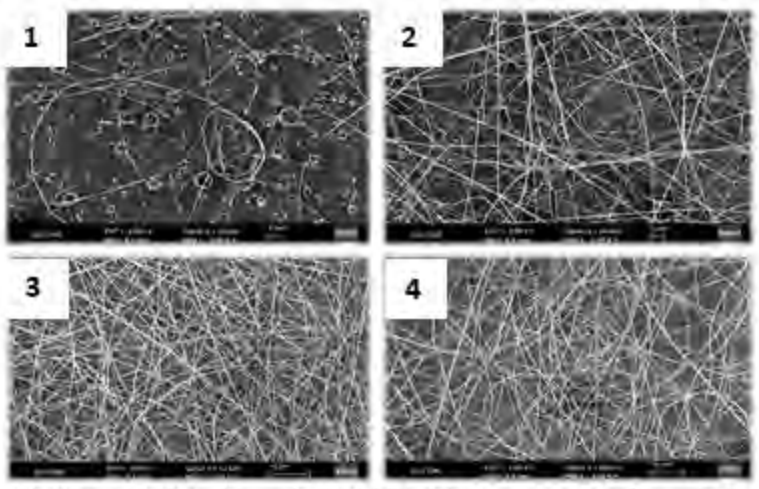
Solvent Select
Molecular weight of Polymer
Polymer concentration
Conductivity
Viscosity
Dielectric constant
Dipole moment
Surface tension
Applied voltage
Distance between needle and collector
Flow rate



Deposition of CA-CHIT on Glassy Carbon Electrode

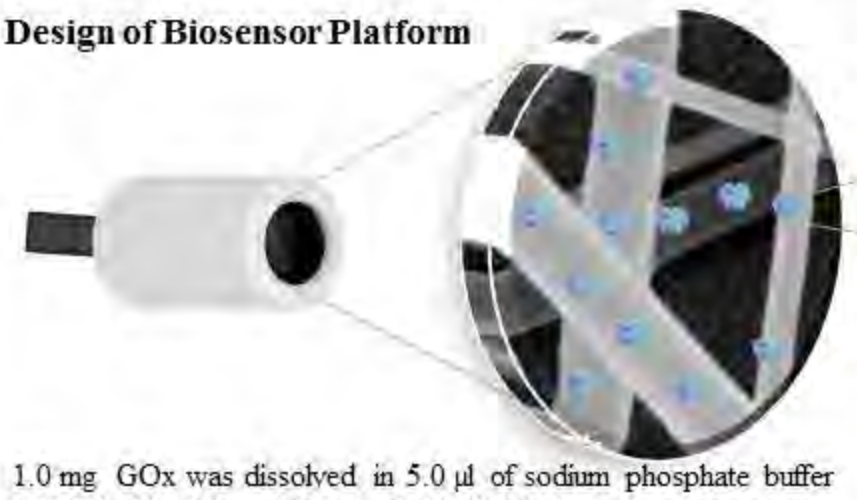
Conditions; %wt 20 CA and %wt 0.5 CHIT were dissolved in TFA/FA/DCM 4.5:0.5:5 (v:v:v), Distance: 17 cm, Flow rate: 1.5 ml/hr.

SEM images of CA-CHIT nanofibers

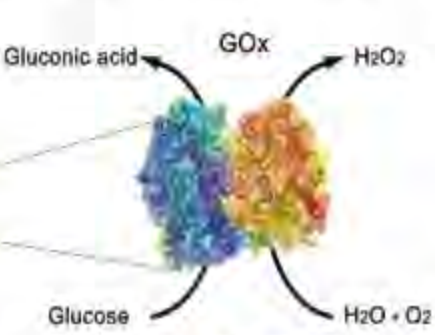


1.) %wt 10 CA- %wt 0.5 CHIT, 2.) %wt 15 CA-%wt 0.5 CHIT, 3.) %wt 20 CA-%wt 0.5 CHIT, 3.) %wt 25 CA- %wt 0.5 CHIT

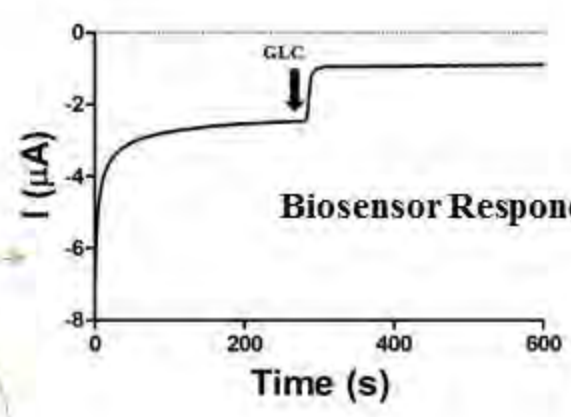
Design of Biosensor Platform

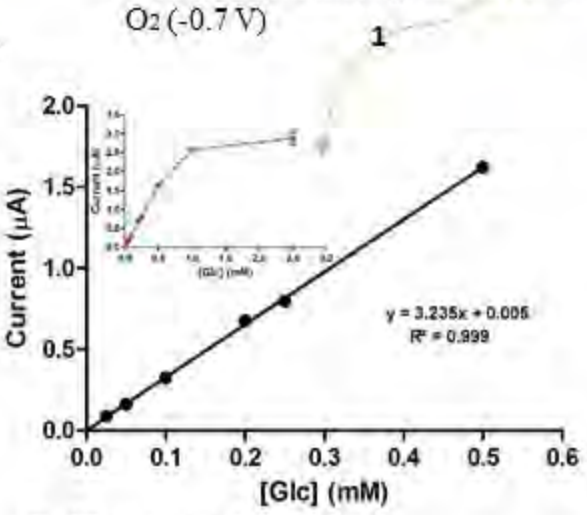


1.0 mg GOx was dissolved in 5.0 µl of sodium phosphate buffer (pH 7.0, 50 mM) and 2.5 µl of GA (0.5 % in sodium phosphate buffer pH 7.0, 50 mM) were added into the enzyme solution. After mixing, the solution was dropped onto a GC surface homogeneously. The electrode was allowed to dry at room temperature.

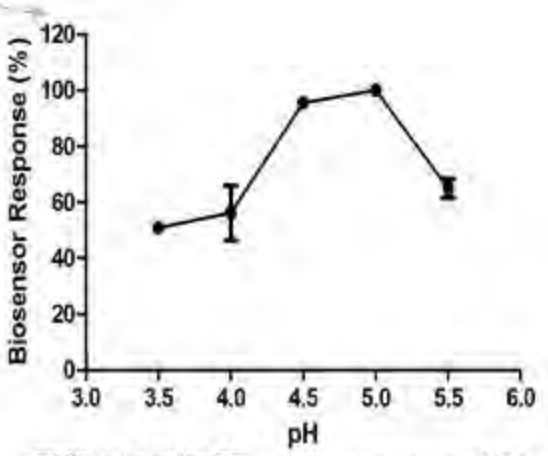


Amperometric Detection of Glucose, Monitoring utilization of O_2 (-0.7 V)





Calibration plots for first-generation glucose biosensors using CA-CHIT/GOx biosensor (in sodium acetate buffer, 50 mM, pH 5, error bars show S.D. of two measurements. Km: 0.79, Vmax: 4.01.



Effects of pH on the response of CA-CHIT/GOx biosensor (in 50 mM sodium acetate and phosphate buffers at ambient conditions, error bars show S.D. of two measurements)

Conclusion

In conclusion, CA-CHIT offer new platforms for enzyme immobilization and CA-CHIT/GOx biosensor can be a alternative to glucose biosensors

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Detection of bacterial toxins via surface plasmon resonance with florescence spectroscopy based biosensor

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Water contamination and access to clean water is one of the important problems concerning both developed and underdeveloped countries. Fast detection of waterborne pathogens would prevent the spread of the related diseases and help to take immediate action against them.

This study aims to construct fast-response biosensor that recognizes toxins of waterborne pathogens. For this purpose, surface plasmon resonance with florescence spectroscopy (SPFS) method was used. Recognition layer was prepared on gold-coated LaSFN9 glasses using three different modifications: Poly(N-isopropylacrylamide) (PNIPAAm)-based terpolymer (containing methacrylic acid and benzophenone methacrylate comonomers), a carboxymethylated dextran (CMD), or 11-mercaptoundecanoic acid (11-MUA). For the detection, sandwich assay format was chosen and truncated form of cholera toxin (Cholera toxin beta subunit; Ch-B) was used as model analyte.

MATERIALS AND METHODS

- ✓ LaSFN9 glasses were coated with 2 nm Cr and 50 nm Au for surface plasmon resonance (SPR) experiments (Figure 1).

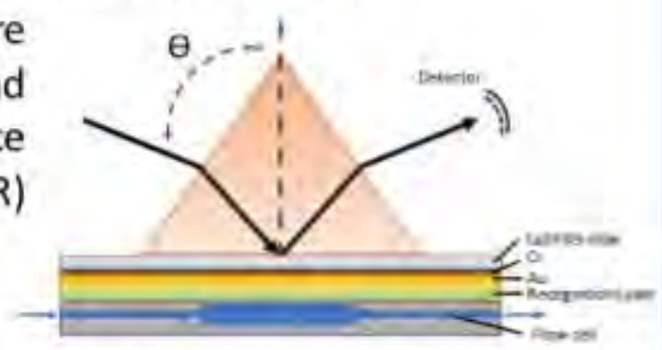

Figure 1: SPR measurement unit

- ✓ Recognition elements were immobilized on 11-mercaptoundecanoic acid (11-MUA) (Figure 2-A), PNIPAAm based terpolymer or carboxymethyl dextran (CMD) (Figure 2-B).
- ✓ PNIPAAm based terpolymer (Figure S1) and CMD (Figure S2) were synthesized and characterized by Nuclear Magnetic Resonance (NMR) spectroscopy (Figures S3 and S4).
- ✓ Polymers were spin coated and crosslinked on SPR slides (Figure S5).

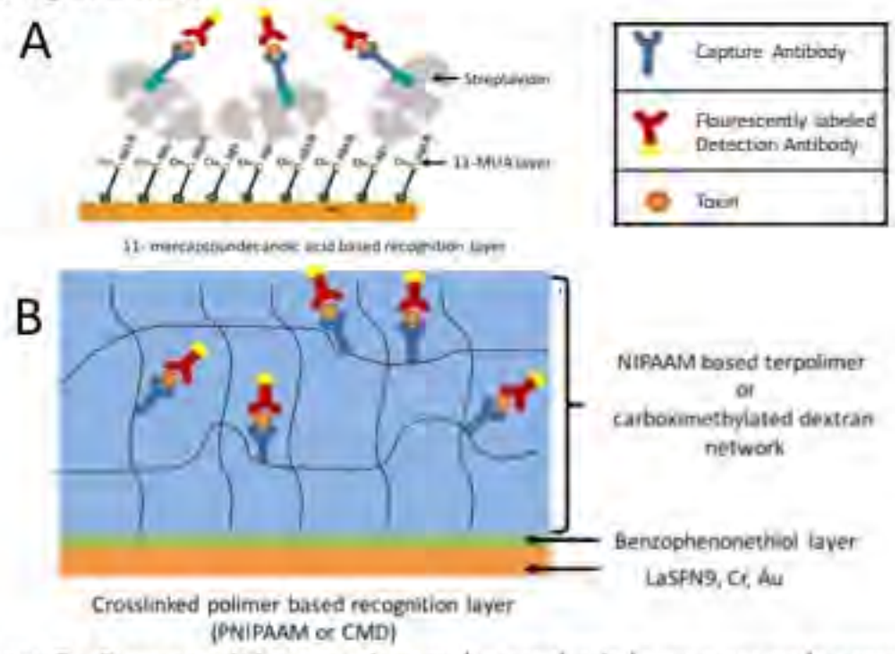

Figure 2: Recognition unit and sandwich assay scheme

- ✓ **Cholera toxin beta** (Ch-B) (Figure 3) was chosen as the model analyte.

Figure 3: Cholera Toxin Beta subunit

- ✓ **Capture antibody** (Ab) was biotinylated Ch-B Ab (B-Ab) for 11-MUA coated surface, and unlabeled Ch-B Ab (U-Ab) for polymer coated surfaces.
- ✓ **Detection Ab** was Cy5 conjugated Ch-B Ab.

RESULTS

- ✓ SPR results for B-Ab/Streptavidin (SA) surface(Figures 4 and 5).
- ✓ SPR results for U-Ab /PNIPAAm surface (Figures 6 and 7).

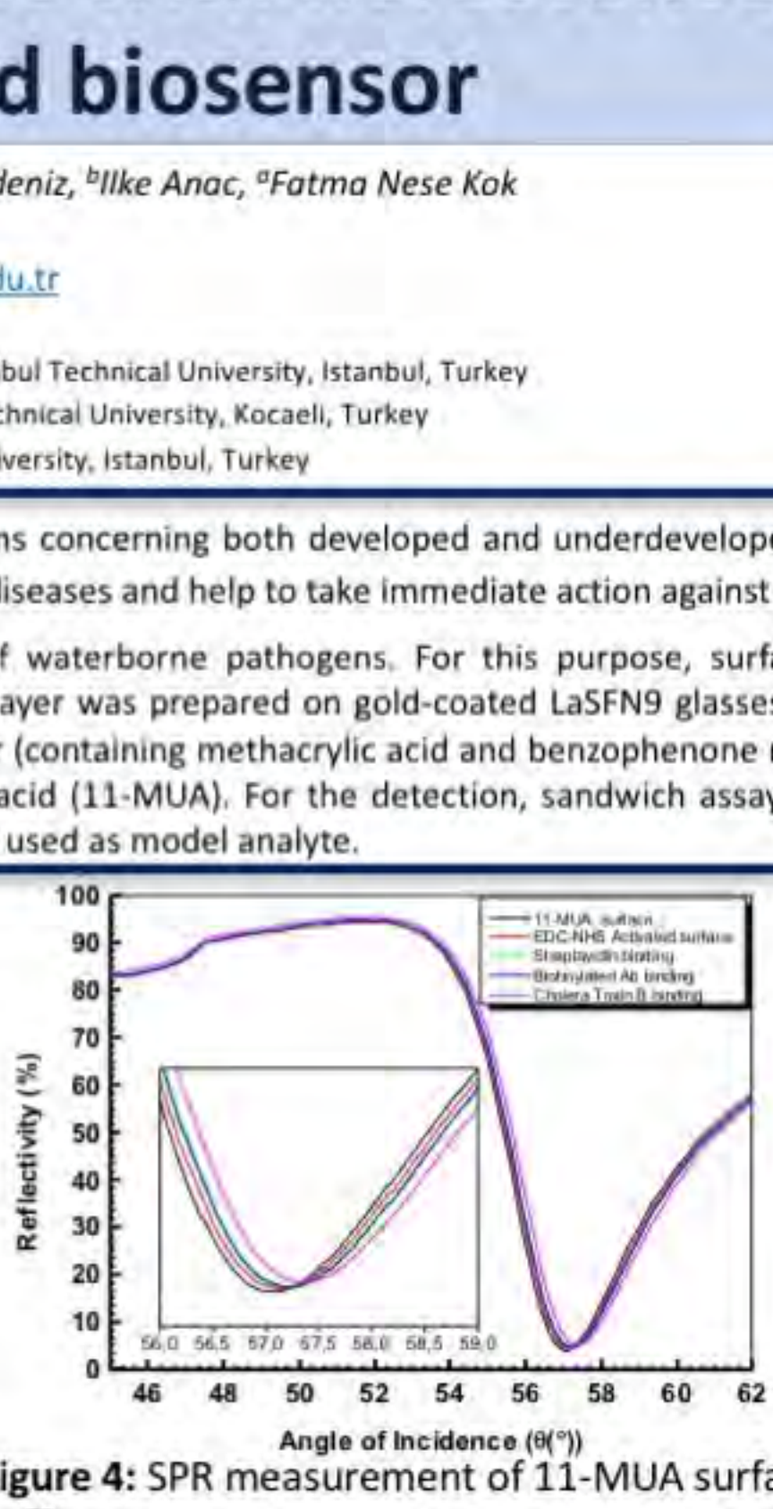

Figure 4: SPR measurement of 11-MUA surface

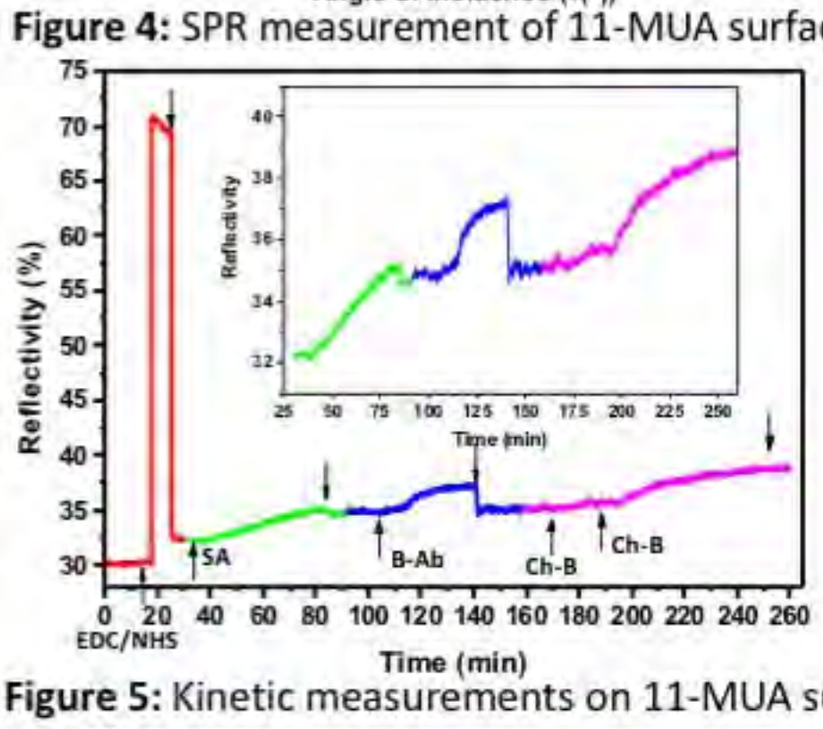

Figure 5: Kinetic measurements on 11-MUA surface

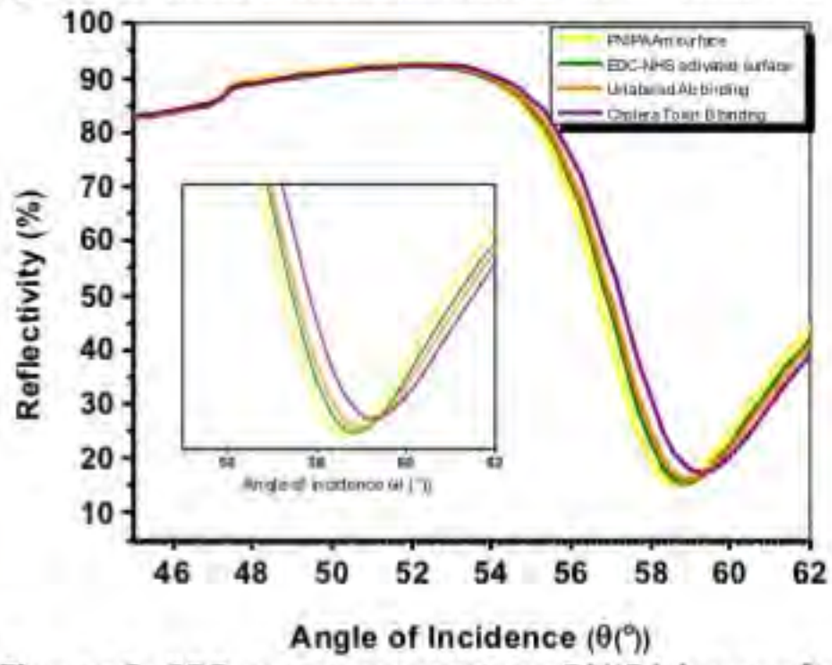

Figure 6: SPR measurement on PNIPAAm surface

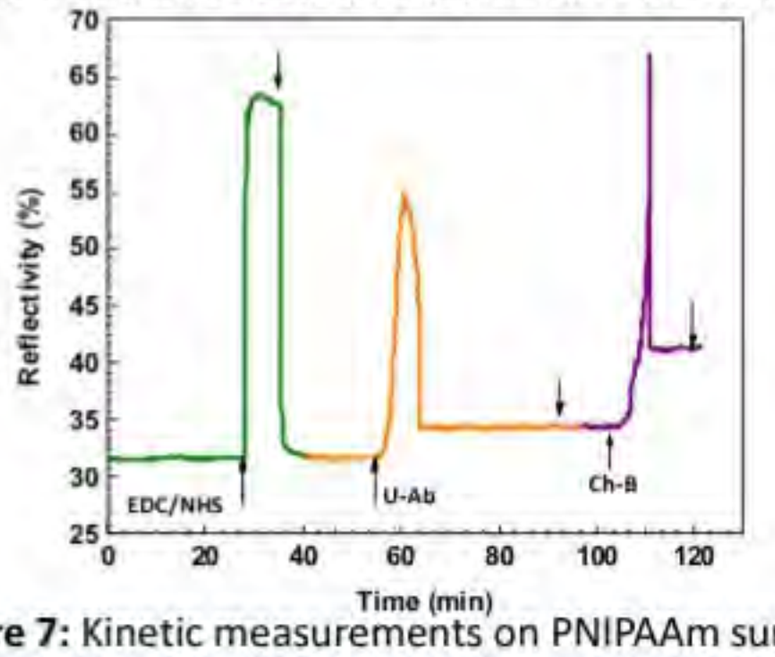

Figure 7: Kinetic measurements on PNIPAAm surface

CONCLUSIONS and FUTURE WORK

- ✓ Capture Ab immobilization was successfully achieved on both 11-MUA and PNIPAAm based terpolymer.
- ✓ System will be optimized for carboxymethyl dextran derivative.
- ✓ Two different Ch-B concentrations were determined (10 & 20 mg/mL) w/o fluorescence measurement. Lower detection limits were expected using fluorescently labelled detection Ab.

ACKNOWLEDGEMENTS

The authors would like to thank The Scientific and Technological Research Council of Turkey (Grant no:115E015) for providing financial support to this project

POLYPEPTIDE WITH ELECTROACTIVE ENDGROUPS AS SENSING PLATFORM FOR THE ABUSED DRUG 'METHAMPHETAMINE' BY BIOELECTROCHEMICAL METHOD

Bilal Demir, Tulay Yilmaz Sengel, Emine Guler, Z. Pinar Gumus, Huseyin Akbulut, Ebru Aldemir, Hakan Coskunol, Demet Goen Colak, Ioan Cianga, Shuhei Yamada, Suna Timur, Takeshi Endo, Yusuf Yagci

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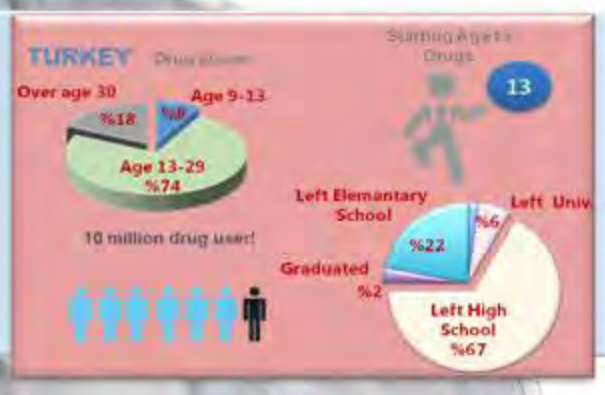
INTRODUCTION

Recently, rapid growth of drug abuse has been a major problem which causes indisputable social and economic damages. Methamphetamine (METH), an illicit drug associated with severe neurological and physical consequences afflicting its users, remains a significant public health concern worldwide. Although METH use in the United States has displayed a stable trend among the general population aged 15–64 between 2010 and 2013; deaths related to METH use have increased by more than 70% between 2008 and 2012 in certain parts of the United States.

Antibody-based analytical techniques enable great advantages with practical, robust and sensitive detection of narcotic drugs by using selective antibodies. Functional and robust polymeric materials have gained attraction in the cost-effective fabrication of novel and sensitive immunosensing systems.
Polymeric structures bearing polypeptides were used widely in various bio-applications such as biosensing, drug delivery, nanoscale self-assembly, as well as tissue engineering.

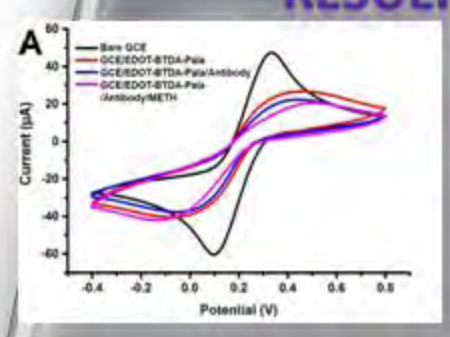
PROBLEM

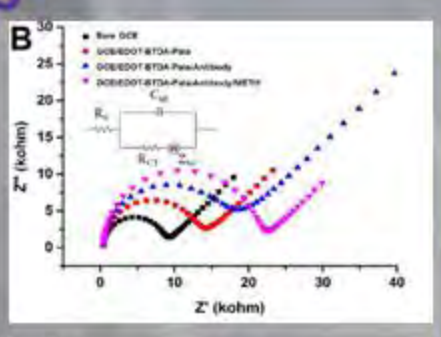


Goal of the Study

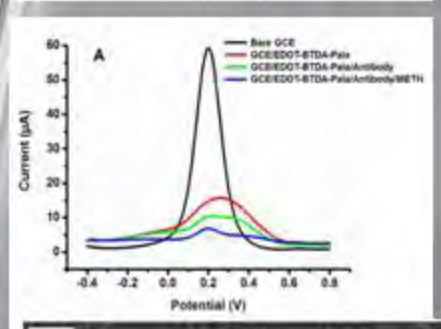
"Construction an abused drug immunosensor with polypeptide-extended polymer which provides diagnosis and tracking of Meth use"

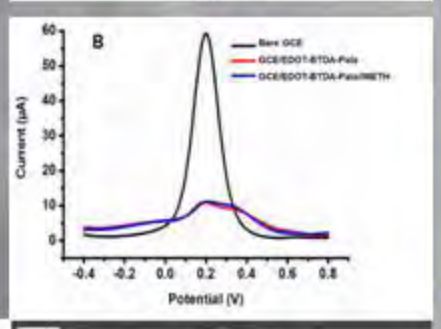
RESULTS





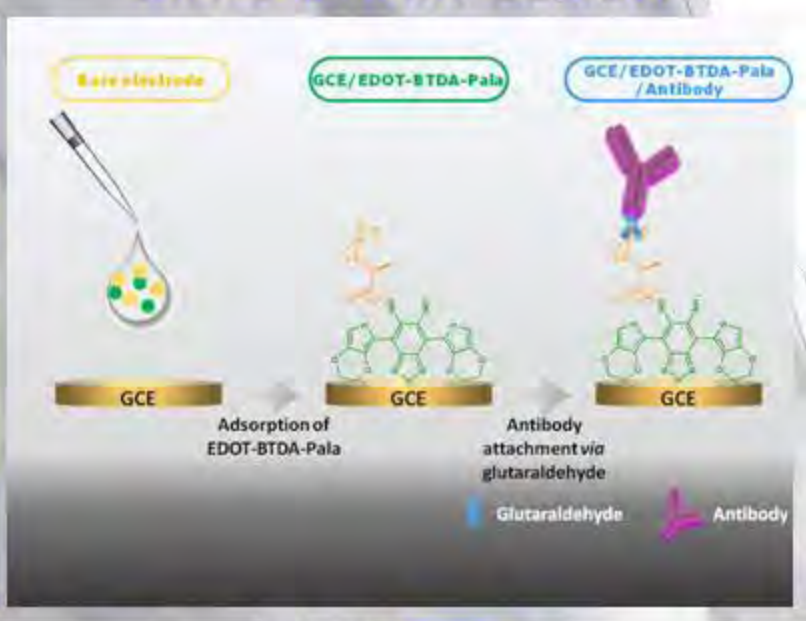
(A) CV and (B) EIS analysis of surface modifications

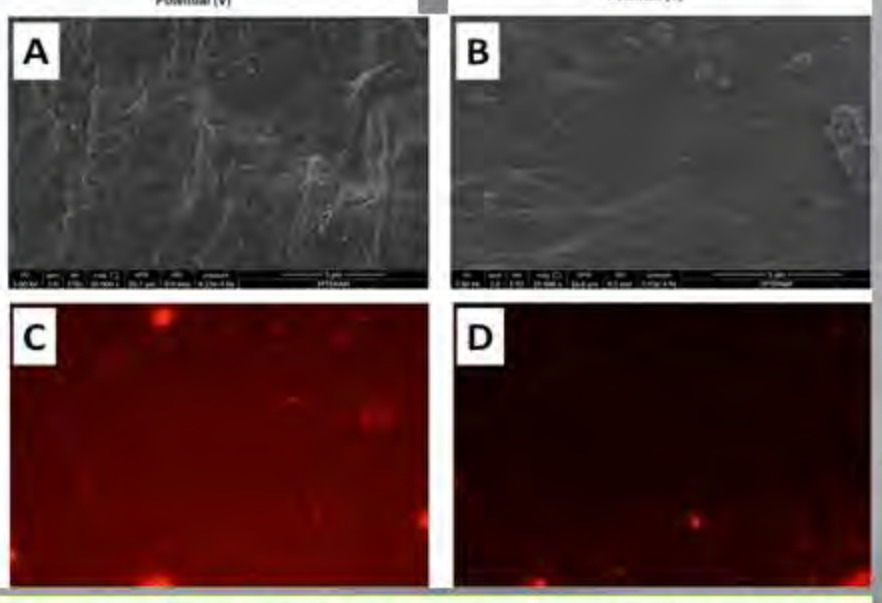




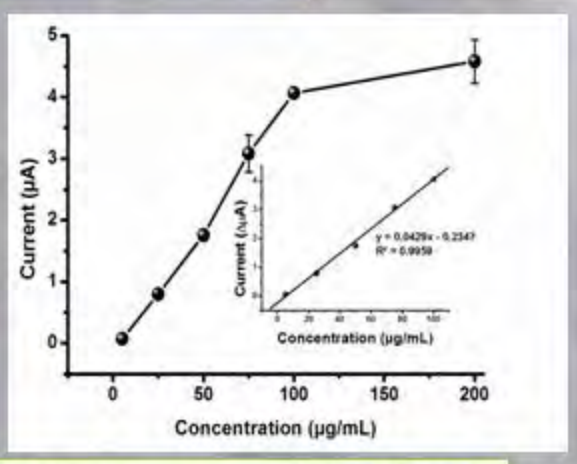
DPV analysis of electrodes in the presenece of (A) METH antibody and (B) only polymer

STATE-OF-ART DESIGN

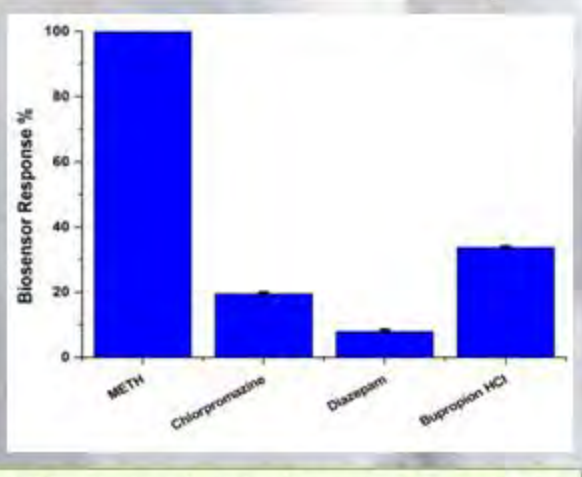




SEM and fluorescence microscopy images of (A, C) ITO/ EDOT-BTDA-Pala and (B, D) ITO/EDOT-BTDA-Pala /Antibody coated surfaces (Red emission; 604-644 nm).



Calibration curve of GCE/EDOT-BTDA-Pala/Antibody/METH generated by DPV method



Influence of interferants over GCE/ EDOT-BTDA-Pala /Antibody biofilm

CONCLUSION

- Illicit drug sensor was improved by using METH antibody for the selective detection.
- Analytical performance of GCE/ EDOT-BTDA/Antibody surface was excellent with its linear range and LOD.
- Comparison of the biosensor and LC-MS/MS results confirmed that GCE/ EDOT-BTDA /Antibody/METH sensor holds reliable usability with great potential and further modifications

B. Demir, T. Yilmaz Sengel , E. Guler et al., Talanta, 2016, 161, 789-796.

Analytical Parameters

Parameters	Values
Linear range (μg/mL)	10–100
Slope (μA mL/μg)	0.0429
Intercept	0.235
S.E of intercept	0.097[a]
S.E of slope	0.002[a]
Correlation coefficient	0.996
LOD (μg/mL)	13.07
Repeatability (± S.D)	0.042
Coefficient of variation	2.38%

*This work is supported by Republic of Turkey, Ministry of Development (Project Grant No: 2016K121190).

Sample application of the biosensor to the spiked biological matrices.

Matrix	Spiked METH (μg/mL)	Found METH (μg/mL)	Recovery	RSD (%, recovery)
Urine	10	9.855 ± 0.015	98.54 ± 0.145	0.152
	25	25.508 ± 1.37	102.03 ± 5.5	5.39
	50	52.995 ± 1.24	105.99 ± 2.49	2.34
Serum	10	10.36 ± 0.041	103.58 ± 0.4	0.395
	25	23.61 ± 0.365	94.43 ± 1.46	1.546
	50	48.514 ± 1.94	97.03 ± 3.876	3.991
Saliva	10	0.931 ± 0.022	93.06 ± 2.2	2.36
	25	22.51 ± 0.007	90.03 ± 0.03	0.034
	50	46.525 ± 0.993	93.05 ± 1.986	2.134

Analytical results of the spiked samples for confirmation via LC-MS/MS.

Matrix	Spiked METH (μg/mL)	Found METH (μg/mL)	Recovery	RSD (%, recovery)
Urine	10	10.47 ± 0.04	103.53 ± 1.50	1.45
	25	27.41 ± 0.02	109.63 ± 0.06	0.05
	50	54.10 ± 0.53	108.2 ± 1.08	0.99
Serum	10	10.63 ± 0.08	106.33 ± 0.75	0.71
	25	27.16 ± 0.50	108.67 ± 2.01	1.85
	50	54.65 ± 0.33	109.30 ± 0.66	0.59
Saliva	10	10.26 ± 0.07	102.56 ± 0.70	0.68
	25	27.11 ± 0.59	108.46 ± 2.38	2.19
	50	54.65 ± 0.73	103.53 ± 1.50	1.45

DEVELOPMENT OF EASY ASSEMBLE IMMOBILIZATION MATRIX WITH MAGNETIC NANOFIBER LAYERS

BBMEC 12th

[a]Serdar Şanlı, [a,b]Emine Güler, [b]Zinar Pınar Gümüş, [a]Dilek Odacı Demirkol, [a,c]Suna Timur

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Introduction

Stabilization of biomolecules on biosensor immobilization matrices is critically important. Many polymers and nanomaterials are used as immobilization matrix with different methods. Direct electro-spinning the fibers on electrode surface could be one of the examples from literature[1,2]. Here in this work it is aimed to form composite nanofibers with superior morphological and magnetic properties by electro-spinning of magnetic nanoparticles in appropriate polymer matrix on collector surface for multiple usages[3].

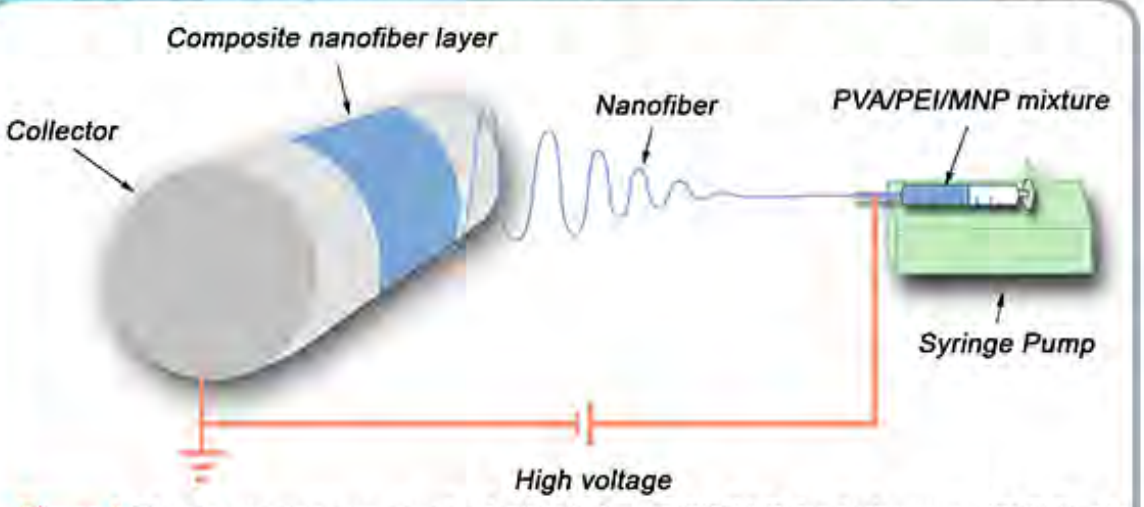


Figure 1.Electrospinning process of polyviniyl alcohol (PVA)/Polyethylenimine (PEI)/Fe_3O_4 magnetic nanoparticles (MNP).(Potential:18 kV, Distance:18 cm, Flow Rate:0,75ml/h)

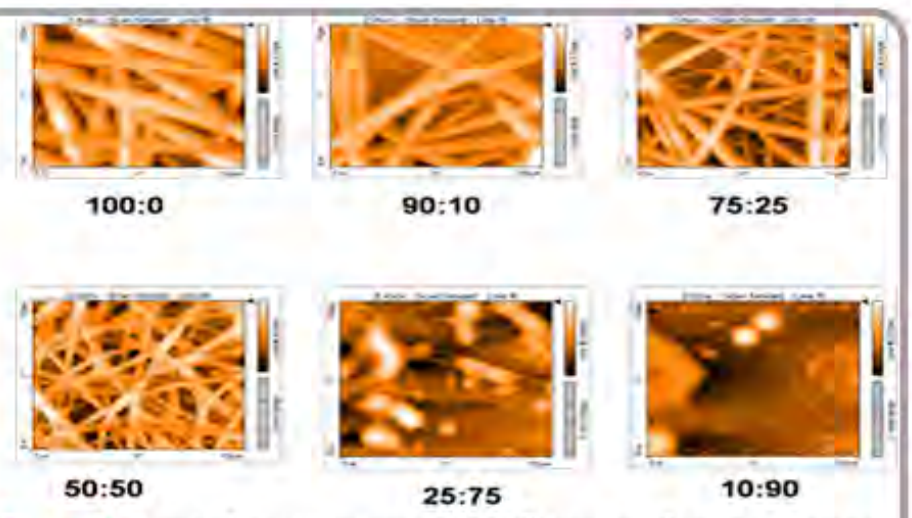


Figure 2. Atomic force microscopy (AFM) images of composite nanofibers with different compostions (mass ratio, PVA:PEI)

Material and Method

Fe_3O_4 (magnetite) is used as magnetic nanoparticle (MNP) with a mixture of polyvinyl alcohol (PVA), polyethylenimine (PEI), to produce a homogenous composite nanofiber layer with electrospinning process (fig.1)[4].Composite magnetic nanofiber(MNF) layer properties were characterized by atomic force microscopy (AFM) (fig.2). As a model enzyme, glucose oxidase (GOx, glucose 1-oxidase, β-D-glucose: oxygen 1-oxidoreductase, EC 1.1.3.4) is immobilized on a piece of produced composite nanofiber layer which cut same size of the working electrode surface, with the usage of glutaraldehyde as crosslinker(fig.3).

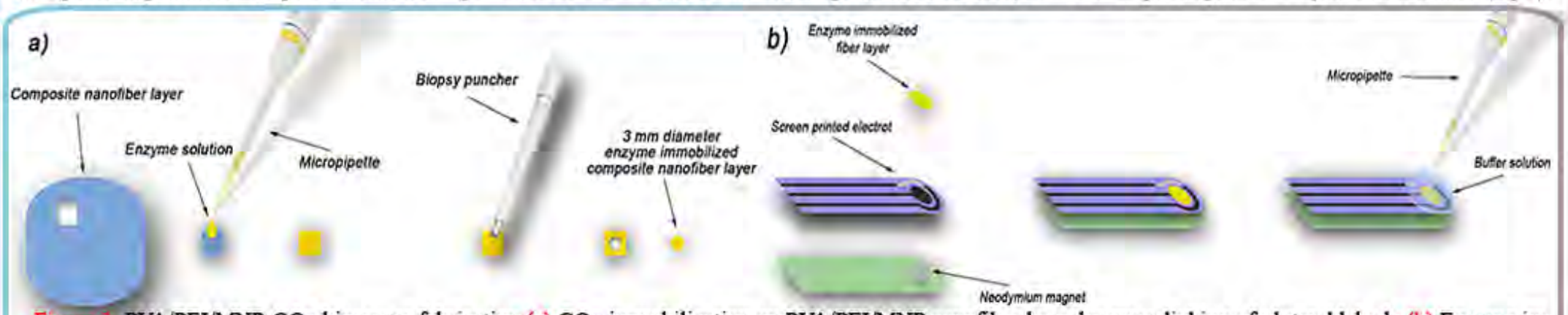
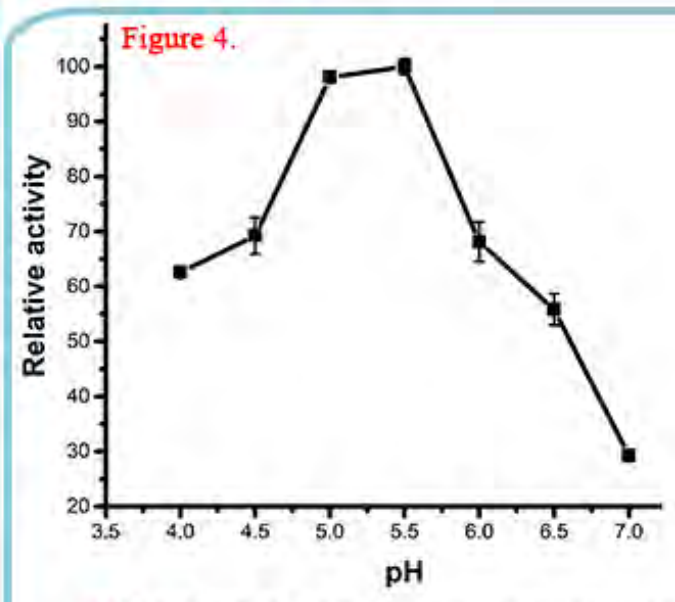


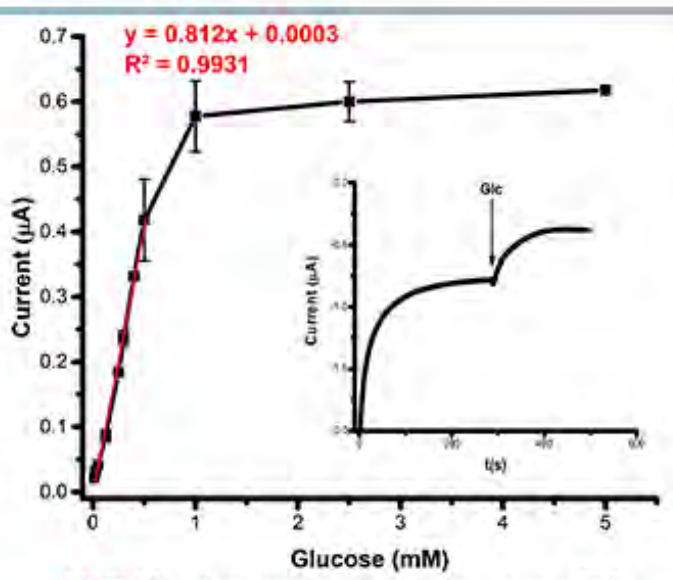
Figure 3. PVA/PEI/MNP-GOx biosensor fabrication (a) GOx immobilization on PVA/PEI/MNP nanofiber layer by cross-linking of glutaraldehyde (b) Enzyme immobilized MNF biofilm fixed on screen printed electrode (SPE) by the help of neodymium magnet for electrochemical detection[5].

Results

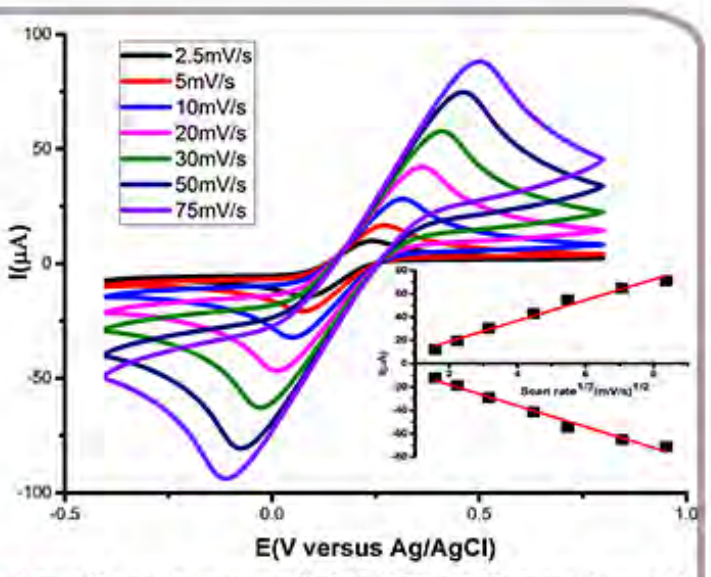
Figure 4.



a) The effect of the pH on the biosensor response of the PVA/PEI/MNP-GOx biosensor.



b) Calibration curves for the detection of glucose of the PVA/PEI/MNP-GOxbiosensor (in 50 mM sodium acetate buffer, pH 5.5, 25 °C, −0.7 V).



c) Cyclic Voltametry of PVA/PEI/MNP-GOx immobilized electrodes presence of 5.0 mM Fe3(CN)$6^{3-/4-}$at the different scan rates, Inset: The correlation between the current and square root of the scan rate.

Conclusion

Magnetic properties of nanofibers are provided to assemble the fiber layer on screen printed electrode by the help of neodymium magnet for electrochemical measurements. This method is prevented handicaps such as reproducibility, excessive chemical and time usage and also allowed to reuse screen printed electrode by cleaning quickly and effortlessly with removing the used MNF layer within the split away of the magnet.

References

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[3] Ye, X.-Y., Liu, Z.-M., Wang, Z.-G., Huang, X.-J. & Xu, Z.-K. Preparation and characterization of magnetic nanofibrous composite membranes with catalytic activity. Mater. Lett. 63, 1810–1813 (2009).
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[5] Pérez-López, B. & Merkoçi, A. Magnetic nanoparticles modified with carbon nanotubes for electrocatalytic magnetoswitchable biosensing applications. Adv. Funct. Mater. 21, 255–260 (2011).

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Detection of *Escherichia Coli* O157:H7 in food products by Impedimetric Immunosensors

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AIM

Rapid, cost effective, highly sensitive and selective label – free impedimetric immunosensors have been developed by different immobilization procedures, in order to detect very low *E.Coli* O157:H7 cells in foodstuffs.

APPARATUS

Gold μ-electrode Micrux

Batch Electrochemical Cell

Autolab PGSTAT 204

MANUFACTURING

a. Not oriented MAb on MBA (11-Mercapto benzoic acid) self-assembled monolayer

b.Oriented Mab on MBA self-assembled monolayer

c.MAb on electrochemically deposited Cysteamine layer

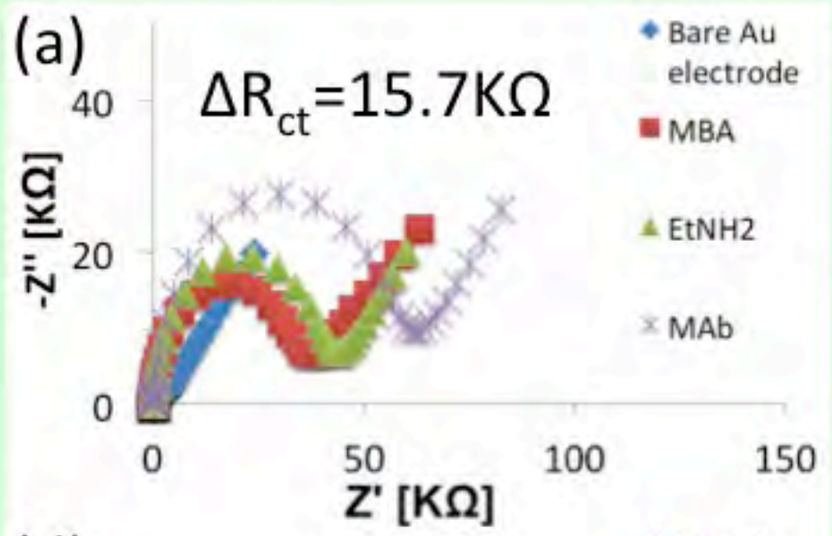
d.MAb on Cysteamine and ferrocene layers

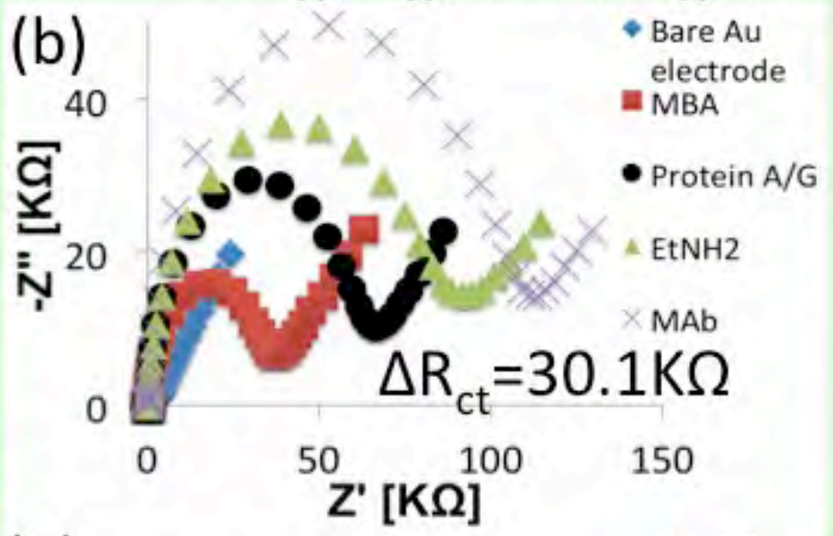
e.MAb on PAMAM dendirmer and ferrocene layers

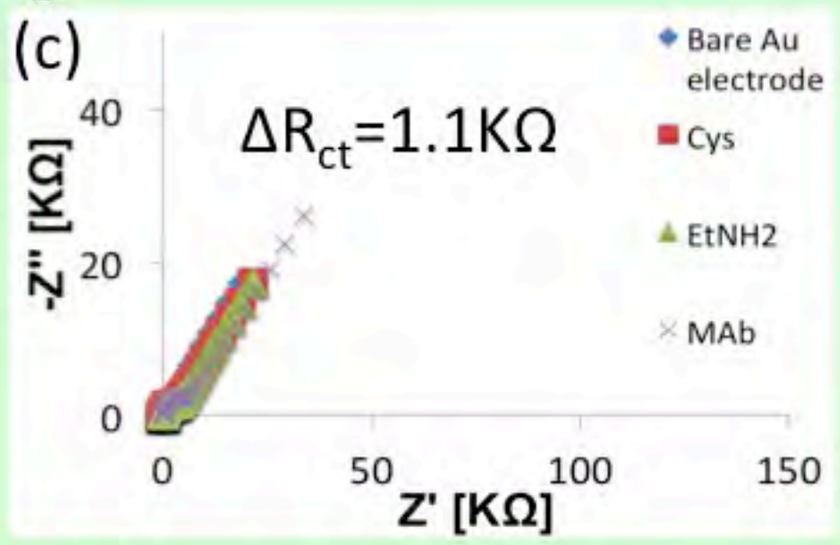
RESULTS

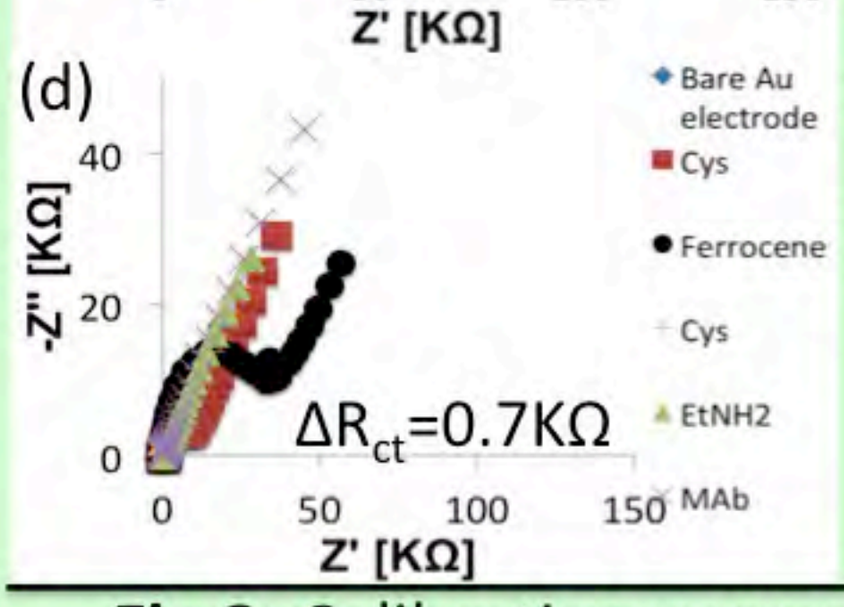
Fig.1: EIS responses to all immunosensors fabrication steps.

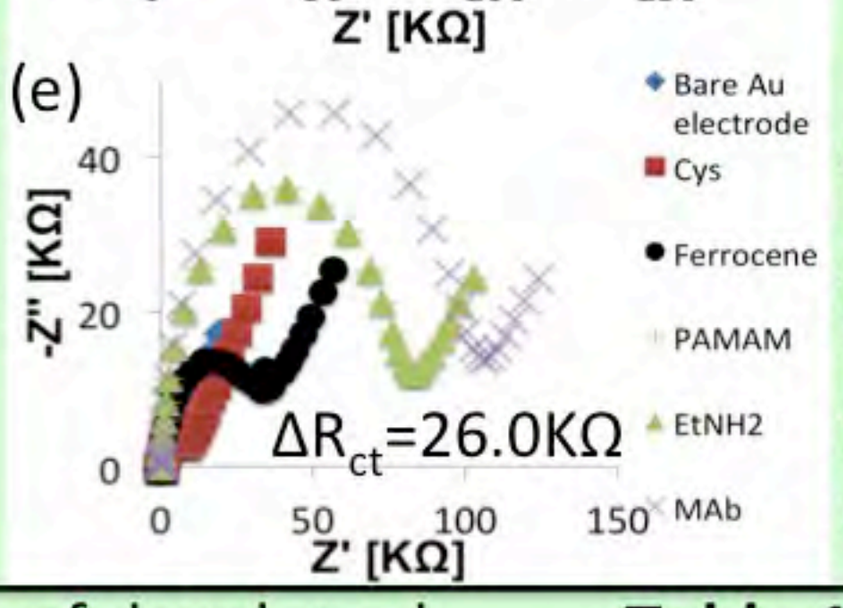
$\Delta R_{ct}=R_{ct}(MAb)-R_{ct}(EtNH_2)$





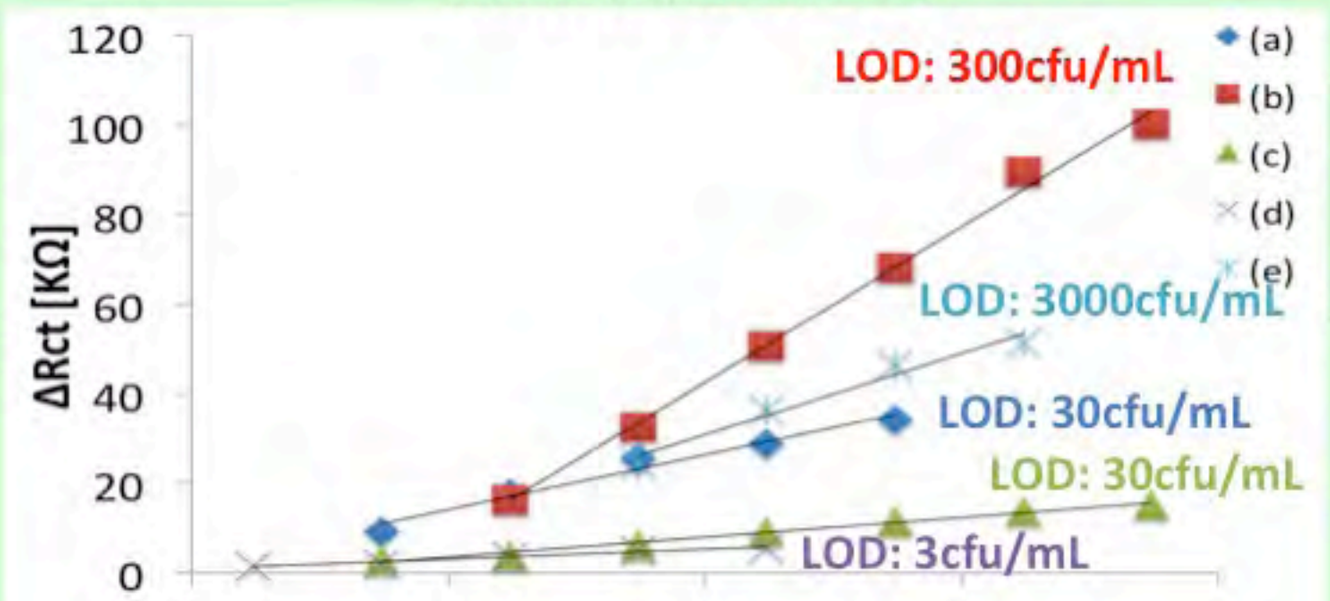






After each step, the Impedance increase due to the reduction of the redox probe diffusion close to the electrode caused by different layers.

Fig.2: Calibration curves of developed immunosensors.



Charge Transfer Resistance $\mathbf{R_{ct}}$ of Randle's Circuit used for fitting of experimental data was investigated: *an increase of R_{ct} with increasing concentration of E.Coli O157:H7 was observed*

Table 1: Comparison between Immunosensor and ELISA Kit in spiked milk and meat samples

	Spiked E.Coli O157:H7 [cfu/mL]	Immunosensor [cfu/mL]	ELISA [cfu/mL]
Milk	1.00×10^3	$0.72\pm0.02\times10^3$	$1.08\pm0.02\times10^3$
	5.00×10^3	$4.90\pm0.08\times10^3$	$4.76\pm0.02\times10^3$
	1.00×10^4	$9.14\pm0.12\times10^4$	$11.69\pm0.02\times10^4$
Meat	1.00×10^3	$0.98\pm\times0.03\times10^3$	$0.89\pm0.02\times10^3$
	5.00×10^3	$5.24\pm\times0.10\times10^3$	$4.16\pm0.02\times10^3$
	1.00×10^4	$9.55\pm0.25\times10^4$	$9.62\pm0.02\times10^4$

➡ The lowest number of immobilized antibodies allows to reach the **lowest detection limit**

CONCLUSIONS

The coupling cysteamine/ferrocene has allow to reach a very low detection limit (**3cfu/mL**) and good results for the detection of *Escherichia Coli* O157:H7 in food samples.

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INTERNATIONAL BIOSENSOR CONFERENCE
25. – 29. September 2017 | Rome, Italy

THE *azoR* GENE PROMOTER: A NEW SENSING ELEMENT FOR THE DETECTION OF TRACE EXPLOSIVES

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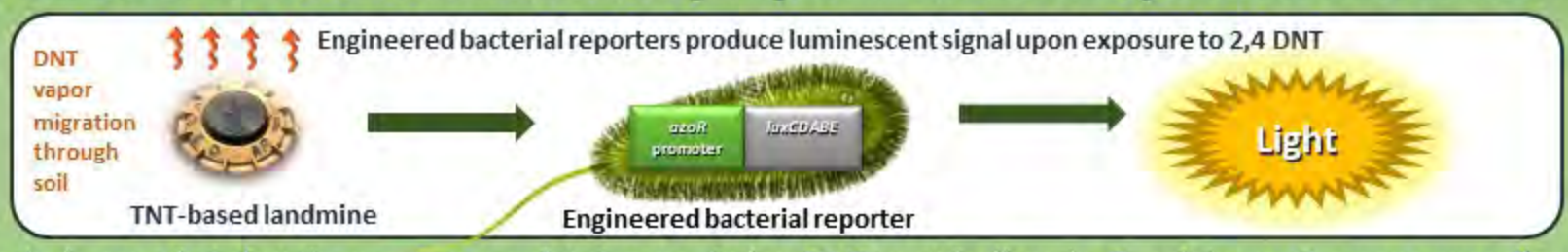
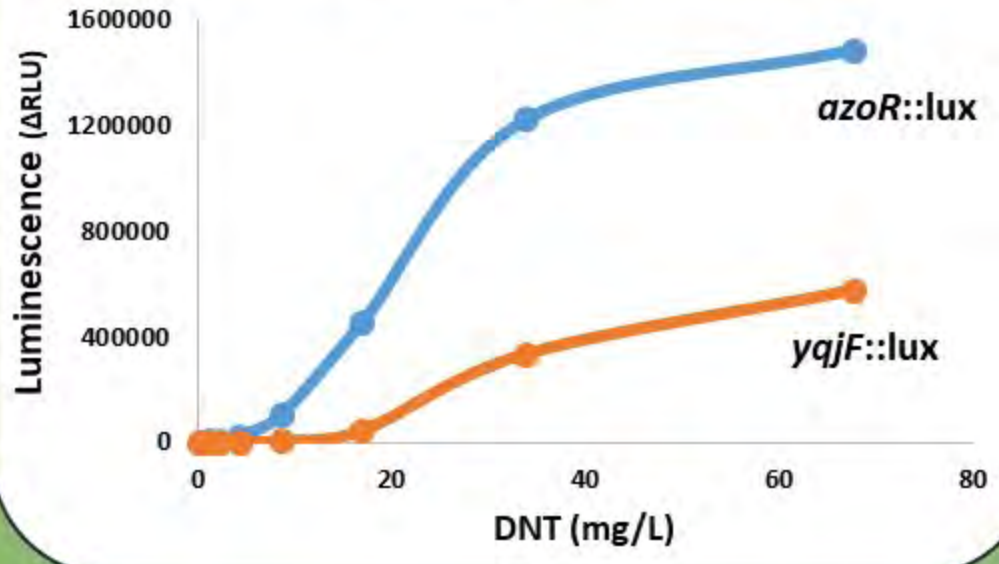
Institute of Life Sciences, the Hebrew University of Jerusalem, Israel

TNT-based landmines contain mainly 2,4,6-TNT but also manufacturing impurities, including 2,4-dinitrotoluene (DNT). Vapors of this compound slowly leak into the soil above the landmine, rendering it a potential indicator of the presence of buried landmines.

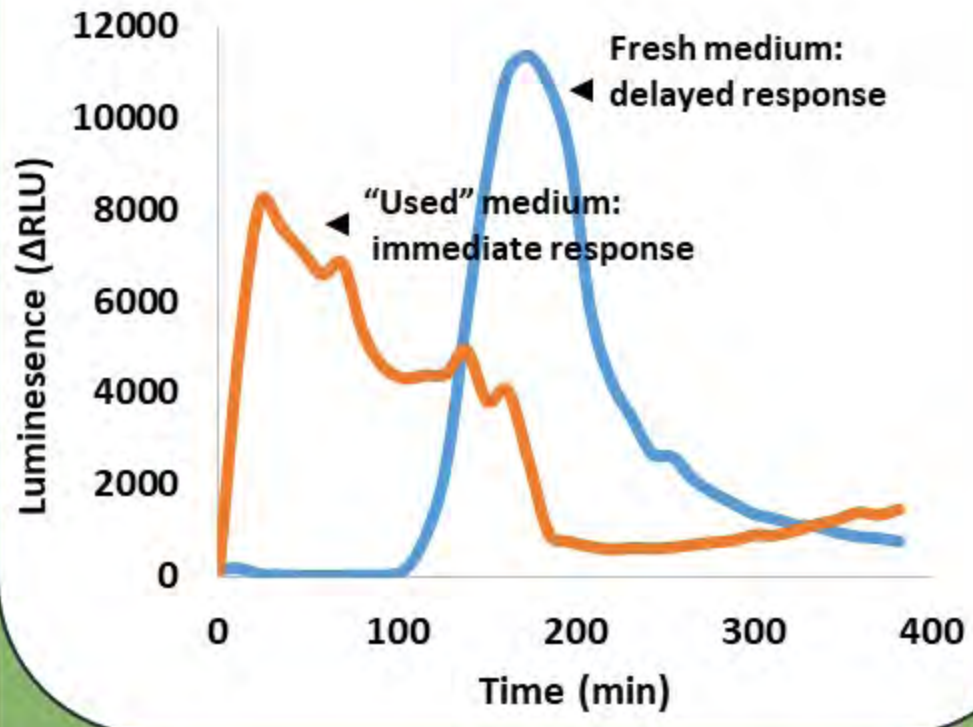


Transcriptome analysis revealed that the most up-regulated gene during DNT metabolism in *E. coli* is *azoR*, a gene encoding an Azo-reductase. A bioreporter based on the fusion of the *azoR* promoter region to *luxCDABE* was designed, and its activity in response to DNT and TNT was investigated.

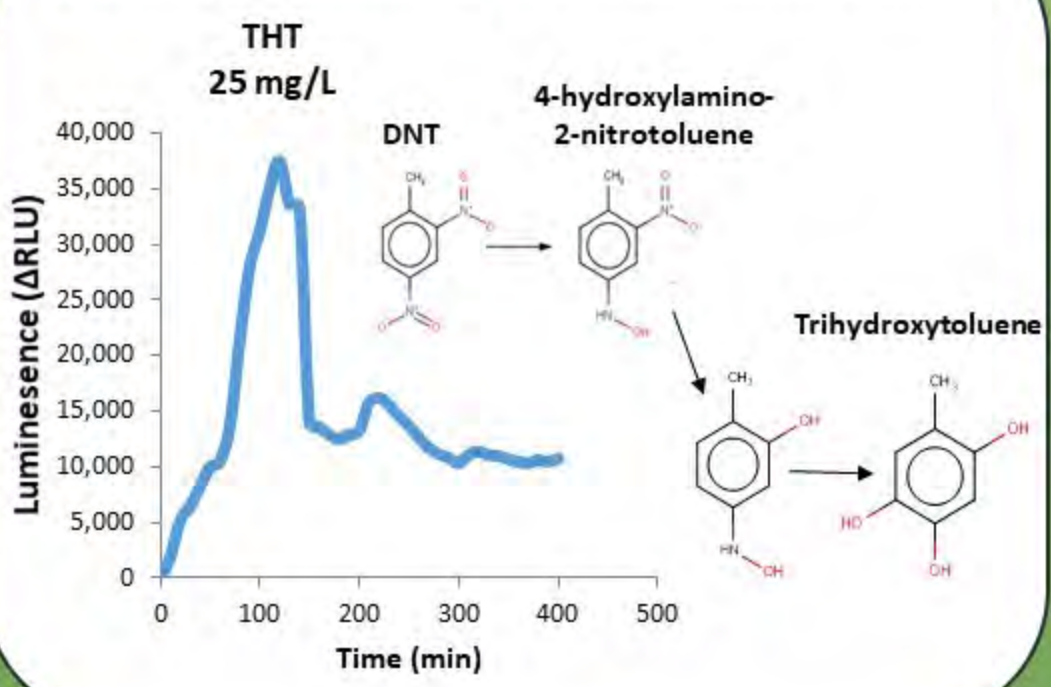
The *azoR* promoter is a more sensitive DNT sensor than the *yqjF* promoter, another DNT-responsive gene promoter



azoR is not directly activated by DNT, but rather by a downstream metabolite



2,4,5-trihydroxytoluene may be the direct inducer of *azoR*



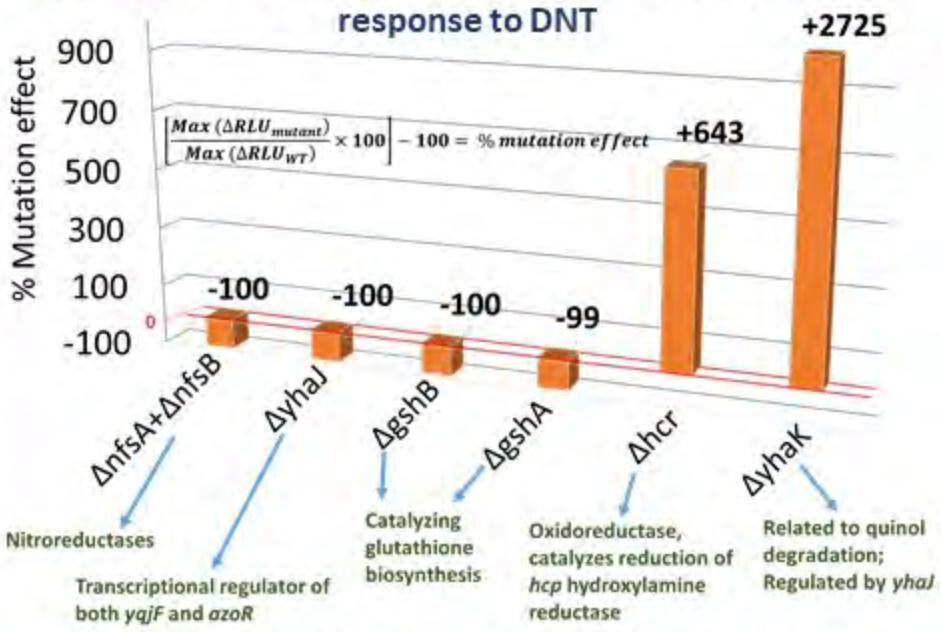
In addition to DNT, TNT and DNB, *azoR* show response to quinol-like compounds

CHEMICAL	RESPONSE INTENSITY
NITRO AROMATIC COMPOUNDS:	
2,4,6-TRINITROTOLUENE (TNT)	+
2,4-DINITROTOLUENE (DNT)	++
2,6-DINITROTOLUENE (2,6-DNT)	-
1,2-DINITROBENZENE (1,2-DNB)	-
1,3-DINITROBENZENE (1,3-DNB)	++
1,4-DINITROBENZENE (1,4-DNB)	-
2-NITROTOLUENE (2-NT)	-
3-NITROTOLUENE (3-NT)	-
4-NITROTOLUENE (4-NT)	-
NITROBENZENE	-
CYCLOTRIMETHYLENETRINITRAMINE (RDX)	+
AMINO AROMATIC COMPOUNDS:	
ANILINE	-
1,3-BENZENEDIAMINE	-
2-AMINOTOLUENE	-
QUINOL COMPOUNDS:	
PHENOL	+/-
HYDROQUINONE	+++
METHYLHYDROQUINONE	+++
RESORCINOL	+
CATECHOL	+++
1 2 4 BENZENETRIOL	+++
4-AMINOPHENOL	++
MENADIONE	++
OTHER STRUCTURALLY SIMILAR AROMATIC COMPOUNDS:	
BENZENE	-
TOLUENE	-
CYCLOHEXANONE	-
2-CHLOROTOLUENE	-
3-XYLENE	+/-
4-NITRO-O-PHENYLENEDIAMINE	-

(-) No response;
(+/-) 3>Ratio>10;
(+) 10>Ratio>100;
(++) 100>Ratio>1000;
(+++) Ratio>1000.

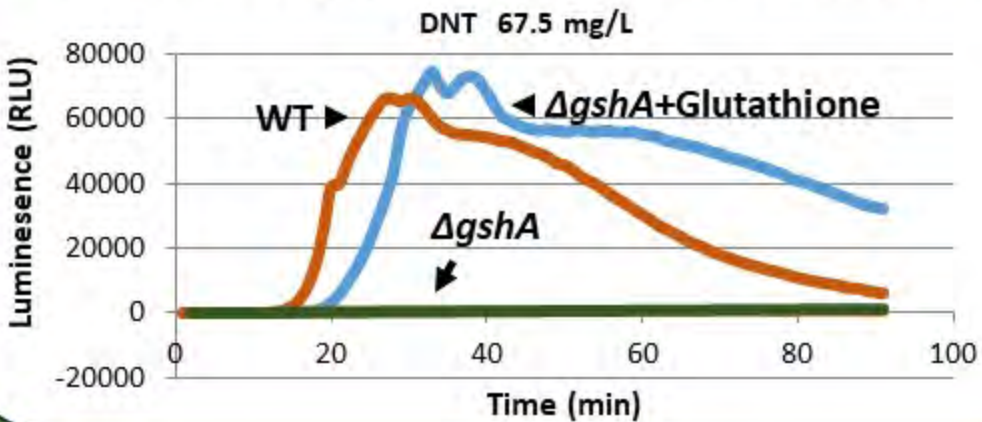
The direct inducer of *azoR* may be a quinol-like compound.

Mutation in several genes strongly influenced the *azoR* response to DNT



It seems that DNT is first reduced by nitroreductases, then further metabolized to produce the *azoR* inducer, possibly a quinol-like compound.

Glutathione has a yet unknown but crucial role in *azoR* activation



Conclusions:

- The *azoR* gene promoter is an effective sensor element for a DNT bioreporter.
- It seems that *azoR* is activated by a degradation product of DNT, possibly a quinol-like in structure.
- Additional genes play an important role in DNT biotransformation and consequently in *azoR* activation, therefore much is left to research on the subject.

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האוניברסיטה העברית בירושלים
The Hebrew University of Jerusalem

Detection of contaminants' residues on surfaces by microbial bioreporters

האוניברסיטה העברית בירושלים
The Hebrew University of Jerusalem

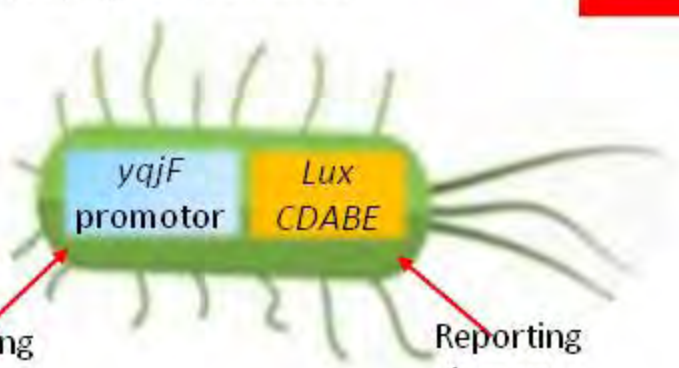
Yelena Bykov, Dror Shakibai, Tal Elad and Shimshon Belkin
Institute of Life Sciences, the Hebrew University of Jerusalem, Israel

We present preliminary results of an innovative approach for the detection of pollutant residues on everyday surfaces, by spraying live bioluminescent bacterial sensor strains and monitoring the ensuing light emission.

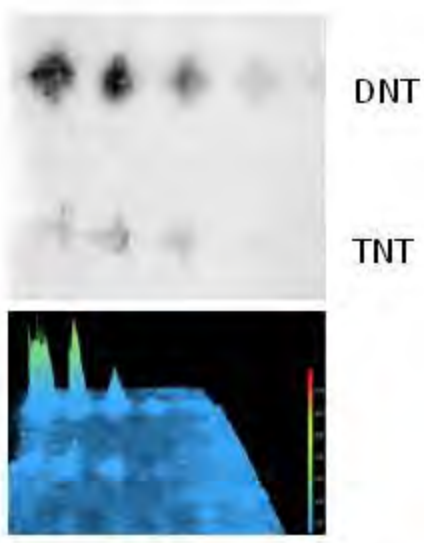
General concept

Surfaces with residues

Manually or semi-automatically spraying bacterial sensor



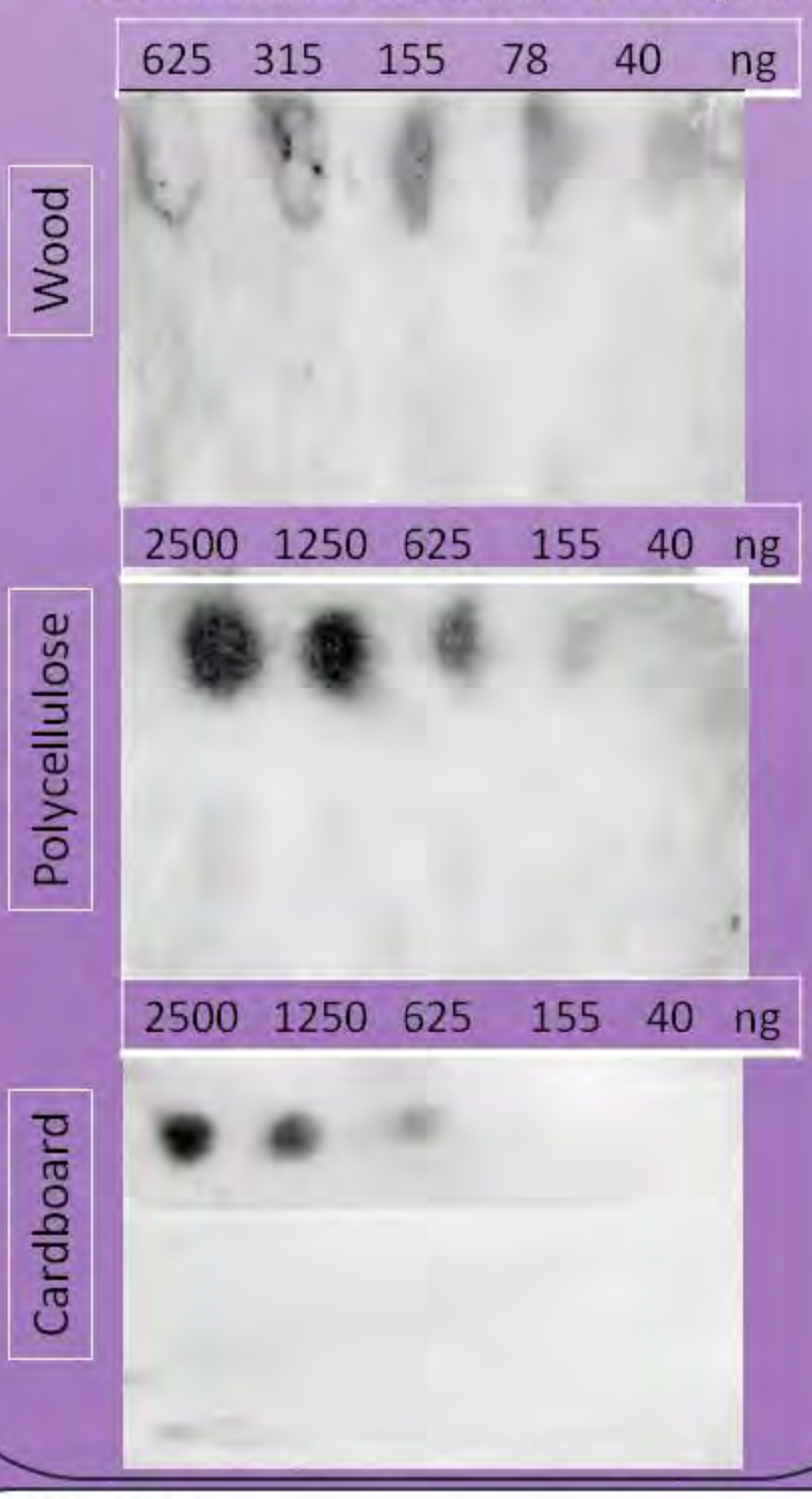
Engineered bacterial reporter



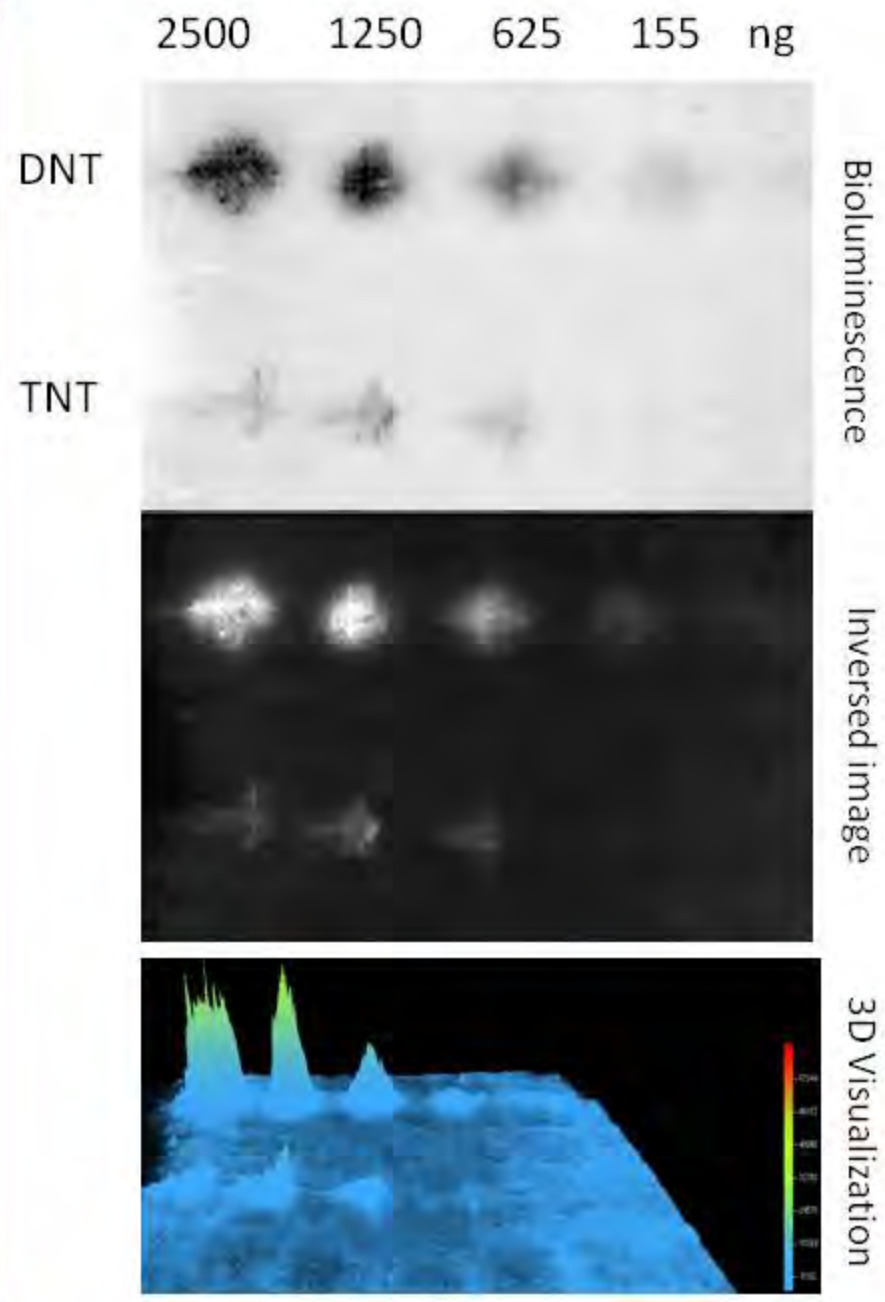
Bioluminescence response

This study demonstrate the method using 2,4,6-trinitotoluene (TNT) and 2,4-dinitrotoluene (DNT) as model targets, and a luminescent *E. coli* bioreporter, genetically engineered to detect these chemicals, as the model sensor. Different types of surfaces exposed to these two compounds were sprayed with a thin layer of the bioreporter, and following a short incubation, luminescence was imaged by bioluminescence Imaging Systems.

DNT detection on different surfaces



DNT&TNT detection on cotton surface



Current detection limits:
155 ng/spot DNT, 625 ng/spot TNT

Future plans:

(1) Improve spraying performance
(2) Increase detection sensitivity
(3) Expand the spectrum of detectable compounds on different surfaces.

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MOLECULARLY IMPRINTED POLYMER NANOPARTICLES AS SYNTHETIC ANTIBODIES FOR CELL IMAGING

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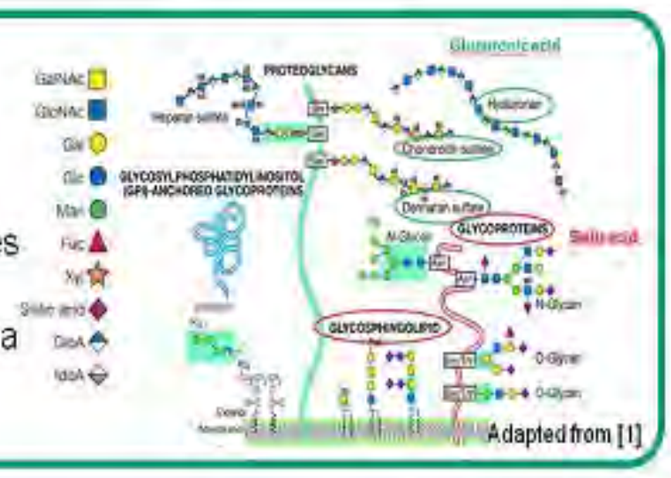
Introduction

✓Altered glycosylation levels or distributions of **sialic acids (SA)** or **hyaluronan** are indicators of pathological conditions.
✓Since the production of antibodies that recognize them is difficult, immunostaining methods for glycosylations are rare.
✓**Molecularly Imprinted Polymers (MIPs)** are tailor-made biomimetic receptors. Their molecular recognition properties and high stability make them interesting substitutes for antibodies in bioimaging.
✓Here we applied MIPs labeled with organic dyes or quantum dots (QDs) on the example of **D-glucuronic acid (GlcA)**, a substructure of hyaluronan, and **N-acetylneuraminic acid (NANA)**, the most common member of SA to human cells.



Adapted from [1]

Synthesis of rhodamine labeled MIPs

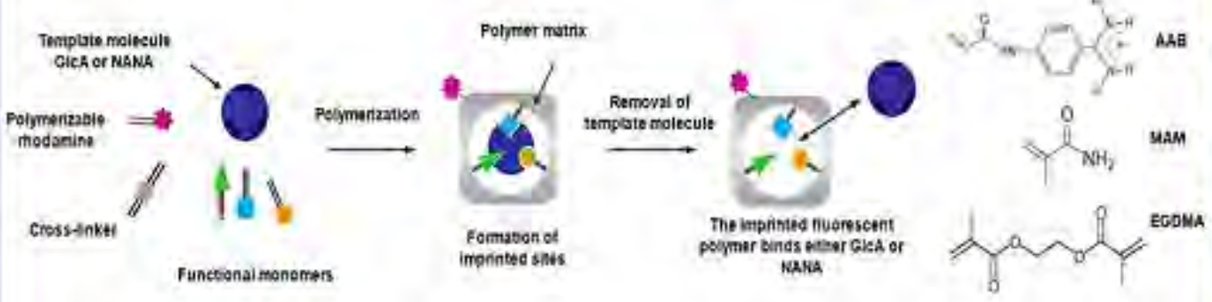


Figure 1. Imprinting of GlcA and NANA with acryloyl benzamidine (AAB) and methacrylamide (MAM) as functional monomers, ethylene glycol dimethacrylate (EGDMA) as cross-linker and polymerizable rhodamine as fluorescent monomer. A control, non-imprinted polymer (NIP) was synthesized in the absence of the template molecule.

Synthesis of MIPs coated Quantum Dots

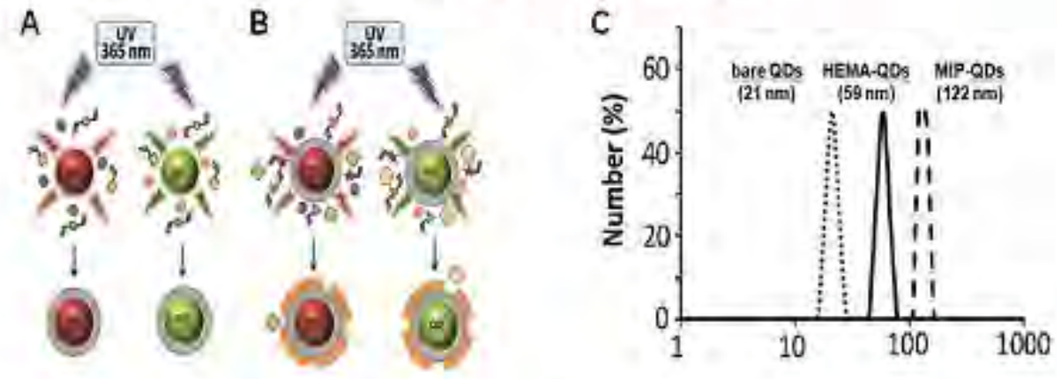


Figure 4. A) An hydrophilic shell was synthesized around QDs using 2-hydroxyethyl methacrylate (HEMA). B) A second shell of MIP was synthesized for NANA with red-QDs and GlcA with green-QDs. (C) Evidence for the formation of polymer shells around the QDs.

Characterization of rhodamine labeled MIP

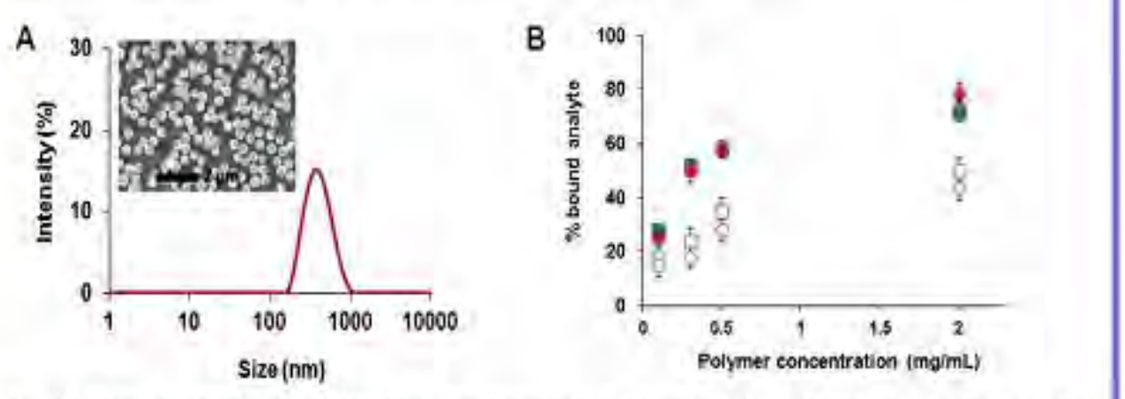


Figure 2. (A) Size of rhodamine MIPGlcA by dynamic light scattering, SEM image (inset). (B) Equilibrium binding isotherms of [^{14}C]D-GlcA and [^{3}H]NANA to MIPGlcA (red circles), NIPGlcA (empty circles), MIPNANA (blue squares) and NIPNANA (empty squares).

Characterization of MIP coated Quatum Dots

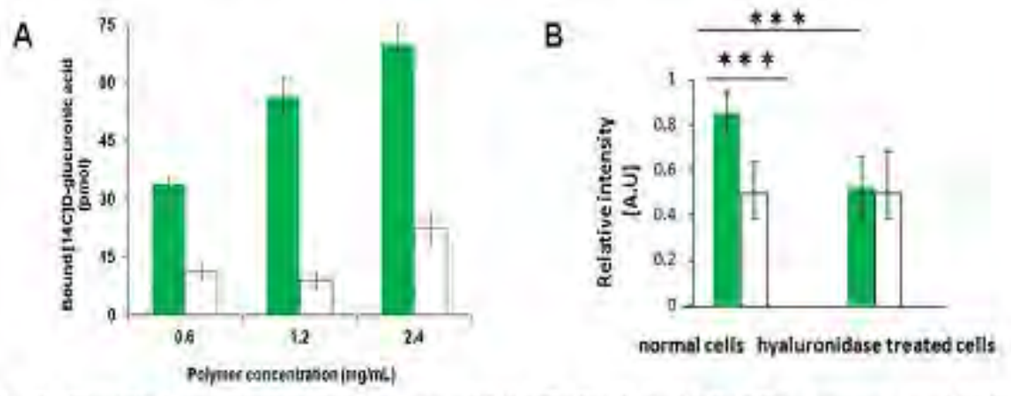


Figure 5. (A) Equilibrium binding isotherms of [^{14}C]D-GlcA to MIP-QDs (green) and NIP-QDs (white). (B) Fluorescence intensity of keratinocytes after imaging with and without hyaluronidase, MIP-QDs (green) and NIP-QDs (white).

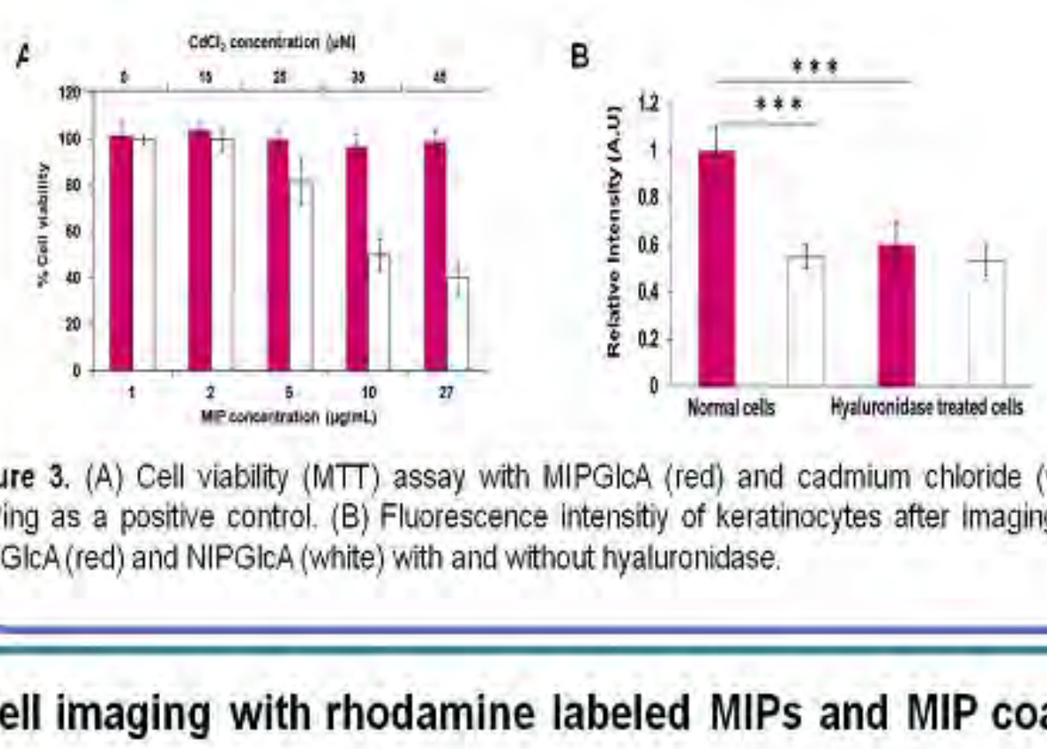


Figure 3. (A) Cell viability (MTT) assay with MIPGlcA (red) and cadmium chloride (white) serving as a positive control. (B) Fluorescence intensitiy of keratinocytes after imaging with MIPGlcA (red) and NIPGlcA (white) with and without hyaluronidase.

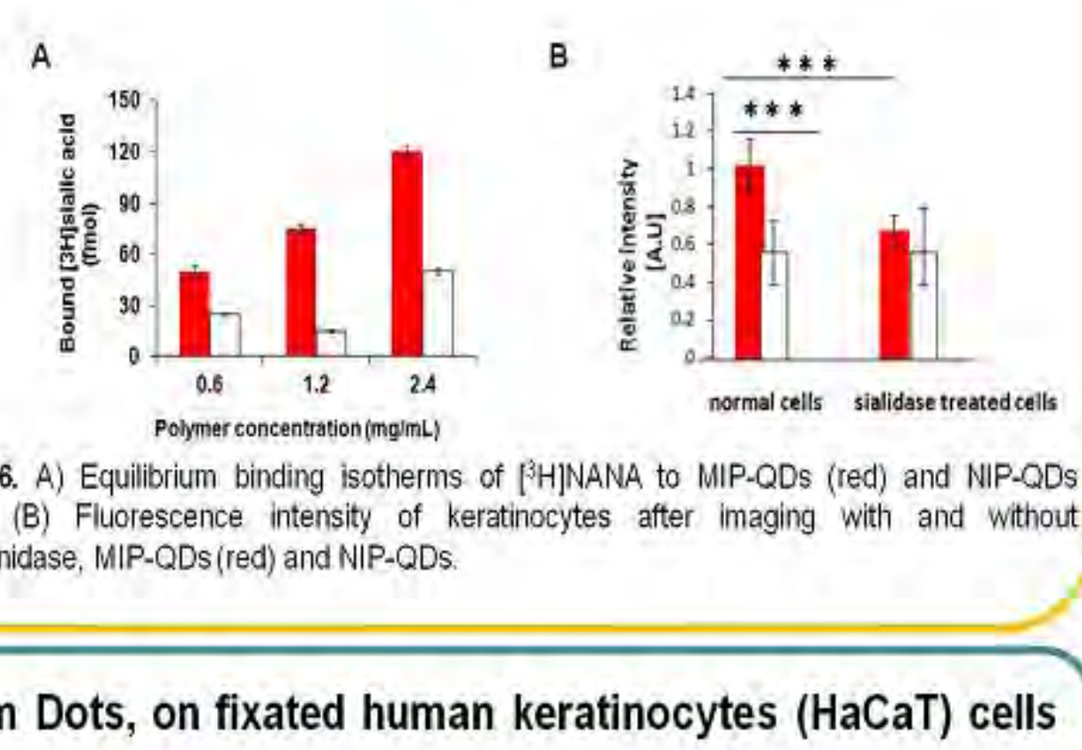


Figure 6. A) Equilibrium binding isotherms of [^{3}H]NANA to MIP-QDs (red) and NIP-QDs (white). (B) Fluorescence intensity of keratinocytes after imaging with and without neuraminidase, MIP-QDs (red) and NIP-QDs.

Cell imaging with rhodamine labeled MIPs and MIP coated Quantum Dots, on fixated human keratinocytes (HaCaT) cells

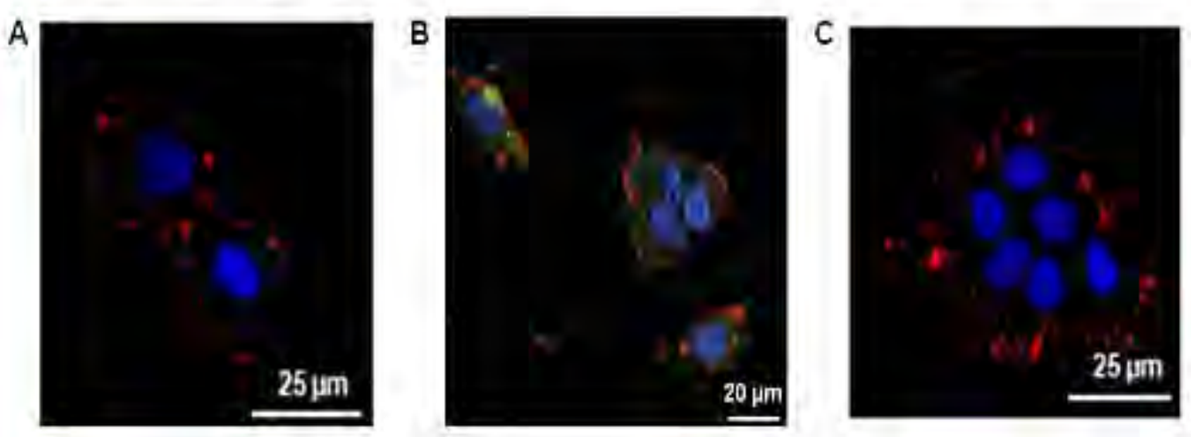


Figure 7. Confocal microscope images of HaCaT cells fixed and stained with rhodamine-MIPGlcA (A,B) and rhodamine-MIPNANA (C) (red signal), cell nucleus stained with DAPI (blue signal) and cell membrane with DiO (green signal in Figure 7B).

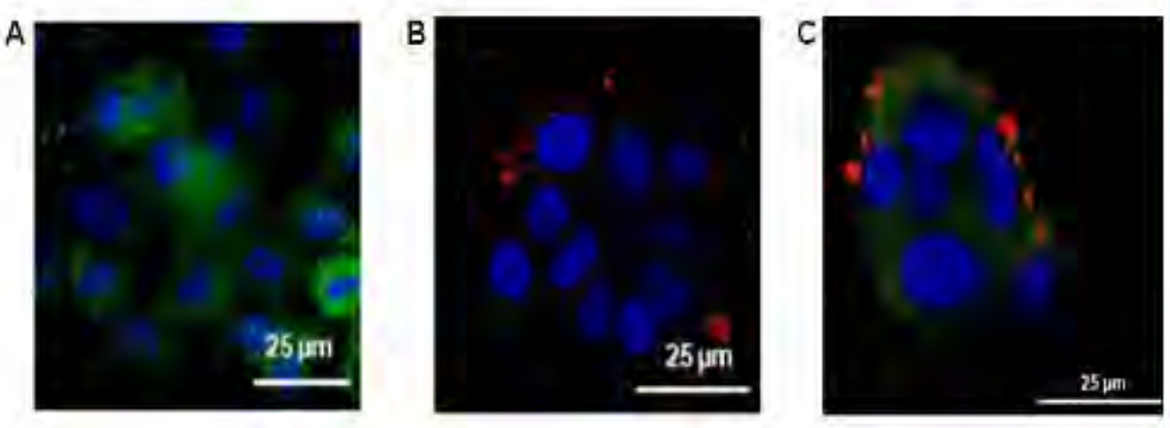


Figure 8. Confocal microscope images of HaCaT cells fixed and stained with MIPGlcA-QDs (A), MIPNANA-QDs (B) and simultaneously MIPGlcA-QDs and MIPNANA-QDS (C), cell nucleus stained with DAPI (blue signal).

Conclusions

- ✓ MIPs labeled with organic dye or QDs were syntheized for imaging glycosylation sites.
- ✓ A standard immunostaining protocol was successfully adapted for MIP staining on cells. No primary and secondary antibodies were needed.
- ✓ Simultaneous dual-color imaging of the cells with two MIP-coated QDS was demonstrated for the first time.
- ✓ The MIPs are not cytotoxic and could be applied to live cell labelling and imaging.
- ✓ These synthetic receptors have potential as bioimaging tool, targeted drug delivery device or specific blocking agent on cell and tissues.

We acknowledge financial support from the Mexican National Council for Science and Technology (CONACYT), the European Union (FP7 Marie Curie Actions: ITN SAMOSS, PITN-2013–607590) and the Region of Picardy (CPER 2007–2013)

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[2] S. Kunath, M. Panagiotopoulou, J. Maximilien, N. Marchyk, J. Sänger, K. Haupt, *Advanced Healthcare Materials* **4**, 1322-1326 (2015)
[3] M. Panagiotopoulou, Y. Salinas, S. Beyazit, L. Duma, E. Prost, A. Mayes, M. Resmini, B. Tse Sum Bui, K. Haupt, *Angewandte Chemie International Edition* **55**, 8244-8248 (2016)
[4] M. Panagiotopoulou, S. Kunath, P. Medina-Rangel, K. Haupt, B. Tse Sum Bui, *Biosensors and Bioelectronics* **88**, 85-93 (2017)

Towards a model of electrochemical immunosensor using silver nanoparticles

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Abstract

The interaction between metal nanoparticles (NMPs) and biomolecules may reveal new material functions, where the NMPs introduce new properties in the molecules, or alternatively, the biomolecules affect the structure or properties of the NMPs.

The aggregation of metal clusters on the surface of a working electrode of screen printed electrodes
In this work, the electroactive properties of silver nanoparticles (AgNPs) have been exploited to obtain an enzymeless electrochemical probe through the functionalization of AgNPs with antibody to recognize specific antigen. With this goal a model immunosystem has been developed using the Ag-NPs as support for immunological chain and the transducer with immobilization of anti-IgG antibody in order to detect the specific IgG.

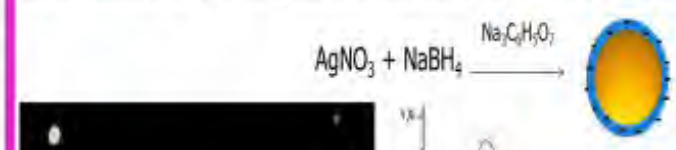
AgNPs possess the dual role of electrochemical markers in electrochemical measurements and backing material for the immunological chain.
A stable interaction between Ab and AgNPs is revealed by scanning electron microscopy (SEM) and by the downturn of the anodic peak current in cyclic voltammetry (CV) while increasing the antibody concentration.

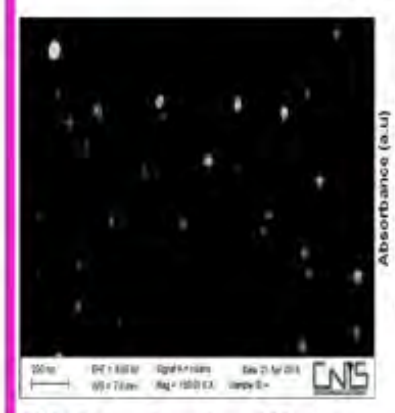
This finding is supported by the anodic stripping of AgNPs, that reveals the area of the oxidative signal ($Ag \rightarrow Ag^+$) increasing while increasing the presence of the target molecule (IgG) on AgNPs. This result stimulates our interest in a research work related to the use of AgNPs as biochemical sensors which highlights the advantages of using this strategy over common analytical methods.

Synthesis of silver nanoparticles

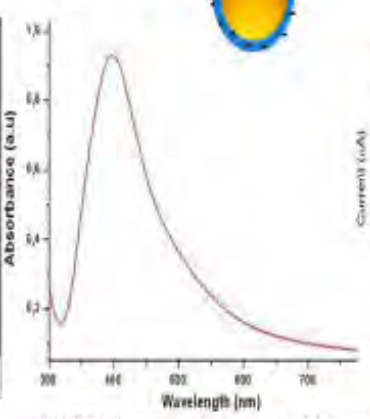
Reduction of $AgNO_3$ with $NaBH_4$ in presence of $Na_3C_6H_5O_7$

The dimensions of nanoparticles, the position of plasmon peak and the electrochemical behaviour were revealed by scanning electron microscopy (SEM),UV-VIS spectroscopy and cyclic voltammetry.

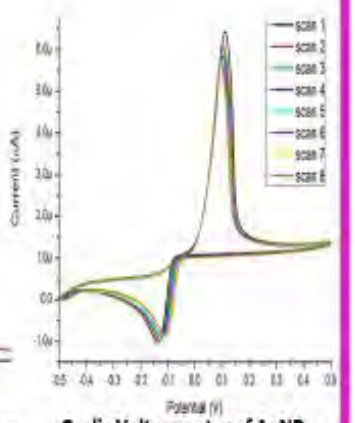




SEM: clusters dimensions of 28 ± 8 nm



UV-VIS: plasmon resonance at 394 nm



Cyclic Voltammetry of AgNPs

Immobilisation of antibody on Silver Nanoparticles

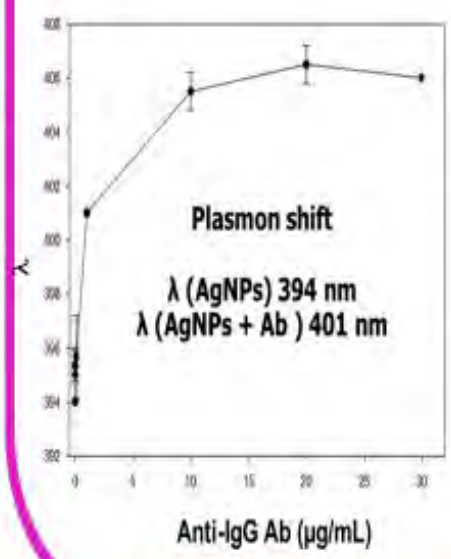
The conjugation of silver nanoparticles with anti-IgG antibody was realized at growing concentrations of anti-IgG antibody (Ab).

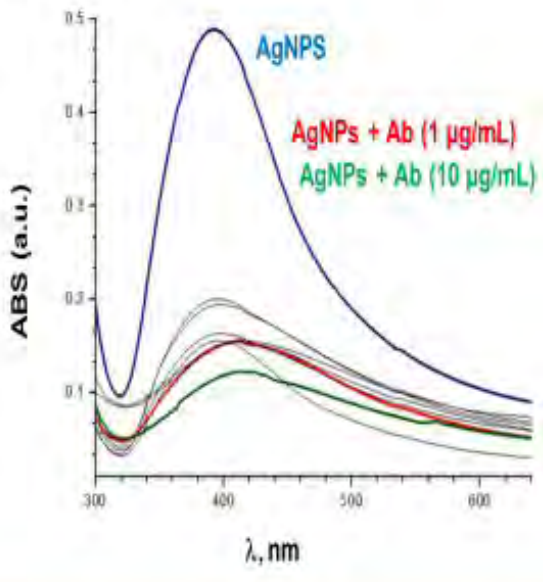
AgNPs solution at fixed concentration was employed and obtained by diluting the initial solution.

Ab concentration was varied between 10^{-3} µg/mL and 30 µg/mL.

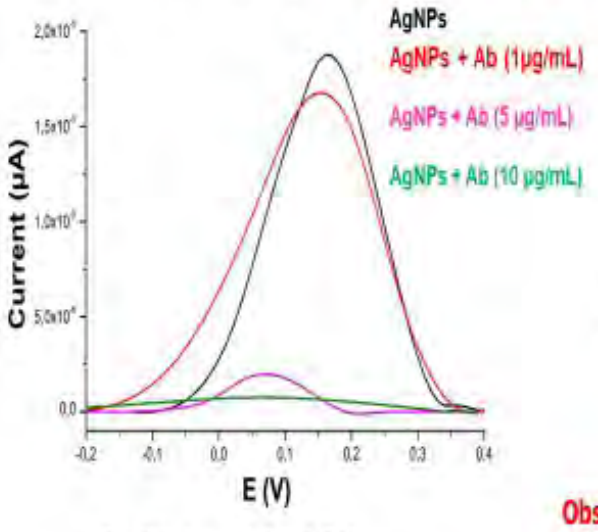
The conjugated silver nanoparticles already analyzed by UV-VIS spectroscopy were transferred on the surface of screen printed electrodes (SPE) and subjected to cyclic voltammetry (CV) and anodic stripping voltammetry (ASV).

UV-VIS results



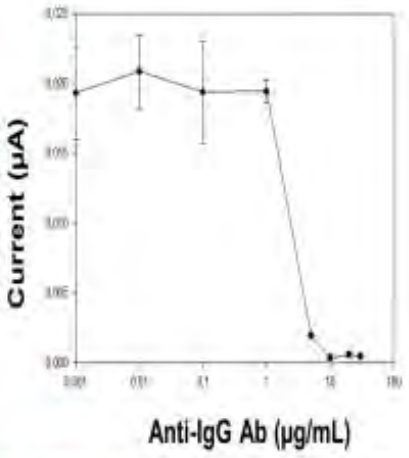


Anodic stripping voltammetry (ASV) results



Conditioning : -0.6 V (15 s)
Deposition : -1,2 V (55 s)
Stripping voltage: -1.2 V÷ 0.5 V at 100 mV/s

Coating curve

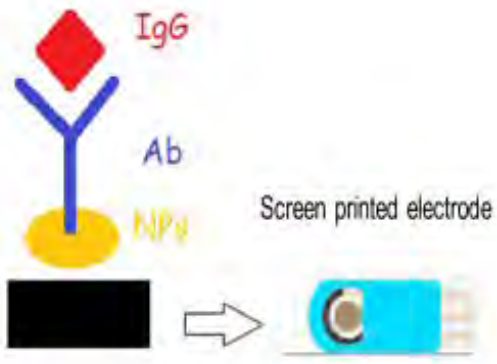


Observations: Growing coverage of AgNPs surface while increasing the Ab concentration, responsible for a growing steric indrance that hampers the oxidative process.

Immunosensor Model of anti-IgG/IgG

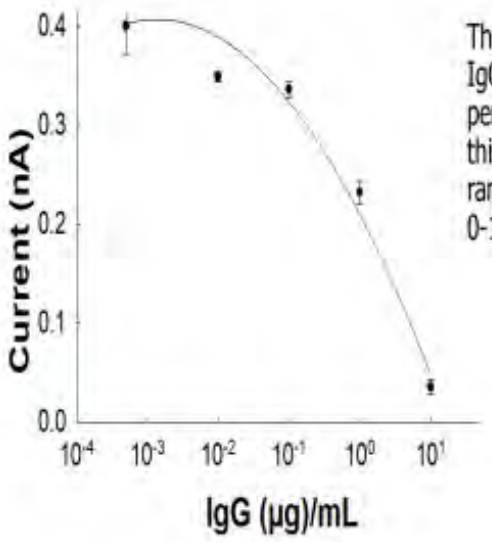
The immobilisation of Ab (anti-IgG) on AgNPs was tested as label-free model of electrochemical anti-IgG/IgG immunosensor.

AgNPs functionalized with 10 µg/ml solution of Ab were incubated with decreasing concentrations of IgG between 0 and 30 µg/mL and transferred on screen printed electrodes (SPEs) for ASV measurements.



Scheme of the immunosensor: AgNPs/anti-IgG/IgG

Binding curve of Anti-IgG antibody at growing concentrations of IgG



The binding curve of IgG demonstrated the permanent binding of this species in the range of concentration 0-10 µg/ml.

Anodic stripping voltammetry

Conditioning : -0.6 V (15 sec)
Deposition : -1,2 V (55 sec)
Stripping voltage: -1.2 V÷ 0.5 V at 100 mV/sec

Conclusions

A novel model of label-free immunosensor was developed exploiting silver nanoparticles (AgNPs) as backing material and electrochemical tracker.
AgNPs were functionalized with anti-IgG molecules and employed to detect IgG.

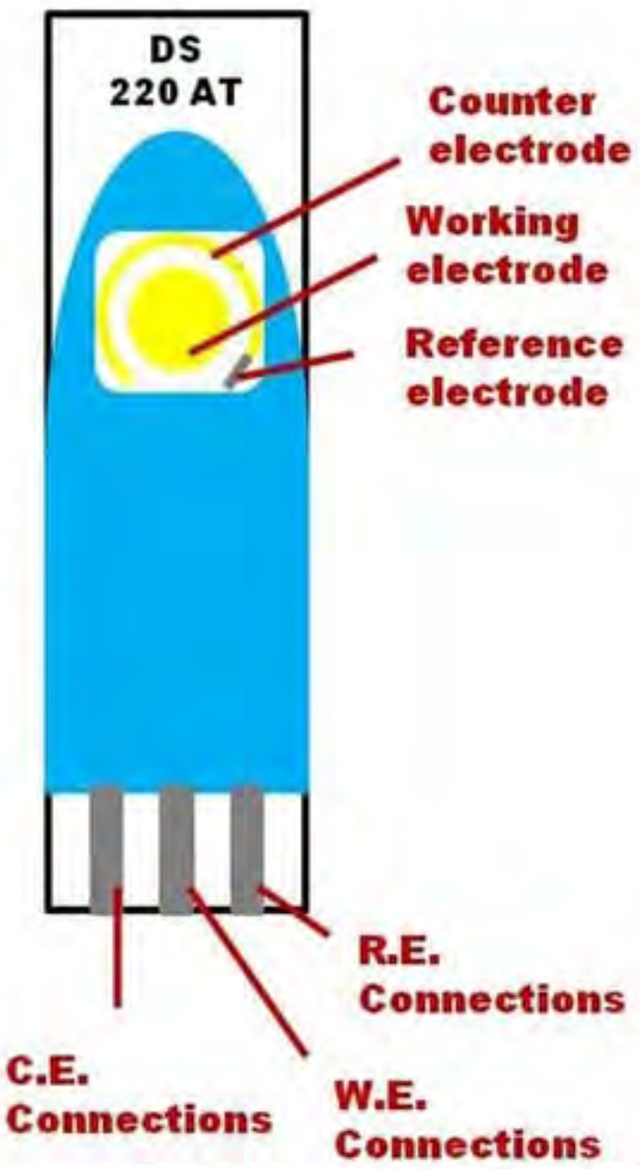
A successful immobilisation of Ab and AgNPs was obtained, allowing the use of this system as enzymeless electrochemical probe for IgG determination.
IgG was successfully detected in the range of concentration 0-10 µg/ml, demonstrating the possibility of using this strategy in the wide family of bioanalytical methods.

The future work is directed to increase the understanding of the system, to extend the range of analysis and evaluate the possibility of use in immunological bioassays for pathogens, microorganisms and their toxins.

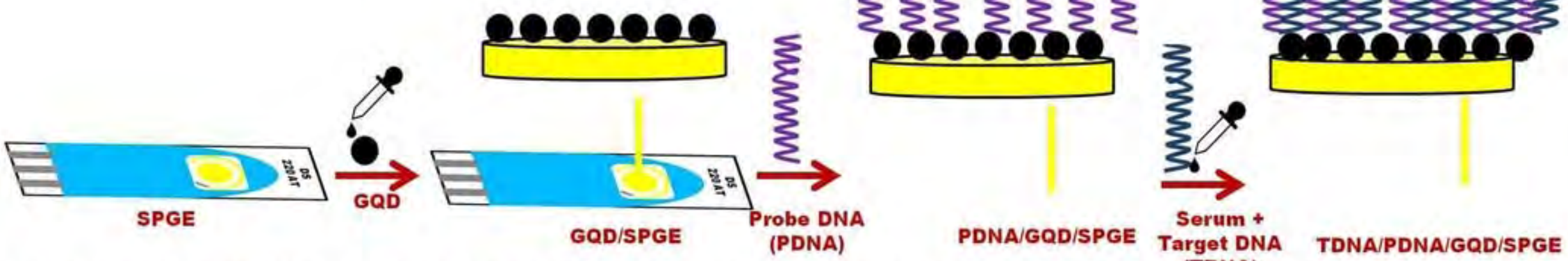
Graphene quantum dots based genosensor for the point-of-care diagnosis of *chikungunya virus*

Chaitali Singhal, O.V. Manila, Subbiah Alwarappan, C.S. Pundir, Jagriti Narang, DK Avasthi

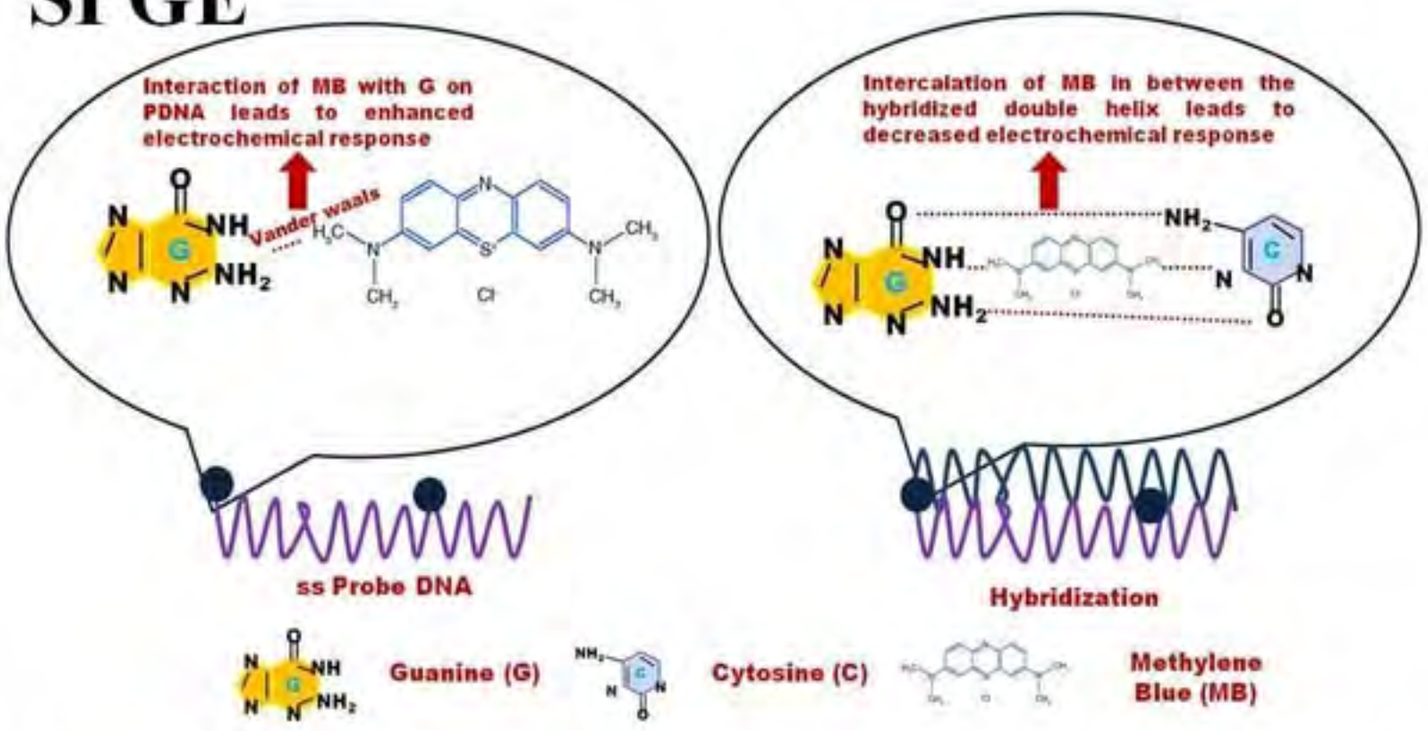
CHIKUNGUNYA

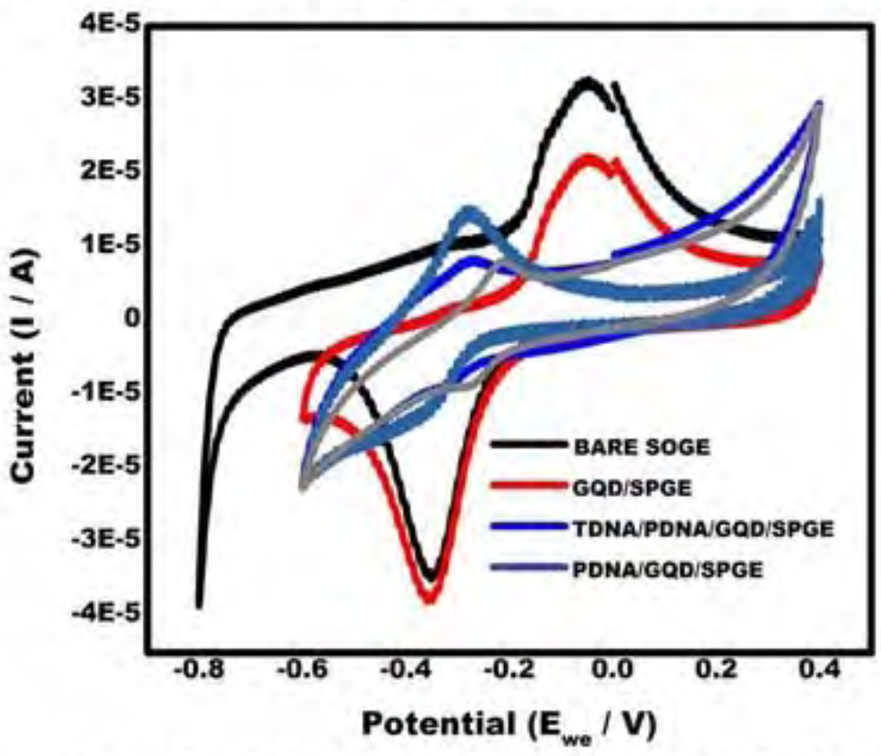


SCREEN PRINTED GOLD ELECTRODE



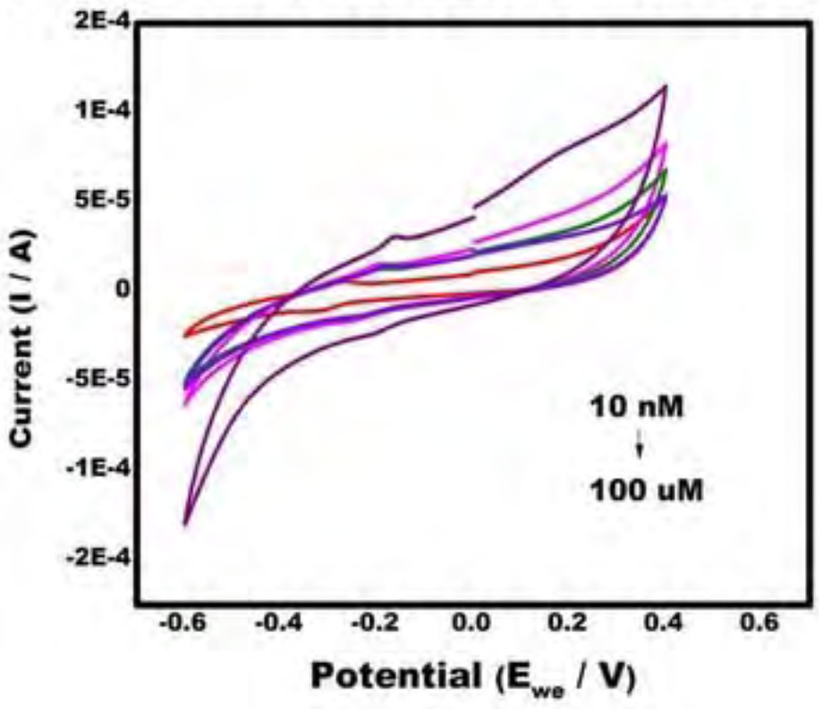
SCHEMATIC REPRESENTATION OF THE FABRICATION OF SPGE





PRINCIPLE OF DETECTION OF HYBRIDIZATION OF TARGET DNA

CYCLIC VOLTAMMOGRAM AT VARIOUS STAGES OF SPGE



CYCLIC VOLTAMMOGRAM AT VARIOUS CONCENTRATIONS OF TDNA

Coupling HPTLC with bacterial genotoxicity whole-cell bioreporters

Dror Shakibai[1], Liat Moscovici[1], Carolin Riegraf[2], Sebastian Buchinger[2], Georg Reifferscheid[2], Shimshon Belkin[1]

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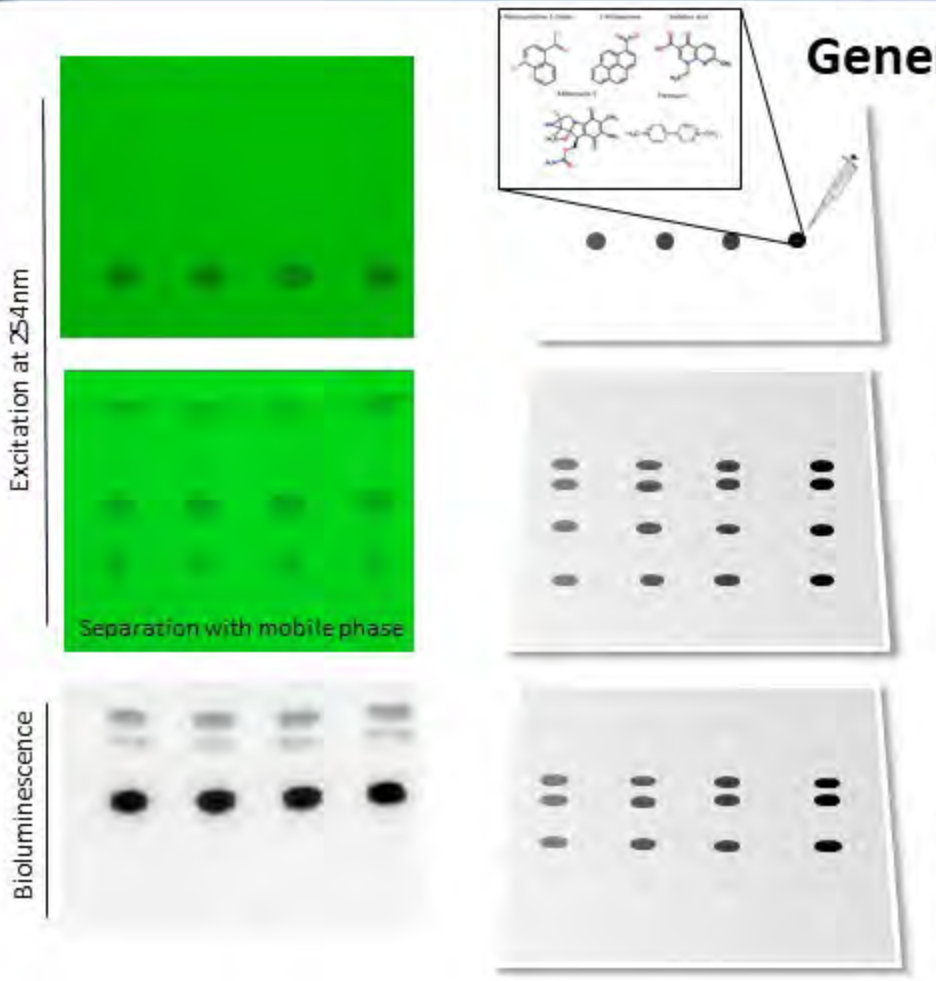
[2] *Federal Institute of Hydrology (BfG), Department of Biochemistry and Ecotoxicology, Am Mainzer Tor 1, 56068 Koblenz, Germany*

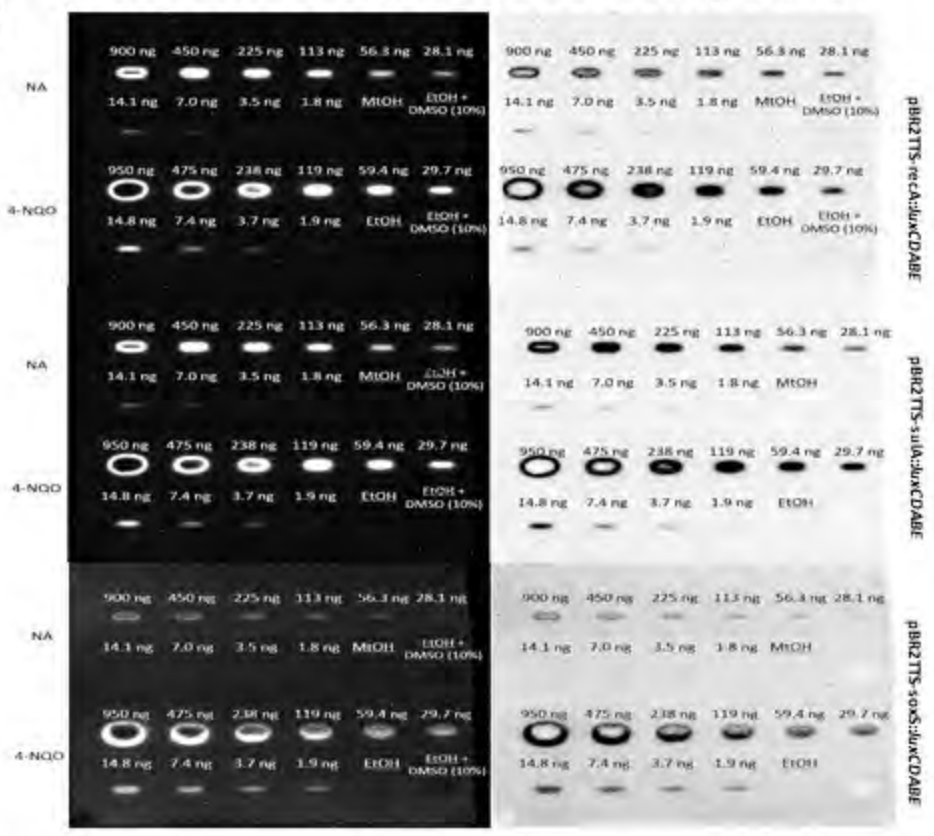
We present an innovative technological platform, combining two complementary approaches for the detection of genotoxic compounds: (a) sample separation by thin layer chromatography (HPTLC) and (b) effect-testing by a bioluminescent genotoxicity bacterial sensors. All *E. coli* reporter strains carrying a plasmid-borne fusion of a stress-responsive gene promoter to bioluminescence reporter genes (*Photorhabdus luminescens luxCDABE*).

General concept



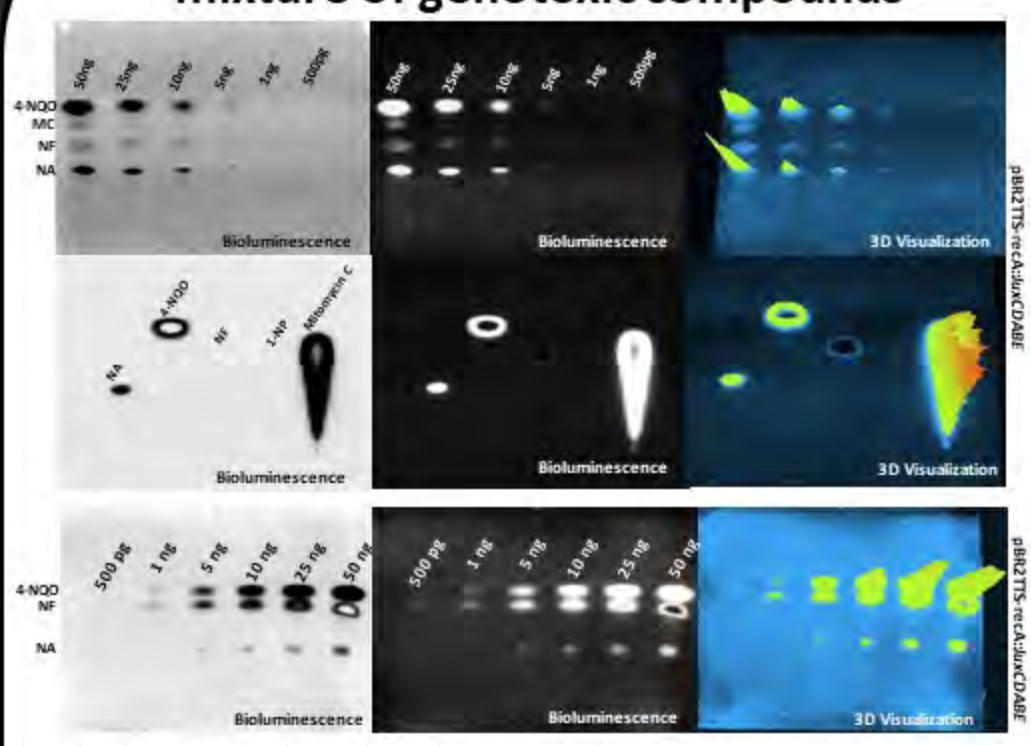
- Sample application
- Separation of sample components with a suitable mobile phase
- Spraying the bacterial strains directly onto the HPTLC plate

Effect-testing of model compounds



HPTLC coupled with three bioluminescent reporter strains, harboring the *recA, sulA and soxS* gene promoters as the sensing elements. Nalidixic acid and 4-Nitroquinoline-1-Oxide served as model genotoxicants.

Separation and detection of a mixture of genotoxic compounds



HPTLC coupled with a bioluminescent genotoxicity reporter strain (*recA'::luxCDABE).* Images were obtained following a chromatographic run and a 1 hour incubation at 37 °C. Nalidixic acid, 4-Nitroquinoline-1-Oxide, Paraquat, 1-Nitropyrene and Nitrofurantoin served as model genotoxicants.

Future directions

- Lowering the detection thresholds by molecular manipulations of the reporter strains
- Developing new strategies for a multi-parallel detection of several adverse biological effects on the same TLC plate
- Implementation of metabolic activation of pro-carcinogens directly on HPTLC
- Testing the system with contaminated water and landfill samples following solid phase extraction

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A novel electrochemical biosensor based on screen printed gold electrode modified with organo-silane for the tumor necrosis factor receptor-associated protein 1 detection

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BBMEC 12

The tumor necrosis factor (TNF) receptor-associated protein 1(TRAP1) was initially identified as a protein interacting with the intracellular domain of the type 1 TNF receptor (TNFR-1). Later sequence analysis revealed that TRAP1 was identical to heat shock protein 75 (HSP75), highly homologous to HSP90. It is well described that the level of heat shock proteins (HSPs) increases in tumors in response to stresses of various kinds in order to restore the normal protein-folding environment and that they are important in maintaining oncogenes in an active conformation. Silane coupling agents are compounds whose molecules contain functional groups that bond with both organic and inorganic materials. It is this characteristic that makes silane coupling agents useful for improving the mechanical strength of composite materials, for improving adhesion, and for resin modification and surface modification.In this study, we designed a novel biosensor to detect TRAP1 biomarker constructed on modified screen printed gold electrode (SPE-Au).

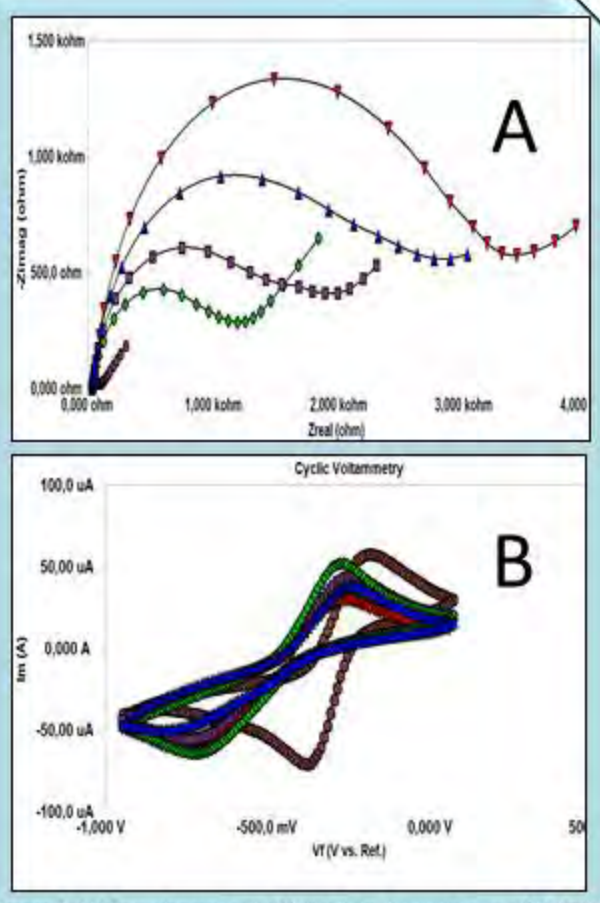


Figure.1. (A) Impedance spectra (B) cyclic voltammograms of the TRAP-1 immunosensor. [-•-•-:SPE-Au (pink), -♦-♦-:SPE-Au /OH (green), -■-■-: SPE-Au/OH/CPTMS (purple), -▲-▲-: SPE-Au /OH/CPTMS/anti-TRAP1(blue), -▼-▼-: SPE-Au /OH/CPTMS /anti-TRAP1/BSA (red)].

3-CPTMS OPTIMIZATION

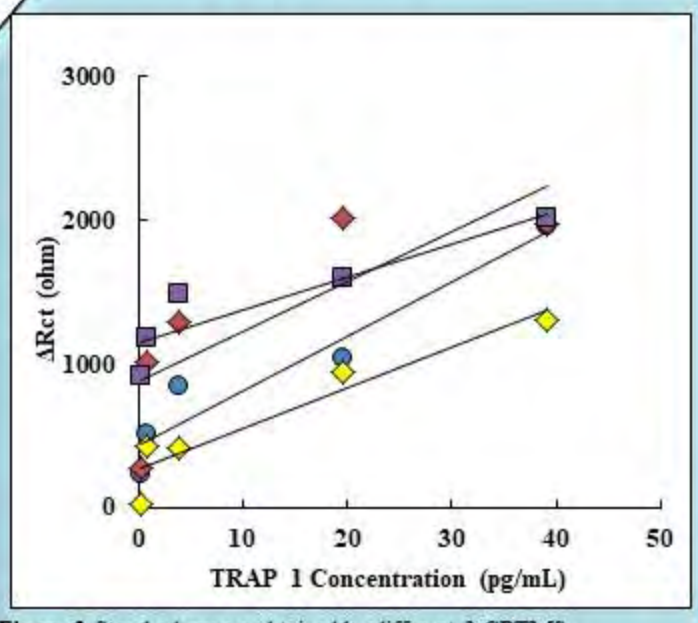


Figure.2. Standard curves obtained by different 3-CPTMS concentrations incelenmesi [-•-•-: % 0.1 CPTMS (blue), -♦-♦-: % 0.25 CPTMS (pink), -♦-♦-: % 0.5 CPTMS (yellow), -■-■-: % 1 CPTMS (purple)].

Anti-TRAP1 CONCENTRATION OPTIMIZATION

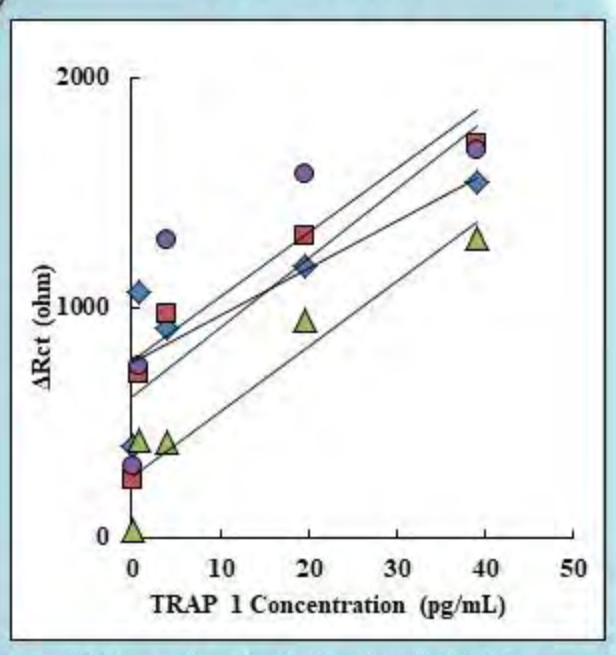
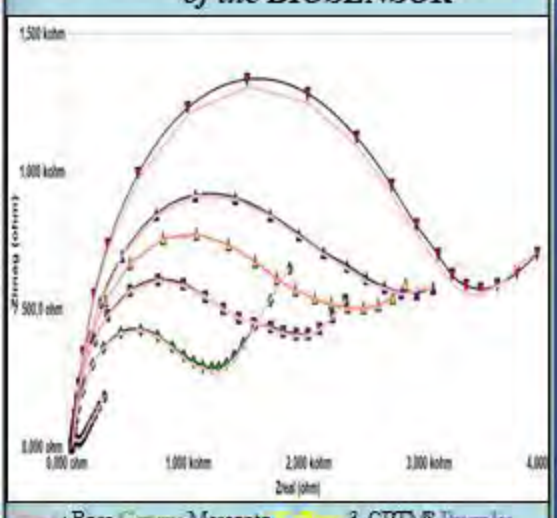


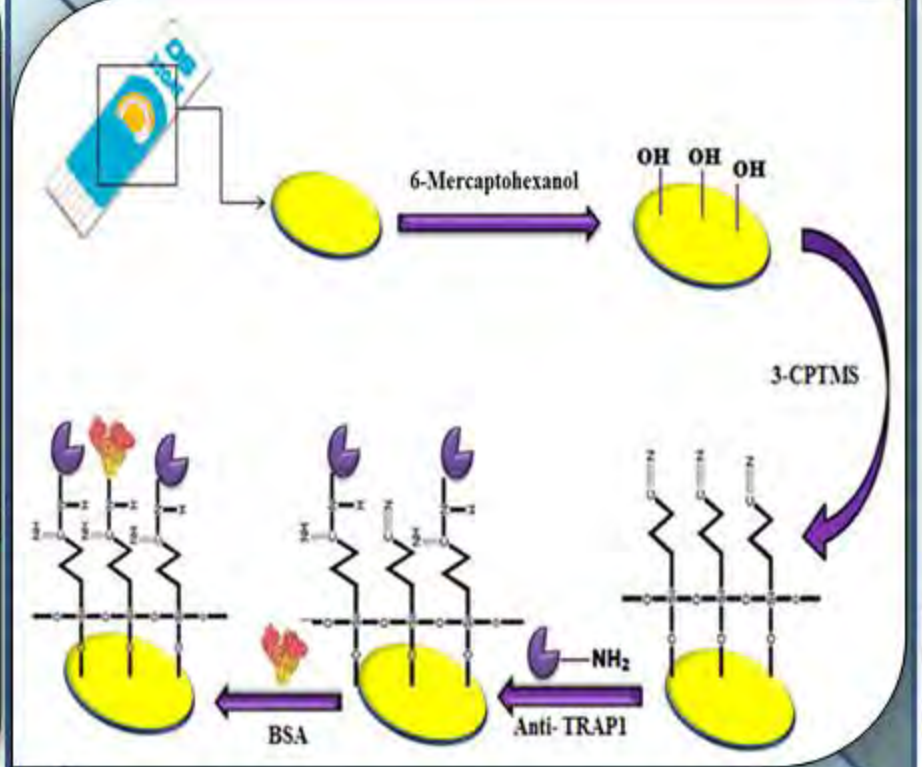
Figure.3. Examination of optimum concentration belonging to anti-TRAP1 antibody [-♦-♦-: 2.5 ng/mL (blue), -■-■-: 5 ng/mL (pink), -▲-▲-: 10 ng/mL(green),-•-•-: 20 ng/mL].

KRAMERS-KRONIG RESULTS of the BIOSENSOR



Blue: Bare Green: Mercapto Yellow: 3-CPTMS Purple: Anti-TRAP1 Blue: BSA Red: TRAP1

Biosensor Surface	Fit values
ITO	24.31
ITO/ OH	75.93
ITO/ OH/3-CPTMS	1.482
ITO/ OH/3-CPTMS/Anti-TRAP1	2.232
ITO/ OH/3-CPTMS/Anti-TRAP1/BSA	6.791
ITO/ OH/3-CPTMS/Anti-TRAP1/BSA/TRAP1	88.23



Anti-TRAP1 INCUBATION TIME OPTIMIZATION

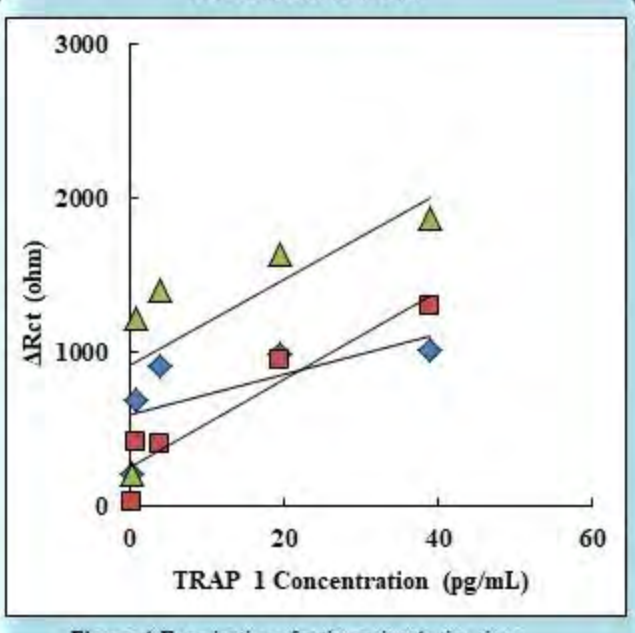


Figure.4. Examination of optimum incubation time belonging to anti-TRAP1 antibody [Incubation times: -♦-♦-: 30 min (blue) , -▲-▲-:45 min (pink), -■-■-: 60 min (green)].

TRAP1 BIOSENSOR

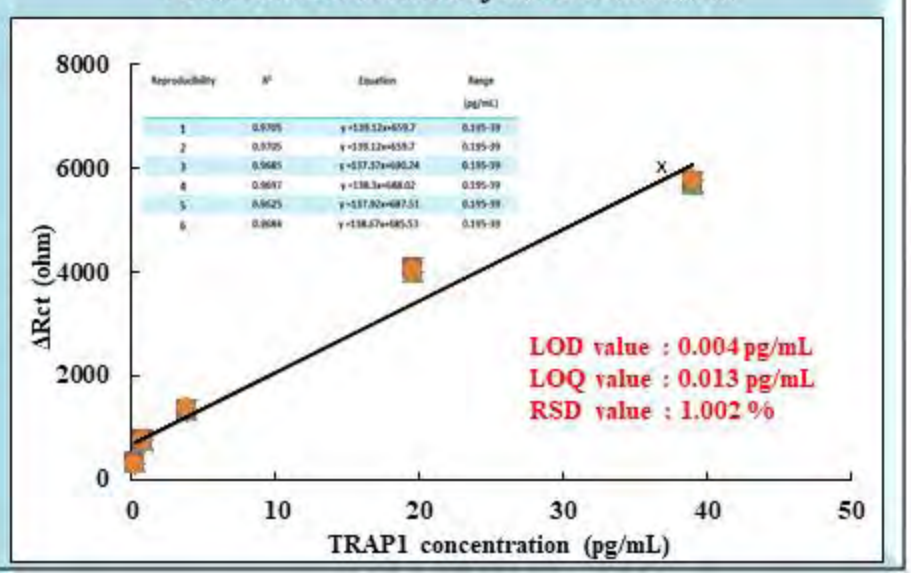
- Optimum 3-CPTMS concentration : 0.5 %
- Optimum anti-TRAP1 concentration :10 ng/mL
- Optimum anti-TRAP1 incubation time : 45 min
- Linear range: 0.135-39 pg/mL

We are grateful for the financial supports of this research from The Turkish Academy of Sciences (Üstün Başarılı Genç Bilim İnsanı Ödüllendirme Programı)(TÜBA-GEBİP, Project number: 58765284-205.02/1489).

REPEATABILITY of the BIOSENSOR

Average Value : 19.26
Standard Deviation : 0.193
Coefficient Variation : 1.0024 %

REPRODUCIBILITY of the BIOSENSOR



CALIBRATION CURVE of the BIOSENSOR

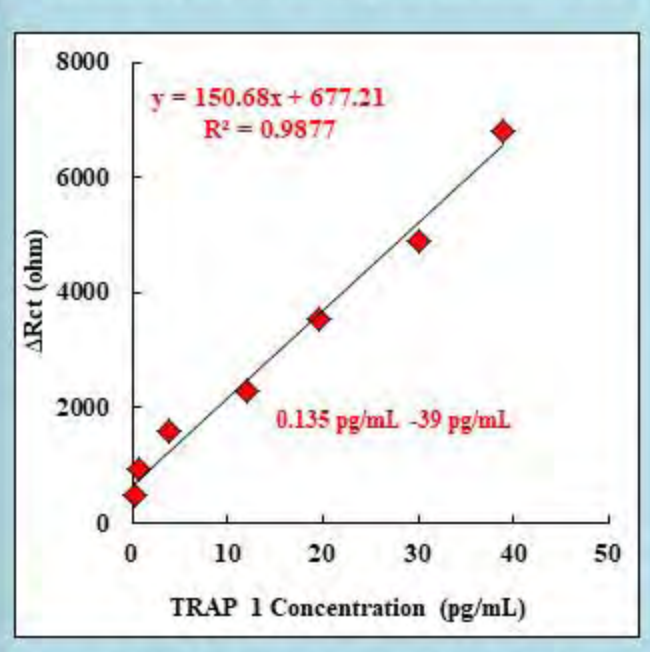


Figure..5. TRAP-1 calibration curve obtained by the present anti-TRAP-1 based immunosensor.

BBMEC 12

Determination of C1 inhibitor human by using screen printed gold electrode modified with silane: higly sensitive and disposable biosensor

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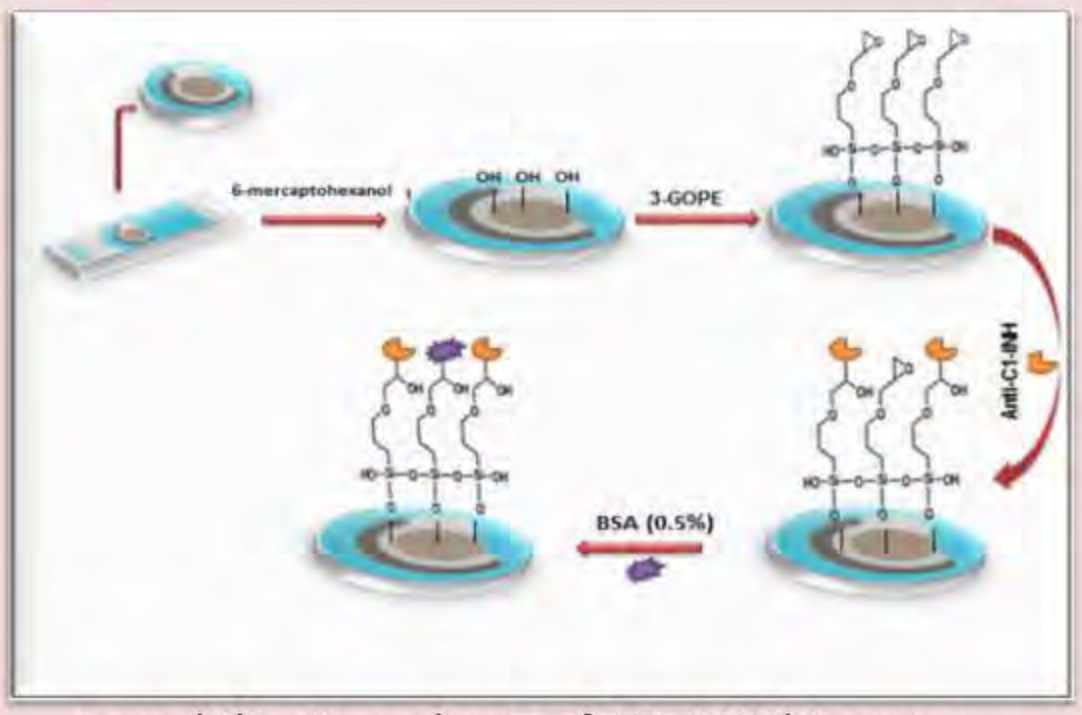
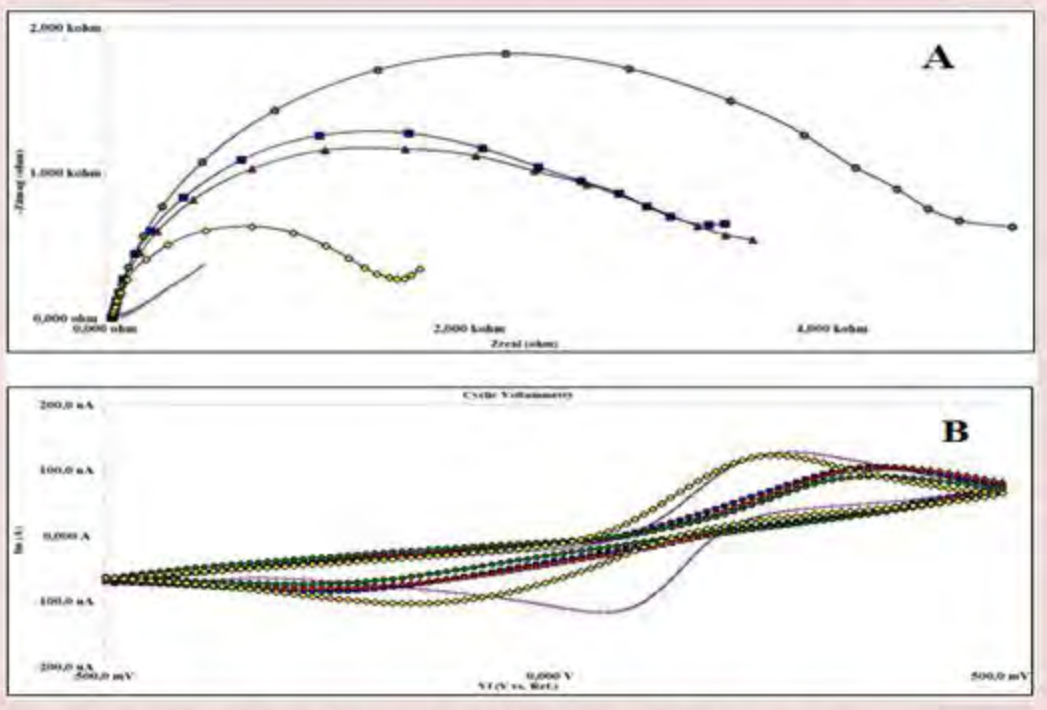
C1 inhibitor (C1-INH) is a serine protease inhibitor (serpin), also known as SERPING1. C1-INH is important in controlling a range of processes involved in vascular homeostasis, including inflammation, blood pressure and coagulation.. In this study, a biosensor based on screen printed gold electrode (SPE-Au) was designed to determine C1-INH.

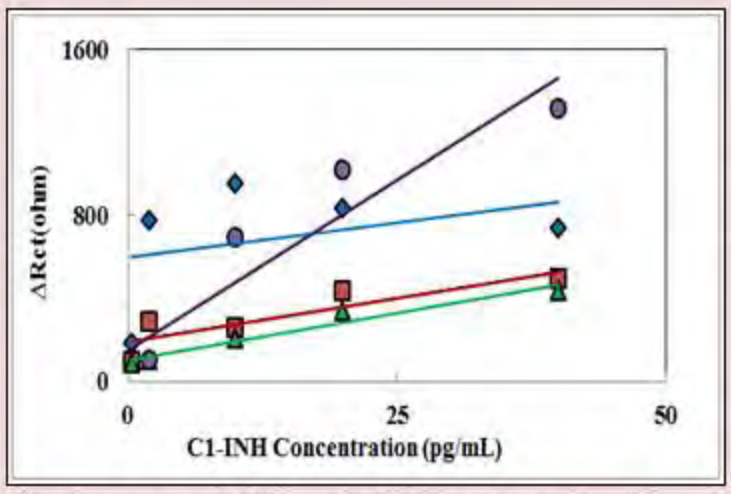
Immobilization steps and binding of C1-INH onto the SPE-Au surface were monitored via cyclic voltammetry and electrochemical impedance spectroscopy. All processes were applied in the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution. Electrochemical experiments were monitored using Gamry Potentiostate/Galvanostate, Reference 600 (Gamry Instruments, Warminster, USA



Immobilization scheme of C1-INH biosensor

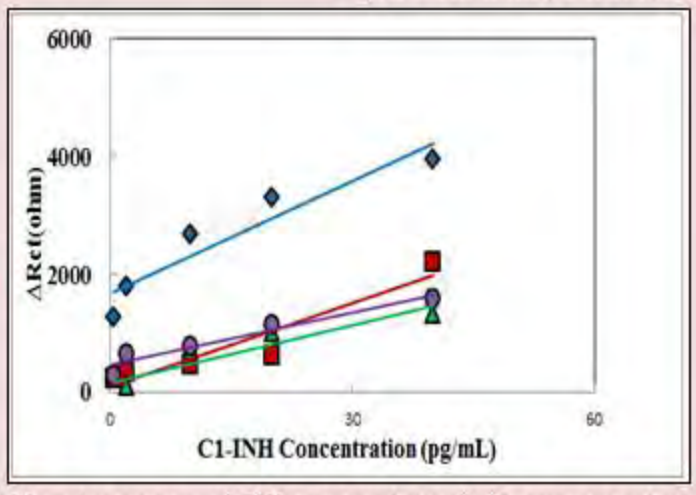


EIS spectrum (A) and CV voltammogram (B) of immobilization steps. purple (-+-+-): SPE-Au, yellow (-♦-♦-): SPE-Au/6-MH, green (-•-•-): SPE-Au/6-MH/3-GOPE, red (-▲-▲-):SPE-Au/6-MH/3-GOPE/anti-C1-INH,blue(-■-■-):SPE-Au/6-MH/3-GOPE/C1 INH/BSA



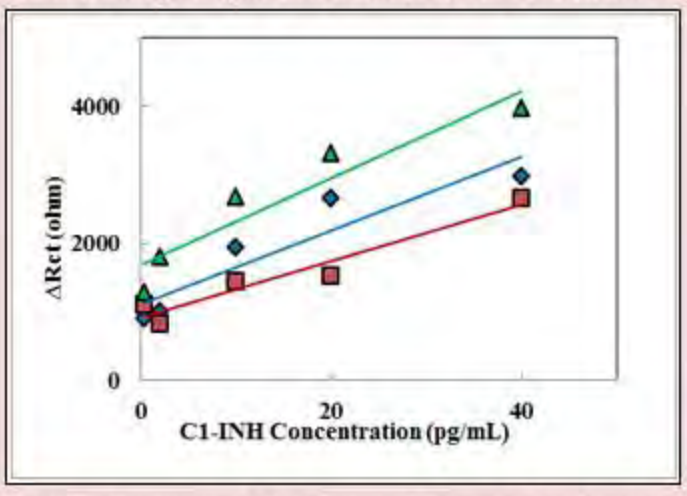
Calibration curves of different 3-GOPE concentrations. blue (--♦-♦-): 0.05% 3-GOPE, purple (-•-•-): 0.1% 3-GOPE red (-■-■-):0.5% 3- GOPE, green (▲-▲): 1% 3-GOPE

Chosen concentration: 0.1% 3-GOPE



Calibration curves of different anti-C1-INH concentrations blue (-♦-♦-): 2.5 ng/mL anti-C1-INH, red(-■-■-): 5 ng/mL anti-C1-INH, green(-▲-▲-):10 ng/mL anti-C1-INH, purple(-•-•-): 20 ng/mL anti-C1-INH

Chosen concentration: 2.5 ng/mL anti-C1-INH



Calibration curve of different anti-C1-INH incubation time. blue (-♦-♦-): 30 min anti-C1-INH, green(-▲-▲):45 min anti-C1-INH, red (-■-■-): 60 min anti-C1-INH.

Chosen incubation time: 30 min

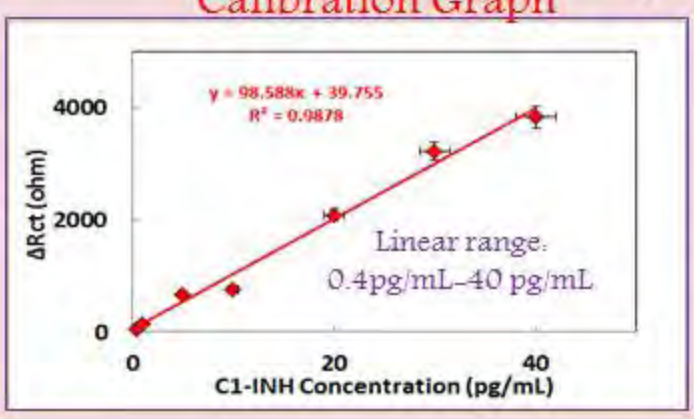
OPTIMIZATIONS

ANALYTICAL STUDIES

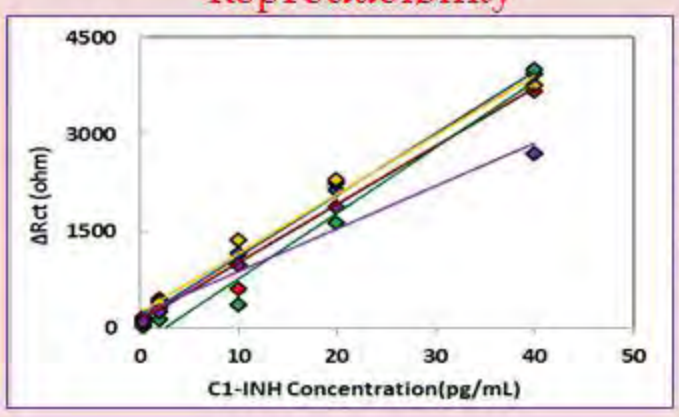
Standard deviation value: 1.317405
Coefficient of variation:6.30%
Average value: 20.87333

LOD:0.04 pg/mL
LOQ: 0.134 pg/mL
RSD% (slope): 13.54%
RSD% (intercept): 0.3%

Calibration Graph



Reproducibility



Reproducibility	R^2 values	Equation
1	0,9987	y = 95.855x + 135.81
2	0,9672	y = 90.211x + 107.54
3	0,9721	y = 101.58x – 255.57
4	0,9815	y = 91.455x + 234.12
5	0,9636	y = 66.316x + 207.54

We are thankful for financial support from the *TÜBA* Üstün Başarılı Genç Bilim İnsanı Ödüllendirme Programı (TÜBA-GEBİP, Project number: 58765284-205.02/1489).

A label-free low cost sensor based on surface-plasmon resonance on plastic optical fiber coupled with a biomimetic receptor for furfural detection in water

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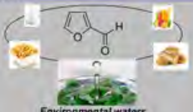
N. Cennamo[b], L. Zeni[b]
[b] Department of Industrial and Information Engineering, University of Campania Luigi Vanvitelli, Aversa, Italy

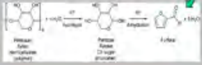
2-FAL proposed as a marker of food for wrong storage and/or degradation and environmental control



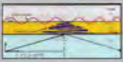
Environmental waters

Formation of furanic compounds in environment and food:

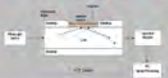
- from *decomposition of the Maillard product*
- from *dehydration of sugars*



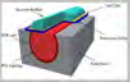
Surface Plasmon Resonance (SPR)



SPR on plastic optical fiber



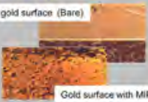
Measurements set up



- Sensing region: 10mm
- Photoresist (Microposit S1813): 1.5µm
- Gold film: 60nm
- Reference: transmission spectrum in air (no resonance)

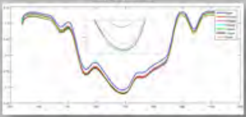
Optical platform on POF for measurement on drop

Halogen lamp emitting from 360 to 1700 nm; Spectrum analyzer detection range: 300-1100 nm



SPR spectra of 2-FAL_MIP at different 2-FAL concentrations in water

SPR transmission spectra at MIP sensor S3, at different 2-FAL concentrations in water, vs bare in air



$$\Delta\lambda_c = \frac{\Delta\lambda_{max} K_{aff} c}{1 + K_{aff} c}$$

Langmuir adsorption isotherm

Plasmon resonance wavelength variation vs 2-FAL C(M): a) vs bare in air; b) vs MIP in air

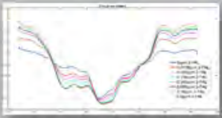
Table [illegible]

	normalization with bare platform in air					normalization with MIP modified platform in air				
	λ_{res}	sensitivity at low conc (nm/M) ($\Delta\lambda_{max} K_{aff}$)	K_{aff}	$\Delta\lambda_{max}$	LOD	λ_{res}	sensitivity at low conc (nm/M) ($\Delta\lambda_{max} K_{aff}$)	K_{aff}	$\Delta\lambda_{max}$	LOD
S1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	754.8	[illegible]	[illegible]	[illegible]	[illegible]
S2	717.5	[illegible]	[illegible]	[illegible]	[illegible]	725.5	[illegible]	[illegible]	[illegible]	[illegible]
S3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

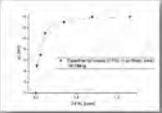
SPR spectra of 2-FAL_MIP at different 2-FAL concentrations in synthetic wine

Parameters of the standardization curve in synthetic wine

	normalization with MIP modified platform in air				
	λ_{res}	sensitivity at low conc (nm/M) ($\Delta\lambda_{max} K_{aff}$)	K_{aff}	$\Delta\lambda_{max}$	LOD
S6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]



SPR transmission spectra at MIP sensor S6, at different 2-FAL concentrations in wine, vs bare in water



Plasmon resonance wavelength variation vs 2-FAL C(ppm)

BBMEC 12° INTERNATIONAL BIOSENSOR CONFERENCE – 25-29 September 2017 ROME

BIOSENSOR FOR THE DETECTION OF BACTERIOPHAGES BASED OF THE MICROWAVE RESONATOR

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[5] - Saratov State University, Saratov 410005, Russia

As long as viral infections remain one of the global problems the studies aimed at the development of new express methods for their determination are very urgent. For the first time the possibility of recording the interaction of immobilized microbial cells with the bacteriophages using the detection system based on the microwave resonator (5 – 8.5 GHz) was shown. The general scheme of the experiments show on the Fig. 1.

Polystyrene films plasma treatment causes a change in surface morphology.

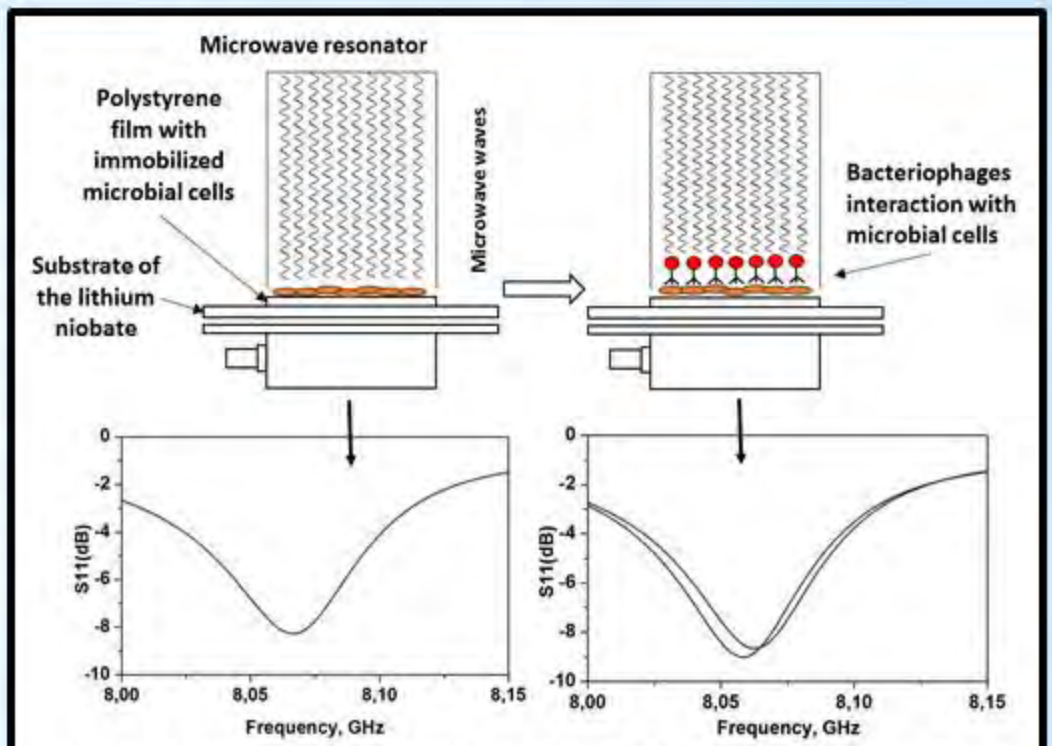

Fig. 1 The general scheme of the experiments

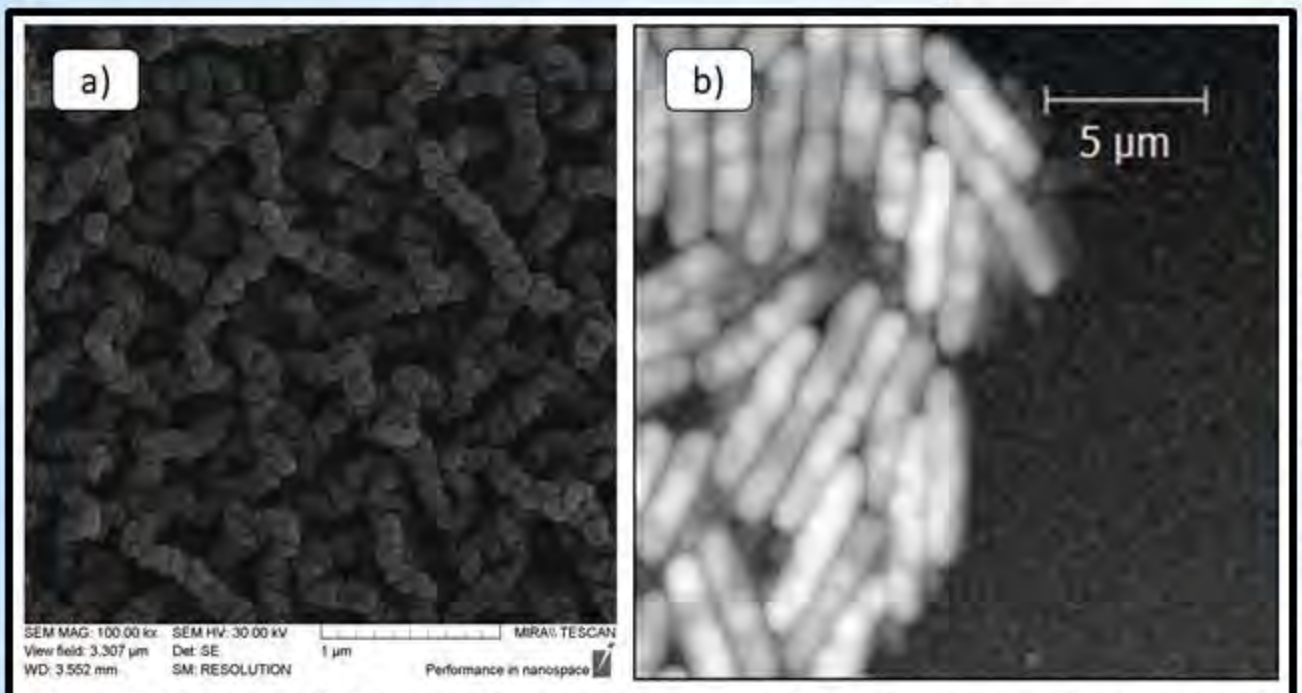

Fig. 2 Image of film surfaces in a scanning electron microscope after modification in plasma for 30 s (a), and films containing immobilized microbial cells obtained by probe microscopy (b).

SEM image of polystyrene film surface after RF plasma treatment is shown in Fig. 2. The surface of the sample was represented by the rods of irregular shape with diameter and length of 0.21 and 1.8 μm, respectively.

It has been demonstrated that the biosensor can differentiate the case of the interaction of bacterial cells with specific bacteriophages from the control experiments, when such interaction is absent.

For the first time the possibility of immobilization of microbial cells on the surface of thin polystyrene films modified by plasma radio frequency argon discharge (13.56 MHz) was shown. The optimal conditions of the immobilization of the cells Azospirillum lipoferum Sp59b, which retained the activity of the cells in relation to specific bacteriophages, were selected. By using the microwave sensor one can determine the content of bacteriophages ΦAl-Sp59b in the analyzed suspension. The sensitivity of the sensor is~106 phage/ml, while analysis time is about 10 min(Fig. 3). At that an additional option of the specified sensor is the determination of the viability of microbial cells after immobilization. The obtained results are novel and promising from the point of view of the development of methods for the determination of the presence of viral particles and the viability of microbial cells by use of the sensor based on microwave resonator.

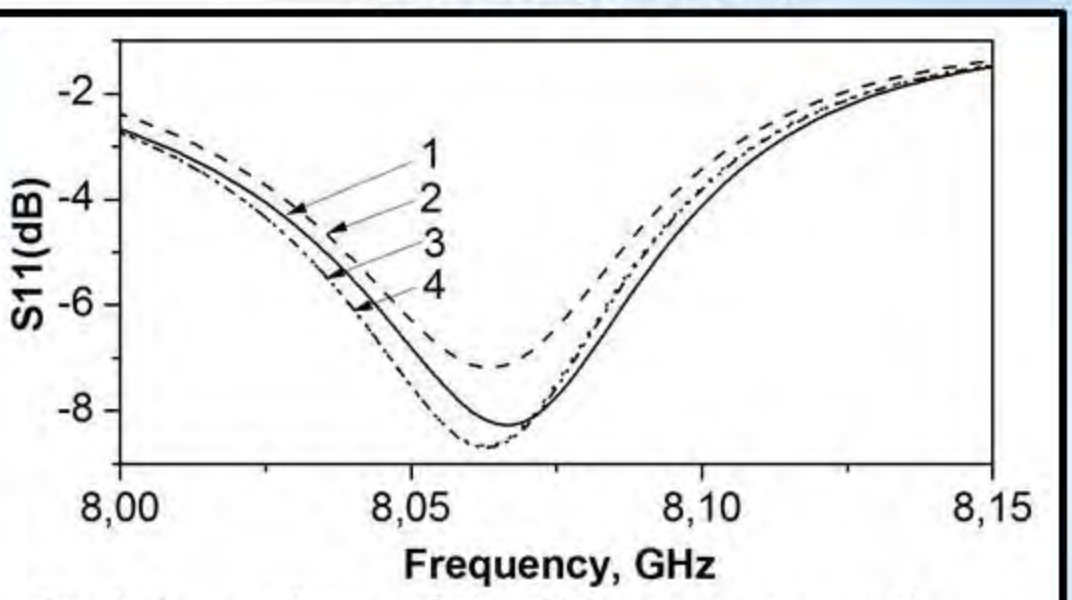

Fig. 3 Dependence of the S11 parameter on the frequency of the resonator with plasma-treated polystyrene film before (1) and after immobilization microbial cells for 10 (2), 20 (3) and 30 (4) min.

This work was supported in part by the Russian Foundation for Basic Research (projects nos. 16-07-00818 and 16-07-00821) and grant of Council Grants of the Russian Federation President (SP-677.2015.4).

Development of novel biosensor system for fish stress monitoring using self-assembled monolayer

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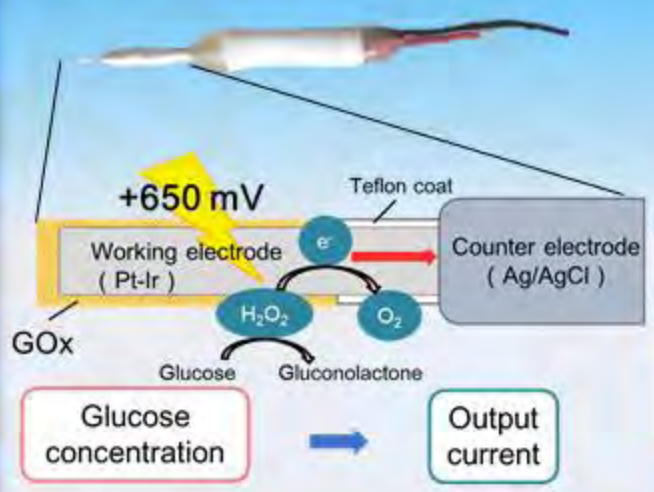
[a]Tokyo University of Marine Science and Technology, JAPAN
[b]Hokkaido Industrial Technology Center, Hokkaido, JAPAN

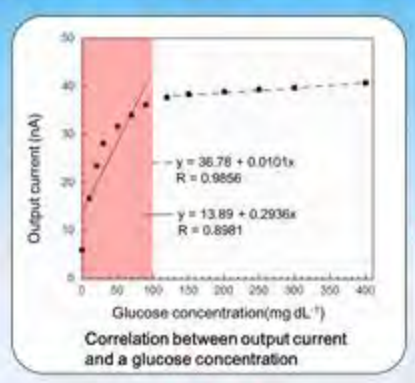
科研費 KAKENHI

Introduction



We developed a biosensor system to measure the glucose concentration as an indicator of fish stress in fish using glucose oxidase (GOx) oxidation reaction.

BUT

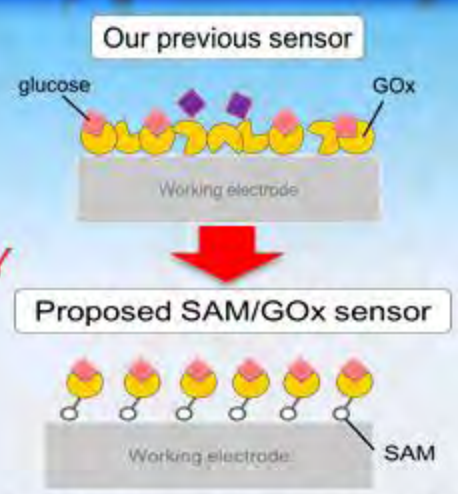


Correlation between output current and a glucose concentration

Only Low concentration range (10-100 mg dl^{-1}) shows high responsiveness

Glucose fluctuation of test fish (Tilapia, *Oreochromis niloticus*) is about 50-150 mg dL^{-1}

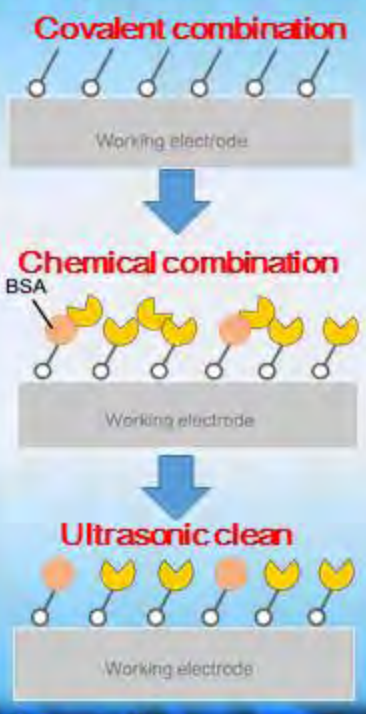
AIM OF THIS STUDY



We attempted to enhance the sensor response by immobilizing the enzymes using a self-assembled monolayer (SAM) in which molecules can spontaneously assemble to form a regular array.

Methods

Preparation of SAM/GOx sensor



① Immersed in MPA solution (SAM) for 8 hours to modify with SAM on the working electrode surface

② Immersed in EDC/NHS solution for 2 hours to replace MPA end with high active ester group

③ Immersed in GOx/BSA solution overnight to immobilize GOx and block the unbound site by BSA

④ Ultrasonic cleaned the sensor for 10 seconds to remove the excess-bounded GOx

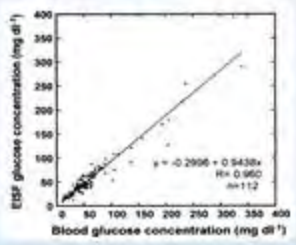
Wireless biosensor system and sensor implant site

①

Wireless transmitter : It is a self-made transmitter. It send data by using radio waves.

②

USB-type Receiver: It is also self-made which programmed for accepting data from particular transmitter.

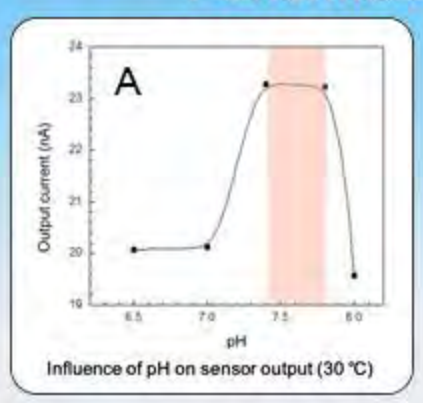


(Y. Yonemori, H. Endo et al. *Anal. Chim. Acta*. 2009; 633: 90-96)

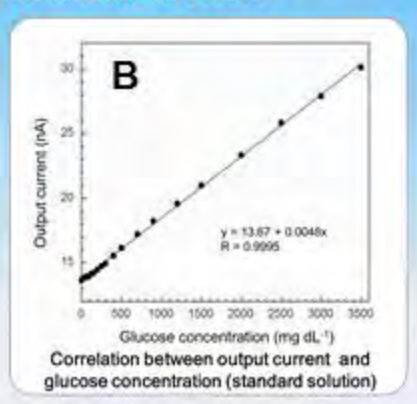
Good correlation between glucose concentration in EISF (Fish Eye Interstitial Fluid) and blood glucose level has been confirmed.

Results

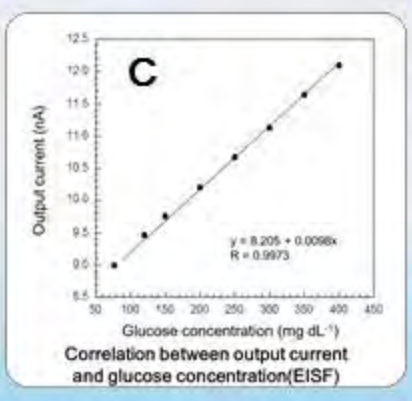
Performance measurement



Influence of pH on sensor output (30 °C)



Correlation between output current and glucose concentration (standard solution)

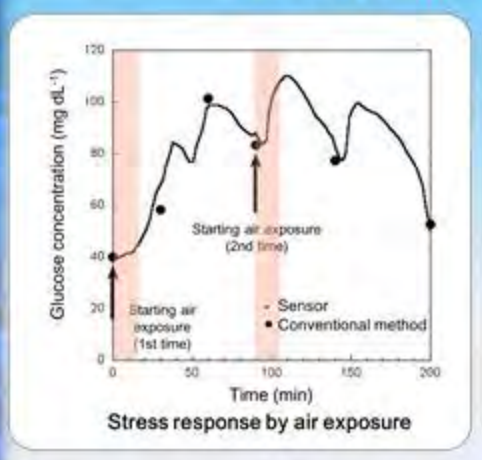


Correlation between output current and glucose concentration(EISF)

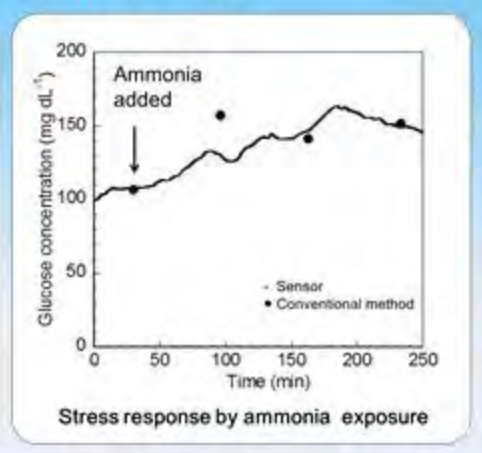
The highest sensor output was found around pH 7.4 to 7.8 (Fig. A). The output current and glucose concentration were well correlated in the range of 10 - 3500 mg dL^{-1} in standard glucose solution (Fig. B) and 90-400 mg dL^{-1} in biological sample (EISF) of fish(Fig. C).

The dynamic measurement range of the SAM/GOx sensor was significantly improved in the high concentration range.

In vivo measurement



Stress response by air exposure



Stress response by ammonia exposure

The proposed biosensor system was attached to the test fish (Tilapia, *Oreochromis niloticus*). The test fish was exposed to air twice (15 min, shaded part in the upper-left figure)and ammonia solution (10 mg L^{-1}, arrow in upper-right figure). As a result, an increase in glucose concentration could be confirmed after each air exposure or ammonia. And the sensor responses were have a closed relate to the results obtained from conventional method (Colorimetric method). These results showed the proposed sensor was able to successfully measure glucose concentration in vivo in fish.

Prospect

Monitoring stress response by other stress factors

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New design of label-free human immunoglobulin G impedance biosensor with a micro-gap parallel plate electrodes

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[2]National Institute of Material Science, 1-2-1 Sengen, Tsukuba, Ibaraki 305-0047, Japan
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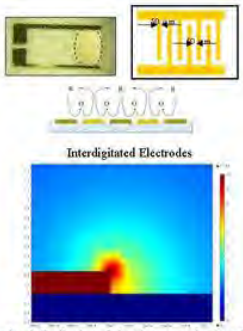
Abstract

In past few years, electrochemical impedance spectroscopy (EIS) attracts considerable attention. Since this technique can detect the affinity binding event without labeling process. To improve the sensitivity of EIS, the interdigitated micro-electrodes (IDE), that have a series of parallel microelectrodes with alternating micro-bands connected together, have gained increasing attraction. Because of the short distance between the anodic and cathodic electrodes of IDE, the speed of oxidation and reduction cycle is extremely enhanced, which results in a high efficiency of data collection. However, there is a serious problem in IDE that the electric current highly concentrated on the edge of electrode, which indicates that impedance signal is determined by only the binding event around edge part. In this research, we develop a new approach of signal enhancement with a parallel plate electrode (PPE) structure. Since PPE utilizes the smooth electrode surfaces for EIS measurement, increasing sensing performance and reproducibility compared with IDE.

Problem

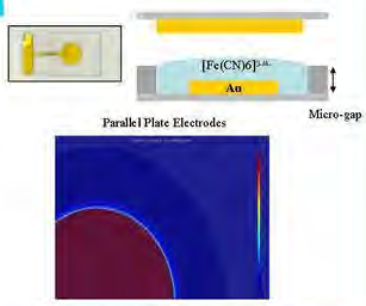
According to our current density analysis on interdigitated micro-gap electrodes (IDE), it was found that the current was highly concentrated on the edge part. However, the edge part has not an identical surface for biosensing because a rough surface is introduced by the lithography and etching processes.



Simulated distribution of electric current density of IDE.

Parallel Plate Electrodes

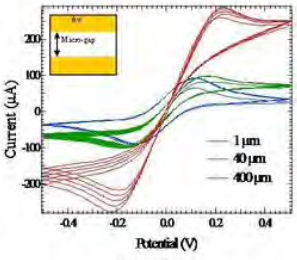
We develop a new electrode structure of parallel plate electrodes (PPE) with a micrometer scale gap. With this structure, the electric current is distributed homogeneously on the top flat surface with a smooth and well-ordered structure.



Simulated distribution of electric current density of PPE.

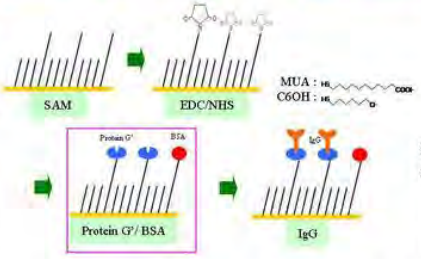
CV measurements

We performed cyclic voltammetry (CV) measurements using the PPE under the presence of $[Fe(CN)6]^{3-/4-}$. The CV profiles were recorded as the function of gap distance between the electrode. It was observed that the faradic current gradually increased with decreasing the gap distance.



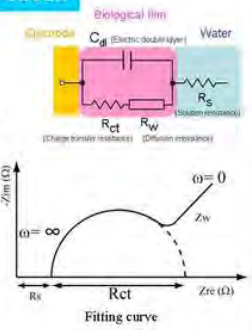
CV profiles

Sensor preparation



Self assembled monolayer was formed on the electrode surface, and Protein G' which selectively adsorbs IgG was chemically bonded.

Equivalent circuit

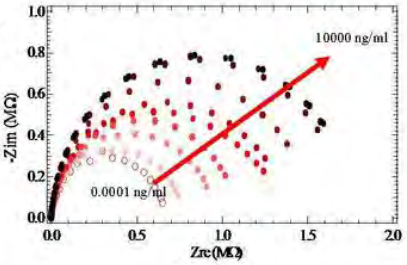


Fitting curve

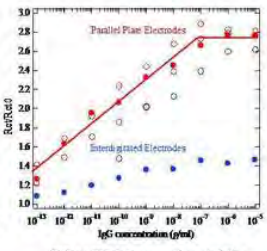
EIS measurements

We prepared human immunoglobulin G (IgG) biosensor. Protein G'was employed to detect IgG. We immobilized Protein G' on the one surface of the parallel electrodes and measured EIS spectra as the function of IgG concentration.

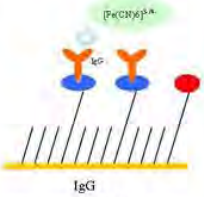
As the IgG concentration was increased, the diameter of the semicircle (Rct) of EIS spectrum increased. The observed diameter increase is explained as the affinity adsorption of IgG.



Nyquist plot



PPE vs IDE IgG sensor characteristics

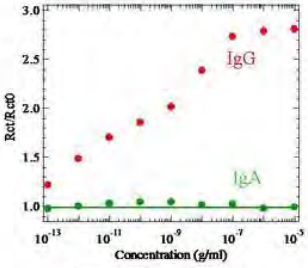


IgG

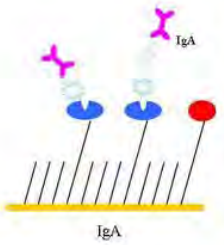
PPE showed higher Rct change rate and higher repeatability result than those of IDE.

IgG specificity

The specificity of PPE sensor was tested by using human immunoglobulin A (IgA) solutions. No increase of Rct was observed for IgA, indicating a high IgG-spesific character of the sensor.



Comparison of sensor characteristics



IgA

Conclusions

- From the CV measurements, we found that the faradic current was highly promoted by narrowing the gap distance between parallel plate electrodes. The result indicates high efficiency of data collection is brought about due to the enhancement of redox cycle.
- From the EIS measurement, the sensor with parallel plate electrode showed higher sensitivity and reproducibility than the sensor with interdigitated micro-electrodes.

Determination of total antioxidant capacity in plant extracts using electrochemical sensors

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[c]*Faculty of Sciences, Valahia University of Targoviste, 130024, Targoviste, Romania*
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Various plants contain high concentrations of antioxidants, which can inhibit the oxidation of biomolecules by the free radicals that are formed in living organisms. As pro-oxidant (ROS), hydrogen peroxide in variable concentrations was used. Optimization studies by means of pH, working potentials, and extract concentration, as well as biosensor performance were highlighted and carried out. The results were validated and correlated using classical methods of spectroscopy.

ROS action: $O_2 \xrightarrow{+1e^-} O_2^{\cdot} \xrightarrow{+1e^-} H_2O_2 \xrightarrow{+1e^-} \cdot OH$

AIM: optimizing a label-free sensor based on gold nanoparticles (AuNP) to determine antioxidant capacity

Plant extracts:

***Hippophae rhamnoides L.* (seabuckthorn)**

***Lavandula angustifolia Mill. si L.* (lavender)**

Extraction methods:

US – ultrasound bath (Langford Sonomatic)

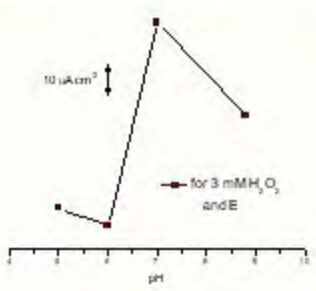
T – pressure enhanced solvent extractor (Timatic)

Extraction solvents:

1 and **2:** Hydroalcoholic extract (1:1)
3 and **4:** Hydroglycerine extract (1:1)
5 and **6:** Hydro-propyleneglicol extract (1:1)

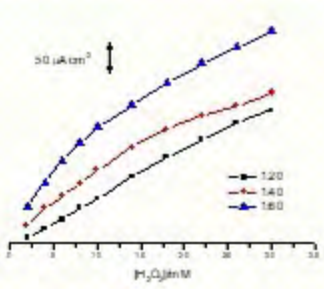
AuNP -based electrochemical sensor optimisation for total antioxidant capacity, by H_2O_2 inhibition in the presence of antioxidants from plant extracts

pH study:

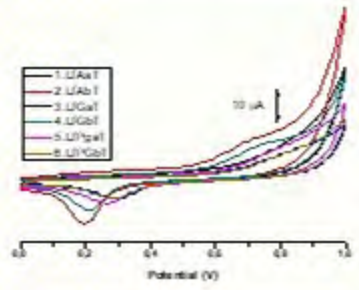


Amperometry in 0.1 M NaPB

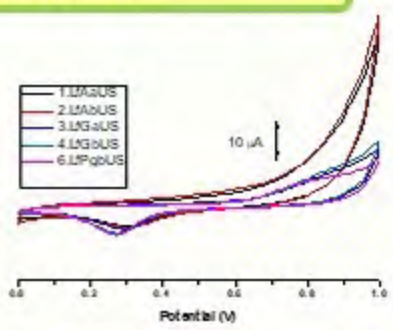
Influence of extract dilution:



Voltammetric characteristics:



Lavender – T extraction



Lavender – US extraction

Optimum parameters:

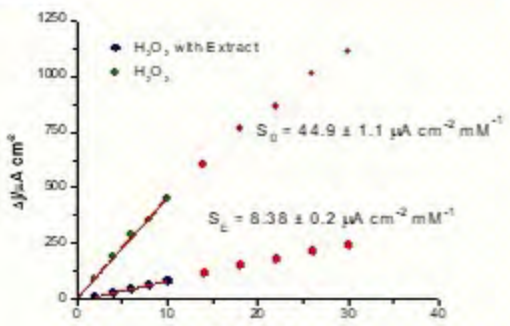
- **pH: 7.0**
- **Working potential: 0.55 V**

Analytical parameters ➢ Relative Antioxidant Capacity :

Amperometry: pH 7.0, at 0.55 V

$$RAC(\%) = \frac{S_0 - S_E}{S_0} * 100$$

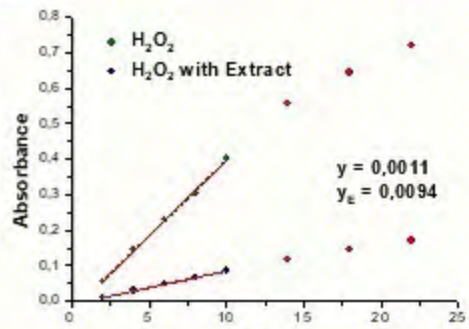
S_0 - sensitivity of the blank
S_E - sensitivity in the presence of the extract



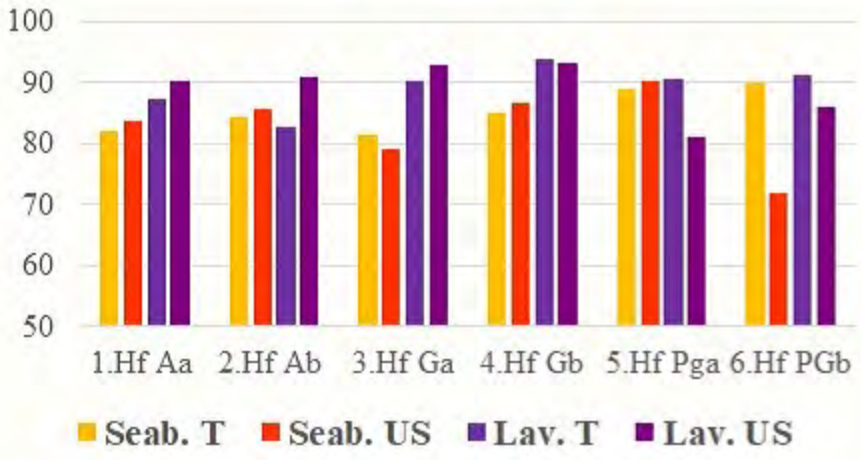
UV-VIS spectroscopy: pH 7.0, λ = 243

$$RAC = 1 - \frac{y_E}{y}$$

y - slope of the blank
y_E - slope in the presence of the extract

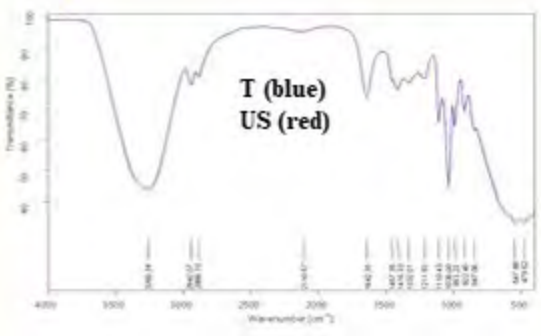


RAC (%) of seabuckthorn and lavender extracts using amperometry:



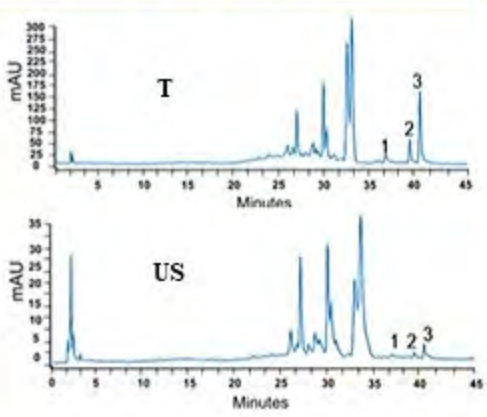
Antioxidant activity correlation:

Lavender: FTIR



Clear signals (peaks at 3300 cm^{-1}) attributed to phenolic compounds.

Seabuckthorn: HPLC analysis



Clear signals (peaks 1, 2, 3) attributed to flavonols for T extraction method.

BBMEC 12th Workshop on Biosensor & Bioanalytical Microtechniques in Environmental, Food & Clinical Analysis
INTERNATIONAL BIOSENSOR CONFERENCE
25. – 29. September 2017 | Rome, Italy

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, PN-II-RU-TE-2014-4-2801.

CONCLUSIONS

- **H_2O_2 sensors are a valid method for measuring antioxidant capacity for plant extracts**
- **Overall, lavender extracts have a higher antioxidant capacity than seabuckthorn extracts**
- **For all extracts, both the extraction method and solvent type have a great influence, in all cases the pressure enhanced extraction method being more qualitative**
- **Good correlation of our results with classical methods has been observed**

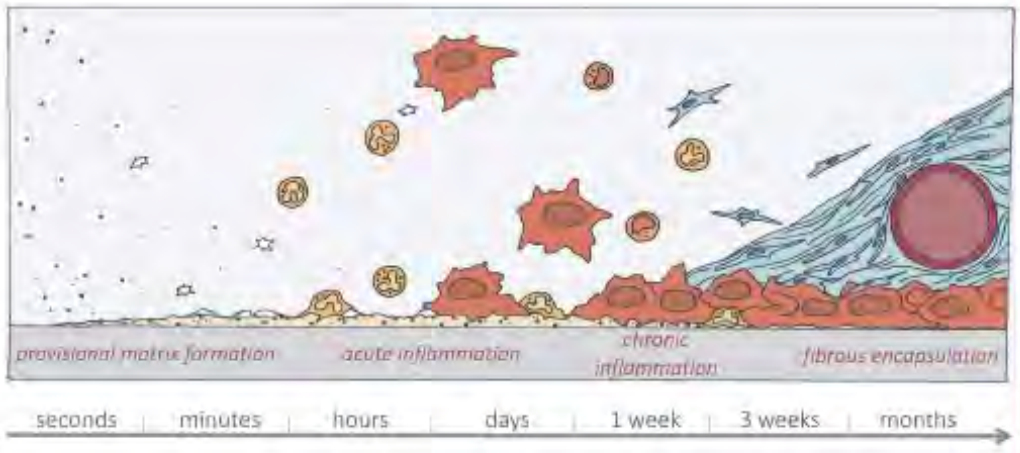
AN OPTICAL SENSOR FOR STUDYING HEMOCOMPATIBILITY OF BIOMATERIALS IN A FLOW SYSTEM

J. Hutterer, Eberhard Karls Universität, Tübingen/Deutschland,
G. Gauglitz, Eberhard Karls Universität, Tübingen/Deutschland

Background

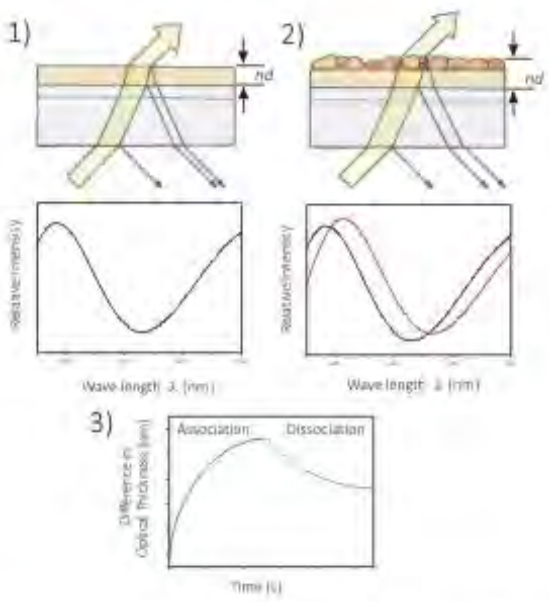
Implants in contact with blood:
- proteins start to adhere to the implant surface within milliseconds.
- amount, type and possible conformational changes initiate various processes such as:
  - platelet accumulation
  - activation of immunological cascades
  - formation of foreign body giant cells or apoptosis of adherent cells.

These unintended interactions of a foreign material with the human body can - inter alia - result in implant failure.
The here presented sensor has been developed to be particularly suitable for testing hemocompatibility.



Method

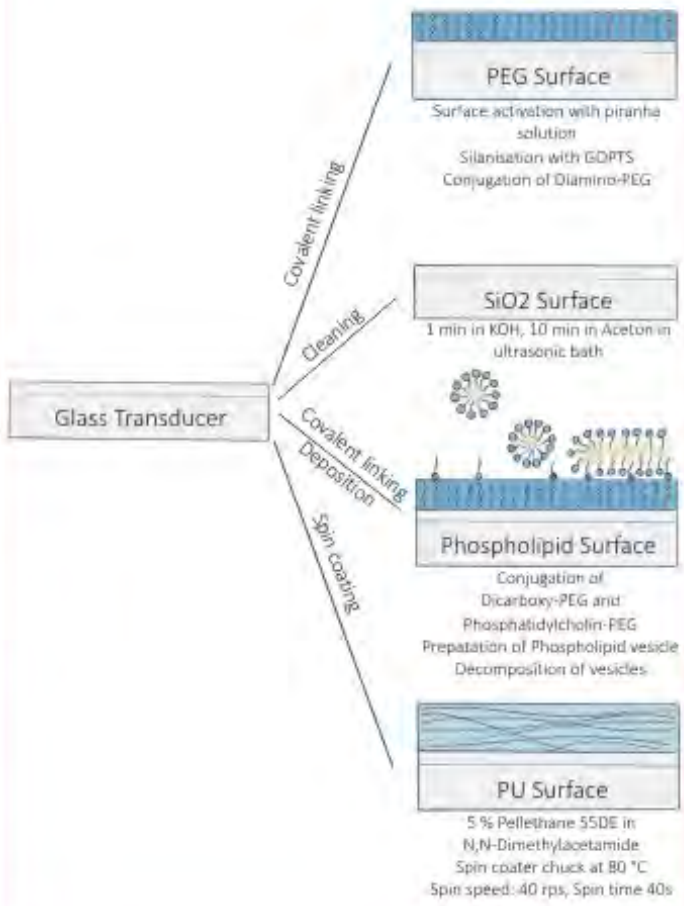
The formation of a protein film that develops on the sensor surface is recorded using reflectometric interference spectroscopy.



1) Using white light, the glas transducer is illuminated from the bottom. The reflected secundary beams superimpose and result in a characteristic interference spectrum.
2) Binding events at the investigated surface lead to a change in the optical thickness (*nd*). Thus, the interference spectrum is shifted.
3) The displacement of an extrema in the interference spectrum is monitored and the change in optical thickness can be calculated

Surface Preparation

The interactions of four different surfaces - polyethyleneglycol (PEG), glass (SiO_2), phospholipids and polyurethane (PU) with 5 % serum (FCS) in PBS were tested.



Analysis

Kinetic analysis of the binding events were carried out for PEG-, glass-, and phospholipid-surfaces.

The binding of the ligand L to the receptor R can be described by a reaction pseudo first order:

$$L + R \longrightarrow LR$$

L: ligand, R: receptor

Binidng equilibrium of ligand and receptor:

$$\frac{dc_{RL}}{dt} = k_a \cdot c_R \cdot c_L - k_d \cdot c_{RL}$$

k_a: *association constant*, k_d: *dissociation constant*, c_R: *concentration of receptor*, c_L: *concentration of ligand*, c_{RL}: *concentration of ligand − receptor complex*

The binding rate at the surface:

$$\frac{d\Gamma(t)}{dt} = k_a \cdot c \cdot (\Gamma_{max} - \Gamma(t)) - k_d \cdot \Gamma(t)$$

Γ: *surface loading*, Γ_{max}: *maximal surface loading*, c: *concentration in the bulk*, t: *time*

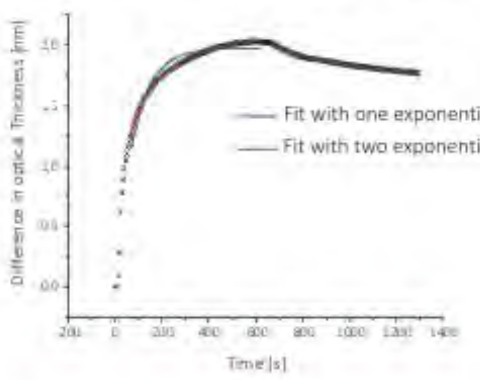
Dissociation (left) and Association (right):

$$\Gamma(t) = \Gamma_{00} \cdot e^{-k_d t} \qquad \Gamma(t) = \Gamma_{00} \cdot \left(1 - e^{-(k_a \cdot c + k_d) t}\right)$$

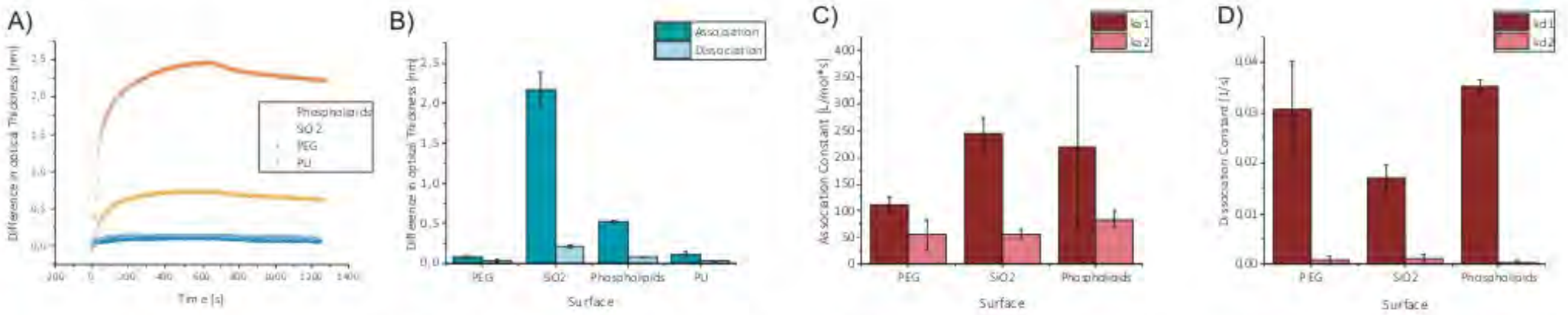
To parameterize for inhomogenities in binding behaviour of different components of the sample, a second equal exponential term was included.

$$\Gamma(t) = \Gamma_{001} \cdot e^{-k_{d1} t} + \Gamma_{002} \cdot e^{-k_{d2} t} + B$$

$$\Gamma(t) = \Gamma_{001} \cdot \left(1 - e^{-(k_{a1} \cdot c + k_{d1}) t}\right) + \Gamma_{002} \cdot \left(1 - e^{-(k_{a2} \cdot c + k_{d2}) t}\right)$$



Results and Outlook



A) Difference in optical thickness over time while applying 5 % serum (FCS) in PBS on one exemplary Phospholipid-, SiO_2-, PEG- and PU-surface, each. B) The mean difference between before and after association (dark blue) and dissociation (bright blue) of the optical thickness (n=3) Errorbars denote for the standard devialtion of the mean. C) Calculated mean association constants (n=3) for different surfaces where errorbars depict the standard deviation of the mean. D) Calculated mean dissocaition constants (n=3) of different surfaces where errorbars depict the standard deviation of the mean

In the future, the here presented method can be extended to adherence analysis of further blood components, such as platelets, erythrocytes and lymphocytes. By testing the adherence of these cellular blood components individually or in combination with other blood components, a more complete picture of the body response to cardio-vascular implant surfaces could be achieved.

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An innovative carbon black modified sensor to detect free chlorine

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[b] *TecnoSens srl, Rome, Italy*
mariarita.tomei@uniroma2.it

Chlorination is the most common treatment for the disinfection of drinking water and swimming pools through sodium hypochlorite, thanks to its characteristics of high disinfection power and cost-effectiveness combined with easiness to use. It is also used in several industrial applications. [1]
In aqueous solution there is an equilibrium between hypochlorite ion and hypochlorous acid, and the sum of these two species is defined as "free chlorine".

The World Health Organization (WHO) established that its concentration in swimming pools should be between 3 and 5 ppm, while Italian legislation fixed a range between 0.7 and 1.5 ppm. [2,3]
Analytical tools are required to control the free chlorine level, and the development of cost-effective and easy to use probes could be really helpful.
Herein, we report a sensitive and cost-effective amperometric sensor to detect of free chlorine in water, using screen-printed electrodes modified with carbon black (CB-SPEs).

Materials and methods

The system comprises a SPE connected to a portable potentiostat (PalmSens Emstat) and a personal computer. The electrochemical properties of SPEs are improved by modifying the working electrode surface with a dispersion of CB N220 (1 mg/mL in a mixture of DMF: water 1:1 v/v) through drop casting method. [4]
The CB is characterised by a large number of edge plane/defect sites that improve the electrochemical properties of the sensor.

Results and discussion

Optimization

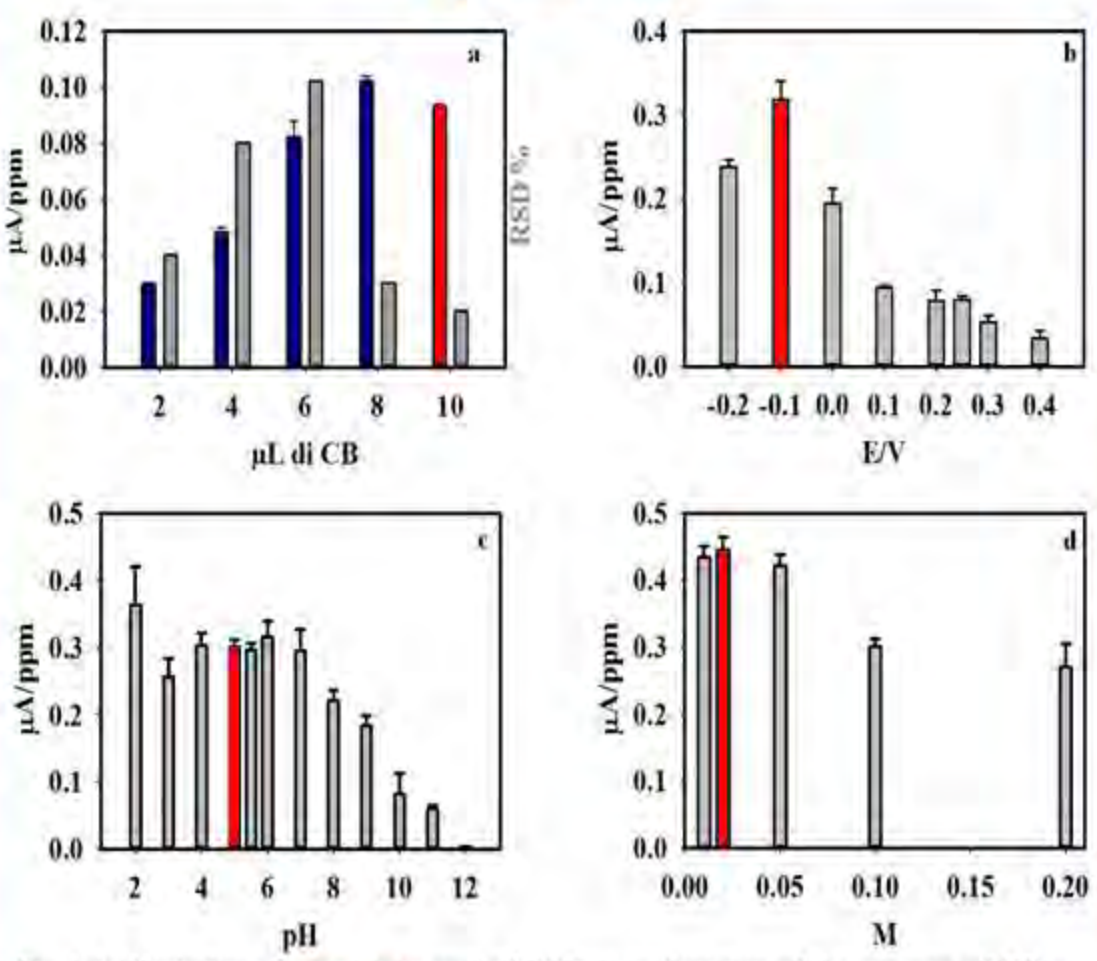

Fig 1:(a)Variation of sensitivity and of RSD % in function of the amount of CB at 0.25 V vs Ag/AgCl in phosphate buffer 0.1 M + KCl 0.1 M and pH 5.5. Variation of sensitivity in function of (b) the applied potential (c) pH (d) the concentration of Britton Robinson buffer solution. ***In red the selected working parameters.***

Analytical features

Linear range: 0.05–200 ppm (R^2=0.995)
Sensitivity: 0.32 ± 0.02 µA/ppm
RSD: 6 %
LOD: 0.01 ppm ($\frac{S}{N} = 3$)
LOQ: 0.03 ppm

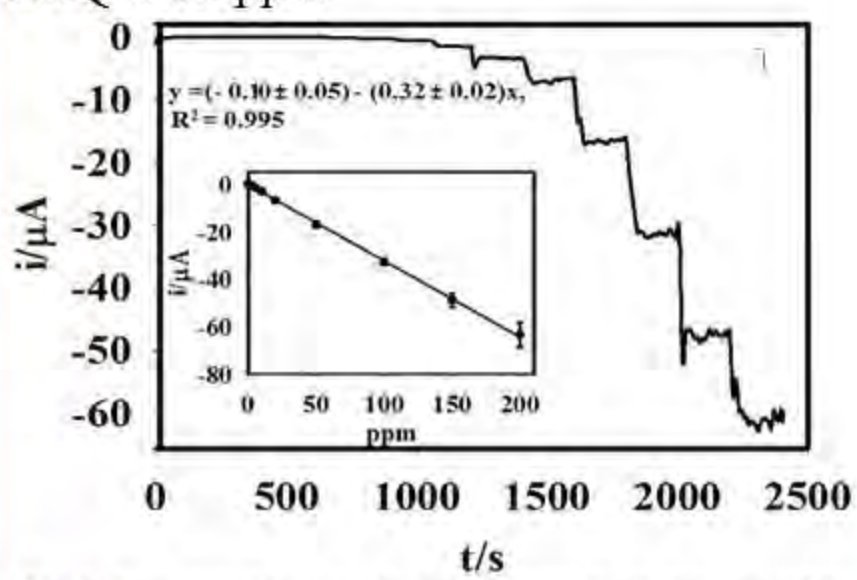

Fig.2: Amperometric curve for inter-electrode studies using CB-SPEs using free chlorine up to 200 ppm (insert the calibration curve) in the selected conditions.

The selectivity and the sensitivity of developed sensor were evaluated testing **trichlorine** as source of free chlorine up to 200 ppm, obtaining a sensitivity of 0.36 ± 0.01 µA/ppm.
The performances of the developed sensor were finally tested in **swimming pool water** (diluted 1:10 (v/v)) reaching a sensitivity of 0.258 ± 0.001 µA/ppm. The accuracy was estimated by recovery method to be **97±10%**.

Conclusions

The developed sensor has showed relevant electrochemical performances with the advantages to be miniaturized and cost-effective, demonstrating the possibility to be employed as sensing tool for free chlorine quantification in water samples.

References:

[1] F. Kodera et al., Anal. Chim. Acta (2005) 293-298; [2] World Health Organization, Geneva, 2006; [3] L. Bonadonna et al., Rapporti ISTISAN 07/11; [4] F. Arduini et al., Electroanal. (2012) 743-751.

POTASSIUM SENSING WITH ION SELECTIVE FIELD-EFFECT TRANSISTOR

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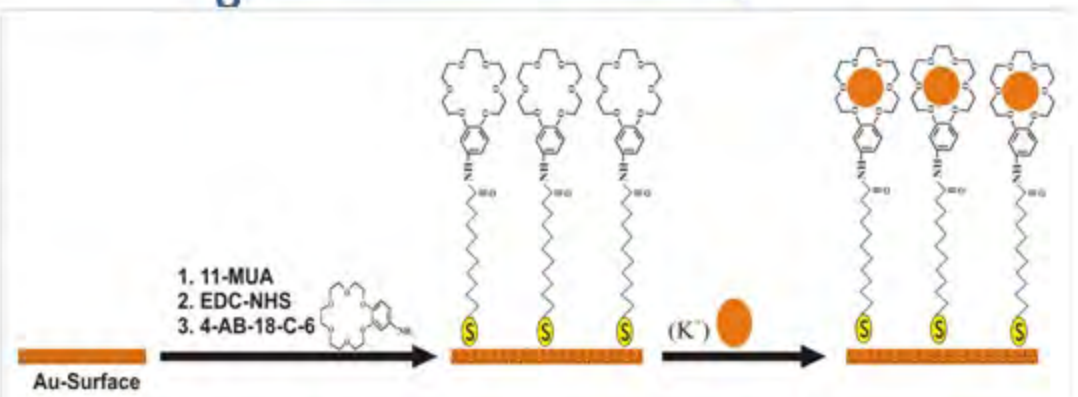
ABSTRACT

Simple voltammetric sensor based on self-assembled 18-crown-6 ether derivative with a positively charged redox probe, hexammineruthenium(III) chloride as ' reporter ion' to transduce the recognition of K^+ ion in solution. Present study demonstrates functioning of electrochemical transducer with nano-thin gold surface functionalized self-assembled monolayer binding of crown ethers based ionophore and K^+ in solution. Technique has been applied to monitor potassium ion in soil samples. The sensor functionalization protocol has potentiality for construction of Ion sensitive field effect transistors suitable for field monitoring of nutrients for soil analysis.

INTRODUCTION

- **Potassium ions in soil** are essential macro elements for growth & development of plants.
- Level of active K^+ in soil (100-6000 ppm): one of the most important issue in precision agriculture.
- Flame Photometry suffers low resolution due to Interference arising from high Na^+ over K^+
- Ion Selective Electrode takes long time to achieve equilibium, hence sensors having real time response are much in demand.
- Self assembled monolayers of crown ether based ionophores represent an attractive method for amperometric sensor fabrication for electrochemically inactive metal ions. We present here electrochemical based Sensor for selective, sensitive and real-time monitoring of potassium in soil samples.

EXPERIMENTAL



Immobilization of 4-aminobenzo-18-crown-6 onto the gold chip as selective ionophore for potassium ion binding

RESULTS AND DISCUSSION

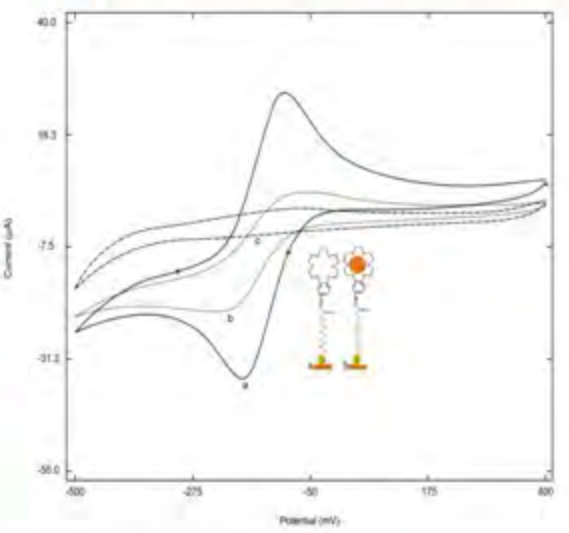
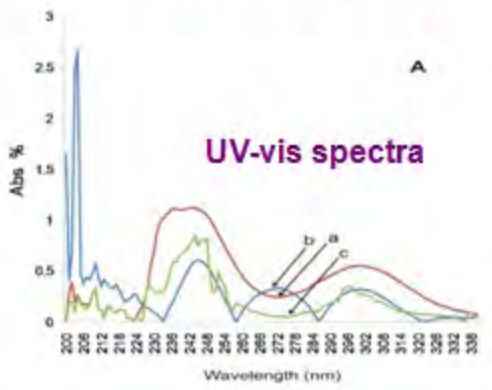


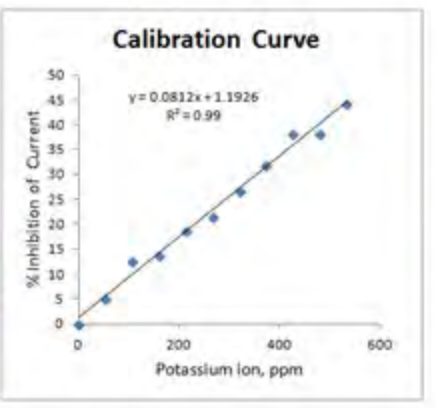
Fig 2
CV of 3mM $Ru(NH_3)_6^{3+}$ in 0.1M TBAB at sensor Au/11-MUA/4-AB-18-C-6

A. Nil Potassium
B. Soil extract
C. Soil extract + 100mM K^+

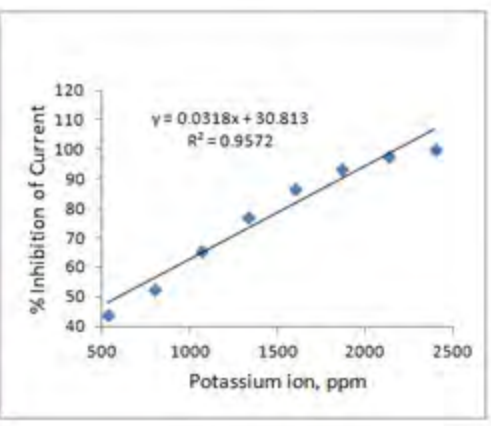
Potassium : 4-aminobenzo-18-Crown-6 Host-Guest Complex

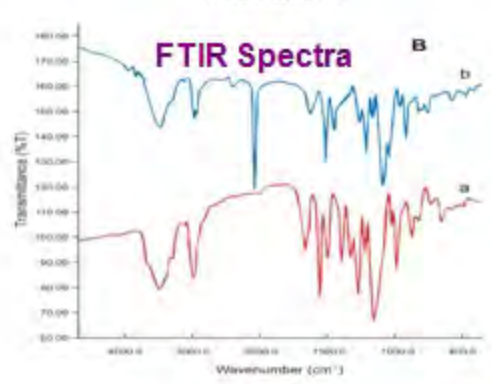


a. 4-AB-18-C-6
b. K -4-AB-18-C-6 Complex
c. Na- 4-AB-18-C-6 complex



Inhibition of DPV current with increasing K ion concentration





EC based Sensors

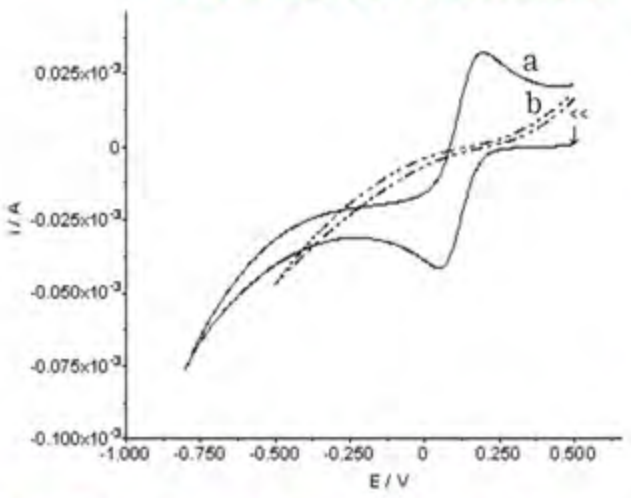


Fig. 1 Ferricyanide/ Ferrocyanide
(a) CV: at bare gold surface
(b) CV: at 11-MUA immobilized gold surface

Functioning of K Sensor: Au/11-MUA/4-AB-18-C-6

- The disappearance of redox signal marks the successful binding of analyte K cation onto the sensor surface. The fact that positevely charged 11-MUA monolayer bearing crown on the electrode surface blocked electron transfer of positevely charged species Ru(NH3)63+ .
- Concentration of reporter ion is a key factor in the working of proposed sensor which can be tuned to optimise the detetion range for K+ ion as per the requirement of sample.
- Differential pulse voltammetric current at −130mV, employing sensor electrode (Au/11-MUA/4-AB-18-C-6) with 5mM Ru(NH3)63+/2+ revealed a gradual decrease in peak current with increasing conc of K ion in the range 50 to 2000 ppm.; with complete disappearance of voltammetric signal at 2500 ppm, covering the concentration range of K in soil.
- The system is promising for real-time selective recognition of potassium over lithium, sodium.

CONCLUSION

Inhibition in voltammetric current confirms the selective and sensitive binding of potassium ion with chosen crown ether based ligands on sensor surface.

International Biosensor Conference: BBMEC-12, Rome, 25-29 Sept 2017

Folat Receptor Targeted Multimodal Engineered Vesicles for Imaging and Therapy: A Magic of Theranostics

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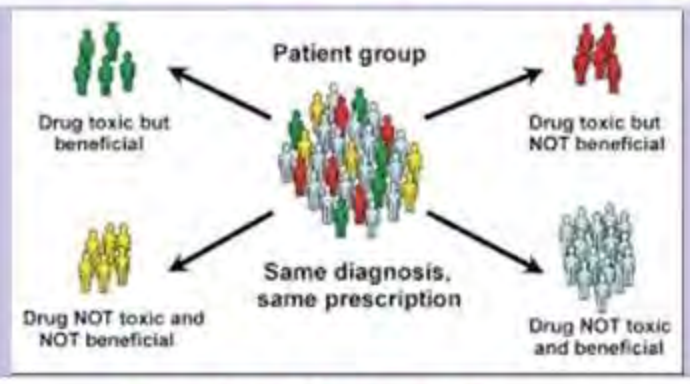
INTRODUCTION

Theranostic is one of the most promising technology in the war of cancer , which simultaneously allows treating and real-time monitoring of cancer [1]. In the present study, a novel material has been developed for theranostic approach. Gold nanoparticles (AuNPs) and Protoporphyrin IX (PpIX) both have encapsulated in niosomes (PpIX-AuNP). Here niosomes have been used for the encapsulation of PpIX-AuNP due to the fact that niosomes possess high biocompatibility, physical and chemical stability.

AuNP and PpIX are good sensitizer for radio therapy (RT) and PpIX is one of the more studied agent for photodynamic therapy (PDT). This developed formulation was also targeted with folic acid (FA) for HeLa (folat receptor +) and A549 (folat receptor -) cell lines.

PROBLEM

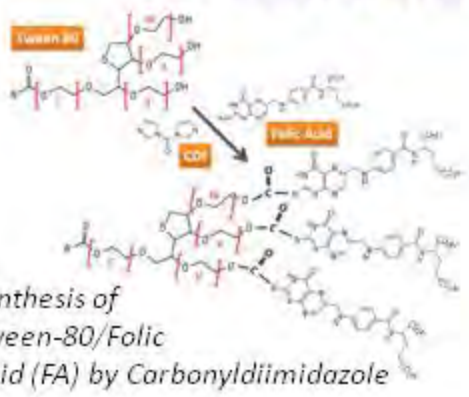


Goal of the Study

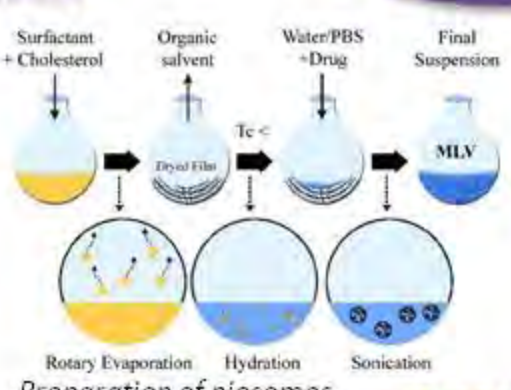
"Generation of novel theranostically engineered nanovesicles to perform effective treatment and monitoring of tumoral cells"

DESIGN

PART-I: SYNTHESIS

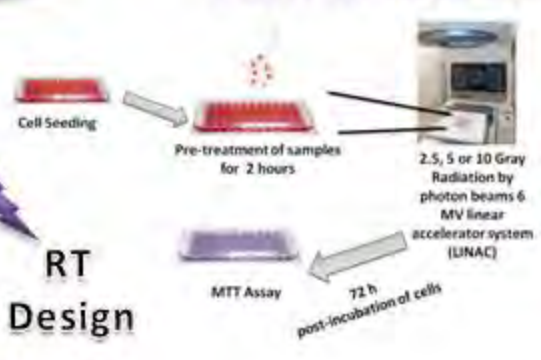


Synthesis of Tween-80/Folic Acid (FA) by Carbonyldiimidazole coupling.



Preparation of niosomes

PART-II: CELL EXPERIMENTS

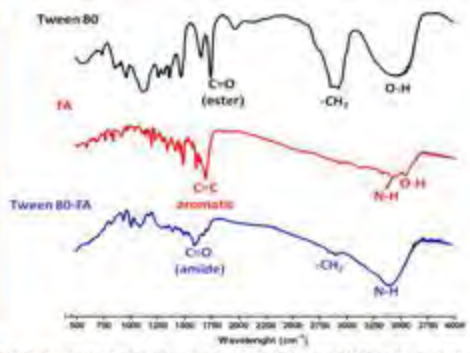


RT Design

PDT Design

RESULTS

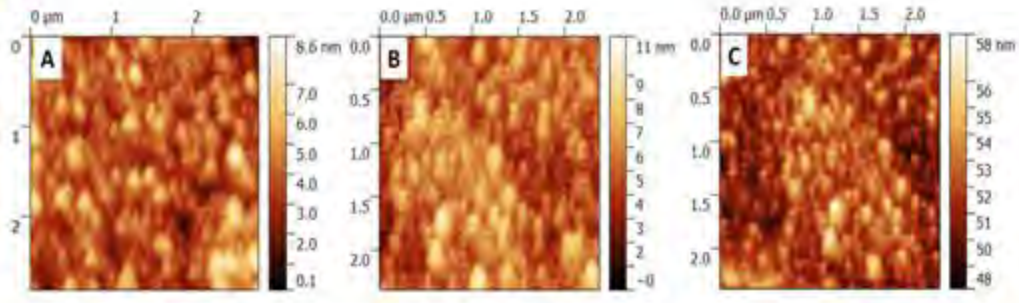
Characterization



FT-IR spectra of Tween 80, FA and Tween 80-FA

	Particle Size (nm)	PDI	Zeta Potential (mV)
AuNP	8±2	0.143	-42±7
PPIX-Au	69±10	0.697	-48.5±8
PPIX-FA	70±13	0.636	-46±8
PPIX-Au-FA	93.6±17	0.546	-50±9

Sizes and surface charges of nanovesicles (n=3)

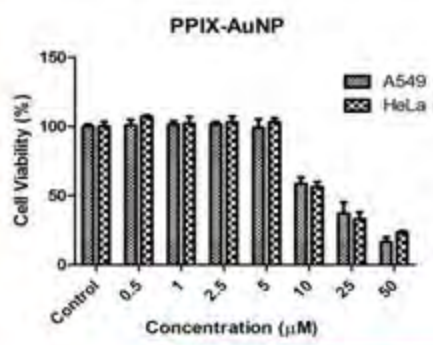


Atomic Force Microscopy histograms of PPIX-Au (A), PPIX-FA (B), PPIX-Au-FA (C)

Encapsulation efficiency (EE) of PpIX (n=3)

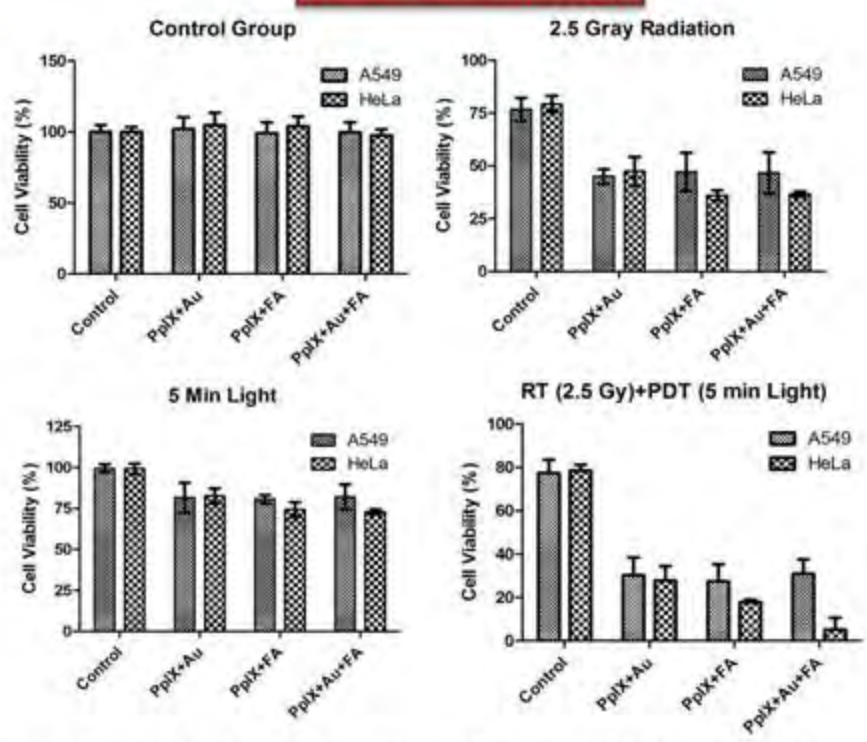
Sample	% EE	% S.S.
PPIX-FA	86.31	1.08
PPIX-Au-FA	86.56	0.96
PPIX-Au	84.15	4.26

Cell Based Tests

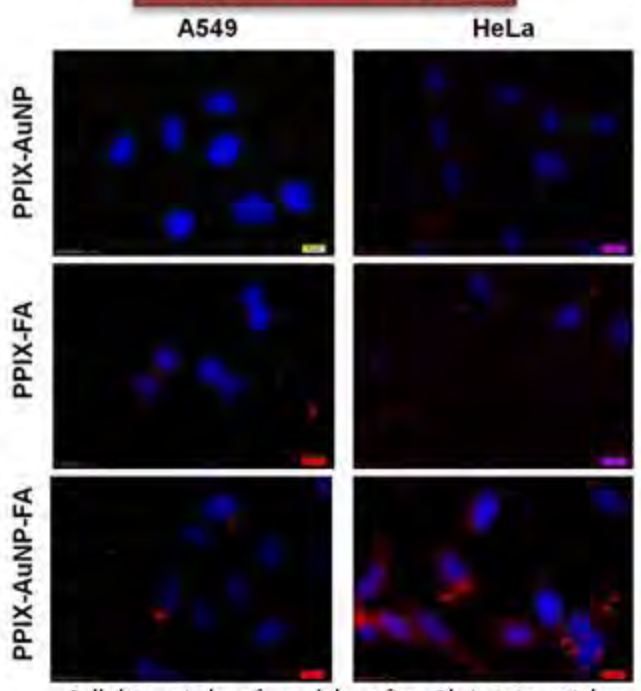


The dose-dependent toxicity of PPIX-AuNP, PPIX-FA and PPIX-AuNP-FA formulated niosomes for HeLa and A549 cells over 24 h. (Concentration is belong to PpIX amounts.

Combined Therapy



Targeted Bioimaging



Cellular uptake of vesicles after 2h treatment by using PPIX fluorescence properties in the RED field. DAPI was used for nuclei staining..

Conclusion

- Novel candidates with high potential for imaging guided therapy applications
- Development of FA-targeted theranostic vesicles with the approach of combined (RT+PDT) therapy modality was successfully established.

*This work is supported by Ege University Research Project (Project No:2017 FEN 007 and 2012 FEN 025)

[1] J. H. Ryu et al., Advanced Drug Delivery Reviews, 2012, 64, 1447–1458.

Agreement between Official control and self-monitoring: data report in an Italian dairy chain

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Background & Aim

Cow milk is a relevant component in the Western Consumer's diet. The nutritional and economic value of raw milk, as well as its physicochemical properties are directly dependent on milk composition, which, in turn, give valuable information on herd nutritional status and general health. Daily measurements of milk components, both at the individual and herd level, is becoming a common tool to assess the safety and economic value of milk production. Following EU Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002, Italian milk processing industries follow a strict self monitoring process per HACCP, and are subject to Official control by the Italian Competent Authority (Ministry of Health, Moh). A network of 10 Experimental Zooprophylaxis Institutes (IZS) provide accredited laboratory services for analyses for raw milk samples.

However, limited scientific evidence is known about measurements agreement between the milk analyses carried on by official control bodies and the internal ones done by milk processing industries. A number of factors could affect this agreement. Different methods in milk component quantifications (different techniques, with different LOD and LOQ, as well as measurement uncertainty) could be a primary source of disagreement. A second difference between the two measurement chains is that, while the internal industrial monitoring routines are carried on immediately after the raw milk arrival at the processing premises, official monitoring procedures are carried on by sampling the milk, and delivering the sample to the accredited laboratory. While this procedure is validated and refrigerated transportation is routinely used, the effect on raw milk samples biological parameters, especially during the hot season, may be still partly understood.

The main aim of the present study is to verify the agreement between Official Control and Self-control measurements in raw milk processing industry, identify any possible discordances or biases, and to possibly point out procedures to overcome these discordances.

Methods

In the framework of the Alert 2015 (http://www.alert2015.it/) project funded by the "Industria 2015" program of the Italian Ministry of Economic Development, a chance was given to collect simultaneous data coming from the official control IZS- LT (Lazio and Tuscany Section) and the industrial Partner Centrale del Latte di Roma Spa (CLR).

Milk production and component measurement data were obtained during a purposely developed monitoring program involving CLR, a FSSC 22000:2010 – certified milk processing plant with an average 350000l/day throughput. The monitoring program started in March 9, 2014 and ended in June 24, 2014. Data collection regarded the daily raw milk collection carried on by CLR on a regional basis, consisting in a daily average 137000 Kg of raw milk collected from local farmers via a fleet of 15-metric tons refrigerated trucks. Traceability of bulk milk was limited to groups of individual farmers. All samples were taken following rules matching both CLR internal procedures and IZS procedures.

The first sample was immediately sent to CLR internal labs for analyses, while the second sample was kept refrigerated and sent to the IZS labs. A total of 114 twin samples were collected and analyzed for 7 twinned variables (Freezing Point, Aflatoxin, Total Bacterial Count, Somatic Cell Count, fat, lactose and proteins percentages). Descriptive statistics, correlation analysis, paired sample Wilcoxon Signed Rank test and a Bland-Altman analysis was performed in order to assess measurement agreement between CLR and IZS determinations. A General Linear model was used to investigate the effect of CLR Total Bacterial count, time between sampling and IZS analysis, truck tank temperature and farmer's group on the difference between CLR and IZS Total Bacterial Count.

No outlier removal procedure was used. For all statistical analyses, the significance threshold was set at .05.

Results

A Shapiro Wilk normality test performed on each variable in the data set showed that almost all variables could not be modeled by a normal distribution, except for CLR Lactose, CLR Proteins, and IZS Proteins. A Wilcoxon signed ranks test on the twinned variables assessed that Freezing Point, Aflatoxin, Total Bacterial Count, Somatic Cell Count, Lactose and Fat data showed a significant difference in the median of the paired samples.

Correlation and Bland-Altman analysis concluded for a sound agreement between CLR and IZS Proteins measurement. A moderate to good agreement was found for Somatic Cell Count, Fat, Lactose and, to a lesser extent, for Freezing Point determinations. Low agreement was found between CLR and IZS Total Bacterial Count determinations. The General Linear Model highlighted that the difference between CLR and IZS Total Bacterial Count was not influenced by time between sampling and analysis and truck tank temperature. A significant influence on the difference was CLR Total Bacterial Count. Farmer's group (i.e. the group of farmers whose bulk milk was collected together in the truck tank) had a lower contribution on the difference between CLR and IZS Total Bacterial Count.

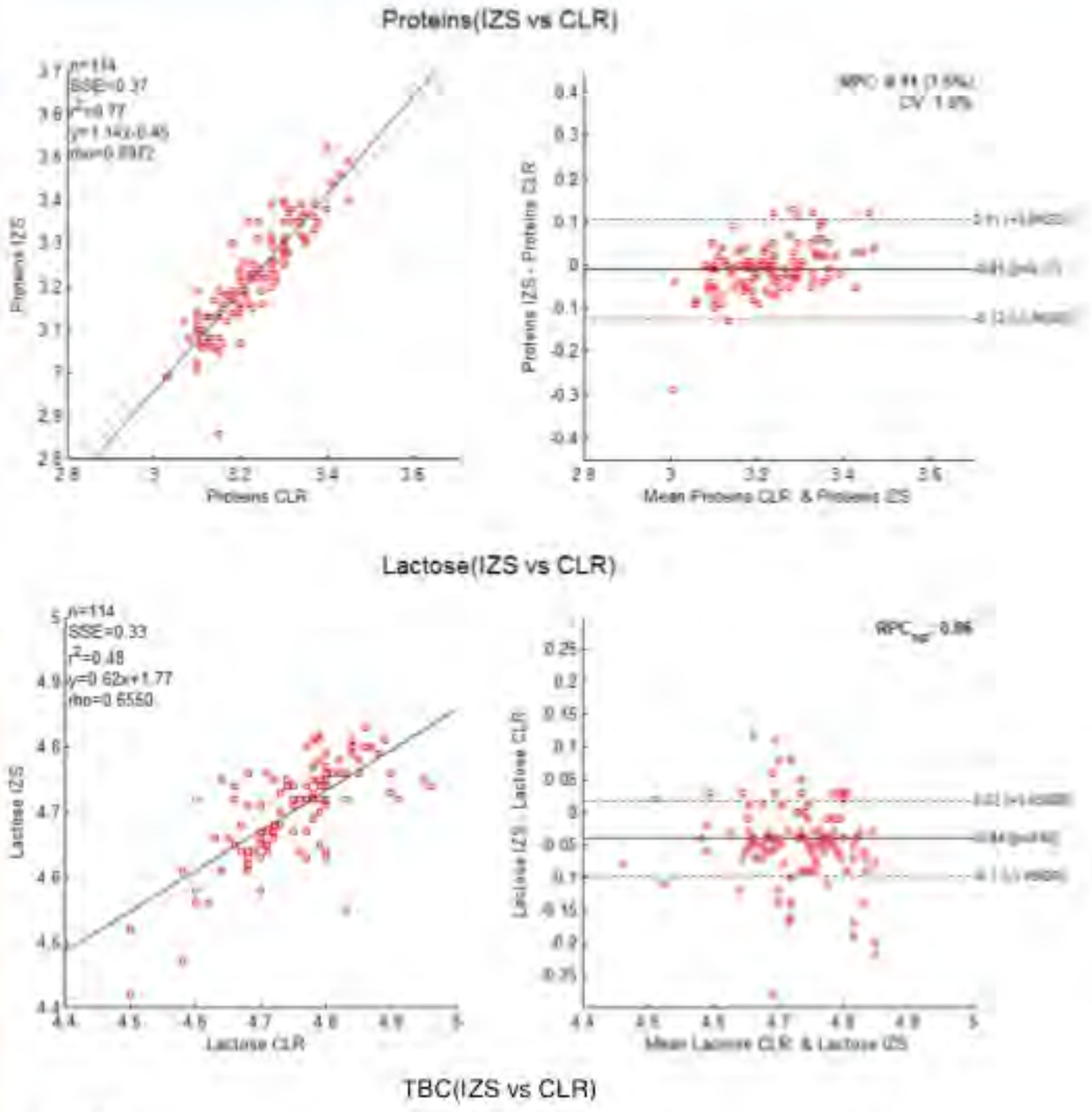

Figure 1 A,B,C: examples showing good, moderate and bad agreement betwheen Official Control and Self Control measuremts

Conclusions

This study confirmed a good/ moderate to good agreement between Official Control and Self-control measurements in raw milk processing, at least for Proteins, Somatic Cell Count, Fat, Lactose, and for Freezing Point determinations. A remarkable discordance was found for Total Bacterial Count, which, in line of principle, could not be attributed to differences in the analytical methodologies. Truck tank temperature, and the number of hours between sampling and IZS analysis did not affect this discordance. In turn, the absolute value of CLR Total Bacterial Count did affect the difference, as well as the milk origin (traced up to groups of farmers). Further research is needed to identify how these factors can influence the observed discordance.

Sandwich NP-based biobarcode assay for C-reactive protein quantification in plasma samples

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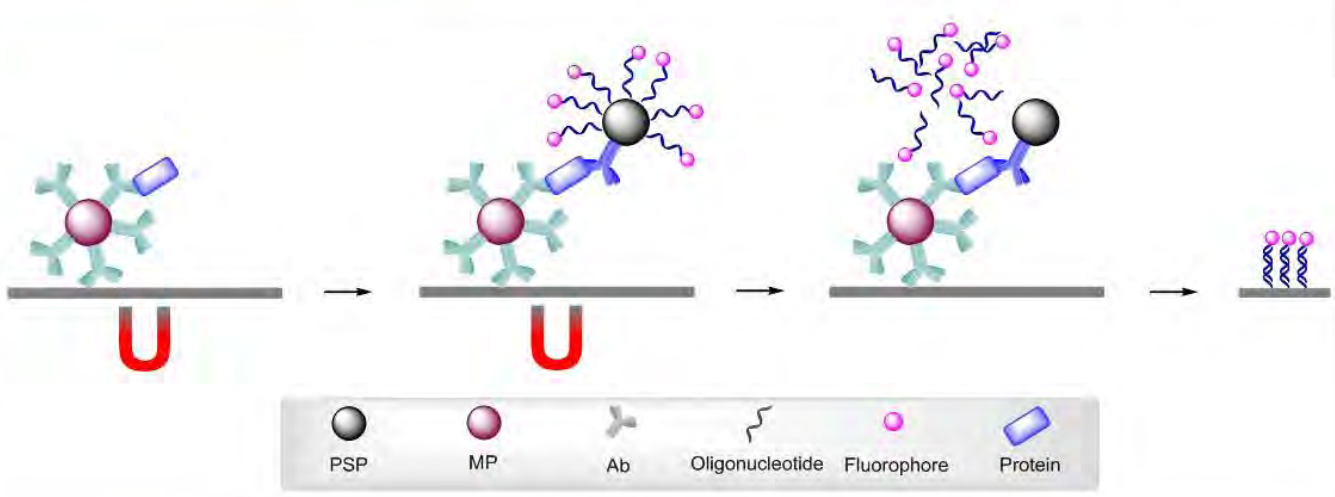
[1]Nanobiotechnology for Diagnostics (Nb4D). IQAC-CSIC, www.iqac.csic.es/nb4d. [2]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN). C/ Jordi Girona, 18-26, 08034 Barcelona. *e-mail: roger.galve@iqac.csic.es

Introduction

C-reactive protein (CRP) is an annular homopentameric protein that participates in the systemic response to inflammation. Between other diseases, high levels of CRP in blood may be related to an inflammation of the arteries of the heart, indicating risk to suffer a heart attack. Because of the high prevalence of cardiovascular diseases (CVDs) in developed countries, routine measurement of CRP levels is being contemplated as a strategy to reduce deaths caused by these diseases.

Assay design

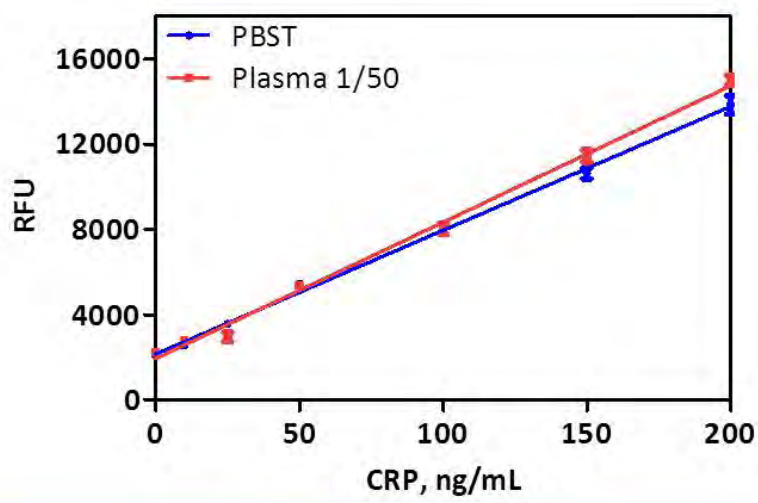
Magnetic beads are added to the sample to form immunocomplexes that will be isolated. Then, the codified particles are added to form a sandwich complex with the CRP. Subsequently, the complexes are treated with dithiothreitol to release the oligonucleotide codes, which are finally hybridized in a fluorescent DNA microarray.



Matrix effect

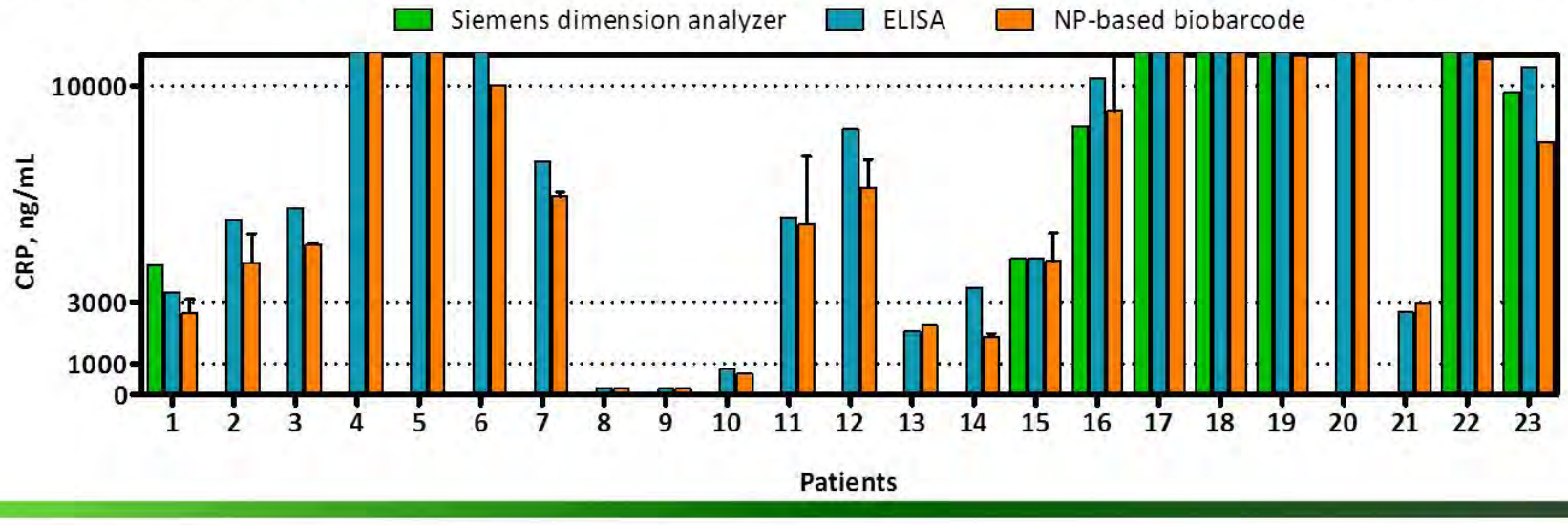
In order to fit clinical requirements we had to dilute plasma samples 50 times.

	Buffer	Plasma 1/50
Slope	63.2 ± 4.49	64.0
Y-intercept	2393 ± 221	1937
R^2	0.957 ± 0.03	0.978
LOL, ng mL^{-1}	250.0	-
LOQ, ng mL^{-1}	18.0	-
LOD, ng mL^{-1}	11.0	-



Clinical samples

Clinical samples were analyzed and compared with ELISA and Siemens dimension analyzer (a reference method).



Conclusions

The sandwich NP-based biobarcode assay developed for CRP shows a detectability (11.0 ng/mL) which is sufficient for directly measuring this biomarker in complex samples such as plasma.

Literature: M. Broto, Analytica Chimica Acta 2017

Acknowledgements

This work has been funded by the ministry of Economy and Competiniveness (MAT2012-38573-C02-01)

High throughput immunoassay for the therapeutic drug monitoring of tegafur

Marta Broto [1,2], Rita McCabe [1,2], Roger Galve [1,2,*] and M.-Pilar Marco [1,2]

[1]Nanobiotechnology for Diagnostics (Nb4D). IQAC-CSIC, www.iqac.csic.es/nb4d. [2]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN). C/ Jordi Girona, 18-26, 08034 Barcelona. *e-mail: roger.galve@iqac.csic.es

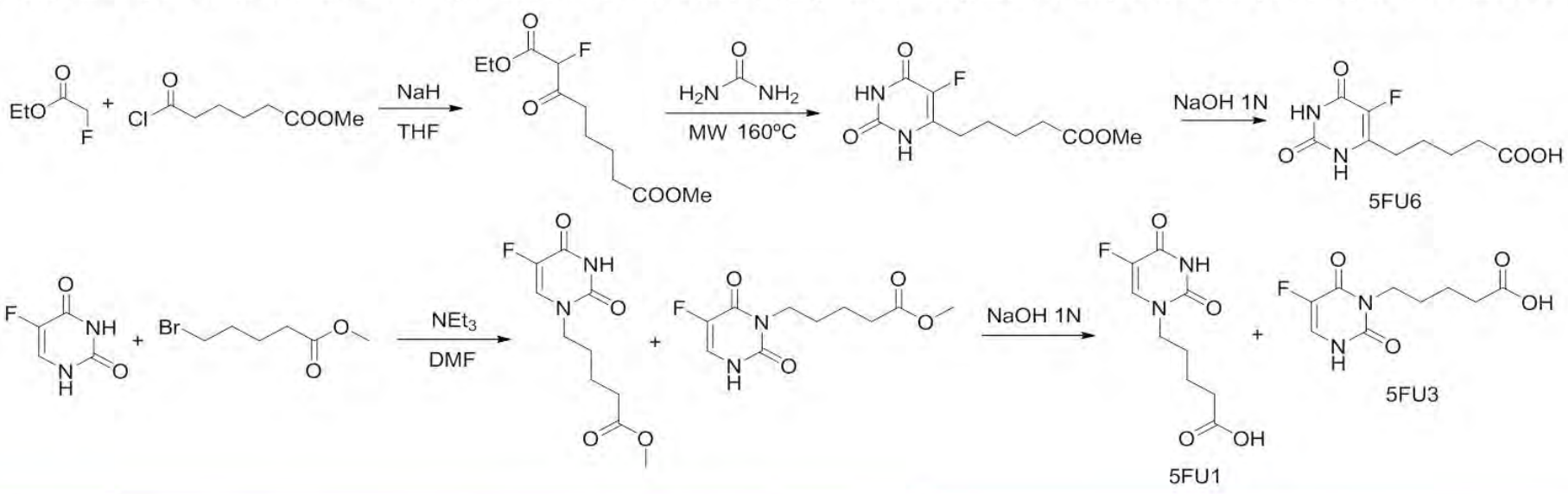
Introduction

Chemotherapy is one of the cancer treatments that uses chemical agents. These drugs are toxic and produce undesirable adverse reactions. They need to be monitored in order to stablish a personalized treatment. Therapeutic drug monitoring (TDM) has the potential to improve the clinical benefit of chemotherapy drugs due to their narrow therapeutic window and highly variable pharmacokinetics.

Tegafur, prodrug of 5-fluorouracil (5FU), is one of the main anti-cancer drugs used worldwide. Herein, a reproducible and sensitive indirect competitive ELISA has been developed and validated in plasma samples.

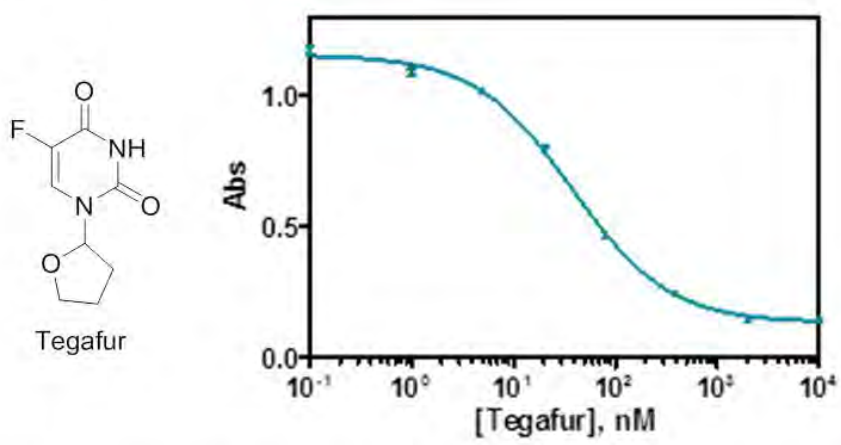
Synthesis of the haptens

Schematic representation of the synthesis of the immunogen (5FU1) and competitor haptens (5FU3 and 5FU6).



Immunoassay features

The assay shows an IC_{50} of 35 nM, achieving a LOD of 2.7 nM, much lower than the concentrations found in blood samples of treated patients and lower than HPLC and LC-MS/MS reported methods.



Parameters of the assay (As337/5FU6-CONA)

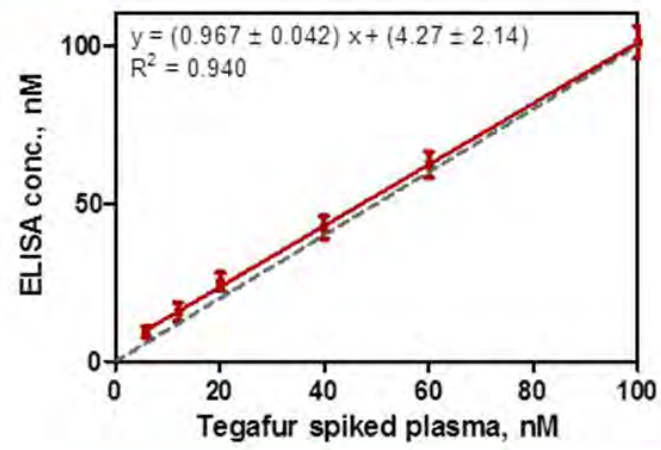
As dilution	1/7000	$Signal_{min}$	0.134 ± 0.010
[Competitor]	0.25 µg mL^{-1}	$Signal_{max}$	1.157 ± 0.010
Competition time	10'	Slope (m)	-0.915 ± 0.043
Agitation	600 rpm	R^2	0.992 ± 0.001
pH	7.5	IC_{50}, nM	35.6 ± 2.6
Ionic strength	15.0 mS/cm	DR[a], nM	from 7.5 ± 1.3 to 157.5 ± 5.1
Tween 20	0.05 %	LOD, nM	2.7 ± 0.7

Specificity of the assay

	IC50 (nM)	% CR
FT, Tegafur	36	100
5FU, 5-Fluorouracil	9900	0.37
5BrU, 5-Bromouracil	13310	0.27
2MeFU, 5-Fluoro-1,3-dimethyluracil	> 10000	<0,05
T, Thymine	> 10000	<0,05
Urd, Uridine	> 10000	<0,05
5FUrd, 5-Fluoro-2'-deoxyuridine	5867	0.62
5BrUrd, 5-Bromo-2'-deoxyuridine	7934	0.46

[a]Cross-reactivity is expressed as a percentatge of the relation between the IC50 (nM) of tegafur and the IC50 (nM) of the other compounds tested.

Serum Evaluation. Recovery studies



Conclusions

The ELISA method, which needs no sample treatment, can be applied for TDM of tegafur since it is well suited for high throughput analysis, reporting a remarkably low detectability. The assay presents an IC_{50} of 35.6 and a LOD of 2.7 nM.

Literature: Analyst, 2017, **142**, 2404 - 2410

Acknowledgements

This work has been funded by CETEMMSA Technological Center (part of the EURECAT Technology Center of Catalonia)

Development of LED-color-switching type biosensor for the visualization of fish stress response

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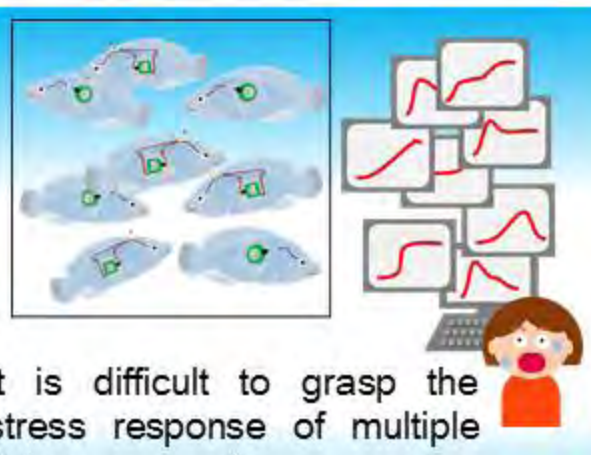
[a]Tokyo University of Marine Science and Technology, JAPAN
[b]Hokkaido Industrial Technology Center, Hokkaido, JAPAN

科研費 KAKENHI

Introduction

It is known that fish were reduced their immunity when they feel stress..

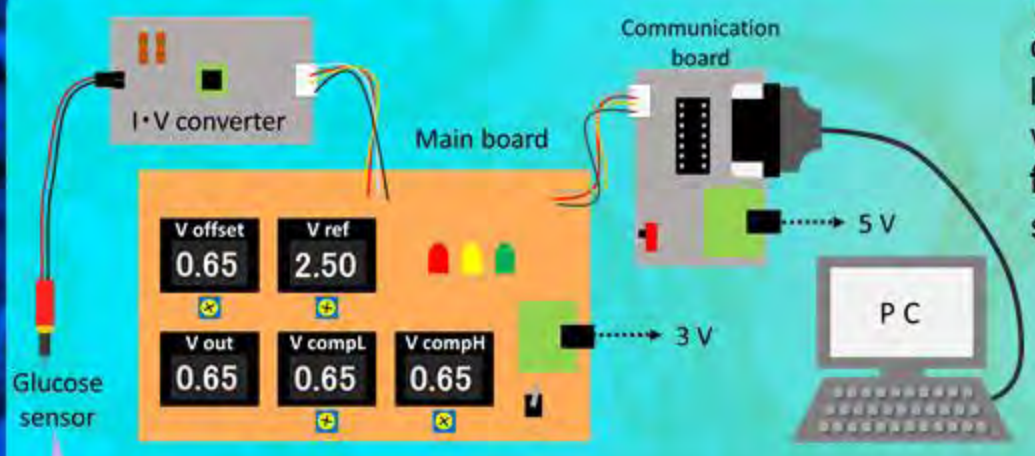
In our laboratory, we measured the glucose concentration as a stress index of fish using wireless biosensor for carrying out physical examination.



It is difficult to grasp the stress response of multiple fish at once due to we can not analyze the massive data from all the fish at same time.

We aim to establish a system that makes it easier to grasp the whole stress response of fish at same time by distinguishing the degree of stress by the lighting different LED.

Methods



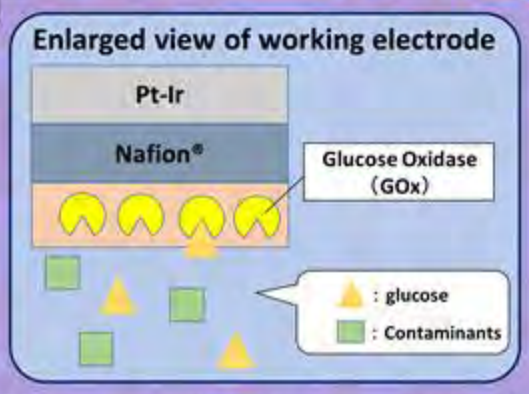
The present system is based on the principle of changing the output voltage value of the glucose biosensor to decide the lighting color of the LED on the main board. When VcompL · H were set and the output voltage value (Vout) changes as follows, the lighting color of the LED on the main board switches to green, yellow or red.

Vout ≦ VcompL	→ Green
VcompL ≦ Vout ≦ VcompH	→ Yellow
VcompH ≦ Vout	→ Red

Glucose biosensor

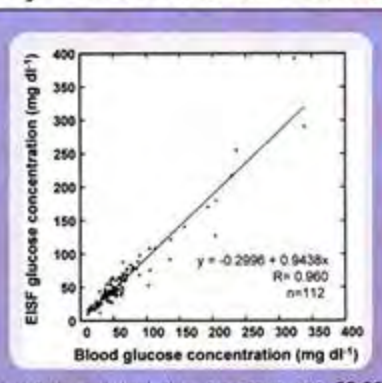
$C_6H_{12}O_6 + O_2 \xrightarrow{GOx} C_6H_{10}O_6 + H_2O_2$

$H_2O_2 \xrightarrow{+650\ mV} 2H^+ + O_2 + 2e^-$

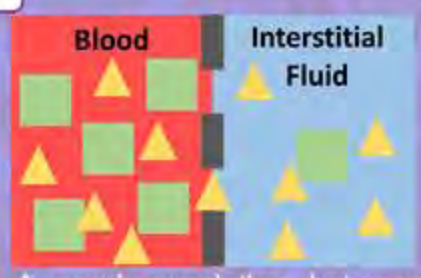


We use oxidation reaction of glucose to measure glucose.

EISF (Fish Eye Interstitial Fluid)

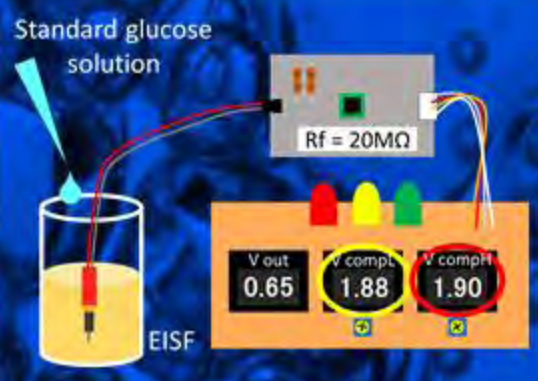


(Y. Yonemori, H. Endo et al. *Anal. Chim. Acta.* 2009 ; **633** : 90-96)



A good correlation between glucose concentration in EISF and blood glucose level has been confirmed.

Results 1: In vitro color switching experiment



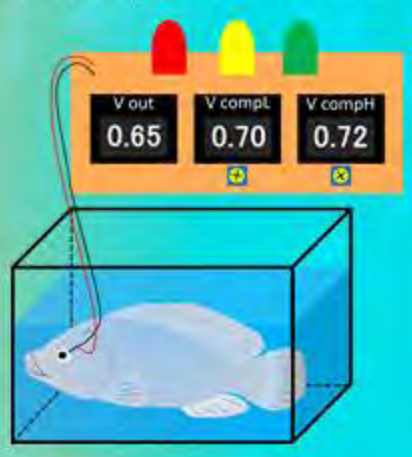
Glucose concentration in EISF was adjusted to 100, 200 mg dl^{-1}.

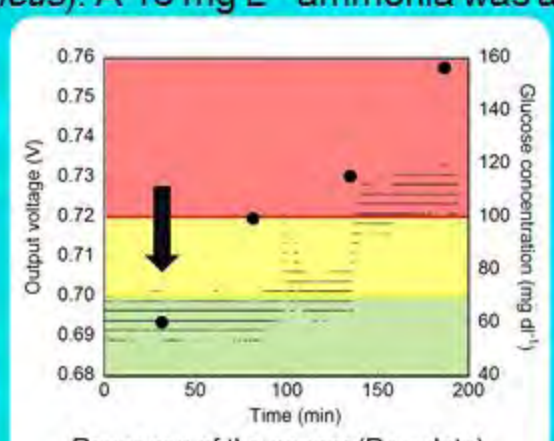
Glucose concentration (mg dL^{-1})	Output Current (nA)	Output voltage (V)	Display voltage (V)	Vcomp (V)	Color
54.07	61.71	1.8842	1.87	-	Green
70	62.07	1.8915	1.87	-	Green
100	62.93	1.9086	1.89	1.88	Yellow
200	63.79	1.9257	1.91	1.90	Red

As the glucose concentration in EISF increased, the output voltage of the sensor increased and the lighting color switched according to the setting value.

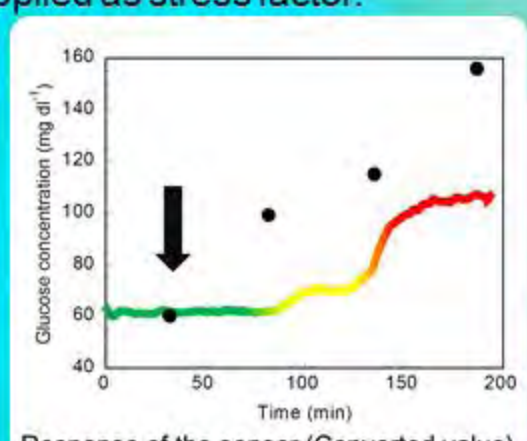
Results 2: In vivo color switching experiment

VcompL · H was set similarly to the in vitro experiment. The biosensor was inserted into EISF of tilapia (*Oreochromis niloticus*). A 15 mg L^{-1} ammonia was applied as stress factor.





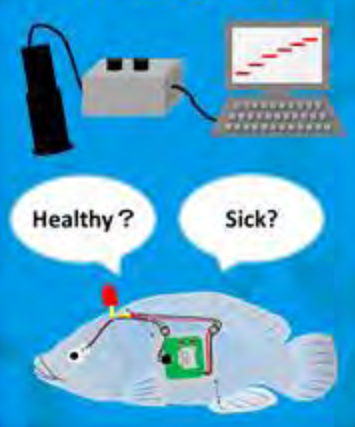
Response of the sensor (Raw data)



Response of the sensor (Converted value)

Prospect

- Miniaturization of the system



*Corresponding author : endo@kaiyodai.ac.jp

Coupling of yeast based biosensors and thin layer chromatography for the detection of endocrine disrupting compounds

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[1]Hebrew University, Institute of LifeSciences, Department of Plant and Environmental Sciences, Jerusalem 91904, Israel
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Background

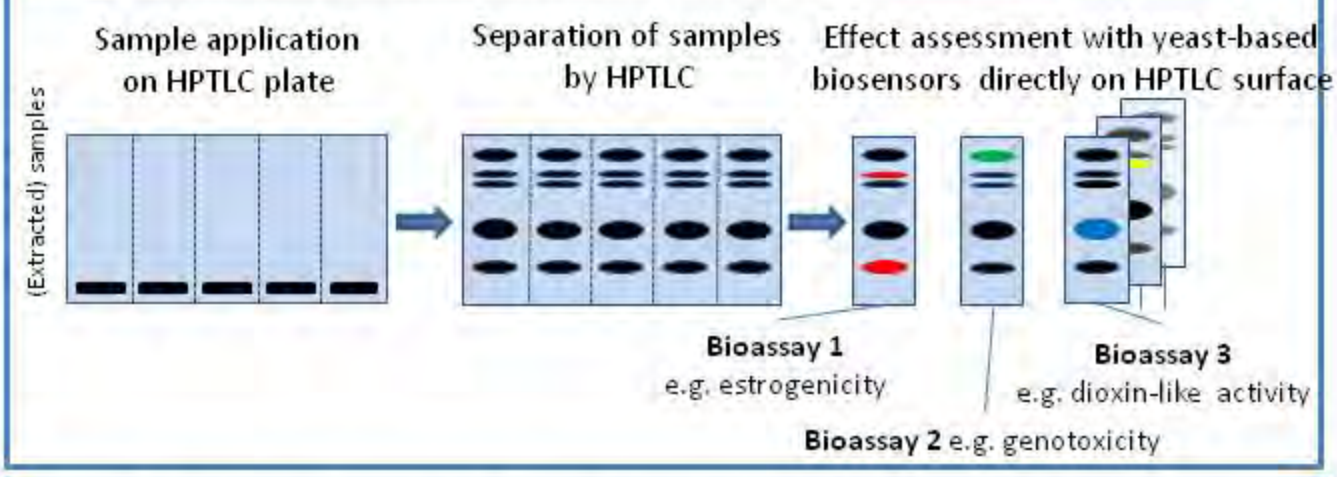
The presence of Endocrine-Disrupting Chemicals (EDC's) in wastewater, surface water, ground water and even drinking water is a major concern worldwide, as it affects human health by disrupting normal endocrine function. Several assay formats using genetically modified yeast sensor strains are available for the specific effect detection of EDC's. However, a direct link between effect and the effect-causing compound is missing because bioassays detect the overall effect of all compounds present in a sample.

To meet the need for a fast and robust method to link the presence of compounds to their respective biological effects, we propose a direct combination of thin-layer chromatography (TLC) and yeast based biosensors designed for the multi-parallel detection of a range of diverse EDC's such as estrogen- and androgen-receptor agonists.

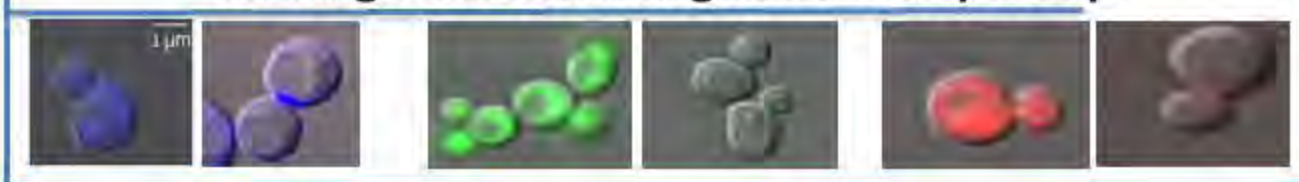
Objectives

- Design and construction of novel EDC-responsive yeast biosensors
- Combination of biosensors with high-performance TLC
- Development of strategies for a multi-parallel detection of adverse effects
- Validation of the developed test system with reference compounds and real samples

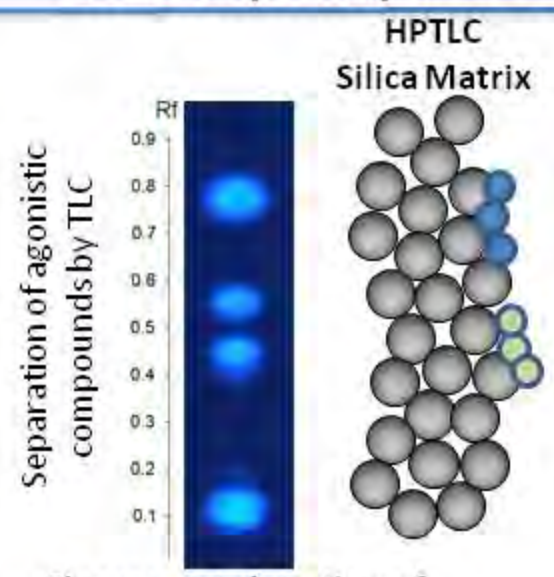
Proposed combination of analytical techniques

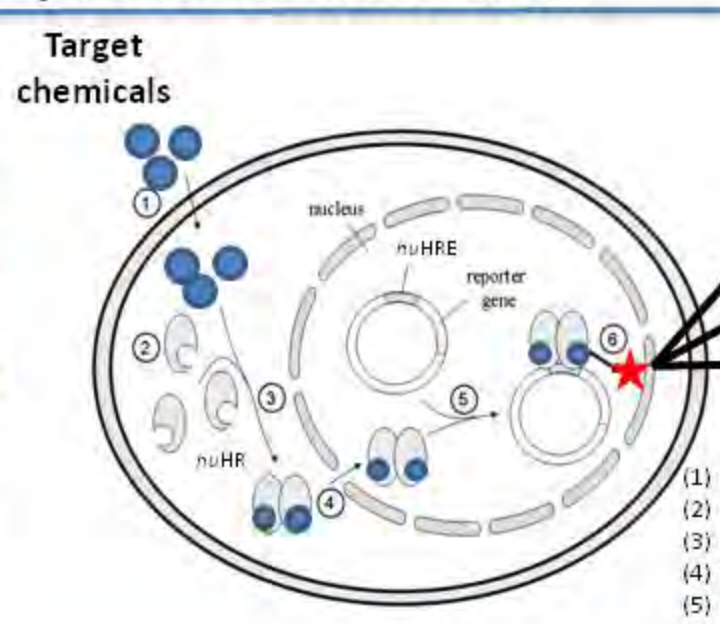


Yeast-optimized fluorescent proteins controls for androgenic and estrogenic activity assays



General principle of TLC / biosensor combination

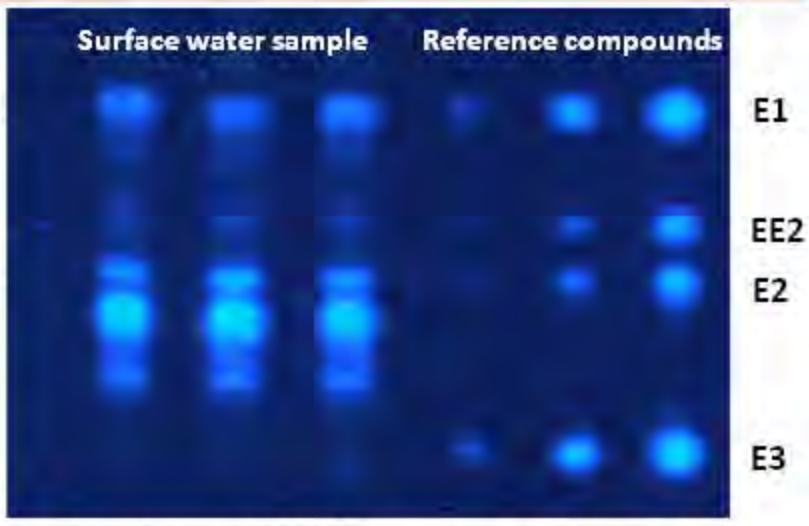




Multiparallel detection using different reporters

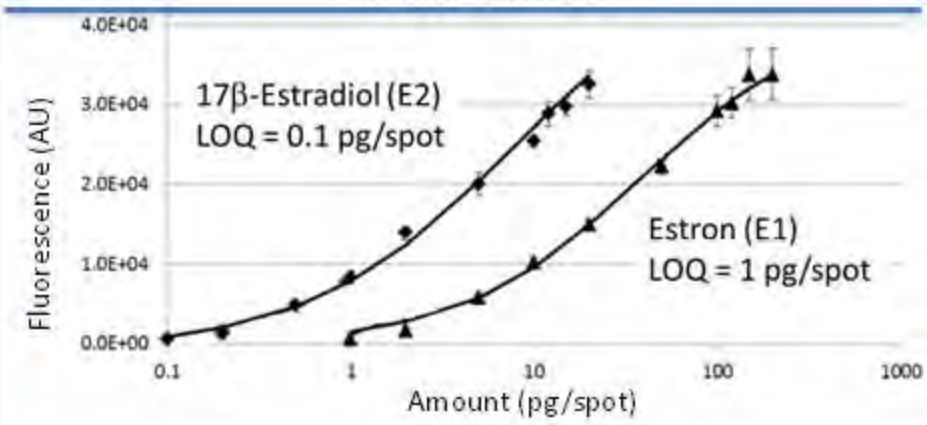
(1) Receptor agonists enter cell
(2) Human hormone receptor
(3) Binding of agonist to receptor
(4) Translocation of complex to nucleus
(5) Binding to human hormone response element
(6) Expression of reporter protein

Estrogenic effects detected in a water sample using YES strain



Surface water samples were 2000-fold concentrated by solid phase extraction (SPE). Estrone (**E1**), 17 β-estradiol (**E2**), 17 α-Ethinylestradiol (**EE2**) and estriol (**E3**) served as reference compounds.

Very low detection thresholds of ER-agonists are obtained



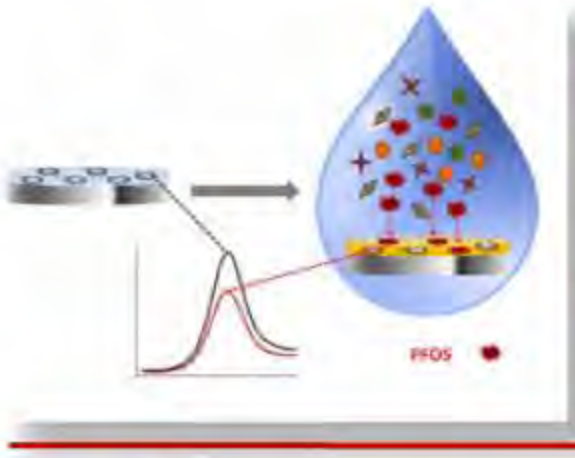
We gratefully acknowledge funding by the Cooperation in Water Technology Program supported by the German Federal Ministry of Education and Research (BMBF) and the State of Israel Ministry of Science, Culture and Sport (MOST).

Literature: [1] Buchinger et al. Anal Chem 85(15): 7248-56;
[2] Schönborn & Grimmer JPC - Journal of Planar Chromatography - Modern TLC 26(5):402-408

Contact: Liat Moscovici, **Hebrew University, Institute of Life Sciences, Department of Plant and Environmental Sciences, Jerusalem 91904, Israel.** moscoviciliat@gmail.com

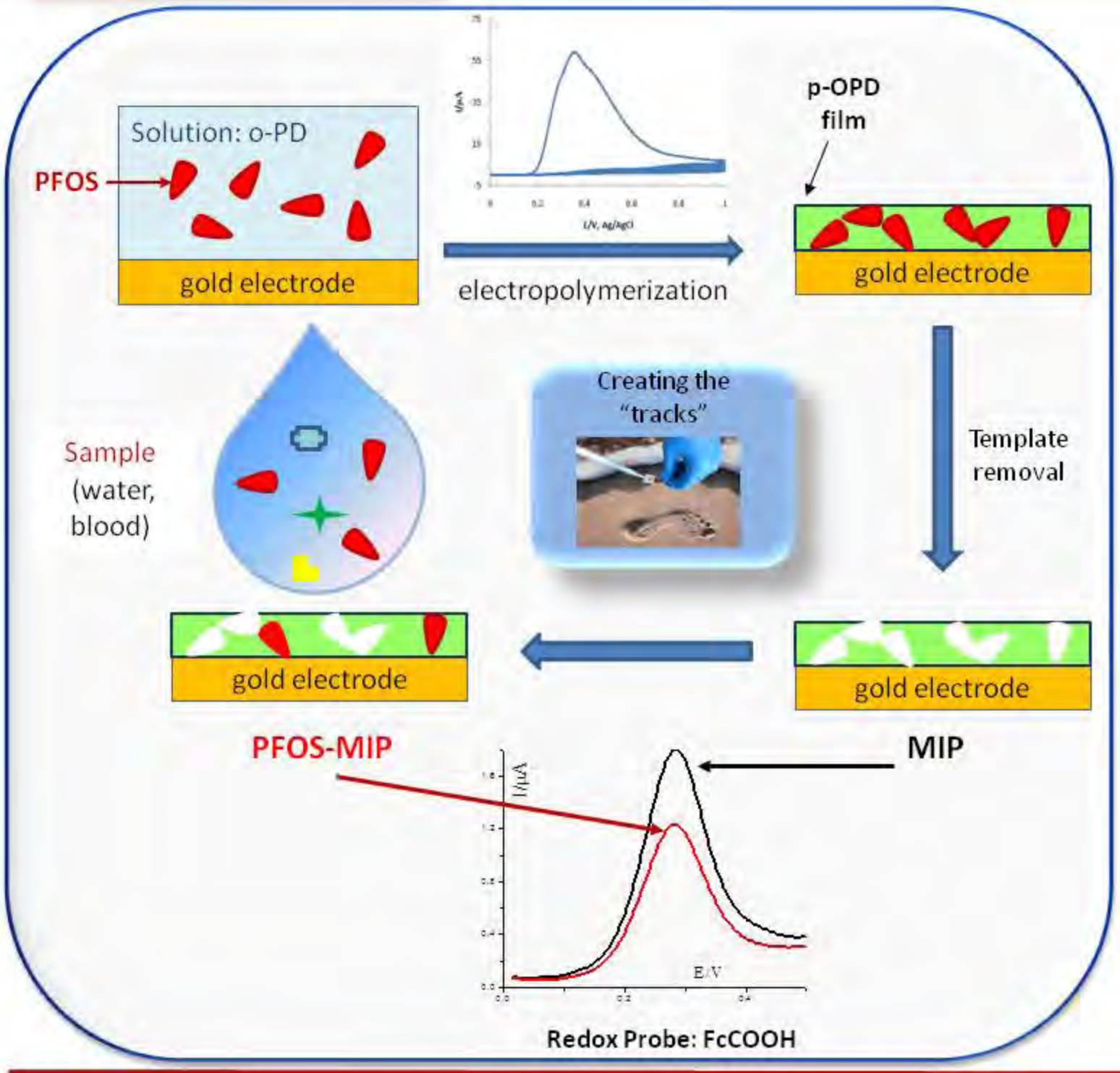
Electrochemical Sensor Based on Ultrathin Nanostructured Coating for Analysis in Biological and Environmental Samples

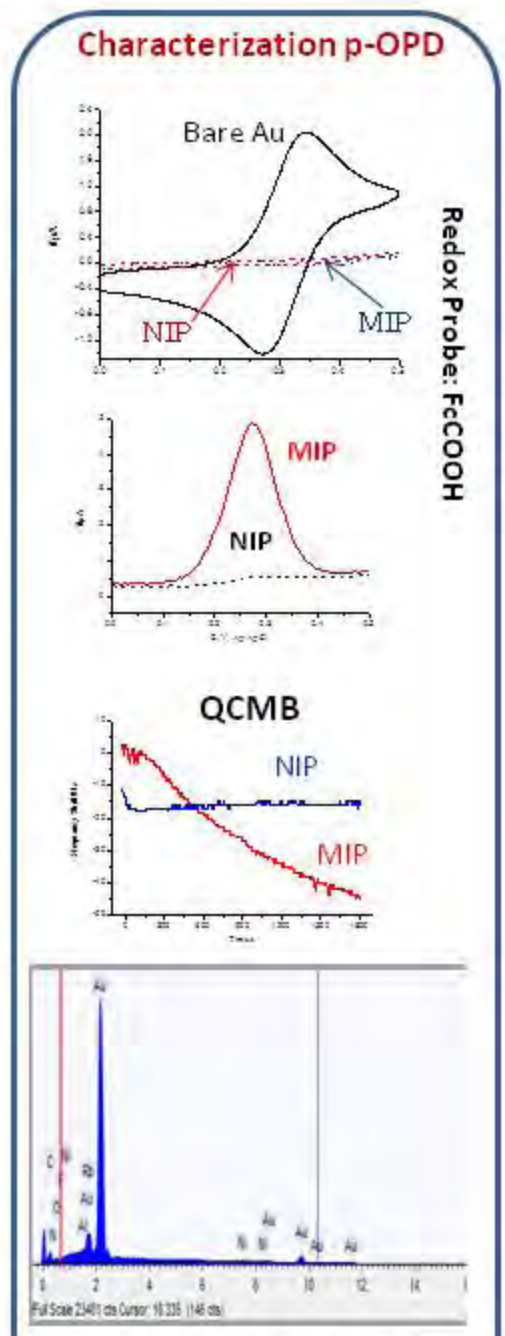
Ligia Maria Moretto, Najmeh Karimian, Angela Maria Stortini, Paolo Ugo
Department of Molecular Sciences and Nanosystems, University Ca'Foscari of Venice, Italy
Contact: moretto@unive.it



Problem: to monitor in a simple and economic way the concentration of perfluorinated compounds (PFCs) in environmental and biological fluids.

Proposal: electrochemica l sensor for PFOS (perfluooctane sulfonate) based on molecularly imprinted polymer (MIPs).





Selectivity to:	Concentration (nM)	Change in current response (%)
Perfluorohexane sulfonate (PFHxS)	2.0	+2.8
Perfluorohexanoic acid (PFHxA)	4.0	+0.93
4-Dodecyl benzene sulfonic acid (DBSA)	4.0	+5.6
Perfluorooctanoic acid (PFOA)	10.0	-6.5

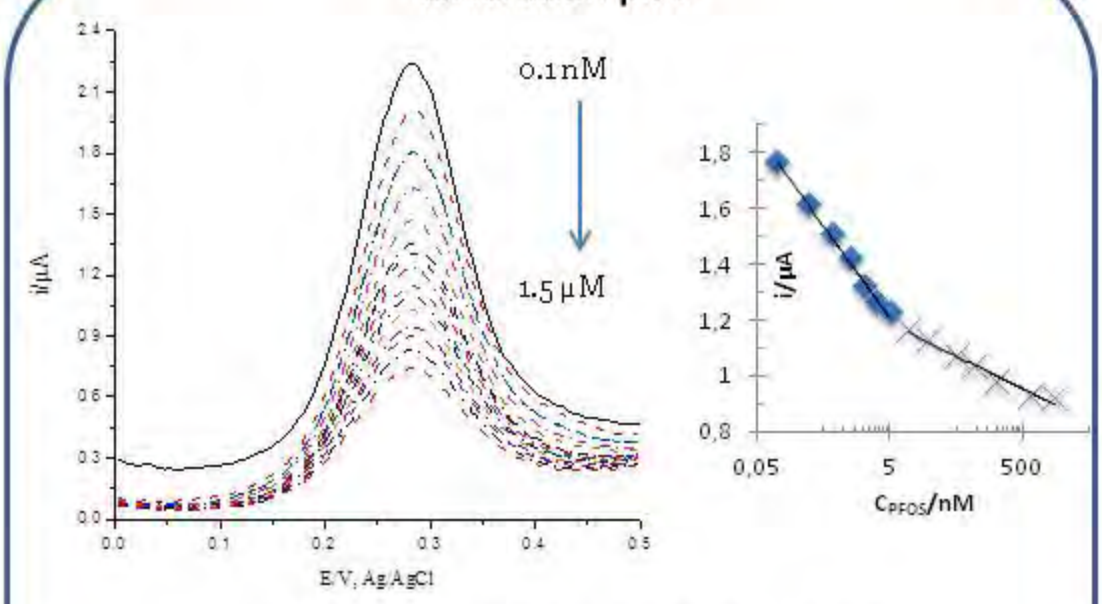
CONCLUSIONS

Electrochemical molecular imprinting is an appealing approach for the development of PFOS sensors.

The sensor is characterized by:

Low cost
Wide linear range
Low DL
High selectivity
Satisfying stability

Calibration plot



DPVs of MIP-modified electrode after 15 min of incubation in different PFOS concentrations.

More details in the "sequel"...

References
1. Ugo, p., Karimian, N., Stortini, A.M., Moretto, L., Patent No. 102017000026417 (03/2017).
2. Miralles-Marco, A., Harrad, S., Environ. Inte.77 (2015) 148–159.
3. Haupt, K., Mosbach, K., TIBTECH 16 (1998) 468.
4. Karimian, N., Turner, A.P.F., Tiwari, A., Biosens Bioelectron 59 (2014) 160.

Multiplexed analytical platforms based on the use of antibodies for monitoring pollutants in marine environment samples

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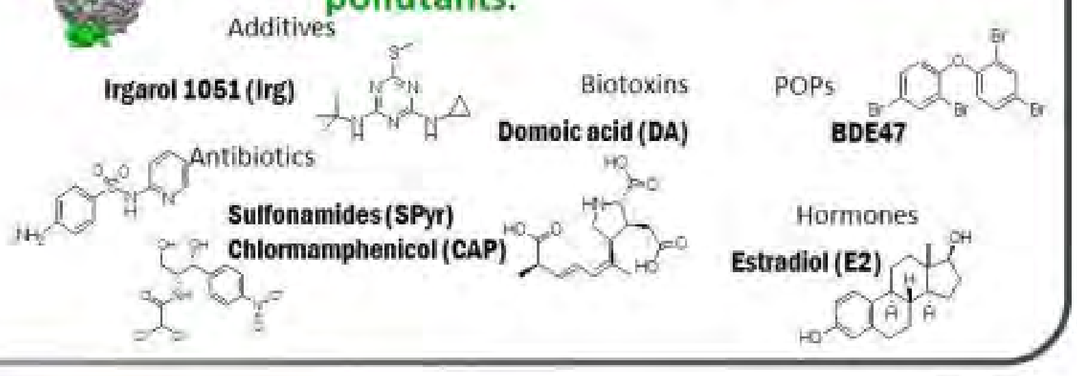
Introduction

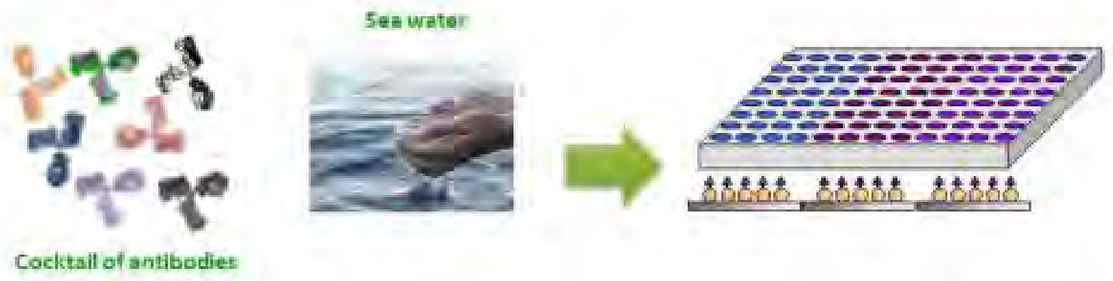
The Marine Strategy Framework Directive (MSFD)[1] and the Water Framework Directive (WFD) support the development of new technologies for monitoring pollution and management of good practices in environmental vigilance and food safety with special interest in **sea water**.

Immunochemical techniques are based on the use of **antibodies** as a biorecognition element for the sensitive and specific recognition of the targeted pollutant. These kind of techniques can be use as alternative or complementary tools in the detection of **environmental pollutants.**



ELISA

A site encoded ELISA platform was proposed for the multiplexed determination of six pollutants at the same time, directly in sea water. Six different pairs of inmunoreagents were used.



Analyte	Irgarol1051	Sulfapyridine	Chloramphenicol	BDE47	β-Estradiol	Domoic acid
Group	Additive	Antibiotic	Antibiotic	Persistent Organic pollutant	Hormone	Algal toxin
IC50 (µg/L)	0.11 ± 0.03	1.84 ± 0.39	0.32 ± 0.09	11.86 ± 3.05	1.02 ± 0.02	3.02 ± 0.33
LOD (µg/L)	0.015 ± 0.007	0.12 ± 0.07	0.02±0.011	0.59±0.08	0.10±0.01	0.24 ± 0.02

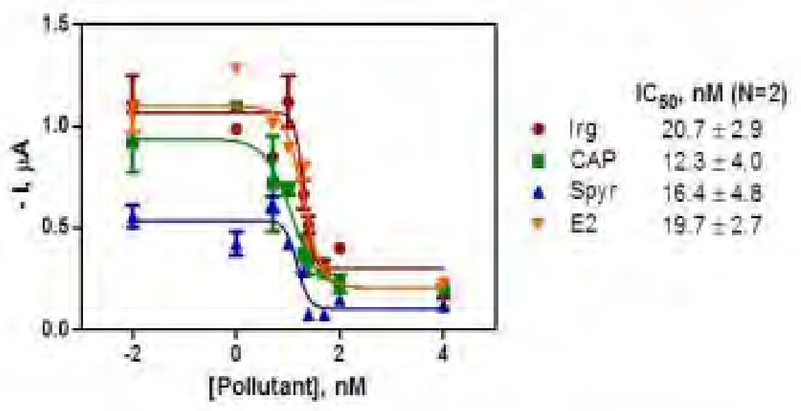
Antibody microarray

A **fluorescent antibody microarray** has been developed for the simultanous determination of six different organic pollutants, measured directly in sea water.

Analyte	Irgarol1051	Sulfapyridine	Chloramphenicol	BDE47	β-Estradiol	Domoic acid
Group	Additive	Antibiotic	Antibiotic	Persistent organic pollutant	Hormone	Algal toxin
IC50 (µg/L)	0.77 ± 0.26	2.77 ± 0.40	1.18 ± 0.15	19.85 ± 2.17	2.95 ± 0.47	10.08 ± 0.30
LOD (µg/L)	0.19 ± 0.06	0.17 ± 0.07	0.11 ± 0.04	2.71 ± 1.13	0.94 ± 0.29	1.71 ± 0.30

Amperometric immunosensor

Multiplexed **amperometric immunosensor** has been developed for the simultaneous determination of four environmental pollutants directly in sea water. The system was design to set-up the immunosensor protocol in a **flow mode**[2].



SEA-on a chip project

All these work was performed in the framework of an European project "*Sea on a chip project, FP7-OCEAN 614168*". Thanks to the whole consortium, it was possible to developed a buoy capable to monitor simultaneously, autonomously and remotely seven environmental pollutant in sea water during 30 days. **FOLLOW THE LINK FOR FURTHER INFORMATION!!!!**

Conclusions

Different analytical platforms has been developed of the simultaneous determination of relevant environmental pollutants based on the use of antibodies as a biorecognition element. Those systems are highly specific, sensitive, with high detectability and robust that allow the measurement directly in sea water. All platforms can be used as a monitoring tool for the monitoring of these representative pollutants in aquaculture.

CONTACT

Literature

1 Shephard, S., et al., *Marine Strategy Framework Directive*. ICES Journal of Marine Science: Journal du Conseil, 2015. **72**(8): p. 2269-2277

2 Salvador JP, Marco MP. 2016. *Amperometric Biosensor for Continuous Monitoring Irgarol 1051 in Sea Water*. Electroanalysis 28:1833-8

Acknowledgements

SEA-on-a-chip project (FP7-OCEAN-2013-614168)

Carbon nanotube modified screen printed electrodes: pyranose oxidase immobilization platform for amperometric enzyme sensors

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BBMEC 12th Workshop on Biosensor & Nanobiotech Microtechniques in Environmental, Food & Clinical Analysis
INTERNATIONAL BIOSENSOR CONFERENCE
25 - 29 September 2017 | Rome, Italy

Introduction

Screen-printing technique has emerging area because of enabling to simple, rapid and inexpensive biosensor preparation in large scale production. Biosensors, which were prepared using screen-printed electrodes (SPEs), have been extensively used for detections of biomolecules, phenolic compounds, pesticides, antigens and anions. Modification of SPEs with various nanomaterials such as graphene oxide, carbon nanotubes (CNTs), palladium nanoparticles, fullerenes etc. to improve analytical performance of electrochemical sensors has been reported nowadays. Carbon nanotubes (CNTs) are members of the carbon-based nanomaterials offering unique mechanical, electronic and chemical stability properties. When CNTs have been used as an electrode, they have a characteristic to mediate electron-transfer reactions with electroactive species. Because of some advantages such as low cost, versatility, and miniaturization in screen-printing technology, the disposable amperometric biosensors based on SPEs has increasing potential. The alteration of SPEs with CNTs has enabled the production of sensitive and stabile sensors [1].

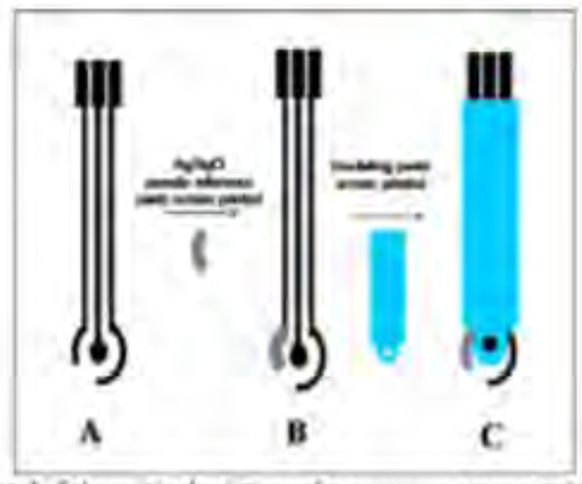
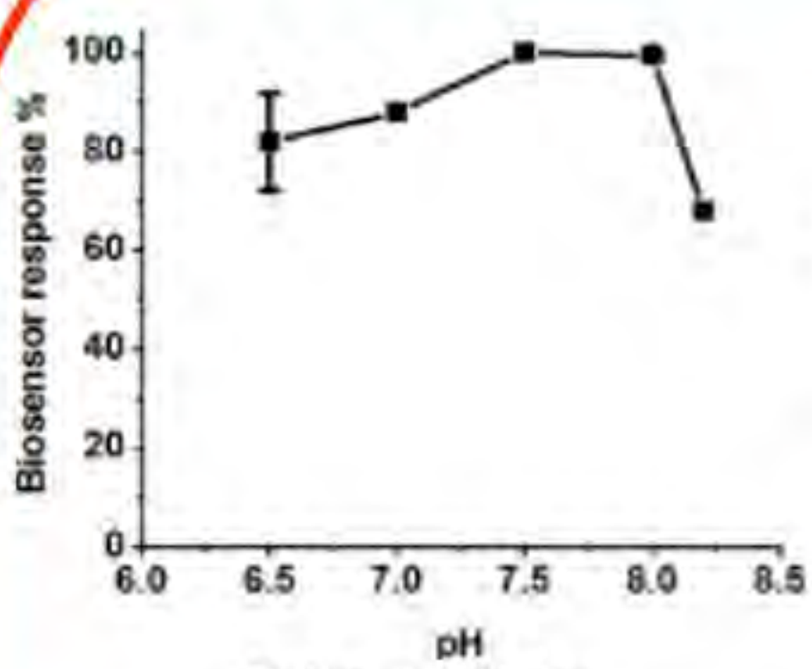
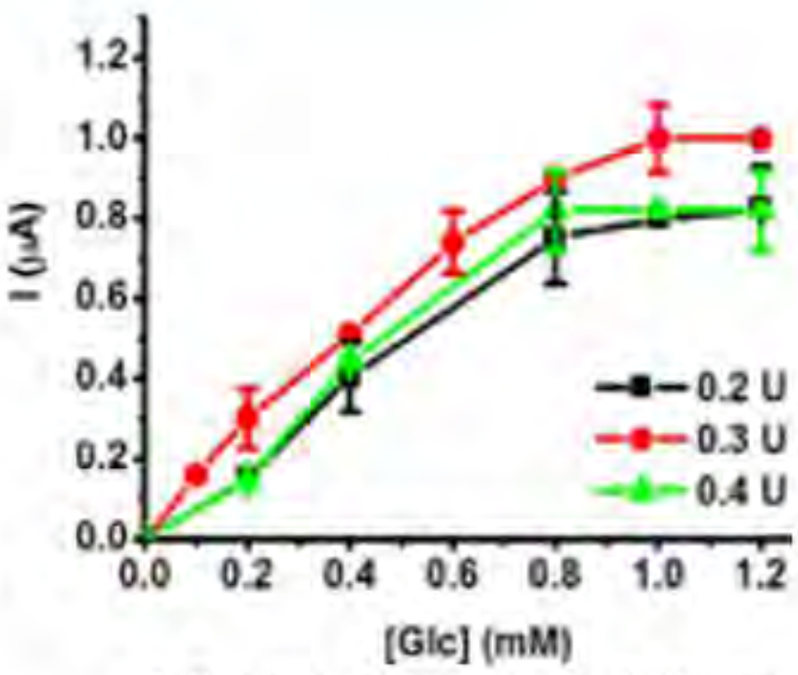


Figure 1. Schematic depiction of a common screen printing procedure using home made three electrodes configuration

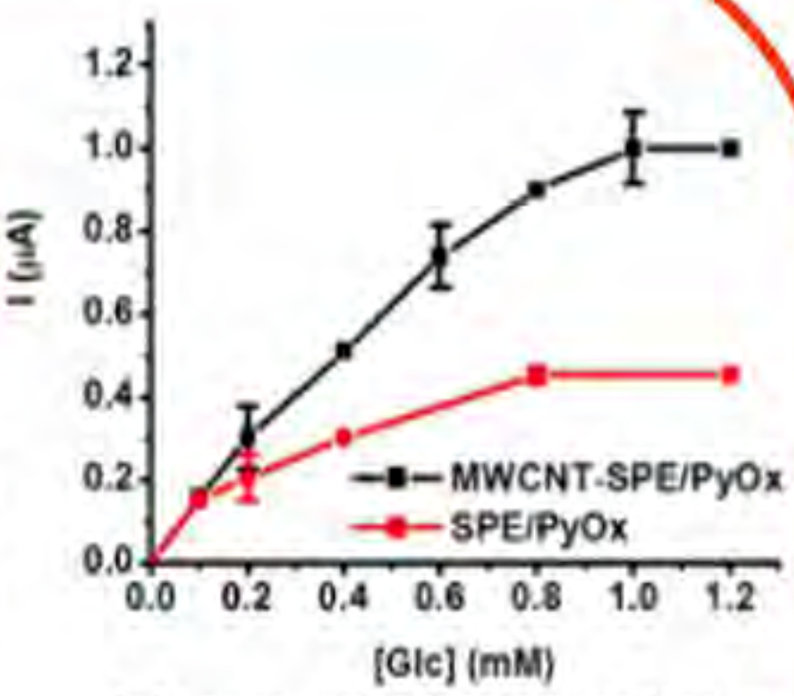
Results



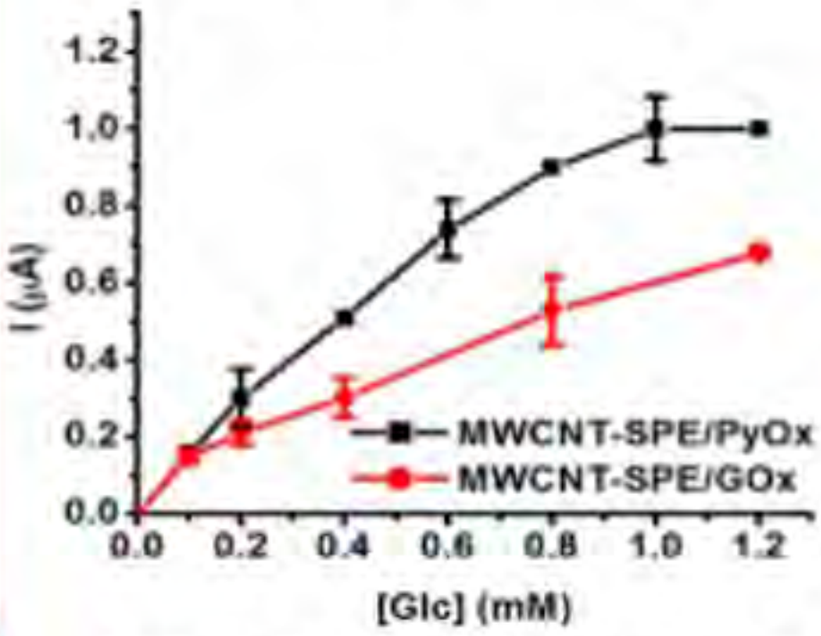
Optimum pH for MWCNT-SPE/PyOx biosensor



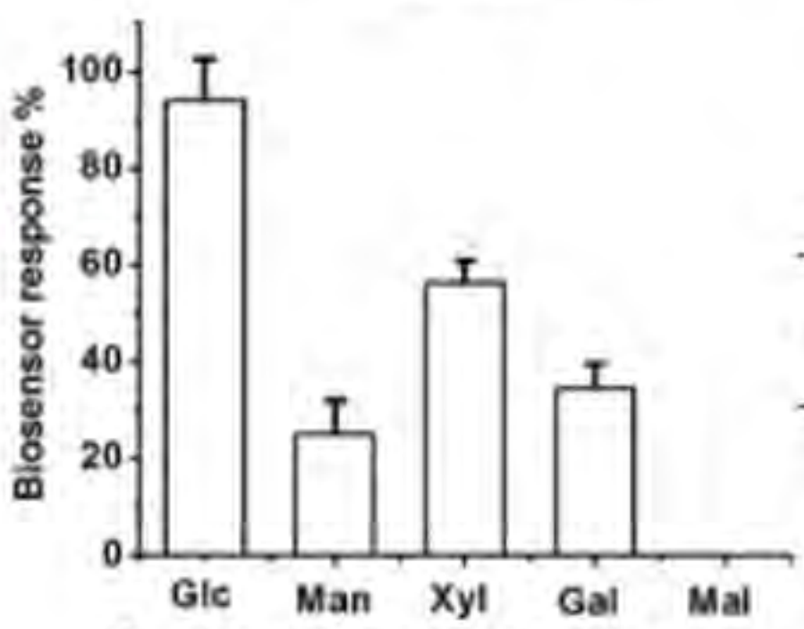
Influence of enzyme activity on the MWCNT-SPE/PyOx response



Influence of carbon nanotubes on the MWCNT-SPE/PyOx response (in working solution; PB; 50 mM, pH7.5, -0.7 V)



Comparison of MWCNT-SPE/PyOx biosensor against to MWCNT-SPE/GOx



Carbohydrate analysis using MWCNT-SPE/PyOx

Data for Glc analysis in samples

Sample	Glc* (g/L)		Recovery %
	MWCNT-SPE/PyOx	Spectrophotometric method	
Fruit juice	12.05±1.04	12.41±1.39	97

Conclusion

In this study, PyOx was immobilized on the surface of the carbon nanotube-modified SPEs by means of gelatin. After optimizing the pH and enzyme loading, MWCNT-SPE/PyOx biosensor was calibrated for glucose detection. The addition of MWCNT to the carbon paste used for printing electrodes resulted in increased sensitivity with respect to electrodes without CNT. Higher sensitivity was also verified for PyOx with respect to GOx immobilized on the same nanostructured electrode surfaces. The use of PyOx instead of GOx for obtaining a novel glucose biosensor resulted in a larger range of substrates allowed to be detected too. For these reasons the MWCNT-SPE/PyOx can be fruitfully used in enzymatic biofuel cells as an alternative to GOx because many other sugars than glucose can be oxidised by PyOx (e.g., lignocellulose hydrolysate). In this paper preliminary determination of sugars in soft drinks was tested obtaining good performance and recovery respect to a reference method.

References
1. D.O.Demirkol, C. Ozdemir, R. Pilloton, S. Timur, Suleyman Demirel University J. of Natural and Applied Sciences, 2017, 21, 286-291.

Acknowledgments
Dr. D.O. Demirkol acknowledges The Turkish Academy of Sciences-Outstanding Young Scientists Award Program-2015 (TUBA-GEBIP). C. Ozdemir thanks Turkish Scientific Technical Research Consultation (TUBITAK) for financial support under program of "2209 National and International Supports for University Students»

Aminothiophenol modified montmorillonite as an immobilization matrix for enzymes in biosensor preparation

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INTRODUCTION

The main component of amperometric biosensors is electrot that modifed with enzyms. Immobilization materials and various techniques have been used in biosensor systems. İmmobilization of enzyms in matrix useful for fabrication of biosensor techniques. Especially clay materials obtain advatages to constitute different surface matrixs for enzym immobilization with their high adsorbant capacity, stabilty, wide surface area and low cost(1,5,6). The flavoenzyme Pyranose Oxidase(PyOx) catalyzes C2/C3 oxidation of various mono and disaccharides, bring forth hydrogen peroxide, particularly D- glucose and FAD have a function as a cofactor in here.). PyOx has high afinity to D-glucose rather than glucose oxidase and can be useful as a glucose biosensor with high stabilty and can be used a test material for analys the amounts of D-glucose in blood quantitatively(2,3). PyOx based biosensor measures amount of H_2O_2 that occur oxidation of substrat by enzym. Clays have been widely utilized in life sciences like biosensing, regenerative medicine, biomedical, cosmetics and drug carrying systems. General structure, physical and chemical properties of clays tender high termal capacity, intercalating, advanced adsorption feature, biocompatibility and cationic ion exchange capacity and stability of mecanic and chemical properties. Within these features modified clays have a important role for biosensing applications like an immobilization platform(4). In this work PyOx was immobilizied in the glassy carbon electrote (GCE) with 4-aminothiophenol(4-ATP)/Mt clay and used for glucose sensing.

METHODOLOGY

Firstly, glassy carbone electrode (GCE) was rubbed with 0.05 micron alumina solution. Electrode was cleaned over 15- 20 min. with ultrosonic cleaning in 1:1 distilled water and ethanol solution for to remove the alumina residues on the electrode. Whereupon, 1.0 mg of PyOx (in 5 µL, sodium phosphate buffer pH 7.0), 5 µL of 4-ATP/Mont solution (1.0 mg mL-1 in sodium phosphate buffer pH:7.0), 2.5 µL of BSA (1.0 mg mL-1 in sodium phosphate buffer pH 7.0) and 2.5 µL of glutaraldehyde (5.0% in sodium phosphate buffer pH 7.0) were mixed. Total volume of the mixture was 15.0 µL. Eventually , the mixture was dropped on the GSE surface and allowed to dry at room temperature for 1 h. The same procedure was used to every step of study. All reactions were studied under outdoor atmosphere, unless nisi noted. The aqueous solutions used in this study were prepared with deionized water passed through a Millipore Milli-Q Plus water treatment system. Amperometric and cyclic voltammetry measurements was performed using a Palm Sens Potentiostat (Palm Instruments, Houten, Netherlands). All experiments were carried out with using three electrode configuration. GCE as a working electrode, Ag/AgCl as a reference electrode (3.0 M KCl, Metrohm, Switzerland) and Pt as a counter electrode Metrohm, Switzerland). All experiments were performed in working buffer (10 mL) in a reaction cell. PyOx immobilized GCE was immersed and initially equilibrated in buffer and after that glucose was added in reaction cell. The answer signal of the consuption of molecular oxygen in the enzymatic reaction was monitored at -0.7 V vs. Ag/AgCl. The measurements were allowed to initially equilibrate and performed 3 times. Data were given with standard deviation (as ±). After each trial electrodes and reaction cell were washed with distilled water and working buffer was refreshed.

RESULTS AND DISCUSSION

Surface characterization of 4ATP/Mt/PyOx biosensors

Herein, CV and EIS techniques were used for the determination of the surface characteristics and electron transfer mechanisms of modified GCE. (Fig. 1).

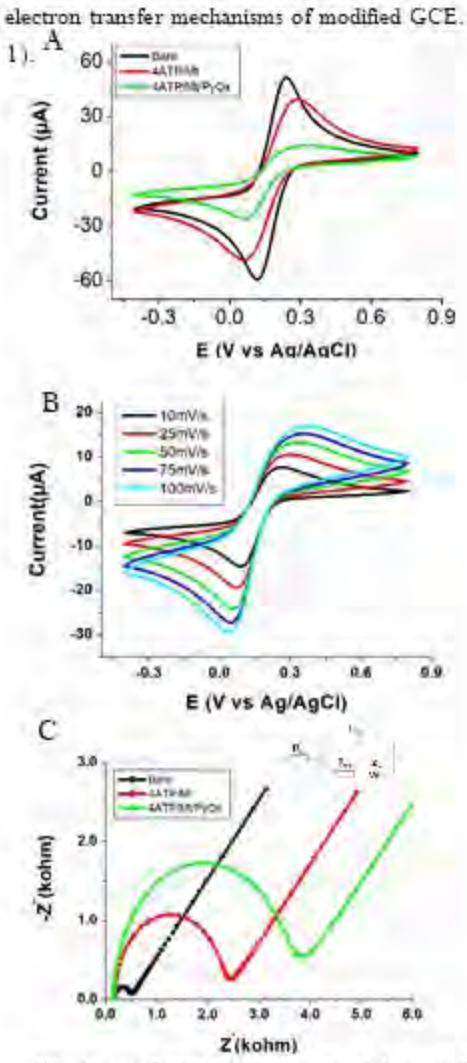

Fig. 1. (A) Cyclic voltammograms of bare, 4ATP/Mt and 4ATP/Mt/PyOx covered electrodes (in 5.0 mM $K_3Fe(CN)_6$ at a scan rate of 5.0 mV s^{-1}). (B) Cyclic voltammograms of and 4ATP/Mt/PyOx modified electrode at increasing scan rates [the inset graph showsthe correlation between the current and square root of the scan rates (10, 25, 50,75,100 mV s^{-1}). (C) Profiles for EIS originated from bare, 4ATP/Mt and 4ATP/Mt/PyOx modified electrodes in 50 mM PB (pH 7.0; including 5.0 mM, $K_3Fe(CN)_6$ and 0.1 M KCl) at 0.18 V, 0.21 x 10^{-2}–100 kHz.

The morphology and dispersion of the samples were investigated by SEM. SEM images of the prepared 4ATP/Mt nanocomposites and 4ATP/Mt/PyOx modified electrodes are indicated in Fig. 2. For the immobilization of PyOx glutaraldehyde was used as a crosslinking agent that binds amino groups of amino acids in the structure of enzymes (here PyOx) and proteins (BSA). The immobilization of PyOx on the surface of the GCE using 4ATP/Mt was carried out covalently. After modification of the surfaces with PyOx using 4ATP/Mt via crosslinking, SEM analysis was carried out to prove the success of immobilization.

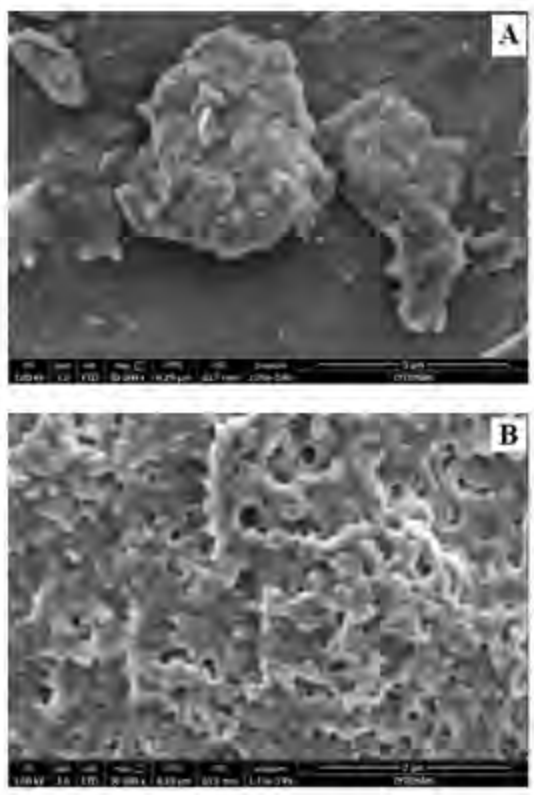

Fig.2. SEM images of (A) 4ATP/Mt, (B) 4ATP/Mt/PyOx

The amount of enzyme to be included in the electrode surface, causes a high sensitivity. For this aim, the electrodes were modified with various PyOx amounts such as 0.5 mg, 1.0 mg and 2.0 mg in order to determine the optimum enzyme amount(Fig.3.). The biosensor response was recorded for different glucose concentrations between 0.01 and 1.0 mM and 1.0 mg enzyme amount was observed that as optimum enzyme amount. Also studing in proper pH is imported situation for enzymatic biosensors. So, sodium acetate (50 mM; pH 4.0–5.5)and soduim phosphate buffer (50 mM; pH 6.0–7.0) were used as a working solution,and the biosensor response for glucose was monitored in different pHs. The optimum pH value was obtain as pH 5.5 sodium acetate buffer(Fig.4.).

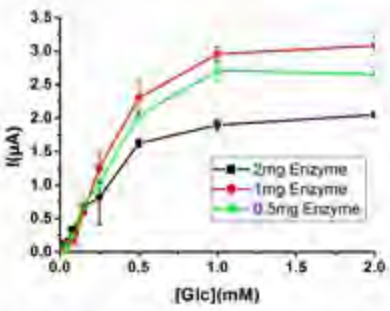

Fig. 3. Effect of enzyme amount on the current response of 4ATP/Mt/PyOx biosensor

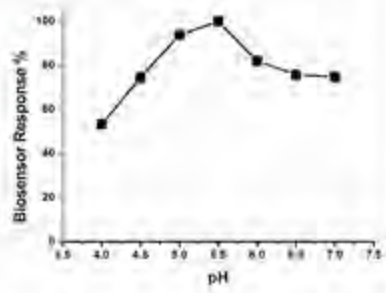

Fig. 4. Optimum pH of 4ATP/Mt/PyOx

Analytical characterization

The linear range obtained with the 4ATP/Mt/PyOx biosensors was from 0.01 to 0.5 mM glucose concentrations with the equation of y = 4.86x + 0.11 (R2 = 0.986). The limit of detection (LOD) for glucose was 1.17 mM glucose. For determination of operational stability, 82 trials were implemented by injecting 0.1 mM analyte (glucose) over 54 hours, and a 42% decrease in the biosensor response of the developed system was observed. Also reproducibility was achieved with a deviation of 0.2%. Most metabolites can be oxidized and interfered with at high working potentials. Thus, several compounds such as uric acid (0.01 mM), ascorbic acid (0.01 mM), ethanol (0.01 mM), and 3-acetamidophenol (0.01 mM) were used to investigate the effects of interferents on the 4ATP/Mt/PyOx biosensor response under the working conditions. According to the results, meaningful interference of the biosensor response for glucose did not ocur.

CONCLUSION

Clays have been widely utilized in life sciences like biosensing, regenerative medicine, biomedical, cosmetics and drug carrying systems. General structure, physical and chemical properties of clays tender high termal capacity, intercalating, advanced adsorption feature, biocompatibility and cationic ion exchange capacity and stability of mecanic and chemical properties. Within these features modified clays have a important role for biosensing applications like an immobilization platform. Here, we reported the preparation of a novel 4ATP/Mt nanocomposite and its application as an immobilization material to fabricate biosensors. PyOx was utilized as a model enzyme. The tproposed 4ATP/Mt/PyOx biosensor was optimized and characterized for glucose detection. Then, it was applied to detect glucose in real samples

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Study for developing an electrochemical tongue to differentiate three types of natural waters, using very simple sensors and the Principal Component Analysis.

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Aim of research

A basical research has been carried out using four probes to develop an electrochemical tongue able to discriminate three different kind of natural waters, i.e. rain, river, and groundwater. Several natural water samples were analyzed to this purpose using a pH glass electrode, a digital thermistor thermometer, an amperometric gaseous diffusion oxygen sensor and a DMFC enzymatic probe. Found data were processed by means of principal component analysis (PCA), obtaining three well differentiate clusters.

Introduction

The study and the analysis of natural waters is a very current topic, especially related to the problems of pollution, to which natural waters are very exposed [1]. The collection for analytical purposes, of many and different samples of natural waters, of numerous types, made some times without a careful discrimination, often creates additional difficulties, also for the purposes of programming further, more in-depth and targeted analysis of various natural water samples [2]. In the present research it was therefore address the problem of recognizing, in a very simple, rapid, and safe way, three different types of natural waters (rain, river and groundwater) by means of sensors of low cost and in addition using a most popular chemometric elaboration, such as the principal components analysis (PCA). This study can represent a basic investigation for the development of a simple, but effective and inexpensive "electrochemical tongue".

Samples

The natural water samples analyzed were: rain samples, collected by us in several places of the city of Rome (Italy) at different times; river waters, sampled by us in the Tiber river in the city of Rome and near Rome, at different times, and in another river, i.e. "Sacco" river, in the central Italy. Lastly groundwater samples were taken by the authors of the present paper from surfacing groundwater in the basement of the oldest center of the city of Rome. All samples were put in specially sealed glass containers, carefully washed and sterilized, finally analyzed, in a hurry after they have been taken.

Methods

All measurements were carried out in the laboratory, except the temperature measurements, which, of course, were done "in situ". pH measurements were made using a combined electrode (glass- calomelane) (Fig. 1 (a)) and a temperature compensating probe, after having performed a manual calibration of the instrument before each set of measurements, using two buffer solutions at pH 7.00 and 4.00, respectively. Each measure was repeated at least three times. Dissolved oxygen content measurements were carried out using an amperometric probe (Clark type) (Fig. 1 (b)) equipped with a thermometer probe for automatic temperature control. Before to each measurements set, the automatic calibration of the measuring apparatus was always carried out, firstly checking the operating parameters of barometric pressure and temperature, secondly using as a reference a sodium sulphite solution (10 g L^{-1}) and atmospheric oxygen. Sample temperature measurements were made directly "in situ" when sampling natural waters, by means of a thermistor digital thermometer (see Fig. 1 (c)). The probe was then immersed in the sample by checking that the immersion level was 4 cm. The reading was done after 10 s and repeated three times.

For alcohol measurement, ethanol (+ methanol if present), the used (DMFC) device was based on the following (Pt-Ru) catalyzed reactions [3,4]:

$$C_2H_5OH + 3\,H_2O \longrightarrow 12\,H^+ + 12\,e^- + 2CO_2$$
$$CH_3OH + H_2O \longrightarrow 6\,H^+ + 6\,e^- + CO_2$$

at the anode the ethanol, or methanol, was catalytic oxidized to carbon dioxide and hydrogen ions, while electrons were released to the anode.

The hydrogen ions permeated the Nafion membrane and reacted with the oxygen to the cathodic section of the cell, giving water; this process generated a difference of potential from the anode and the cathode. To reduce the response time of the fuel cell, an immobilized enzyme (alcohol dehydrogenase) was introduced in the anodic section of the fuel cell (as described in detail in previous paper [3]). It was possible to record the supplied current (SC) of the fuel cell, at the opimized applied potential (AOP) [3,4]. The measurement of the supplied current, at the steady state was read off and correlated with the alcohol concentration in the fuel cell [3,4].

Results and discussion

After having pointed out all the analytical methods, previously validated several times [5,6], for the measurement of the pH, the dissolved oxygen, the alcohol content and the temperature (see main features collected in the Table 1), three different types of natural waters have been measured. All different analyzed real natural water samples (i.e. rain, river, and groundwater), were experimetally checked for pH, dissolved oxygen concentration, temperature values, and alcohol content, using methods described in previous paragraph (see all found values collected in the Table 2). Therefore it was possible after "standardization", to process all these obtained experimental values, coming from the measures of four different considered parameters, for a chemometric application, processing all data by the Principal Components Analysis, using a MVA software (*MultiVariate Analysis* of Prof. Richard Brereton, Les Erskine and Tom Thurston) [7] for Microsoft Excel.

The application of PCA, to data collected, has shown that it is possible to obtain an excellent separation into different clusters, which enable the classification of different three types of natural waters, with great simplicity and remarkable effectiveness.

The "scores" of PCA representation show that the first two principal components (reported in the Fig. 2(a)) are able to separate, with about 67% of the total variance, all the samples into three clusters, one of which groups the samples of rain water, the other two groups the river and groundwater samples respectively, while the loadings representation (see Fig. 2(b)) show that the pH values give the main contribution to clusters separation, along principal components PC1, however the temperature, and alcohol concentration, allow the separation along the PC2 component. The representation along principal component 1 and 3 are on the contrary unable to separate completely three respective kind of samples (see Fig. 3 (a) and (b)). While, the Fig. 4 (a) shows as, the scores representation along the second and third principal components is still able to discriminate three different type of samples in three respective clusters. In this case the temperature and O_2 content give the main contribution to a still acceptable separation (as show the loading representation of Fig. 4 (b)). On the other hand it can be observe (see Fig.5 and Table 3) as each one of three eigen values are higher than one, therefore all three principal components, on the basis of the "mean eigen value criteria", would be theoretically significatives, but the more high value, i.e. that one of the first principal component, in practice is the more effectively significant (Tab.3).

Conclusions

Using four inexpensive, quick and simple to apply probes, in the first instance, able to operate also in situ [3], it was possible to measure four different parameters in three different types of the most common natural waters, i.e. rain water, river water and groundwater, with sufficient precision (see Table 1) and accuracy, since all four methods used were already validated and standardized.

Lastly it was possible to demonstrate as the PCA application to only four different kind of data (all simply measured using four different common sensors), are able to separate each one type of three examined natural water from the other two water kinds. Owing the simplicity and practicality of the sensor methods described, the conducted research can be considered as a useful and necessary study for the realization of a simple and inexpensive electrochemical tongue, which allows to effectively discriminate between the different types of natural waters, above all for the purpose of further different types of targeted analyses, which was believed to be later necessary for the total characterization of each of all these aqueous samples [8,9].

Table 1. main analytical features of four utilized probes.

Sensor type	Measured parameter	range	precision	sensitivity	Response time	Life time
Digital thermometer	Temperature (°C)	-50 to +150 °C	±0.3 °C	0.1 °C (between -20 and +90 °C)	10 s	1 year
Combined pH electrode	pH (pH units)	0 to 12 pH units	±0.05 pH units	0.01 pH units (at 25 °C)	20 s	6 months
Gas diffusion amperometric O_2 probe	O_2 (ppm O_2)	0 to 20 ppm O_2	(±0.02) ppm O_2	0.005 ppm O_2 (between +15 and +40 °C)	5 min	6 months
Enzymatic DMFC probe	Alcohol concentration (M)	5×10^{-3} to 6×10^{-1} M	(±0.005) M	2×10^{-3} M (at room temperature)	20 min	10 days (enzymatic); or >3 months (if not enzymatic)

Table 2. All experimental values of four measured different parameters in all the analyzed natural water samples (each value is the mean of three determinations).

Rain, river, ground-water sample n.	pH (pH units) SD%≤2.3	Dissolved O_2 (ppm) SD%≤2.9	T(°C) SD%≤1.7	Total alcohol concentration found value, using fuelcell (mg/L) SD%≤2.8
1 (rain)	5.4	8.89	20.8	7.6
2 (rain)	5.6	8.38	21.3	0.0
3 (rain)	5.7	8.78	21.1	9.5
4 (rain)	5.6	8.65	21.2	7.2
5 (rain)	5.3	8.78	20.7	3.9
6 (rain)	5.4	8.81	20.9	0.0
7 (rain)	5.5	8.45	21.2	6.1
8 (rain)	5.5	8.53	21.1	5.3
9 (river)	7.6	8.50	19.2	0.0
10 (river)	7.5	8.40	19.3	3.7
11 (river)	8.3	8.70	19.3	5.2
12 (river)	7.8	6.50	19.2	0.0
13 (river)	7.7	6.01	19.4	3.6
14 (river)	7.6	7.60	19.5	3.0
15 (river)	7.8	7.90	19.3	0.0
16 (river)	7.9	8.10	19.2	0.0
17 (groundwater)	7.2	7.60	22.9	3.8
18 (groundwater)	7.6	5.40	23.1	3.1
19 (groundwater)	7.8	5.90	23.4	3.2
20 (groundwater)	8.0	6.70	23.3	3.2
21 (groundwater)	7.3	7.40	23.0	3.6

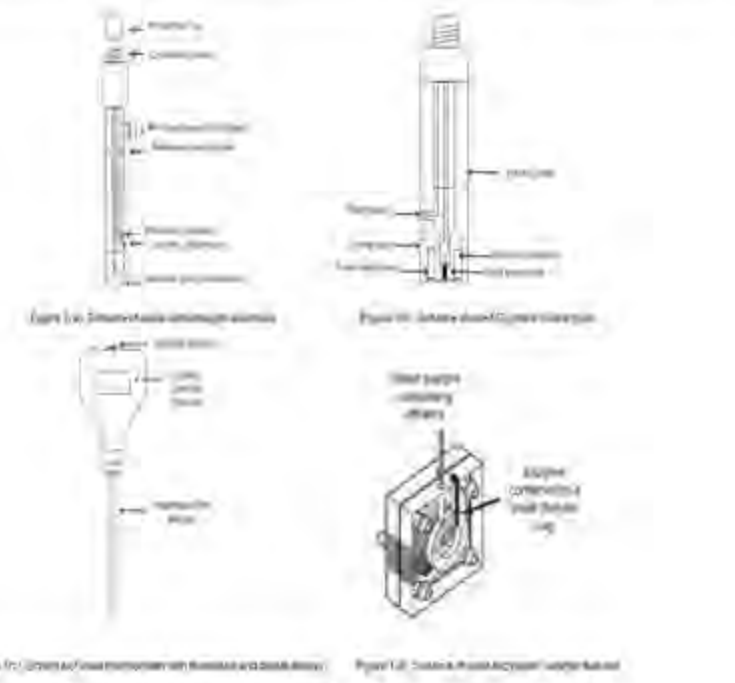

Figure1. Representation of four used probes.

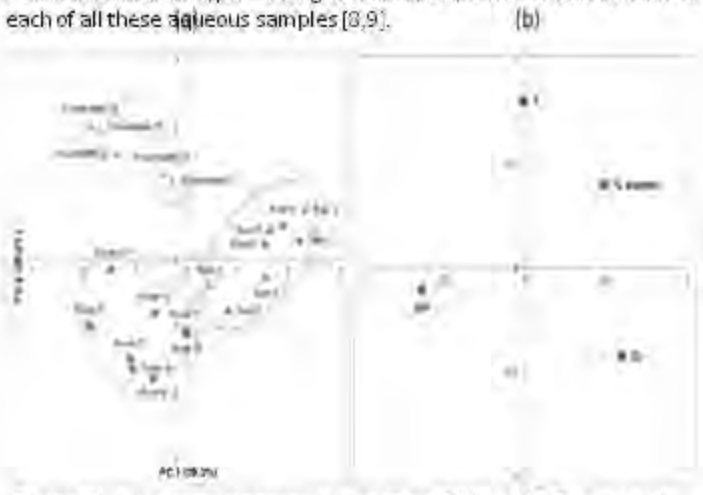

Figure 2: (a) scores representation along PC1 and PC2 principal components; (b) loadings representation.

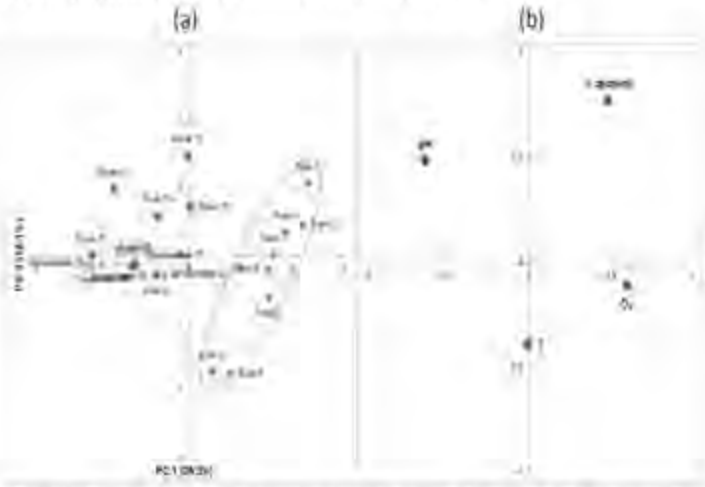

Figure 3: (a) scores representation along PC1 and PC3 principal components; (b) loading representation.

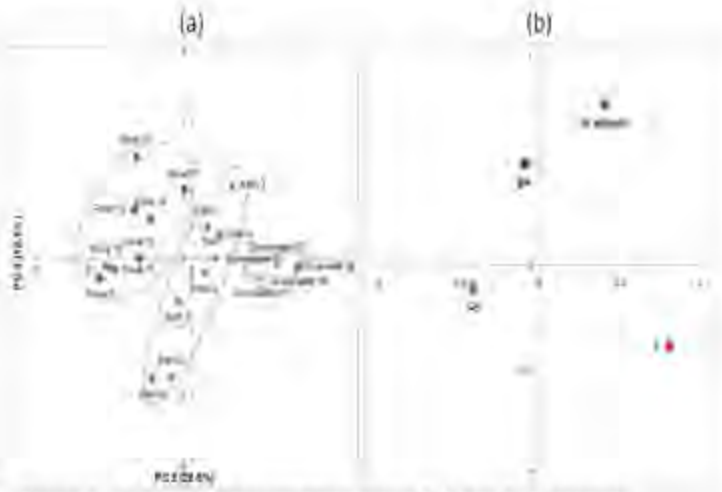

Figure4: (a) Scores representation along PC2 and PC3 principal components;
(b) loadings representation.

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Figure5. Mean eigenvalue criterion

Table 3. *RSS* (Residual Sum of Squares) and *PRESS* (Predictive Residual Sum of Squares), as a function of principal components number.

PCA - Principal Components Analysis (Standardized)		
N. PCs	Cross validation	
	RSS	*PRESS*
Total SS	1126.89	1126.89
#1	138.73	171.88
#2	68.04	82.84
#3	5.82	n/a

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BBMEC 12th Workshop on Biosensor & Bioanalytical Microtechniques in Environmental, Food & Clinical Analysis

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Electrochemical detection of hydrogen peroxide by free standing nickel oxide-plastic electrode

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Abstract

The transition metal oxides have attracted the attention of research in sensors development as the catalytic properties of NiO are exploited to optimize non-enzymatic sensors i.e. for H_2O_2 or glucose determination. NiO powder alone or mixed with carbon-based derivatives and/or conductive polymers and/or ion-exchange polymers are distributed on conventional electrodic surfaces (i.e. glassy carbon). NiO particles holds to the electrode surface after solvent evaporation. Alternatively, NiO is mixed with carbon paste blend then lodged into a specific holder. The preparation of a freestanding graphite-based plastic electrode (PE) modified by NiO nanoparticles is here presented with the aim of optimizing a new catalytic plastic surface which is tested in H_2O_2 determination.

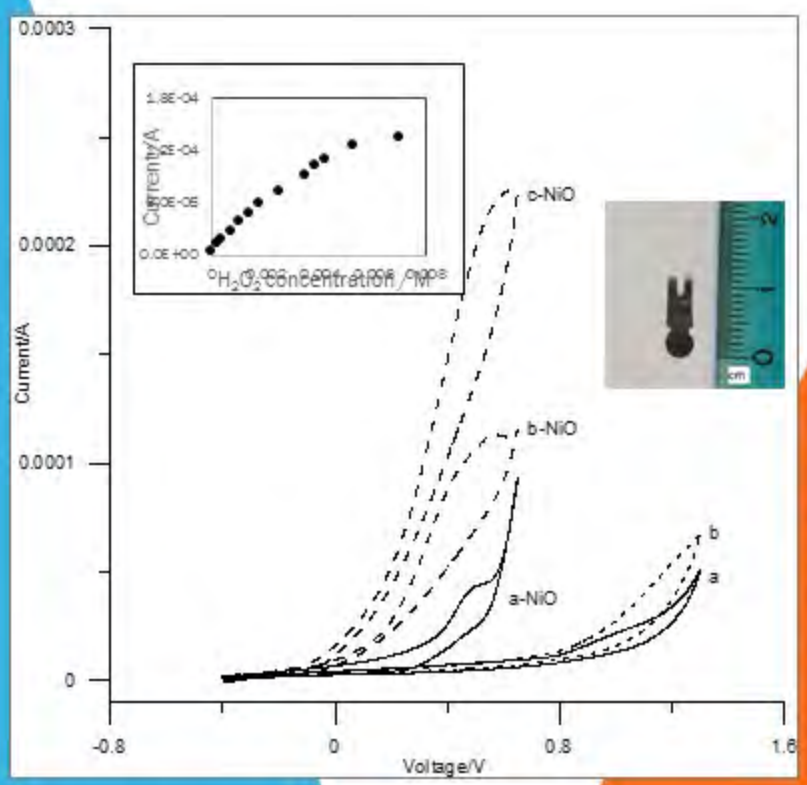
Materials & Methods

Figure 1a: NiO-PE

The electrically conductive plastic film was prepared as reported in [1]. Briefly, synthetic graphite, PVC powder and an adipate-based plasticizer were mixed with tetrahydrofuran (THF), under stirring (**Figure 1, a**). An homogenous, electrically conductive, flexible and free standing film of about 300 µm was obtained. NiO-plastic electrode (NiO-PE in **Figure 1, b**) was obtained by adding NiO nano-powder to the standard formulation. The plastic electrodes were easily cut with a paper cutter (geometrical area of 0.25 cm^2, **Figure 2, insert**). Cyclic voltammetry (CV) and amperometry (CA) were performed into a conventional electrochemical cell. RE= $Ag^+/AgCl,Cl^-$, CE = platinum electrode (CE). CHI-400 potentiostat was used to perform the measurements.

Figure 1b: NiO-PE

Results & Discussions



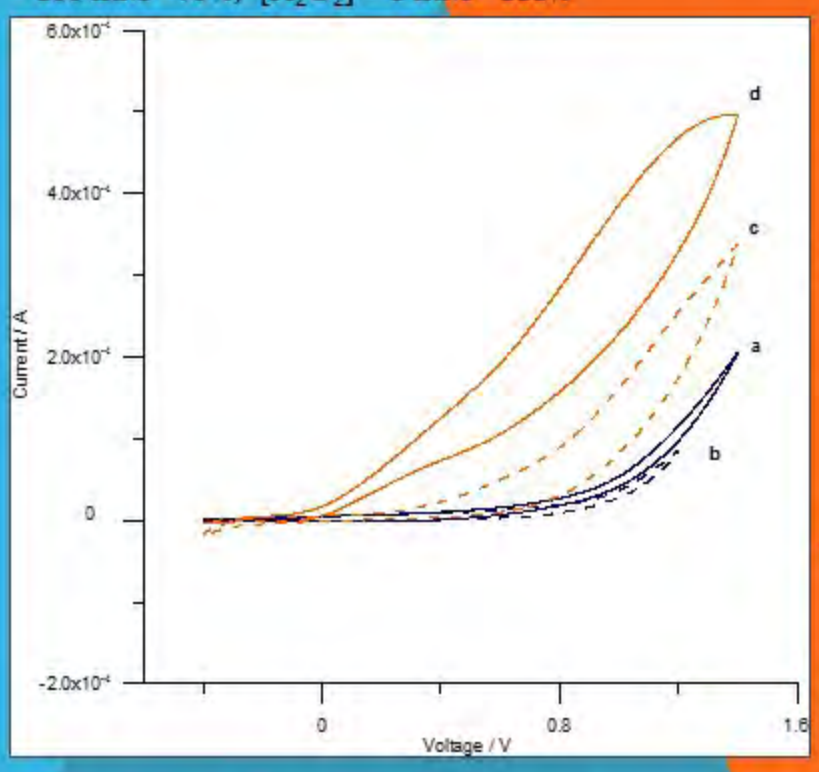
The cyclovoltammograms of PE (**Figure 2,a**) and of NiO-PE (**Figure 2, a-NiO**) were obtained in 0.1 M of NaOH at a scan rate of 50 mVs^{-1}. The redox process of NiO nanopowder embedded into the plastic film can be observed at $Ep/2 = (Epa+Epc)/2 = +0.43$ V, which is in good agreement with literature data at conventional graphite electrodes.

NiO-PE was cycled 30 times adopting as potential limit -0.4 V and +1.2 V, and consecutive voltage cycling leads to a progressive increase in the current values of both anodic and cathodic peaks (*see page 2*). The charge (Q) passed during NiO-PE cycling was used to estimate an immobilized active species of about 8.2×10^{-8} mol cm^{-2}.

The catalytic activity of $Ni(OH)_2$ /NiOOH was tested in presence of hydrogen peroxide (H_2O_2). Figure 2 (b-NiO, c-NiO), shows the CV of the NiO-PE activated with 30 seewps in presence of 1 mM and 2mM of H_2O_2 , respectively. It was confirmed that, as conventional graphite based electrodes, no signal due to H_2O_2 (5 mM) at bare-PE was measured (**Figure 2, b**). The "activated" electrode NiO-PE was polarized at + 300 mV and H_2O_2 was added to the NaOH solution 0.1 M in a concentration range from 20 mM to 5 mM (**Figure 2, insert**). The range was linear up to 4 mM with $y = 2 \times 10^{-8} + 9 \times 10^{-6}$ (A/mM)($R^2 = 0.990$) and LOD ($3s_{blank}$) of 5 µM.

Figure 2: Cyclic voltammograms of PE in NaOH 0.1 M (a) and in presence of 5 mM H_2O_2 (b) and of NiO-PE in NaOH 0.1 M (a-NiO) and in presence of 1 mM H_2O_2 (b-NiO) and 2 mM H_2O_2 (c-NiO). Scan rate 50 mVs^{-1}.

A low fat milk (1.5% of fat) was used the test NiO-PE performances. A sample of milk was added with NaOH 0.1 M to obtain pH 10.0. No sample pretreatments were required. CVs were recorded with both PE and NiO-P and no peaks were observed (**Figure 3**). A new sample of milk (pH 10.0) was prepared to obtain a calibration curve by amperometry with an "activated" NiO-PE polarized at + 200 mV (**Figure 4**). A recovery test was carried out adding H2O2 0.1 and 1 mM as well as glucose and ascorbic acid (both 1 mM) (**Figure 4**). Recovery tests: $[H_2O_2] = 100$ mM =95%; $[H_2O_2] = 1$ mM =111%



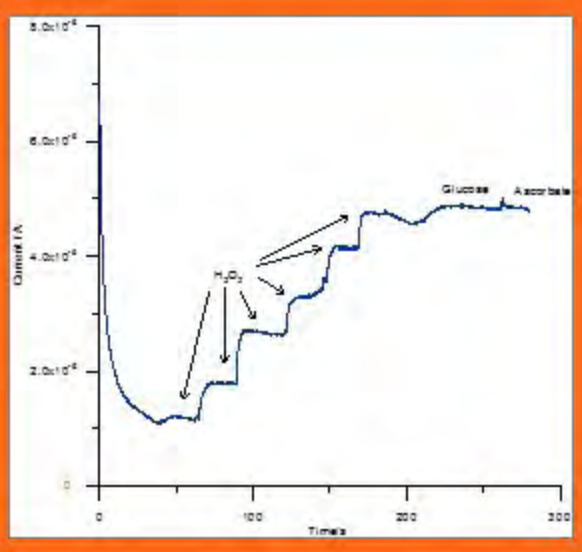


Figure 4: 1) Amperometry performed in milk sample with "activated" NiO-PE in NaOH 0.1M polarized at +200 mV and the 2) calibration curve y = 0.00191 x + 1.01 in milk pH 10.0 .

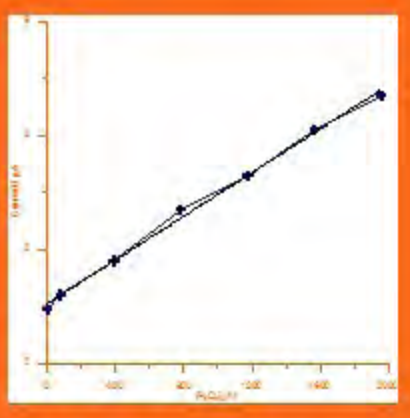


Figure 3: CV performed in low fat milk added with NaOH to pH 10.0. WE: «activate» NiO-PE (c) in milk pH 10.0 and (d) added with H_2O_2 and bare-PE (b) in milk pH 10.0 (a) added with H_2O_2 . Scan rate 50 mVs^{-1}.

Conclusions : An easy way to prepare a free-standing composite graphite-based plastic electrode is here proposed. Moreover, the mechanical features (i.e. flexibility) make this material attractive for applications where a high morphological fitting is required. Further investigations are now in progress to characterize the morphology of the material with the aim of improving its functional performances, however maintaining the features of low cost and easiness of preparation.

Rapid detection of *Escherichia coli* in marine water samples by a remote optical biosensor system

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Introduction

Conventional cultural methods defined by the European Directives require 18-24 hours for the statutory assessment of the bacteriological water quality and 1-2 days more for results confirmation. Since delayed results can threaten public health, cutting-edge technologies able to speed up analyses are needed. In this context, biosensors represent suitable devices for easy, rapid and on site analyses in various applications. Their advantage is the highly specific target's identification ability, which reduces and simplifies the analytical steps. On this basis, a novel, completely automatic optical biosensor system called COLISENS (patent pending), able to rapidly detect *Escherichia coli* (*E. coli*) in marine water, so revealing dangerous microbial contaminations of fecal origin, has been developed (**Fig. 1**).

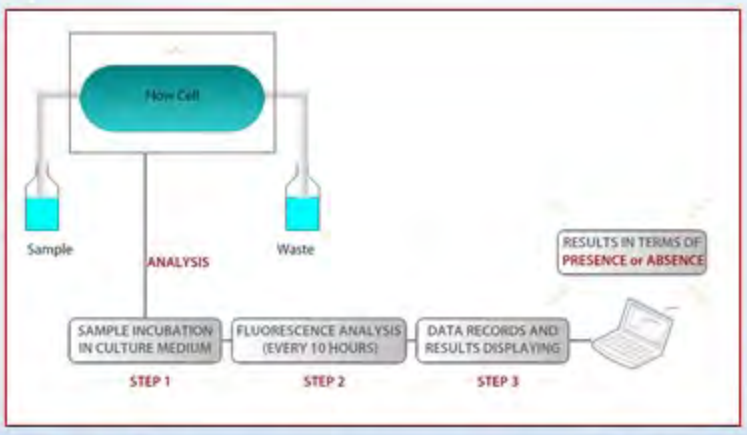


Fig.1. Schematic representation of COLISENS

Method

The optical biosensor system can perform the entire analytical procedure and its autonomy is guaranteed by the provision of a stand-alone supply box, including substrate stock and a communication system that constantly interacts, by UMTS network, with a web server where results are shown for remote control (**Fig. 2**).

Fig.2. Equipment

The principle of the analysis is based on the hydrolysis of the substrate beta-D-glucuronide contained in the culture medium added to the sample. The hydrolysis, operated by the microbial enzyme beta-D-glucuronidase, leads to 4-methylumbelliferone, whose fluorescence is measured, giving evidence of the presence of *E. coli*. In order to validate the system, results were compared with those gained with a standard method (ISO 9308-1:2012) able to quantify *E. coli* growth.

Results

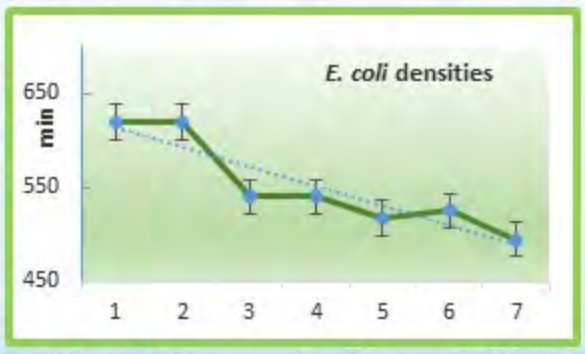
To evaluate the biosensor response to increasing bacterial densities, a great number of analytical tests (n=150) have been performed on water samples added with *E. coli*. The time required by the biosensor, to definitely signaling a microbial pollution over the allowed concentration, has been identified as the time where the fluorescence curve drawn by the biosensor, and corresponding to growth curve of the bacteria, increased upper 4%, indicating the moment where *E. coli* culture moves from the latency to the exponential phase. **Figs. 3** and **4** show the relation between the time required to reach the exponential growth phase of the bacterial culture, and *E. coli* initial densities: as shown, there was a considerable reduction of this interval at the highest *E. coli* concentrations (>50 CFU/mL). The maximum mean time needed to reach the exponential phase was 11 hours. The repeatability of the measures was around 85%.

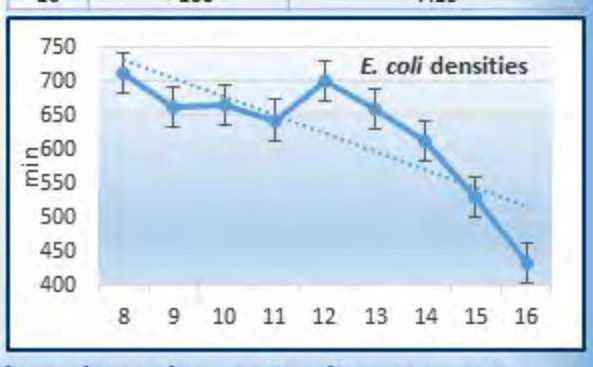
Fig.3.

Rif.	*E. coli* densities [CFU/mL]	Time to log phase [mean] h
1	1-2	10:30
2	6-9	10:30
3	19	9:00
4	31-44	9:00
5	32-70	8:38
6	80	8:45
7	172-325	8:15

Fig.4.

Rif.	*E. coli* densities [CFU/mL]	Time to log phase [mean] h
8	0,28-0,81	11:52
9	1,1-1,5	11:02
10	1,6-2	11:05
11	2,5-3,2	10:43
12	3,5-4,5	11:40
13	5,1-5,5	11:00
14	6-8,7	10:13
15	32-80	8:50
16	>100	7:13





Figs.3-4. Time required to reach the log phase depending on *E. coli* concentration

Conclusions

The developed biosensor system reliably detected *E. coli* as low as 1 CFU/mL, in marine water, in a maximum of 10-12 hours. This enables a minimum of two analyses per day, therefore a tighter control of water conditions and near real-time results. This study paves the way for the development of an automatic monitoring platform, remotely controllable, intended for the management of water resources and acting as an early warning system launching alarm signals when fixed threshold values are exceeded.

Fluorescent microfluidic device based immunoassay for therapeutic drug monitoring: acenocoumarol case

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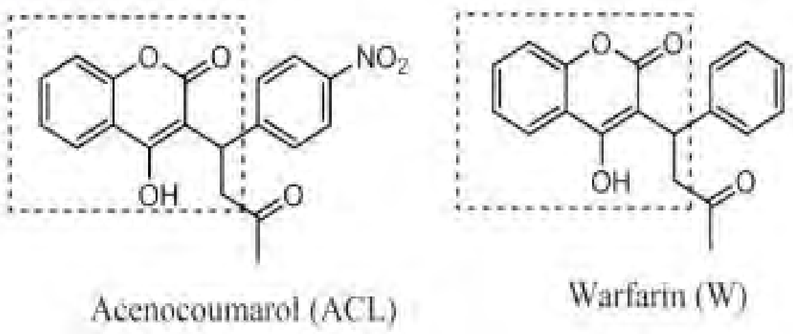
Introduction

Therapeutic Drug Monitoring (TDM) aims to accomplish a more personalized medicine by assisting on adjusting doses, according to each patient needs, reducing toxicity due to unnecessary overdosing, and follow-up of the treatments..

Warfarin (W) or **Acenocoumarol (ACL)**: are Vitamin K antagonist inhibiting coagulation. These drugs are used on several cardiovascular diseases to stop or prevent thrombosis, embolism, stroke, myocardial infarction and dead[1].

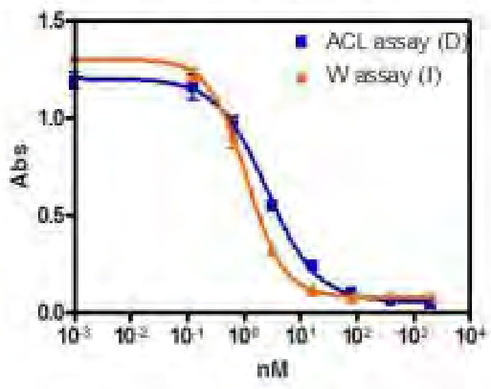
Side effects: hemorrhages (inappropriate dosage)

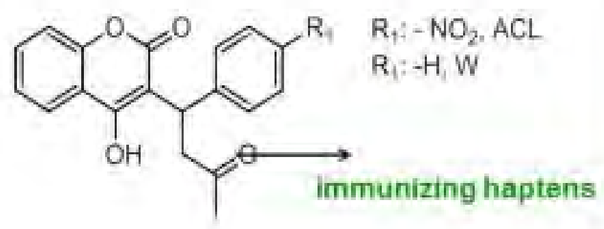


Acenocoumarol (ACL) Warfarin (W)

Antibody production

The aim of the antibody production against **oral anticoagulant compounds** (OAC) is to produce family antibodies that will recognize the main OAC using the same antibody. A **hapten design** was done and an immunizing hapten were synthesize[2]. After immunization and characterization of produced antibodies, an immunochemical procedure was set-up of the TDM of OAC.



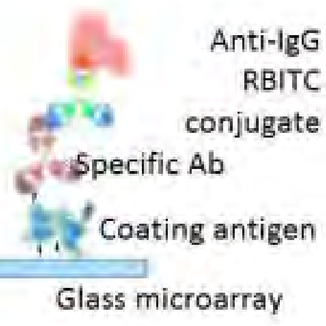


	ACL assay	W assay
Amin	0.056	0.080
Amax	1.207	1.305
Slope	-0.988	-1.305
IC_{50}	2.594	1.126
R^2	0.995	0.996

	W assay		ACL assay	
	IC_{50}	%CR	IC_{50}	%CR
ACL	4.3	84	2.7	100
W	3.7	100	0.8	326
4'-NH_2-ACL	46.9	8	5.0	53
4-OH-COU	>2000	<0.2	285	1
Testosterone	>2000	<0.2	>2000	<0.13
CAP	>2000	<0.2	>2000	<0.13

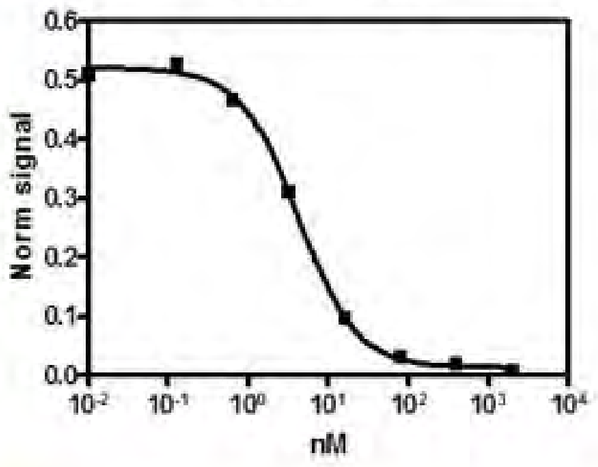
Antibody microarray

A **fluorescent microarray** has been developed for their implementation on a point of care device. An internal standard has been implemented in order to maximize the reproducibility of the assay.



INDIRECT FORMAT: A2BSA/As233
+ Control (hA_BSA/As138)

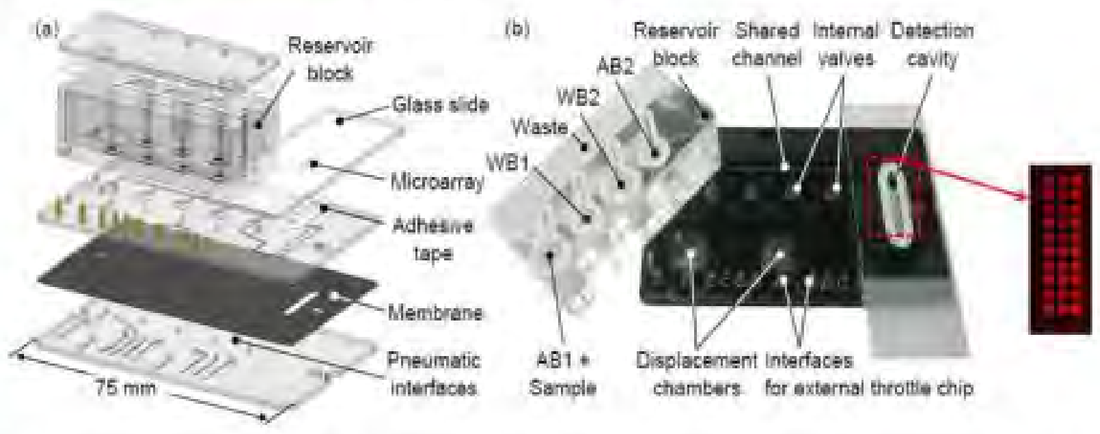
A2BSA/As233



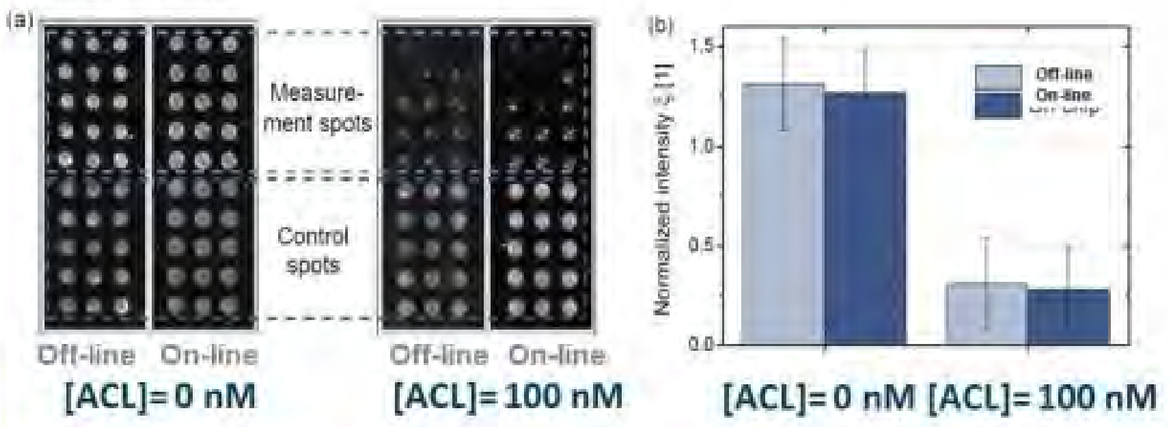
	ACL
$NORM_{min}$	0.01304
$NORM_{max}$	0.5203
Slope	-1.199
IC_{50}	4.249
N	4
R^2	0.998

Point of care device

As a proof of concept, a **point of care device** has been developed for the monitorization of ACL using a external fluorescent read-out. The model system allows the immunochemical procedure just by **finger pressing**. The device works **automatically** all the steps involved.



The PoC developed allows the accurate measurement by external callibration from the developed fluorescent microarray.



Conclusions

Different analytical platforms has been developed of the simultaneous determination of relevant environmental pollutants based on the use of antibodies as a biorecognition element. Those systems are highly specific, sensitive, with high detectability and robust that allow the measurement directly in sea water. All platforms can be used as a monitoring tool for the monitoring of these representative pollutants in aquaculture.

CONTACT

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Acknowledgements

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Yeast-based amperometric bioprobe for simazine detection in agricultural water and raw cow's milk samples

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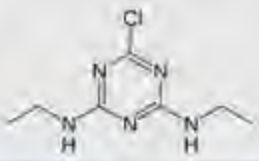
Weed management practices in farming systems associated with chloro-s-triazine herbicides use or misuse can result in chemical contamination of environmental compartments and foodstuffs. Simazine is a chloro-s-triazine herbicide of great agronomical importance worldwide in particular in the United States, Canada, Brazil and China (the world's largest producer and consumer of simazine). Due to its moderate environmental persistence, simazine can contaminate surface waters and groundwaters and thus it can be transferred e.g. into the milk chain through cow consumption of contaminated forage crops and/or contaminated water. Such environmental and food simazine contamination events are rising public health concerns, in particular for reported both short- and long-term toxic effects on non-target organisms health (e.g. endocrine disrupting). Against this background, simplified, sensitive and rapid screening methods and portable sensoristic devices for detection of chemical contaminants (including simazine) in environmental and food matrices are increasingly required. In this work, a *Saccharomyces cerevisiae*-based amperometric biosensoristic device (hereafter referred as yeast-based bioprobe) was utilized to detect the presence of simazine in fortified agricultural water and raw cow's milk samples. Percentage interference (%ρ) with aerobic cellular catabolism (as biomarker of exposure) was assessed by measurements of oxygen consumption of exposed yeast cell suspensions compared with control yeast cell suspensions. More specifically, positive %ρ values indicate cellular respiratory inhibition and negative %ρ values indicate cellular respiratory hyper stimulation (i.e. over the maximum physiological rate) of exposed yeast cells.
Considering the European legal concentration limits for simazine residues in drinking water (0.1 ppb) and cow's milk (10 ppb), various concentrations (0.1, 0.2, 0.02 ppb for agricultural water, 200, and 2 ppb for cow's milk) were tested. The results obtained from short-term (3h) tests showed that the yeast-based bioprobe is able to detect the presence of simazine in agricultural water (14.0%, 36.8% and 39.1% for 0.02, 0.1 and 0.2 ppb respectively) and in raw cow's milk (20.3% and -24.5% for 2 and 200 ppb respectively) without any necessary sample pretreatments. In particular, yeast-based bioprobe was able to detect this herbicide up to concentrations 5 times below the legal limits for both tested matrices. The proposed yeast-based probe for simazine proved to be very sensitive and the absence of samples pretreatments make it potentially employable as field screening method. Thanks to simplicity of execution of tests and suitability of cellular aerobic respiration of *S. cerevisiae* as biomarker of exposure to various organic and inorganic contaminants, this bioprobe lend itself to automation and integration in the patented technological platform BEST (PCT WO/2010/001432): (Bio)Sensors' system in Food Safety as general toxicity monitoring system for environmental and food surveillance at critical control points.

Simazine



- Is a **chemical of great agronomical importance worldwide** (widely used in **U.S., Canada, Brazil and China**) [1]
- Its use as herbicide was **banned in the European Union countries**
- **No more in commerce in Italy** since 2005 but it is **still present in surface and groundwater** [2]
- Moderately persistent in the environment → simazine **can be transferred** e.g. into the milk chain through cow consumption of contaminated feed and/or contaminated water

- **EU legal concentration limit** for **single pesticide** in **drinking water** is **0.1 µg/L** (or ppb) [Council Directive 98/83/EC]
- **EU legal concentration limit** for simazine in cow's milk is **10 µg/Kg** (or ppb) [Annexes II and III to Regulation (EC) No 396/2005]

Aim of this work:

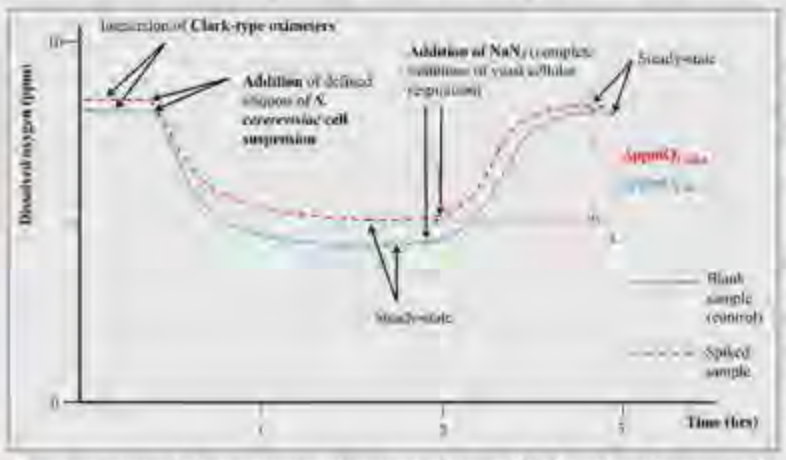
Use a ***Saccaromyces cerevisiae*** **(yeast)-based bioprobe** to **detect** the presence of chloro-s-triazine herbicide **simazine** in spiked **agricultural water** and **raw cow's milk samples**.

Yeast-based amperometric bioprobe

Cellular aerobic respiration of *S. cerevisiae* was previously found to be **very sensitive** to other contaminants, including herbicide **diuron** in **aqueous samples** at **concentration 4 times below EU legal concentration limits** [3]

Monitoring the **change of dissolved O_2 concentration** (linked to **mitochondrial activities**) → **Rapid assessment** of the **presence** of **herbicide** in **samples**

Example of kinetic respirometric curves



$\Delta ppmO_2$ blk = mean of variations of the dissolved O_2 (in ppm) for blank sample.

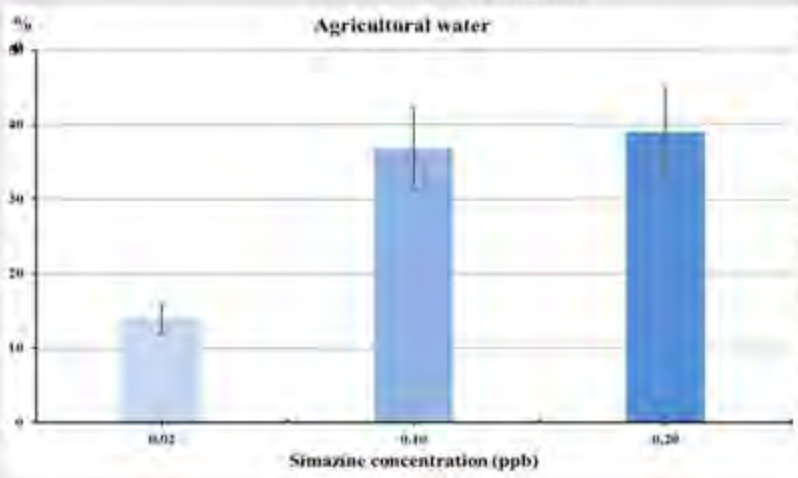
$\Delta ppmO_2$ spkd = mean of variations of the dissolved O_2 (in ppm) for spiked sample.

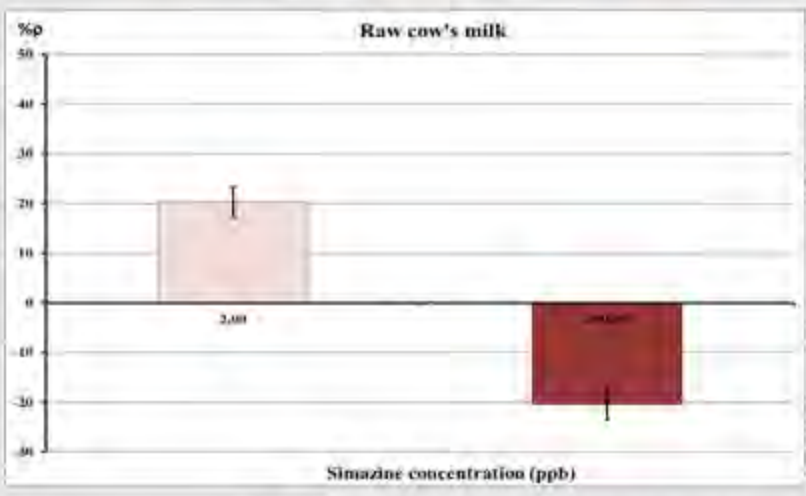
The experimental respirometric curve shows the dissolved oxygen (ppm O_2) as a function of time in the case of interference with the respiration of the yeast cells.

The **percentage interference of cellular respiration** (**%ρ**) was calculated as follow:

$$\%\rho = [1 - (\Delta ppmO_{2\ spkd} / \Delta ppmO_{2\ blk})] \cdot 100$$

Results



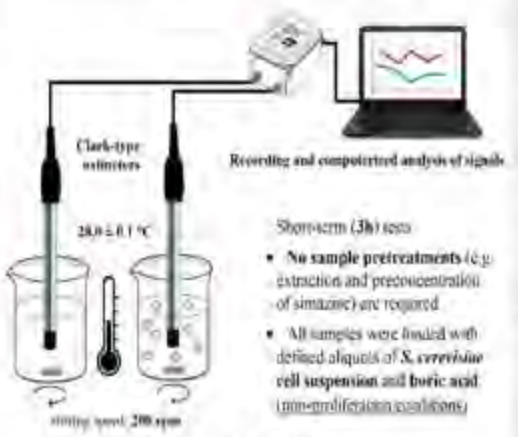


Each control and spiked samples had 4 replicates for each experiment; RSD% ≤ 20% were calculated for blanks and spiked samples for each experiment. ANOVA for Randomised Block Design test: significant relationship between spiked and blank samples for all concentrations

Effects on cellular respiration : **%ρ values >0 → inhibition**; **%ρ values <0 → hyper stimulation** [4]

Respirometric test in agricultural water samples

Respirometric test in raw cow's milk samples



Conclusion and future perspectives

- ✓ The proposed yeast-based probe is proved to be **very sensitive** and **able to detect** (without any necessary sample pretreatments) **herbicide simazine up to concentrations 5 times below the EU legal concentration limits** for both tested matrices).
- ✓ This bioprobe lend itself to **automation** and **integration** in the **patented technological platform BEST (PCT WO/2010/001432): (Bio)Sensors' system in Food Safety** as **general toxicity monitoring system** for **environmental** and **food surveillance** at **critical control points**.

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Design of a bioprobe for total estrogenicity levels determination in environmental and food matrices: a biotechnological approach

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Background and Aims

Endocrine active substances (EASs) are chemicals that can interact or interfere with normal hormonal activity in the endocrine system. They include natural hormones and phytoestrogens but also endocrine disrupting compounds (EDCs). EDCs are a large class of structurally diverse compounds (both of natural and anthropic origin) whose interaction or interference with normal hormonal functions results in adverse effects.

In particular, estrogenic-like EASs (including estrogenic-like EDCs) can cause hormonal imbalances, associable with onset of serious adverse health effects like testis, prostate and breast cancers. Examples of estrogenic-like EDCs are residues of chemicals like pesticides (e.g. chloro-s-triazines and organochlorines) polychlorinated biphenyls (PCBs) and various chemicals (e.g. monomers and additives from plastics like phthalates and bisphenol A) migrating from food contact materials. Both animals and humans are widely exposed to all these compounds both through interaction with environmental compartments and through consumption of foodstuffs contaminated.

Several bioassays and screening methods for the determination of estrogenic-like EASs levels in environmental and food samples are described in literature. In particular, in yeast-based in vitro estrogen assays a two plasmids cloning strategy (one containing the gene for the responsive element and the other for the reporter element) is frequently used. Moreover, the yeast cells are principally engineered with ER-α or ER-β estrogenic receptors, linked to genomic signaling events. In this work the developing of a genetically modified yeast-based (*Pichia pastoris* strain GS115) bioprobe for the determination of the total estrogenicity levels (linked to the presence of estrogenic-like EASs) in food matrices (e.g. raw milk) and environmental matrices (e.g. zootechnical sewages) is described.

Methods

Starting from available data in literature, a preliminary study was focused on G protein-coupled estrogen receptor 1 (GPER-1). GPER-1, who can work as "stand alone" receptor, contrary to ER-α and ER-β receptors i) it is mainly localized in endoplasmic reticulum membranes (and not in the nucleus) and ii) it is recognized as the major mediator of rapid cellular effects linked to estrogens through non-genomic signaling events.

Contrary to yeast-based in vitro estrogen assay previously described, a one plasmid-based genetic engineering strategy was adopted. In particular, used plasmid possess two main features: the presence of a constitutive moderate promoter (that allows for continual transcription in non-toxic levels for the cells) and the absence of a secretion signal (that allows an intracellular expression of the chimeric protein). Such strategy and such receptor were chosen because they could reduce response time of genetically modified *P. pastoris* cells in presence of target estrogenic-like EASs.

Plasmid was designed and constructed with the human gene of the membrane estrogenic-receptor (GPER-1) tagged with the gene encoding fluorophore mCherry. Thus, genetically modified *P. pastoris* cells harbor a fluorescently-tagged GPER-1 whose interaction with target estrogenic-like EASs could result in fluorescence signal variations (e.g. by conformational changes).

Results

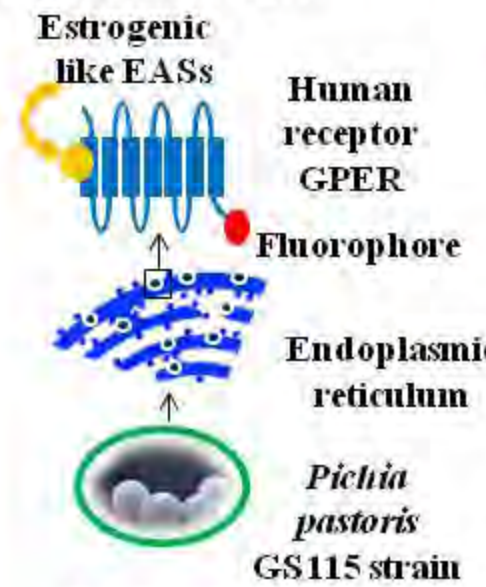
Before the transformation of *P. pastoris* cells the plasmid sequence was checked by double-digestion and sequencing. After cellular growth of positive P. pastoris transformed cells, an extraction protocol was done and fluorescence measurements were performed on the extract samples to verify the spectral optical properties of mCherry-tagged GPER-1.

Conclusions

Following positive results obtained with *P. pastoris* transformation, performances of the bioprobe will be pre-validated with fluorescence measurements on genetically engineered *P. pastoris* cells exposed to estrogenic-like EASs (both as single chemicals and as mixtures) first in simulated milk solutions and simulated wastewater solutions and then in fortified real samples. Following a laboratory validation, the possibility to integrate the designed bioprobe in the patented technological platform BEST (PCT WO/2010/001432): (Bio)Sensors' system in Food Safety will be evaluated.

DESIGN OF THE BIOPROBE

Whole cell-based biotechnological bioprobe for the **determination** of **total estrogenicity levels** of **real matrices** samples (e.g. **raw cow's milk** and **zootechnical sewages**).



Biological mediator:
Engineered *Pichia pastoris* cells;

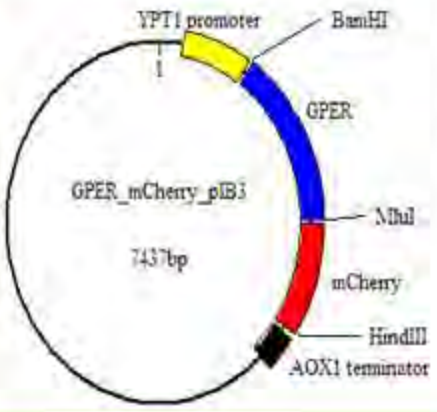
Fluorescence transduction

GPER is a membrane receptor linked to estrogenic effects principally due to **non-genomic signaling events.**

BASIC ASSUMPTION:
Estrogenic-like EASs-receptor GPER interactions could modify the intensity and/or shift the wavelength of fluorescence (e.g. by conformational changes)

One plasmid strategy

- Responsive element: (GPER)
- Reporter element: mCherry fluorophore



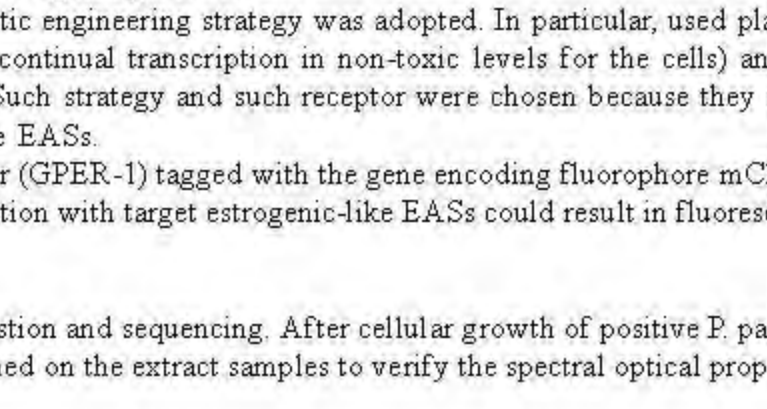
The chosen yeast vector (pIB3) has these features:

- **YPT1 promoter**: moderate constitutive;
- **HIS4 gene**: integration in the yeast genome and positive transformants selection for auxotrophy after transformation in *Pichia pastoris* GS115.

EXPECTED RESULTS:
Suitable analytic performances and a reduced response time.

POSSIBLE FUTURE APPLICATIONS OF THE BIOPROBE

Chemicals detectable with this bioprobe are all the estrogenic-like EASs, including the estrogenic-like EDCs. Examples of this last category are several pesticides (e.g. chloro-s-triazines and organochlorines), various chemicals migrating from food contact materials (e.g. monomers and additives from plastics) and dioxins.



Food safety

Environmental health

The bioprobe will be evaluated also like device for:

Animal metabolism monitoring

Animal metabolism reflects:
- Health;
- Treatments;
- Diet;
- Living environment in livestock.

Farm management and HACCP self-monitoring plans

Technological integrated bioelectronic system and relevant control charting for early intervention on food chain and the environment: the BEST Platform

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Abstract. The Hazard Analysis and Critical Control Points (HACCP) approach is the widely used on-enterprise strategy to control and manage the safety of food production process as well as to support traceability and liability. The milk chain is particularly challenging and challenged by practices (e.g. water dilution of milk or mixing with milk from different species) possibly hindering safety issues, or unintended/unexpected contaminations. Indeed, the milk chain would benefit of early identification of anomalies to shield from commercial frauds and guarantee both authenticity and traceability of the food product.
Innovative technologies for monitoring such factors as farm animal health, productivity, food wholesomeness and traceability can make a substantial difference.
The BEST Platform (PCT WO/2010/001432) is a system for environmental diagnostics and monitoring and toxicological self-monitoring and traceability in the food chain, including primary production.
BEST comprises: i) a detecting unit, having a plurality *n* of independently selectable probes; ii) a data processing unit for acquiring data from said probes, that is connectable to a data collecting module provided with user interface means. The detecting unit is provided with a plurality *m* of biological media ($0 \leq m \leq n$), and each one of said n probes is suitable to detect a biological, chemical, or physical parameter relevant to quality and/or safety thereby generating a suitable signal. The data-collecting module is suitable to store data, and the data processing unit simultaneously control chart the signal of the said probes, thus identifying and determining concurrent anomalies of said parameters in control charts and/or other diagrams. A warning indication is produced via the said interface means based on at least one alert threshold or at least one intervention threshold through the comparison of data with external or self-educated thresholds, in one or more Critical Control Points (CCP) and/or points of particular attention of a food chain and/or a environmental compartments.
The system comprises a plurality n of electronic boards, each one connected to one respective probe and to the data collecting module, and suitable for carrying out a pre-processing of the signal of the respective probe. The probes and the relevant electronic boards are connected in parallel, so that the system is suitable to carry out diagnostic, environmental monitoring and toxicological self-monitoring and traceability in the food chain, including primary production, by early identification, control, monitoring and managing of anomalous variations of an integrated grid of indexes, with respect to a variation range which is considered normal. Finally, the data processing unit is suitable to carry out a statistic analysis, such as a multivariate analysis, to assess integrated indexes.
The BEST Platform was applied to the milk chain monitoring in the ALERT project - www.alert2015.it- (Consortium integrates 3 public scientific bodies: Istituto Superiore di Sanità, Consiglio Nazionale delle Ricerche, Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, and 7 qualified Italian Italian small medium-sized enterprises encompassing the fields of dairy production, sensor technologies and marketing).
Through these applications, BEST patent aim for a fundamental step forward: from invention to innovation through the encounter between Technology Industry and Public Health.

BEST Technological Platform (PCT WO/2010/001432)

Integrated Toxicity (Bio) Sensors' System for hazard analisys and management in the food chain and the environment

Application in the **milk chain**:

- ✓**Monitoring** the **wholesomeness** and **quality** of **cow's milk**
- ✓**Early identification** of **anomalies** in **production**
- ✓**Milk chain traceability**

BEST system consider milk as:

- a food **product**;
- a **bioindicator** of **livestock health status**;
- a **process indicator** (useful for company management)

Acquisition of a multiparametric real-time and daily "fingerprint" of milk

(**simultaneously** acquired) **chemical, physical-chemical and biological parameters** linked to:

- The presence of **contaminants**;
- **Milk quality** (composition and wholesomeness)

→ metabolomics approach

Control chart → to highlight **non-physiological anomalies** of specific **parameters** within a given milk **production**

Use of **indexes**, **(bio)indexes** and **integrated markers** (derived from more indexes) traceable in continuously (through the entire production chain)

Usable by **livestock operators** (even **on the Web**)
→Complete transferability to productive practices and to on-farm realities

Agreement between Official control and self-monitoring: data report in an Italian dairy chain

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Background & Aim

Cow milk is a relevant component in the Western Consumer's diet. The nutritional and economic value of raw milk, as well as its physicochemical properties are directly dependent on milk composition, which, in turn, give valuable information on herd nutritional status and general health. Daily measurements of milk components, both at the individual and herd level, is becoming a common tool to assess the safety and economic value of milk production. Following EU Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002, Italian milk processing industries follow a strict self monitoring process per HACCP, and are subject to Official control by the Italian Competent Authority (Ministry of Health, Moh). A network of 10 Experimental Zooprophylaxis Institutes (IZS) provide accredited laboratory services for analyses for raw milk samples.

However, limited scientific evidence is known about measurements agreement between the milk analyses carried on by official control bodies and the internal ones done by milk processing industries. A number of factors could affect this agreement. Different methods in milk component quantifications (different techniques, with different LOD and LOQ, as well as measurement uncertainty) could be a primary source of disagreement. A second difference between the two measurement chains is that, while the internal industrial monitoring routines are carried on immediately after the raw milk arrival at the processing premises, official monitoring procedures are carried on by sampling the milk, and delivering the sample to the accredited laboratory. While this procedure is validated and refrigerated transportation is routinely used, the effect on raw milk samples biological parameters, especially during the hot season, may be still partly understood.

The main aim of the present study is to verify the agreement between Official Control and Self-control measurements in raw milk processing industry, identify any possible discordances or biases, and to possibly point out procedures to overcome these discordances.

Methods

In the framework of the Alert 2015 (http://www.alert2015.it/) project funded by the "Industria 2015" program of the Italian Ministry of Economic Development, a chance was given to collect simultaneous data coming from the official control IZS- LT (Lazio and Tuscany Section) and the industrial Partner Centrale del Latte di Roma Spa (CLR).

Milk production and component measurement data were obtained during a purposely developed monitoring program involving CLR, a FSSC 22000:2010 – certified milk processing plant with an average 350000l/day throughput. The monitoring program started in March 9, 2014 and ended in June 24, 2014. Data collection regarded the daily raw milk collection carried on by CLR on a regional basis, consisting in a daily average 137000 Kg of raw milk collected from local farmers via a fleet of 15-metric tons refrigerated trucks. Traceability of bulk milk was limited to groups of individual farmers. All samples were taken following rules matching both CLR internal procedures and IZS procedures.

The first sample was immediately sent to CLR internal labs for analyses, while the second sample was kept refrigerated and sent to the IZS labs. A total of 114 twin samples were collected and analyzed for 7 twinned variables (Freezing Point, Aflatoxin, Total Bacterial Count, Somatic Cell Count, fat, lactose and proteins percentages). Descriptive statistics, correlation analysis, paired sample Wilcoxon Signed Rank test and a Bland-Altman analysis was performed in order to assess measurement agreement between CLR and IZS determinations. A General Linear model was used to investigate the effect of CLR Total Bacterial count, time between sampling and IZS analysis, truck tank temperature and farmer's group on the difference between CLR and IZS Total Bacterial Count.

No outlier removal procedure was used. For all statistical analyses, the significance threshold was set at .05.

Results

A Shapiro Wilk normality test performed on each variable in the data set showed that almost all variables could not be modeled by a normal distribution, except for CLR Lactose, CLR Proteins, and IZS Proteins. A Wilcoxon signed ranks test on the twinned variables assessed that Freezing Point, Aflatoxin, Total Bacterial Count, Somatic Cell Count, Lactose and Fat data showed a significant difference in the median of the paired samples.

Correlation and Bland-Altman analysis concluded for a sound agreement between CLR and IZS Proteins measurement. A moderate to good agreement was found for Somatic Cell Count, Fat, Lactose and, to a lesser extent, for Freezing Point determinations. Low agreement was found between CLR and IZS Total Bacterial Count determinations. The General Linear Model highlighted that the difference between CLR and IZS Total Bacterial Count was not influenced by time between sampling and analysis and truck tank temperature. A significant influence on the difference was CLR Total Bacterial Count. Farmer's group (i.e. the group of farmers whose bulk milk was collected together in the truck tank) had a lower contribution on the difference between CLR and IZS Total Bacterial Count.

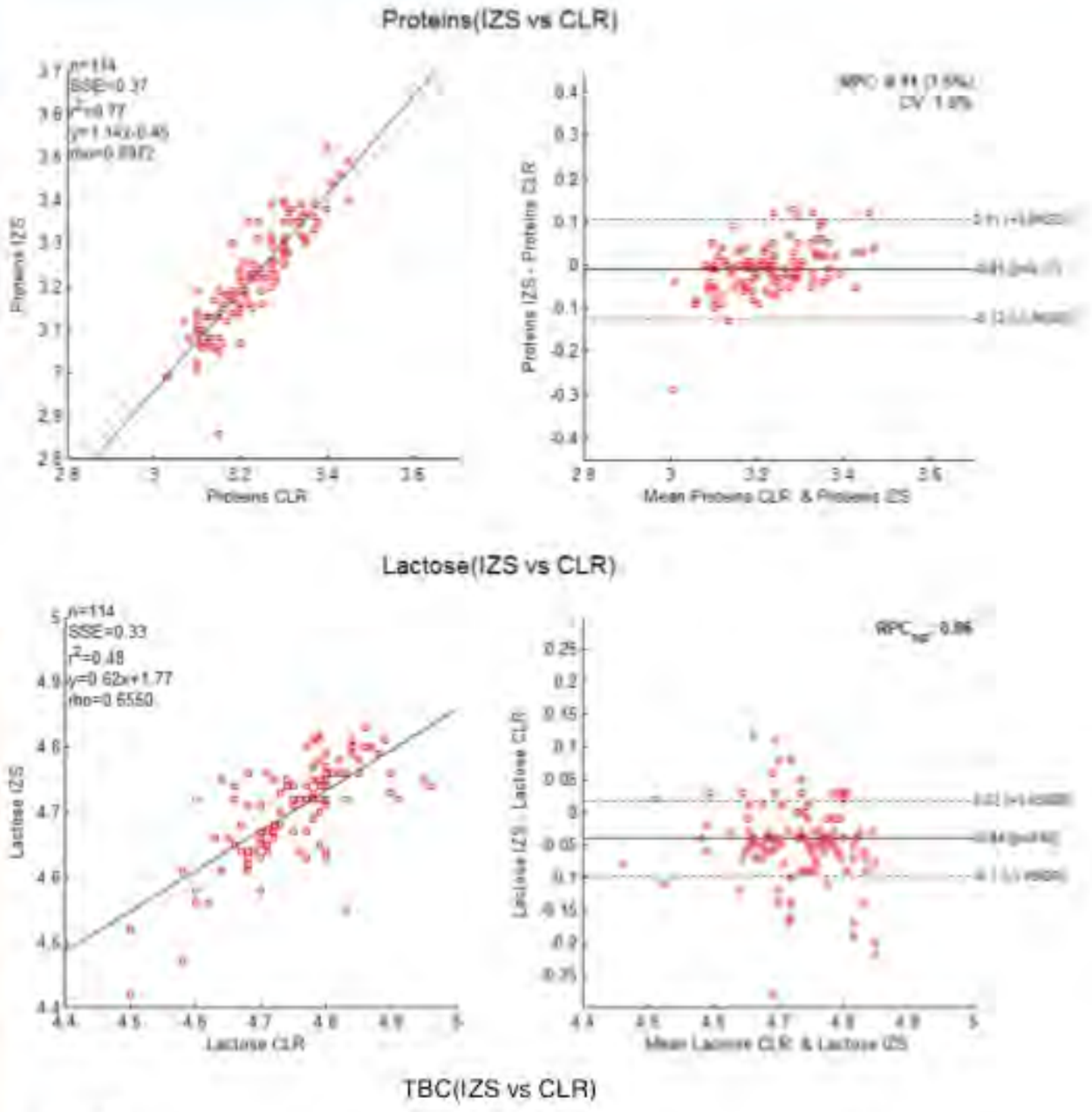

Figure 1 A,B,C: examples showing good, moderate and bad agreement betwheen Official Control and Self Control measuremts

Conclusions

This study confirmed a good/ moderate to good agreement between Official Control and Self-control measurements in raw milk processing, at least for Proteins, Somatic Cell Count, Fat, Lactose, and for Freezing Point determinations. A remarkable discordance was found for Total Bacterial Count, which, in line of principle, could not be attributed to differences in the analytical methodologies. Truck tank temperature, and the number of hours between sampling and IZS analysis did not affect this discordance. In turn, the absolute value of CLR Total Bacterial Count did affect the difference, as well as the milk origin (traced up to groups of farmers). Further research is needed to identify how these factors can influence the observed discordance.

Authors

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