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International Conference on Chemical Sensors

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University of Warsaw, Warsaw, Poland
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National University of Singapore, Singapore
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Abstracts of oral presentations

Importance of Viruses in spite of a Pandemic

Karin Moelling

Institute for Medical Microbiology, University Zurich, Zurich, Switzerland
and Max Planck Institute for Moleculr Genetics, Berlin, Germany
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Viruses are ubiquitous in every habitat and are the most abundant species on our planet. Most ancient are RNA-containing viruses. They may have contributed to the origin of life, since naked RNA-only viruses, the viroids, lack genetic information. They fulfill several criteria of life. Could such structures have contributed to life on exoplanets somewhere in the Universe?

The enzymatic activity of viroids as ribozymes are the essential motors of protein synthesis in ribosomes and must have preceded the protein world. Viruses are innovative, can integrate into genomes thereby supplying novel information and immunity to the host, protecting against superinfection by exogenous viruses. They are the drivers of evolution. As such they contributed e.g. to the placenta in mammals. The human chromosomes consist of about 50% viral elements. Sub-viral structures such as transposable elements can contribute to genetics beyond Darwinian mutations.

Bacterial viruses, the phages, can help to recycle nutrients in the oceans and our intestines and can destroy multi-drug resistant bacteria as phage-therapy if antibiotics fail. Antibiotic resistance my soon become critical. Virus-like structures are required for gene transfer for gene-therapy, vaccines, and gene-modified organisms including animals and plants for food production.

Viruses are opportunists and cause diseases if environmental conditions change, many of which are based on our life-style and cannot be easily modified. Two parameters promote pandemics, high population densities and high mobility. Megacities and traveling as evidenced by events, festivals and air pollution are man-made problems in favor of pandemics. Mankind suffered many diseases and pandemics – which changed history. Even efficient new surveillance systems may fail to prevent the appearance of new pandemics.

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1. Moelling K, Viruses more friends than foes, World Scientific Press, 2nd ed, Singapore, **2021**.
2. Moelling K, Viruses to kill bacteria, a way out of the antibiotics crisis with phages – the viruses of bacteria. Dr. Friedrich Pfeil Press, Munich, Germany, **2022**.

Engineered Proteins From Gene to Diagnostics

Elizabeth A H Hall[1], Dushanth J Seevaratnam[1], Francis Krampa[1], Madhuri Manohar[1], Antony P Jackson[2], Samir W Hamaia[2], Gordon A Awandare[3], and Felix Ansah[3]

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Biosensor-diagnostics used in low and middle income countries (LMICs) are often imported from high income countries as a finished device or assembled locally from kits produced elsewhere. The result is that the devices are high cost when taken in the local context. The required biological reagents for a diagnostic are often the largest proportion of its total cost (typically 50-85%). Without making innovative changes to the end measurement we have revisited the 'unaffordable' diagnostics and considered the challenge of end-to-end local manufacturing of the biosensor, redesigning the biological reagents for direct integration into the final device or reagent kit. We will report on a design that can be manufactured locally, with basic infrastructure. We have used synthetic biology and incorporated locally resourced materials targeted to easy local production in resource poor areas.

Taking a 'gene to diagnostic' approach, a rational design will be discussed for a diagnostic platform that uses multifunctional fusion enzymes for point-of-care diagnostics. The platform is demonstrated for a BST large fragment (BST_{LF}) DNA polymerase fusion protein which can be isolated on silica via a fused R5 silica-affinity peptide and used in nucleic acid diagnostics. Data from a clinical study of malaria are presented raising some new questions about primer base sequence in loop-mediated isothermal amplification (LAMP) performance, possibly as a result of different binding of β- and γ-phosphates of the dNTPs. In malaria testing, the limit of detection depended on Plasmodium species and primer set. For example, 1000 copies of *P. knowlesi* 18s rRNA could be detected with the P.KNO-LAU primer set, with Si-$R5_2$-mCh-FL-BST_{LF} but even 10 copies of *P. ovale* 18s rRNA could be detected with the P.OVA-HAN primer set. The results are discussed in comparison with qPCR and sampling protocol and show that the Si-BST_{LF} polymerase can be optimised to meet the WHO recommended guidelines.

The principles are further applied to a device for sarcosine determination in urine (as a marker of early-stage prostate cancer), while other enzyme fusions have been engineered with luciferase and using silk-like proteins. Most recent engineering has used single chain antibody (scFv) fusions to produce self-labelling lateral flow devices using distributed low cost green manufacturing. We have the first step towards providing low cost diagnostics in resource poor areas, which could deliver a sustained improvement in healthcare, while also developing the local economy.

Wearable Bioelectronic Devices

Joseph Wang

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The rapid development of wearable sensing and interfacing electronics is facing challenges in sustainability and energy independence. Wearable bioelectronic devices have demonstrated considerable promise for on-body wearable applications ranging from non-invasive continuous biomarker monitoring to epidermal energy harvesting the realization of self-powered health monitoring systems. This presentation will discuss our recent efforts towards developing wearable bioelectronic systems, capturing non-invasively molecular information, for obtaining comprehensive information about the wearer health, nutrition and wellness. Particular attention will be given to epidermal devices for monitoring sweat and ISF. By leveraging the advantages of flexible electronics, biocatalysis, and electrochemistry, and addressing key challenges, wearable bioelectronic devices could have a tremendous impact on diverse fields.

Nanozymes Defeating Natural Enzyme for Electrochemical Sensing

Arkady A. Karyakin, **Elena Daboss**

Chemistry faculty of M.V. Lomonosov Moscow State University, 119991, Moscow, Russia
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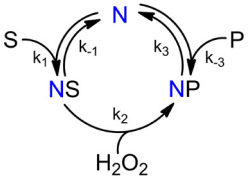
The term 'nanozyme' is generally accepted for catalytic nanoparticles. Compared to the enzymes, their advantages are (i) high stability and (ii) low cost in case they do not contain noble metals.

We synthesized Prussian Blue nanoparticles through catalytic reaction involving hydrogen peroxide activation. The resulting nanoparticles display the size-dependent catalytic rate constants in H_2O_2 reduction, which are significantly (up to 4 orders of magnitude) greater compared to those for natural enzyme peroxidase. The advantages of the reported PB nanoparticles over the known peroxidase-like nanozymes are their true enzymatic properties: (i) enzymatic specificity (an absence of oxidase-like activity) and (ii) an ability to operate in physiological solutions [1].

In contrast to the enzyme peroxidase, whose active site first interacts with hydrogen peroxide, in the case of the nanozymes, H_2O_2 oxidizes their complex with reducing substrate. Slow release of the product is the last step. The interaction of substrates with the nanozymes is 100 times faster than with enzyme peroxidases, and the rate-limiting constant for the nanozymes is also 2 orders of magnitude greater. Thus, the discovered novel advantage of nanozymes over the corresponding enzymes is the 100-fold greater bimolecular rate constants [2].



Simple drop-casting of the nanozymes with subsequent annealing results in modified electrodes with high electrochemical and electrocatalytic activity. In contrast to bienzyme biosensors, we propose the nanozyme-enzyme based ones substituting the enzyme peroxidase with the more active and stable nanoparticles "artificial peroxidase" [3].

Acknowledgements

Financial support through Russian Science Foundation grant #19-13-00131 is greatly acknowledged.

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Electroanalytical Bioplatforms in Their Unstoppable Race to Prove Their Competitiveness and Versatility: Control, Assessment, and Monitoring of COVID-19 Infection and Immunity to Emerging SARS-CoV-2 Variants of Concern

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The changing nature of SARS-CoV-2 mutant strains results in the emergence of Variants of Concern (VOCs), whose ability to evade natural or vaccine-induced immunity is of major concern in controlling and managing SARS-CoV-2 infection [1]. In this regard, tracking the adaptive immune response to SARS-CoV-2 infection by monitoring specific immunoglobulin (Igs) isotypes against the most antigenic viral proteins may provide insightful information which could help to implement personalized vaccination and prevent potential future pandemic outbreaks. Being fully conscious of the supreme priority of practical analytical tools to oversee the upcoming waves of the COVID-19 pandemic, we are pleased to share here one of our most recent and versatile electrochemical bioplatforms offering superb capacities for SARS-CoV-2 infection interrogation, vaccine efficiency evaluation, and immune response monitoring against the most predominant VOCs. Multiplexed bioplatforms were devised by the rational merging between protein engineering protocols and electrochemical transduction mode, by using commercial magnetic microparticles as solid supports onto which SARS-CoV-2 Spike (S) and Nucleocapsid (N) viral proteins were anchored to capture specific serum SARS-CoV-2 Igs, followed by amperometric reading, based on the HRP/H2O2/HQ redox system. The potential of developed bioplatforms was thoroughly examined in control and PCR-confirmed SARS-CoV-2 infected subjects, as well as in non-immunized and immunized individuals. And last but not least, to go a step further, our bioplatforms have successfully tackled the evaluation of some of the most worrisome mutant-specific S variants [2], in a pioneering way with one of the simplest but most powerful detection devices: electrochemical biosensors.

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"Non-conventional" Methods to Detect Viruses

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Viruses can be detected either directly or indirectly through the host immune response (e.g. detection of virus-specific antibodies generated in response to the infection). Direct detection certainly prevails to characterize and standardize virus preparations, and is also used for medical diagnostics because it enables genotyping and is more specific than the measurement of host immune responses (e.g. it is not restricted by seroconversion times, co-morbidities or treatments). However, the majority of direct detection methods target only a single component of the virus, i.e., characteristic nucleic acids and proteins even though virus pathogenesis involves whole virus particles. Thus the measurement of intact virus particles may be considered already non-conventional given the current landscape of virus analytics. We previously worked out methods based on nanopore sensing[1] and fluorescent nanoparticle tracking analysis[2] to approach the quantitation of viruses even in clinically relevant settings. These require both extremely sensitive detection methods, i.e. detection of single virus particles, and highly selective and affine ligands to bind characteristic proteins exposed on the virus surface.

This talk will introduce a novel, seemingly general, enabling concept, to make nanoparticles that are "invisible" to electrochemical detection quantifiable by nanoimpact electrochemistry.[3] The essence of the concept is a surface induced chemical encapsulation process, by which nanoparticles, including viruses, can be enclosed in a redox active metallic shell. The proof of concept is shown through the detection of Influenza A that suggest advantages also in terms of stochastic sensing by optical means.

The talk will be rounded by introducing also a new class of synthetic ligands for the selective detection of SARS-CoV-2 based on epitope imprinted polymers.[4] In this respect we managed to address the deficiencies of the highly empiric development methodologies by implementing a chip-based platform for both high-throughput synthesis and label-free characterization of the synthetic ligands. Already a single chip screening led to polymeric ligands with dissociation constants in the lower nanomolar range for the receptor binding domain (RBD) of the SARS-CoV-2 spike protein, which surpasses the affinity of RBD for its natural target, angiotensin-convertase 2 enzyme.

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The Electrochemical Modulation of Single Molecule Fluorescence

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The electrochemistry of single molecules is one of the frontiers in electrochemistry. The challenge in exploring single molecules is getting a signal from that molecule. There are three common approaches to doing this which are 1) monitoring single or a few electrons, 2) converting electrons into photons or 3) using a single molecule to modulate the flow of charge in an electrochemical system as performed with nanopore sensors [1]. In this talk we will present our findings using the latter two strategies.

In this presentation we will discuss how we can use total internal fluorescence microscopy (TIRF) to follow the fluorescence of single Alexa Fluor-647 labelled bovine serum albumin molecules adsorbed onto indium tin oxide (ITO) electrode surfaces. What was observed was the fluorescence of the Alexa-647 could be reversibly modulated as a function of the potential applied to the ITO. The fluorescence intensity of the Alexa Fluor 647 decreased, or even disappeared, at negative potentials but returned to similar levels to open circuit potential when the potential was swept back positive [2]. An observed pH dependence in the fluorescence strongly suggested the involvement of electron and proton transfer in the switching of the fluorescence. A mechanism for the potential modulating of fluorescence is shown. We then surveyed a variety of other fluorescent dyes and the switching behavior is correlated with molecular structure. The importance of this electrochemical control over the fluorescence of single molecules for the super-resolution light microscopy method, single molecule localisation microscopy will be discussed.

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Aptamer-Modified Nanopipettes for Neurochemical Sensing in Localized Brain Regions

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Advancing our understanding of brain dys(function) necessitates novel nanotools that can monitor chemical signaling at spatial resolutions that approach nanoscale synapses. While advanced methods to record electrical signaling from neurons are prevalent, tools to monitor neurochemical signaling have been limited. We have tackled this challenge by coupling the inherent selectivity of DNA-based recognition elements termed aptamers, with nanoscale pipettes with openings of *ca*. 10 nm. Aptamers are systematically designed oligonucleotide receptors that exhibit highly specific and selective recognition of targets. Aptamers that recognize small-molecule neurotransmitters, including serotonin and dopamine, have recently been isolated [1]. Upon reversible target binding, aptamers undergo a rearrangement of the negatively charged backbone, and these dynamic structural changes can be transduced as measurable changes in current through the nanoscale orifice of the sensors [2]. Nanoscale confinement of the sensor surface reduces biofouling for long-term recordings in complex environments, overcoming a critical bottleneck for clinical biosensors [3]. We have demonstrated the capacity to detect physiologically relevant differences in neurotransmitter amounts released by live neurons in complex media with unprecedented sensitivity [4]. In parallel to conducting measurements of chemical flux in localized brain regions, we aim to target diverse targets through this generalizable method.

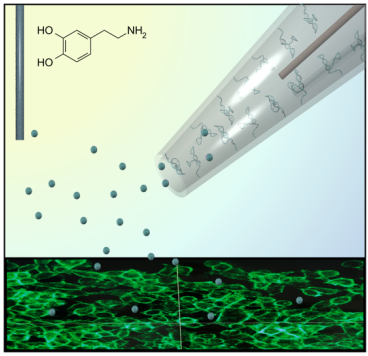

Figure 1. Dopamine aptamer-modified nanopipettes with ~10 nm pore openings can approach neurons in vitro and be inserted into brain slices for real-time, selective dopamine sensing with nanoscale spatial resolution.

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Digital Sensing with CMOS-Based Nanocapacitor Arrays: Formation of Lipid-Supported Bilayers as Case Study

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CMOS-based nanocapacitor arrays allow locally probing the impedance of an electrolyte in real time and with sub-micron spatial resolution. At sufficiently high frequencies the electric field penetrates beyond the electrical double layer caused by screening ions, allowing a form of electrochemical imaging of micron-sized synthetic and biological entities [1,2]. For nanoscale analytes, on the other hand, the response takes the form of discrete, step-like changes in impedance upon binding to the surface of an electrode [3]. Here we illustrate these capabilities by monitoring in real time the formation of a lipid bilayer from the fusion of lipid vesicles [4]. Several nanoscale vesicles are detected as they impinge upon the surface of each individual electrode and gradually coat its surface. Even though the impedance signal at each of the 2^{16} electrodes is stochastic in nature, the total response exhibits the smooth behavior expected for the formation of a macroscopic lipid bilayer. This work is a collaboration with NXP Semiconductors.

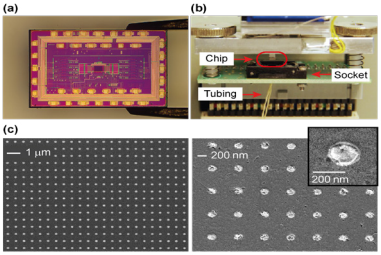

Figure 1. CMOS chip enabling real-time monitoring of supported lipid bilayer formation at sub-micron resolution. Changes in the capacitive response of the ~65,000 separately addressable electrodes in the array allow visualizing individual lipid vesicles as they interact with the surface and create the supported lipid bilayer.

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Electrokinetic Enrichment of Analytes Integrated with Label-Free Electrochemical Sensing

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Sensors that leverage the influence of a biorecognition event on charge transport, such as field-effect transistors and nanoporous membranes, are among the most sensitive because they translate localized binding into a change in a system-scale property. However, fabrication and custom functionalization of these sensors is not trivial, and their integration with protocols that pre-enrich target species and facilitate their transport to the biorecognition site is an active area of research. In this presentation, we demonstrate that ion concentration polarization (ICP) in the presence of fluid flow drives focusing and efficient capture of target nucleic acids within a bed of oligoprobe-conjugated beads embedded in a microfluidic channel. A key finding is that ion conduction along the surface of the bioconjugated beads is the dominant contributor to current through this channel segment under an applied voltage bias. Therefore, hybridization of a target nucleic acid (a polyanion) to the bead surface leads to a shift in the slope of the current-voltage curve. This approach is versatile in that a target nucleic acid can be detected electrically, in the absence of a label. The resulting approach allows for a plug-and-play nucleic acid sensor using off-the-shelf bioconjugated beads and simple electronics, making it advantageous for point-of-care testing. Finally, we share preliminary results for enrichment and sensing of other classes of analytes including viral RNA and antibodies.

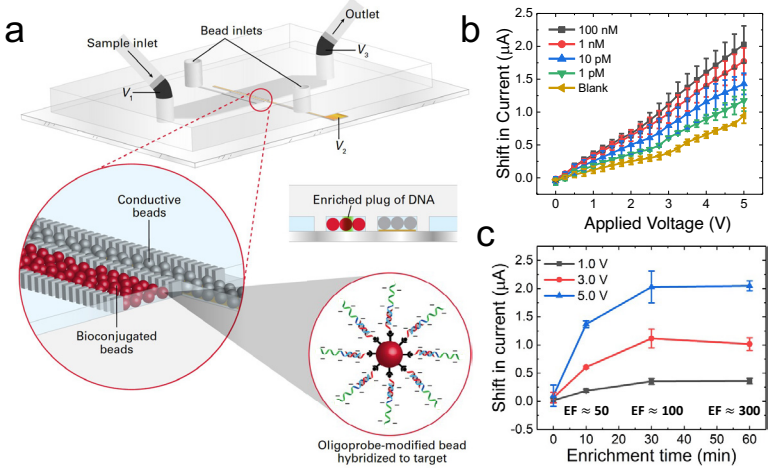

Figure 1. a) Illustration of the microfluidic device with voltage applied to metal interconnects at the inlet and outlet versus a 3D electrode comprising conductive beads. The callout shows detail of the packed beds of conductive and bioconjugated microbeads. b) Plot of the shift in current observed following 30 min enrichment of target DNA from the initial concentrations shown. c) Plot of the shift in current observed at 1.0, 3.0, and 5.0 V as a function of enrichment time. The enrichment factor (EF) is approximately 50-, 100-, and 300-fold at 10, 30, and 60 min of enrichment.

Modified Deoxyuridine Triphosphates for Direct Electrochemical Detection of Double-Stranded DNA Produced by Polymerase Chain Reaction or Recombinase Polymerase Amplification

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Deoxyribonucleic acid (DNA) carries genetic information in all living things and serves as a reliable biomarker of bacterial or viral contamination. To selectively and sensitively detect DNA, various amplification approaches such as polymerase chain reaction (PCR) and isothermal amplification techniques, both producing double-stranded DNA (dsDNA) amplicons, have been developed. Yet, the formation of a double helix hinders the direct electrochemical oxidation of dsDNA through guanine or adenine residues which become inaccessible for reactions on the electrode surface [1]. The aim of this work was to combine the *in vitro* DNA amplification with electrochemical detection of the generated dsDNA amplicons by incorporating electroactive 'labeled' nucleotides with the enzymatic reaction. To solve the problem of dsDNA electroactivity, several derivatives of 2'-deoxyuridine-5'-triphosphate (dUTP) modified via various linkers by aromatic groups of tyrosine, tryptophan [2], 4-nitrotoluene, fluorescein, or rhodamine were studied. By the square wave voltammetry on carbon screen printed electrodes, the oxidation or reduction peaks of all modified dUTP were obtained, similar to their free 'labels'. The tested derivatives of dUTP showed acceptable compatibility with PCR, when the electroactive 'label' was introduced into dsDNA amplicons with a length of 100–200 base pairs. However, in some cases, dsDNA products were not obtained by using modified dUTP instead of dTTP, probably due to specific DNA sequences. Among nucleotides under study, only a few modified dUTP compounds revealed good compatibility with the broadly used isothermal recombinase polymerase amplification. Generated dsDNA fragments with modified nucleotides demonstrated electrochemical signals at micromolar concentrations while no peaks were observed for unmodified dsDNA under the same conditions.

Acknowledgment

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Sulfate Bionsensor for Environental Applications

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Here we report on a novel microscale biosensor for sulfate based on the conversion of sulfate to sulfide by *Desulfovibrio desulfuricans* (DSM 9104). A H_2S microsensor (Unisense A/S) functioned as transducer.

In the bacterial chamber, interposed between the sensor opening and the transducer, the co-presence of the aerobe *Staphylococcus xylosus* maintains anoxic conditions necessary for sulfate reduction, thereby enabling the application of the sensor also in oxic media.

A sensor with a 420 μm long bacterial chamber and tip outer diameter of 90 μm exhibited a linear response up to 1.8 mM and a detection limit of ~1 μM. The 90% response time after variation in sulfate concentration varied between 80 and 220 s, depending on the sulfate concentration. Sensitivity and liner range could be modulated by electrophoretic migration of anions into the sensor, as shown in the figure below. Ferrous iron diffusing from anoxic media could, in principle, lower the sensor response by binding H_2S within the bacterial chamber; however, this effect was proved negligible at concentrations up to 1 mM of Fe^{2+}.

The sensor was successfully applied to characterize sulfate concentration and the depth-distribution of sulfate reduction in freshwater sediments, where very little free sulfide is present due to reaction and/or precipitation with iron compounds.

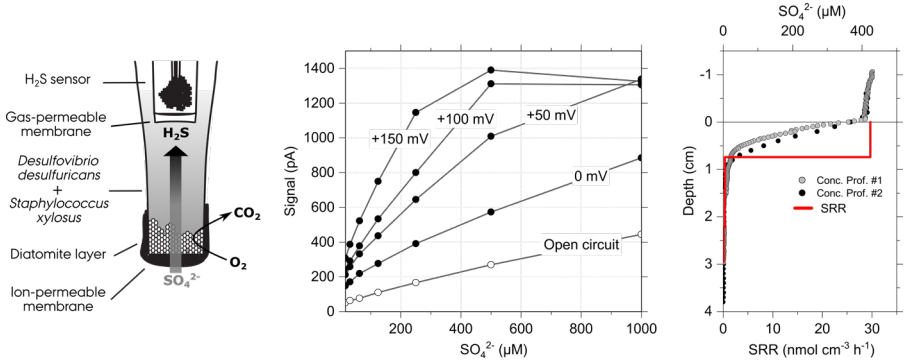

Figure 1. Left. Schematics of the sensor tip region. Center. Calibration curves of sulfate biosensor when operated at various applied potentials between an Ag/AgCl electrode in the bacterial chamber and an external reference electrode. Right: Depth distribution of sulfate in a freshwater sediment. Shown are two profiles measured a few cm apart and the rate of sulfate reduction (SRR) that can be calculated from the data.

Innovative Chemical Sensors Applied to Trace Metal Ecotoxicity Assessment and Natural Water Quality Management

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Assessing the impact of trace metals on aquatic ecosystems and ultimately human health is challenging. Trace metals are distributed in a variety of redox states and chemical species that may vary continuously in space and time [1,2]. Only some trace metal species are bioavailable. The development of robust and adaptive submersible sensitive trace metal bioavailability-assessment tools is therefore required to support the establishment of environmental quality standards and guidelines based on realistic risk assessment to protect aquatic life and biodiversity, and ultimately human health.

Toward this aim, we developed on-chip chemical sensors consisting of an array of interconnected iridium-based microdiscs that are electroplated with appropriate sensing elements and covered with a hydrogel as efficient antifouling membrane [2,3]. Incorporated in in-house submersible probes and interrogated by Square Wave Anodic Stripping Voltammetry, these gel-integrated microelectrode arrays (GIME) allow for the direct *in situ* quantification of the dissolved metal species that are available for uptake by phytoplankton (first chain of the food-web) [2.3]. To date, only trace metals that can be electrochemically reduced and pre-concentrated at the surface of the electrode could be measured. A wider range of trace metals may become accessible by adsorptive cathodic stripping voltammetry, as this approach allows for preconcentration by electrochemical adsorption of trace metal complexes upon adding a selective complexing agent to the sample. This technique has been mainly applied for the mercury hanging drop electrode or with renewable mercury or bismuth film electrodes. However, the renewal of the sensing element after each measurement is difficult to adapt for *in situ* measurements.

We report here on the optimization and evaluation of adsorptive cathodic stripping voltammetry for the direct quantification of the sufficiently labile Co/Ni dissolved species in aquatic systems towards a ligand on a time scale less than 10 minutes. This development forms the basis for the quantification of Technological Critical Elements such as Pt and Cr whose source, behavior and eco-toxicity are today not sufficiently understood.

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In-Situ Acidification in the Tip of a Microsensor: Measurement of pH Dependent Analytes Without Sample Pretreatment or pH Monitoring

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Some analytes are in a pH dependent equilibrium between their ionic and molecular forms. Two examples are the carbonate and sulfide systems, which are both important monitoring parameters in the environment as well as in industrial settings. While there are sensors for individual species in the pH dependent equilibria (e.g., CO_2 and H_2S), the measurement of the total pool is more complicated and cumbersome. Conventional methods usually rely on *ex-situ* methods that require sample pretreatment to convert the different species to the measurable one. In an attempt to facilitate *in-situ* sensor-based measurements, calculation of the total pool by simultaneous pH measurement is often applied. While measuring with two sensors is theoretically simple, combined inaccuracies and differences in response times may lead to erroneous estimates. Spatial heterogeneity may also induce errors.

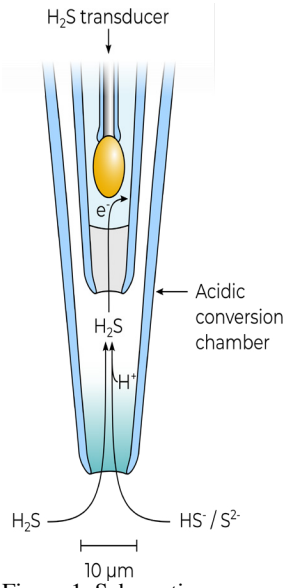

Figure 1. Schematic representation of a total sulfide microsensor.

To overcome these problems, we have equipped Clark-type microsensors with an acidic outer chamber to convert all ionic species to the measurable molecular form, which is then oxidized or reduced at a transducer electrode. This technique facilitates measurement of the total pool of pH dependent analytes at high spatial and temporal resolution even at pH values where most analyte is in the ionic form.

Applying this principle, we developed an microsensor for total dissolved inorganic carbon (DIC) by equipping an amperometric CO_2 sensor with an acidic conversion chamber. Both dissolved CO_2 and ionic carbonate species (HCO_3^- and CO_3^{2-}) can penetrate the tip membrane and the latter get converted to CO_2, which in turn is reduced at a silver cathode in the transducer. The sensor (tip diameter 10 μm) shows a linear response over a wide range (0-8 mM DIC) with a calculated LOD of 5 μM and a 90 % response time of 150 s. Since the sensor did not show any cross sensitivity to ionic strength it could be successfully used to determine DIC in bottled mineral water and seawater [1].

In a similar way we modified an amperometric H_2S microsensor with an acidic outer chamber to convert ionic sulfides (HS^- and S^{2-}) to H_2S, which is oxidized at the transducer electrode (Figure 1). First preliminary experiments in freshwater and seawater sediment profiling showed promising results. Additionally, this sensor could also be used for monitoring sulfide in sewage systems, where high levels of sulfide can lead to severe corrosion problems.

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Symmetry with Solid-Contact Membrane Electrodes

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Throughout the history of potentiometric sensors, the concept of symmetry has been central for assuring optimal signal stability. With pH electrodes, for example, the internal Ag/AgCl element at the indicator electrode side is accompanied with a similar Ag/AgCl electrode at the reference electrode side. The overall cell potential no longer depends on the specific redox couple because the two are compensated. Commercial pH probes of high E^0 potential reproducibility and stability are designed for the two redox elements to be similarly configured and placed within the sensor body so that temperature fluctuations affect both elements at very similar rates. This concept of symmetry was also used by Simon for the realization of a calibration-free potentiometric sensor where the observed value of the cell potential gives direct information about sample ion activity [1].

Unfortunately, this symmetry is broken when the internal Ag/AgCl element of an indicator electrode is replaced with a solid contact transducing material because the reference element is now formed by a different redox couple. This is unfortunate because a major research effort of recent years has been the aim to realize highly stable all-solid-contact potentiometric probes, which is not helped by the asymmetric nature of the cell.

This talk will underline the importance of symmetry in such electrochemical sensors and show examples on how it can be restored with modern all-solid-state potentiometric probes. Examples will include a recently published example for the detection of the nutrient nitrate [2] where some nitrate is added to the bridge electrolyte of the reference element so that a second nitrate electrode of the same design, rather than an Ag/AgCl can be used. This study will then be extended to solid contact pH probes that may behave in complete analogy to commercial pH probes, with a zero potential value at pH 7. It will be evaluated whether symmetry may also help to avoid the lengthy conditioning periods often required for best stability. Finally, the concept of symmetry will be applied to the design of submersible aquatic sensing probes.

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Conjugated Redox Polymer as Solid Contact in Potassium-Selective Electrodes

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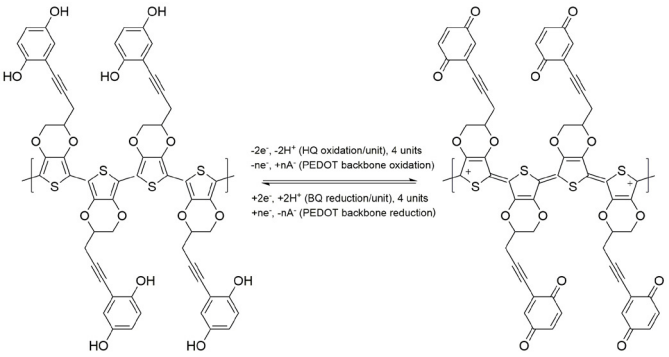
It is reported for the first time that a conjugated redox polymer with hydroquinone (HQ) pendant groups covalently attached to the poly(3,4-ethylenedioxythiophene) (PEDOT) backbone [1] has been used as the solid contact (SC) in PVC-based solid-contact electrodes [2]. The SC itself has ca. 25-30 times higher redox capacitance than unsubstituted PEDOT due to the HQ groups. We show that it is possible to precisely adjust the potential of the SC to ±0.4 mV (n=5) prior to application of the K^+-selective membrane (ISM) on top of it. However, after that, the potential reproducibility of the solid-contact electrodes decreased to ±2.8 mV. Our study reveals that only a minor fraction of the redox capacitance of the PEDOT-HQ solid contact can be utilized when it is placed under the PVC-ISM. This makes us conclude that the PVC-ISM compositions adapted from the liquid contact ISEs may not necessarily be the most suited for solid-contact electrodes.



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Solid-Contact Ion-Selective Electrodes Based on Redox-Functionalized Carbon Nanotubes for Improved Potential and Batch-To-Batch Reproducibility

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Solid-contact ion-selective electrodes (SCISEs) have long been the focus of interest during the development of low-cost, compact, miniaturized chemical sensors. [1] Most typically, different electrically conducting polymers and high surface area carbon materials (e.g., carbon nanotubes, graphene, carbon black etc.) have been tested as solid-contacts. However, the need persists for the appropriate material to enable a reliable large-scale fabrication process that provides sensors with excellent potential reproducibility and stability before SCISEs can be widely commercialized as ready-to-use wearables sensors in medical or sport devices with minimal maintenance. [2]

Here, we introduce redox-functionalized multi-walled carbon nanotubes (MWCNTs) as a novel solid-contact type that combines the advantageous properties of purely capacitive materials and redox-couples. [3] To introduce the redox-active functional group we modified MWCNTs with either ferrocene (Fc) or (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO), and used the thus formed material as solid-contact in K^+-ISEs as proof of concept. We applied different approaches (polarization, short-circuiting) to improve the potential reproducibility and stability of the SCISEs. Our investigation has shown that the presence of the redox group enables the E^0 adjustment via pre-polarization before the functionalized MWCNTs are applied as a solid-contact on the substrate electrode, making any subsequent potential improvement step after ISE fabrication unnecessary. The process not only improved the potential reproducibility for a single set of electrodes (SD=0.43 mV for n=6) but proved to provide excellent batch-to-batch E^0 reproducibility as well (SD=2.13 mV for three batches of 6 electrodes based on TEMPO-MWCNT solid-contact) with a span of one year between the first and the last batch. The manufactured K^+-SCISEs showed excellent potentiometric response and selectivity with no sensitivity to ambient gases (O_2, CO_2) and light and also without any sign of an aqueous layer formation.

Acknowledgment

This research was funded by the National Research, Development, and Innovation Fund of Hungary under Grant TKP2021-EGA-02. Further support was received from National Research, Development and Innovation Fund through ÚNKP-21-3 New National Excellence Program of the Ministry for Innovation and Technology.

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Bubbles in an Ion-Selective Membrane: New Approach to Control Accumulation of Water at Solid-Contact | Membrane Interface

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Transmembrane ion fluxes have earlier been identified as a source of potential instability in solid contact ion-selective electrodes (SC-ISEs). In this work, foamlike structures were intentionally introduced into a potassium-sensitive plasticized poly(vinyl chloride) ion-selective membrane (ISM) near the membrane|solid contact interface by controlling the temperature during membrane deposition. Foamlike structures in the ISM were shown to be effective at physically tailoring the transport of ions in the ion-selective membrane, greatly reducing the flux of interfering ions from the sample to the membrane|solid contact interface. The drifts during a conventional water layer test were hence able to be greatly mitigated, even with SC-ISEs incorporating a relatively hydrophilic poly(3,4-ethylenedioxythiophene) doped with poly(styrenesulfonate) (PEDOT:PSS) solid contact. In solutions with a high background concentration of interfering ions, equilibrated ion-selective electrodes with foamlike membranes were able to reproduce their initial potentials within 0.6 mV uncertainty (n = 3) from 0 to 18 h. This was achieved despite sensor exposure to solutions exceeding the selectivity limit of the ISEs in 3 h intervals, allowing improvement of the potential reproducibility of the sensors. Since the introduction of foamlike structures into ISM is linked to temperature-controlled membrane deposition, it is envisaged that the method is generally applicable to all solid contact ion-selective electrodes that are based on polymeric membranes and require membrane deposition from the cocktail solution [1].

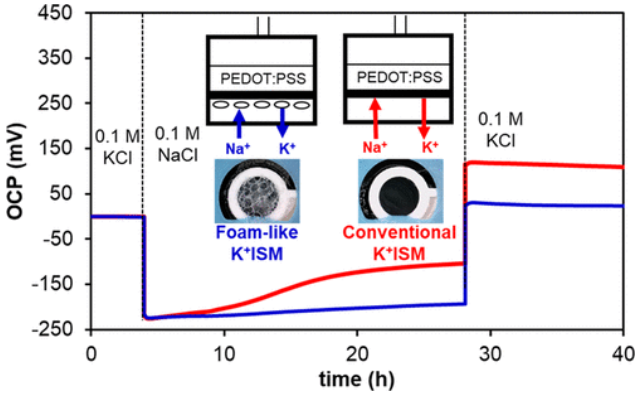

Figure 1. Water layer test for K^+-ISEs with conventional and foamlike membranes.

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Solid-Contact Sensors with Covalent Attachment of Ionophore and Ionic Sites to the Polymeric Sensing Matrix

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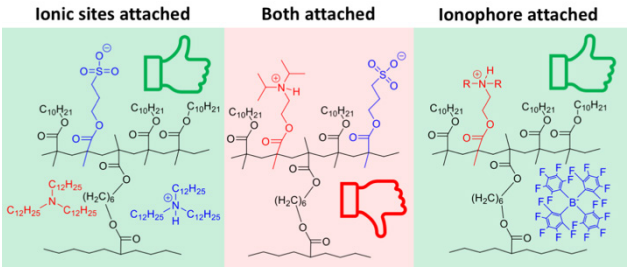
With a view to improving the sensor lifetime, solid-contact ion-selective electrodes (ISEs) were prepared with a plasticizer-free and cross-linked sensing matrix to which either only the ionic sites, only the ionophore, or both the ionic sites and ionophore were covalently attached. In earlier work with covalently attached ionophores or ionic sites, it was difficult to discount the presence of ionophore or ionic site impurities that were not covalently attached to the polymer backbone because the reagents used to introduce the ionophore or ionic sites had high hydrophobicities. In this work, we deliberately chose readily available *hydrophilic* reagents for the introduction of covalently attached H^+ ionophores with tertiary amino groups and covalently attached sulfonate groups as ionic sites. This simplified the synthesis and made it possible to thoroughly remove ionophores and ionic sites not covalently attached to the polymer backbone. Our results confirm the expectation that hydrophobic ISE membranes with both covalently attached ionophore and ionic sites have unpractically long response times. In contrary, ISEs with either covalently attached H^+ ionophore or covalently attached ionic sites responded to pH with quick Nernstian responses and high selectivity [1]. Both conventional plasticized PVC-based ISEs and the new poly(decyl methacrylate) membranes were exposed to 90 °C heat for 2 h, 10% ethanol for 1 day, or undiluted blood serum for 5 days. In all three cases, the poly(decyl methacrylate) ISEs exhibited properties superior to conventional PVC-based ISEs, confirming the advantages of the covalent attachment. Further work is ongoing to covalently attach the sensing membranes themselves to the underlying electrode body [2].



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Novel Design of a Flow-Through Potentiometric Sensor

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A new design of a flow-through potentiometric sensor is presented. The objective of this work was to construct a durable ion sensor that is suitable for wearable sensor applications where the sample volume is very small. The design principle is shown in Figure 1. This planar ion-sensor design allows a large contact area between the solid-contact and the ion-selective membrane (ISM) and a large membrane volume, which are favourable for potential stability and sensor lifetime. The sample volume can still be kept small. Additionally, this design allows free sample flow through the sensor without any additional microfluidics.

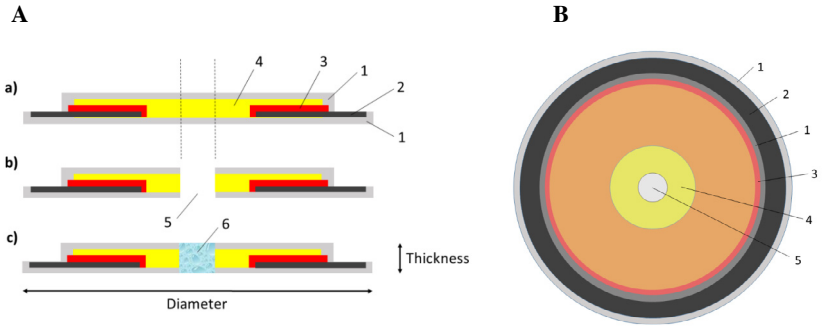

Figure 1. Design principle of a planar solid-contact ISE. 1) electrical insulator, 2) electronic conductor, 3) ion-to-electron transducer, 4) ion-selective membrane, 5) sample compartment, 6) sample. A) cross section: a) encapsulated, b) ready to use, c) with sample. B) top view.

The proof-of-concept will be presented for a solid-contact K^+-ion-selective electrode (K-SCISE), using carbon cloth as the ion-to-electron transducer and a plasticized PVC-based ISM with valinomycin as ionophore. The planar K-SCISE was characterized by potentiometry and electrochemical impedance spectroscopy.

The K-SCISE showed a Nernstian potentiometric response and no water-layer formation at the solid contact was detected. The selectivity coefficients (log $K_{i,j}$) were -3.2 (j = Li^+), -3.3 (j = Na^+), -4.3 (j = Ca^{2+}), and -4.7 (j = Mg^{2+}). Depending on the electrode geometry, the impedance of the planar K-SCISE was in the range of 45-275 MΩ.

The obtained results prove that that this concept of sensor design is feasible, while more work is needed to include also a solid-state reference electrode.

Acknowledgments
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Non-Constancy of the ISE Membrane Bulk Resistance: Origin and Possible Practical Application

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It is known that ionophore-based solvent-polymeric membranes of ion-selective electrodes (ISEs) contain droplets of water absorbed from solution. Therefore, these membranes, rigorously speaking, are essentially heterogeneous, consisting of a continuous organic phase and a dispersed aqueous phase. However, in theoretical models, the ISE membranes are considered homogeneous, and the presence of the dispersed aqueous phase is neglected. These models result in equations which nicely describe the ISE potentials and selectivity. These models also predict a constant value of the membrane bulk resistance within the Nernstian response range of an ISE. In contrast with the latter, our study revealed a significant variation of the membrane bulk resistance along with the concentration of the solution, and the magnitude of the effect increases at lower concentrations. Furthermore, the resistance correlates with the water uptake by the membranes [1, 2]. The explanation of these facts in view of the membrane heterogeneity will be presented. In a membrane the composition of the organic phase proper is constant within a certain range of the solution composition, and therefore the ISE obeys the Nernst equation, within this range. Lipophilic ion-ionophore complexes and ion-exchanger sites are confined to the membrane organic phase and avoid water droplets. Therefore, the presence of the droplets results in a decrease of the effective cross-section of the membranes. Additionally, if charged species encounter droplets, they must circumvent them, so the path-length of the species across the membrane increases. These two effects result in increase of the membrane bulk resistance along with water uptake [3]. A theory will be presented to explain why water uptake increases along dilution, although the chemical potential of water in diluted solutions is almost constant. It will be shown that the variation of the ISE membrane bulk resistance makes possible measurements of the concentration of the analyte in samples with an unknown ionic strength.

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Enhancing Quality Control in Analyzers for Clinical Applications

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Application of blood analyzers in the clinical laboratory and point-of-care (POC) environments has placed increased demands on instrument manufacturers to develop systems that are easy to use, require little maintenance and offer enhanced quality control to generate reliable test results [1, 2]. Most modern critical care analyzers have a high degree of instrument self-diagnostic/ hardware checks for proper functioning of mechanical, fluidics and electronic components. However, these hardware checks provide no information about the analytical measurement process, which includes the sensors and reagents. Assays of liquid quality controls (QC) provide a means for monitoring of the analytical system and the methodology has been automated by packaging the controls within the same measurement cartridge along with some simple automatic corrective actions. These corrective actions may include flushing of the fluidic pathways, sensor recalibration and parameter shut-off for persistent failure. Although automated QC systems require no user intervention to perform QC, the QC testing is still discrete and may not detect transient errors that may occur between QC assays or during sample analysis.

Published documents by the Clinical and Laboratory Standard Institute and by the International Organization for Standardization [3, 4] have provided guidance for managing quality in clinical devices. Suggested methodologies rely on understanding sources of error during each phase of testing process with the goal of designing integrated QC methods for rapid detection and correction of errors. The control procedure must detect immediate errors that occur due to test system failure, adverse environmental conditions and operation performance [5].

This report presents designing an integrated and intelligent QC method in GEM Premier blood gas analyzer for identifying detectable error patterns and devising targeted corrective actions. Timely detection of such error patterns is achieved through continuous monitoring of the measurement system. Methods for gathering and identifying error patterns is accomplished through detailed examination of past end-user data which is facilitated by having a closed analytical system and ability to collect extensive information during every stage of analytical operations.

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Continuous and Polarisation-Tuned Redox Capacitive Ion Sensing at Electroactive Interfaces

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Continuous, real-time ion sensing is highly sought-after across various environmental and medical scenarios but remains underdeveloped.[1] Herein, the potential of redox capacitance spectroscopy as a novel, sensitive and highly adaptable ion sensing methodology is demonstrated, exemplified by the continuous flow sensing of anions at redox-active halogen bonding ferrocenylisophthalamide self-assembled monolayers.[2] Upon anion binding, the redox distribution of the electroactive host interface, and its associated redox capacitance, are reversibly modulated, providing a simple and direct sensory readout. Importantly, the redox capacitance can be monitored at a freely chosen, constant electrode polarization, providing a facile means of tuning both the sensor's analytical performance and the anion binding affinity, by up to 1 order of magnitude. In surpassing standard voltammetric methods in terms of performance and adaptability,[3] these findings pave the way for the development of highly sensitive and uniquely tunable supramolecular ion sensors. More generally, this methodology also serves as a powerful and unprecedented means of simultaneously modulating and monitoring the thermodynamics and kinetics of host–guest interactions at redox-active interfaces, a capability that will undoubtedly be relevant to the investigation of a wide range of interfacial supramolecular systems.

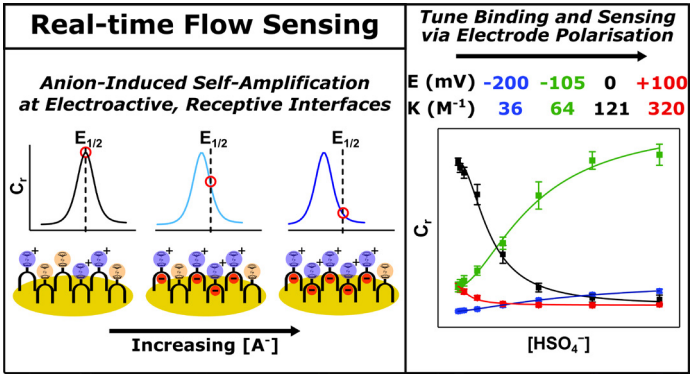

Figure 1. Redox capacitance spectroscopy at electroactive anion receptive interfaces enables sensitive, real-time, continuous HSO_4^- sensing in a flow cell.

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Investigating Electrochemical Devices with the Synergistic Combination of Experimental, Numerical and Machine Learning Methods

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Understanding the fundamental working principles of electrochemical devices is crucial in developing novel detection principles or describing the electrochemical response of devices in various experimental settings. When we have a sufficiently sophisticated theoretical model of the system, we can use the device's experimental response to an external stimulus to assess the most essential kinetic and thermodynamic parameters that govern their behavior. In many cases, this can be accomplished by using a simplified model of the system – or even an explicit equation – and fitting it to the experimental data. However, in a large number of situations, simplified models are inadequate. For example, the electrochemical response of many conventional electrochemical devices, such as the complex impedance of a sensor or charging-discharging of a battery, is governed by a complex interaction of the electric field and the diffusing-migrating species. The time-dependence of the response is not well-described by a simple equation. For these situations, we must resort to more sophisticated models, such as finite element numerical simulations.

Unfortunately, the simulation time of finite element models is on the order of several minutes, which can make the parameter estimation, where thousands of simulations are required, impractical. We have developed a fast and reliable method for speeding up the simulation of the electrochemical response in nonlinear and time-dependent systems to make parameter estimation feasible. We replace finite element simulations with supervised machine learning algorithms that can provide results six orders of magnitude faster. Two types of algorithms are presented. Feed-forward neural networks are black-box machine learning models, so exact knowledge of the internal mechanism and reasons behind the output of the network is not accessible to us. However, it is possible to use white-box machine learning models that provide results that are understandable for experts. Symbolic regression – an accurate white-box machine learning technique – automates the process of finding human-readable symbolic expressions that match the observed data.

In this work, we experimentally investigate electrochemical devices (e.g., sensors and batteries) and use trained models to predict their response. Our method speeds up parameter estimation and helps device development by providing a way to rapidly and accurately visualize the effect of kinetic and thermodynamic parameters on the electrochemical response.

Capillary-Flow Driven Microfluidic Sensors

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A major push in the field of sensor development is production of inexpensive, easy to use sensors that require minimal external equipment. Traditional microfluidic devices can handle small samples and automate many processes. However, they still require pumps and external equipment, limiting their use outside of laboratories and controlled environments. Microfluidic Paper-based Analytical Devices (mPADs) have received significant attention because they are inexpensive (costing pennies per device), easy to use, generate flow without pumps, and can carry out a wide range of chemical assays. mPADs are normally made from porous hydrophilic materials patterned with hydrophobic materials to create flow barriers to direct flow from a sample inlet through sample pretreatment zones to a detection zone. Furthermore, functional elements like reagent pads and electrodes can be readily integrated to provide additional functionality and assay selectivity. Despite their advantages, mPADs are frequently limited to long analysis times due to slow capillary flow and poor limits of detection for naked eye measurements. Our group has recently reported the development of capillary flow driven microfluidic devices that use channels formed using laser patterning of hydrophilic polyester films and double-sided adhesive combined with paper-based pumps and reagent storage systems to enhance the performance of mPADs [1,2]. The system can be adapted to use a wide range of detection modes, including electrochemical and optical methods. We have also applied the system to biological samples including whole blood and nasal swabs [3-4]. This talk will focus on the fundamental development of the capillary-flow driven sensors as well as applications for clinical and environmental diagnostics, including COVID-19 assays.

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Traffic Light Type Paper-Based Analytical Device for Intuitive and Semi-Quantitative Naked-Eye Signal Readout

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Due to increasingly aging societies, there is a strong need for point of care testing (POCT) devices suitable for self-diagnostics performed at home. In this context, microfluidic paper-based analytical devices (μPADs) have attracted great attention due to their user-friendliness. A variety of μPADs enabling semi-quantitative and equipment-free assay readout have been reported. However, most of them still face risks of result misinterpretation since they either require additional operation to read a result or involve a process of subjective judgment, posing an obstacle for true POCT by untrained users [1].

Here, we provide the proof-of-concept for a μPAD for highly intuitive semi-quantitative naked-eye assay result interpretation in the form of a traffic light signal. Traffic light signaling is undoubtedly known around the globe and hence, poses nearly zero risk of misinterpretation. The targeted analyte is hydrogen peroxide generated by the reaction between various metabolites and their oxidase-family enzymes. The traffic light display was achieved by integrating a method of controlling sample flow through a 3-dimensional network of paperfluidic channels of a multi-layer origami type device using a H_2O_2-responsive boronic acid pinacol ester derivative undergoing a hydrophobic to hydrophilic phase-switching process [2]. A traffic light type **μPAD** was designed and evaluated for its response to aqueous H_2O_2 solutions. Control of the concentration threshold resulting in the display of specific traffic light color(s) and the detection range was achieved.

The detection of glucose as a model target was selected to show the possibility of practical application. To analyze glucose spiked into artificial urine, an enzyme layer was integrated into the originally designed device. As a result, a traffic light type μPAD capable of semi-quantitative analysis of glucose concentrations within the clinically relevant range for urine analysis was obtained [3]. It is expected that traffic light type **μPADs** can be adapted to the detection of other metabolites in aqueous biological samples by using other oxidase-family enzymes and integrating the threshold control method. Due to their ultimate simplicity in use and signal interpretation, such systems could become useful for daily routine monitoring of potential lifestyle-related diseases by untrained end-users in home healthcare situations.

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Development of Electrochemical POCT System Based on Cartridges Fabricated with a Use of Polyester Film Technology

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The need for development of diagnostic tools, which allow rapid and reliable analyses is still actual. Recently more and more attention has been paid to diagnostic systems, which can be used for point-of-care testing (POCT) of biomarkers and pathogens, assuring uncomplicated operation and quick results. The cost of such portable devices and especially their disposable elements should be as low as possible to be available to a wide audience. Therefore, the polyester film-based technology becomes more and more popular, as it can allow for a development of low-cost elements of a complex geometry, which can be fabricated on a massive scale with the use of laser cutting and patterning giving the *lab-on-a-foil* microfluidic systems.

In a framework of this presentation the details on the development of electrochemical POCT system will be presented. The proposed system consists of a microelectronic reader, which can be used by doctors, nurses and paramedics under non-laboratory conditions; disposable diagnostic cartridges containing a network of microchannels, bioreactor, specially designed carbon-based electrodes and other elements (fabricated using a foil-to-foil technique), which can be applied for the determination/detection of bacterial, viral and fungal antigens, as well as cardiovascular and hormonal biomarkers (in blood, urine and nasal swabs and other biological samples), and a measurement data management system with accompanying infrastructure. We will discuss the most important issues and challenges, which must be faced when designing and manufacturing such devices. The applicability of developed POCT system for the determination of certain biomarkers e.g. C-reactive protein will be demonstrated and evaluated.

Acknowledgement

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Epitope-MIPs for Peptides and Proteins – Mimicries of Antibodies

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In this talk, we present epitope-imprinted polymer nanofilms for the recognition of the **Receptor binding domain** (RBD) of the spike protein of SARS-CoV-2, **Strep-tagged Membrane Bound Hydrogenase** (MBH) and for **Hemoglobin**. The MIPs were synthesized on gold electrodes by electropolymerization of scopoletin around the chemisorbed template peptides. All steps of MIP synthesis, template removal and rebinding were analyzed by evaluating the permeation of ferricyanide through the MIP by voltammetry, Surface Enhanced IR Spectroscopy and Atomic force microscopy [1,2].

- MIP mimicking the ACE2 Receptor for recognition of the RBD of SARS-CoV-2

The peptide *GFNCYFP* from the binding region of the RBD to the ACE2 receptor was used as the template. The RBD was bound in the lower nanomolar concentration range with a $K_D$value of 15 nM and the signal decreased by only 10 percent in 1:20 diluted splitting buffer of COVID antigen tests.

- Strep-Tag II-MIPs as platform for bioelectrocatalysis

The electrosynthesized MIP based on the C-extended Strep-Tag II WSHPQFEKC bound the C-free Strep-Tag II and Strep-tagged MBH with KDvalues of 3.05 nM and 33.8 nM, resp. thus outperforming Strep Tactin. Pronounced catalytic currents were established for MIP-bound MBH and bacterial Alkaline Phosphatase.

- "Out of pocket" binding prevents discrimination between Hb and $Hb_{A\text{-}1c}$

In contrast to effective discrimination of the glycated N-terminal pentapetide by the MIP based on the template*CVLSPAD* the proteins Hb and $Hb_{A\text{-}1c}$ are bound with comparable affinity.

Acknowledgment

The research has been supported by DFG (EXC 2008/1–390540038–UniSysCat, German Ministry of Education and Research (BMBF, 01DH20018)and the NRDI Fund (TKP2020 IES, BME-IE-NAT) of the Ministry for Innovation and Technology. Hungary 01DH20018).

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Surface Imprints on the Micro- to Nanoscale: Spectrometric and Nanomechanical Characterization and Standardized Rebinding Studies

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Surface molecular imprinting has become popular in designing sensitive layers for (bio)analytes on the micro- and nanoscale [1]. Still, there are limitations in scaling the process to commercial amounts not least due to limited reproducibility [2]. One reason for this is that imprinting is still somewhat empiric lacking exact knowledge of the binding processes.

Tackling this issue means going beyond characterizing recognition layers "just" by their sensor responses. One way to do so is combining Raman Microscopy with quantitative nanomechanical measurements (QNM) on an Atomic Force Microscope (AFM): the latter shows that the adhesion properties of the surfaces within an *E.coli* imprint differ from those of the surrounding polymer after template removal. Carrying the experiments further by recording the Raman spectra, it is possible to actually discern between imprints and surrounding polymer depending on the respective matrix: in "Heavy Duty Ink", an acrylate-based commercial UV-printable resin, chemometric analysis of Raman spectra recorded inside and outside imprinted cavities reveal statistically significant differences between those two areas. Furthermore, the corresponding PLS-DA model can even discern imprinted cavities of different bacteria species, namely *E.coli* and *B.cereus*: it correctly classifies 85% of the respective bacteria based on the spectra of their imprints. Replacing the acrylate system with a copolymer of styrene and divinyl benzene (DVB) annihilates these differences. This clearly indicates that imprinting indeed alters the density/distribution of functional groups on the polymer surface: acrylate-based systems show functionality especially in the spectral range of carbonyl and carboxy derivatives, whereas styrene and DVB obviously lack them.

Nanomechanical studies are also feasible for smaller analytes, such as engineered nanoparticles: peak-force QNM images of stamp-imprinted styrene-DVB copolymer reveal submerged Ag nanoparticles completely covered in the polymer. In such a case, it is thus preferable to generate MIP in situ to avoid complete immersion of the template. Immobilizing a RAFT (reversible addition-fragmentation chain transfer) agent on a sensor surface allows for both controlling MIP film growth from this surface and generating cavities that selectively and irreversibly bind magnetite nanoparticles reaching saturation at roughly $6*10^{15}$ particles/L. They also allow for systematic selectivity studies: switching from particles stabilized by poly vinyl pyrrolidone (PVP; zeta potential: -41 mV) to oleate-stabilized ones (zeta potential: -4 mV) reduces the corresponding QCM sensor responses by a factor of 2.

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Nanoparticle Imprinted Matrices (NAIM): Speciation of Nanomaterials

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This contribution deals with a new exciting approach that aims to detect selectively nanoparticles (NPs). With the benefits that NPs bring due to their unique properties that are a result of their size, they also pose a threat. Nanotoxicology is a new discipline, which deals with the adverse effects of NPs. Evidently, the detection of NPs requires the development of appropriate tools. The toxicity of NPs is affected by the core, size, shape, and stabilizing shell of the NPs. Hence, speciation of NPs, is becoming of utmost importance.

We have developed a new concept for the selective recognition and detection of NPs termed nanoparticle imprinted matrices (NAIM).[1-5] It is analogous to the well-known concept of molecularly imprinted polymers (MIP) in which the molecular analyte is imprinted in a polymer by polymerization of proper monomers with which it chemically associates. The removal of the template forms complementary cavities capable of selective recognition of the analyte. Instead of molecular species, we imprint NPs in various matrices (see for example Figure 1 in a diazonium matrix). The NPs are then removed to form nanometric voids that can selectively recognize the originally imprinted NPs. The NAIM approach works so well that we can detect NPs that are stabilized by different carboxylic acid short molecules.

We will present a few new systems by which we show how NPs can be imprinted inside a matrix. How we can study the NP-matrix interaction, the imprinting of non-spherical NPs, and the detection of NPs from the gaseous phase.

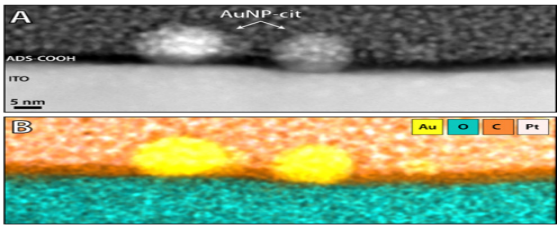

Figure 1: NPs embedded in a very thin matrix on ITO

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How to Minimize Electrode Passivation

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Electrode passivation is one of the biggest problem complicating practical applications of modern voltammetric or amperometric methods for monitoring of biologically active organic compounds [1]. This presentation will be focused on our recent approaches to minimize this problem by simple renewal of electrode surface [2], by using electrode materials resistant to passivation [3], measuring in flowing systems [4], using membranes preventing access of passivating compounds to electrode surface [5], or by novel materials/measuring protocols less amenable to complications connected with electrode passivation [6]. An attempt is made to predict further development in this field and to stress the need for more systematic and less random research.

Acknowledgments

The research was supported by the Czech Science Foundation (GA CR project No.20-01417J). Technical, material and intellectual support from Metrohm Czech Republic is gratefully acknowledged.

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Functionalized Carbon Nanomaterials as Building Blocks for the Development of Affinity Biosensors

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This communication is focused on the design, characterization and analytical applications of nanobiosensors based on the use of rationally functionalized carbon nanotubes and graphenaceous materials. Special attention was given to the influence of nanostructures functionalization and biorecognition/transduction events on the analytical performance of the resulting nanoarchitectures for the quantification of cancer and Covid-19 biomarkers.

The electrochemical (bio)sensors were obtained by modification of glassy carbon, screen printed carbon and gold electrodes modified with multiwalled carbon nanotubes (MWCNTs) non-covalently functionalized with (bio)molecules or graphenaceous materials modified with polymers as platforms to allow the selective anchoring of the biorecognition molecules or to directly work as biorecognition elements..

We propose a new strategy to build impedimetric genosensors connected to the use of MWCNTs dispersed in avidin as platform to support the DNA probes in a mode analogue to the magnetic nanobeads. The resulting architectures were used for the non-label and non-amplified quantification of BRCA1 and SARS-CoV-2 RNA at femtomolar and atomolar levels, respectively. We also propose the development of highly sensitive and selective impedimetric and plasmonic microRNA-21 nanobiosensors based on the use of graphenaceous materials modified with chitosan as platform to immobilize the DNA probe.

The results demonstrated that the rational design of the bioanalytical platforms through the critical selection of the nanomaterial functionalization, biorecognition event and transduction scheme are key aspects for the development of highly sensitive and selective sequence-specific detection biosensors. The proposed schemes not only represent a model for further design of biosensing layers and bioaffinity biosensors, but also for the development of novel *in-situ* amplification schemes.

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Fluorescent Chemosensors and Imaging Agents

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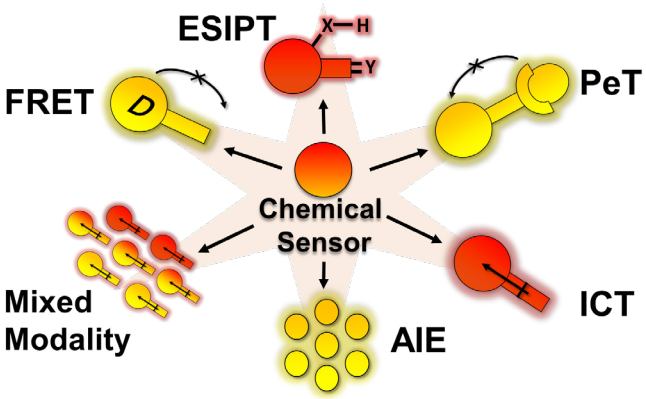
The ability to monitor analytes within physiological, environmental, and industrial scenarios is of prime importance. Given that recognition events occur on a molecular level, gathering and processing the information poses a fundamental challenge. Therefore, robust chemical molecular sensors "chemosensors" with the capacity to detect chosen molecules selectively and signal this presence continue to attract considerable attention. This presentation will concentrate on fluorescent probes developed for enzymes, diols, anions, and redox imbalance. The aim of the research is to mimic nature's level of sophistication in designing and producing chemosensors capable of determining the concentration (and location) of a target species in any medium.



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Chemical Imaging – Recent Advances in Real-time Visualization of Small Analytes in Complex Biological Systems

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Seeing is believing, as the saying goes. Unfortunately, our vision (inbuild sensor) is limited when it comes to seeing chemical species in real-time. Therefore, we need tools to help us. Optical chemical sensors, so-called optodes, are in many ways, at least in my opinion, the perfect tools for this endeavor.

In this contribution I will highlight our recent advances in the field and discuss future directions. With the ambition to understand complex systems and to untangle the connection between the biotic and abiotic world, I aim to develop optodes fit for purpose.

To name an example, we recently developed a wide dynamic range NH_3 optode[1]those measurements fail to unravel the local processes and spatial heterogeneity at the soil air interface. We report a two dimensional (2D. This optode enables us to study NH_3 emission from agriculture; the main source of NH_3 in the atmosphere at a sub-mm resolution. Using this optode in combination with other sensors and analytical methods can help understand the fate of N in soil at unprecedented spatial resolution.

In another project we use O_2 sensitive particles in combination with particle image velocimetry and developed a method called SensPIV. SensPIV tracks individual particles using fast imaging approaches, consequently allowing the visualization of flow fields. The concurrent imaging of the O_2 sensitive luminescence of these particles enables for the first time a simultaneous measurement of flow and O_2 concentrations. This brings us a significant step closer to understanding how organisms exchange nutrients and interact with the surrounding environment (see figure 1).

Those two examples just highlight some of the advances we recently made using chemical imaging and as I will discuss in this presentation this might just be the beginning.

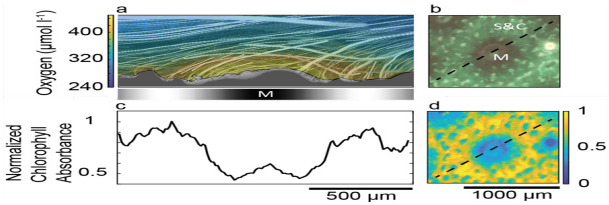

Figure 1: Combined flow dynamics and surface O_2 measured along a single polyp of the coral *Porites lutea*. a Flow dynamics (depicted by the particle trajectories) and O_2 concentration (color map) extracted from the SensPIV results along the dashed line shown in b. Black and white panel below indicate different regions of the coral: Septa and coenosarc (white), Mouth opening (black) (Pacherres et al. unpublished data)

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Advanced Photoluminescence-Based pH and O_2 (Bio)Sensors for Cell Analysis

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A variety of different optochemical pH and O_2 sensors have been described in recent years [1,2], but when it comes to their use for multi-parametric detection and live cell analysis, such systems show significant drawbacks. These drawbacks include: non-optimal spectral characteristics and detection schemes; unstable calibrations, toxic action on cells; low multiplexing potential due to cross-talk and optical interferences; complex sensor chemistry and fabrication process.

In this talk, I will present several new multi-analyte sensor systems developed in our lab, which are specifically tailored for cell analysis and which overcome many of the above limitations. Examples include: i) dual O2/pH sensor system based on one meso-substituted phosphorescent Pt-porphyrin dye [3]; ii) two dual sensing approaches using pairs of fluorescent and phosphorescent porphyrin dyes, which provided internally referenced readouts of both pH and O2 concentrations (patent pending). Uses of the new sensors for detailed multi-parametric assessment of mammalian cells via measurement of their extracellular acidification (ECA) and oxygen consumption (OCR) rates under different physiological conditions will also be presented.

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Emission Readout of Ion-Selective Sensors

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Classical ion-selective sensing applies different membrane compositions, with respect to intended readout: electrochemical or optical. In the latter case the presence of an optical transducer: hydrogen ions chromoionophore is typically required, on top of analyte selective ionophore. The differences in composition and pretreatment of ion-selective membrane intended for potentiometric or optical sensing limit applicability of typical ionophores containing receptors to just one methodology, which can be an obstacle from application point of view. Application of optical readout to emerging current triggered sensing of electrochemically inactive ions using ion-selective membranes remains a challenge for these (classical) systems. Due to the high resistive nature of ion-selective membranes application of optical readout of electrochemically triggered signals is likely to offer advantages such as a higher sensitivity or to extend the analytically useful range.

We propose ion-selective membrane system that can be used in both modes: electrochemical and optical, moreover in each of them offering competitive analytical parameters. The proposed system contains particulates of redox and emission active ion-to-electron transducer – polyoctylthiophene (POT) dispersed within plasticized PVC []. Under potentiometric conditions incorporation of analyte ions into the membrane results in open circuit potential change, as in a classical system. However, due to the presence of lipophilic positively charged ions, polymer backbones, full saturation of the membrane is prevented even for long contact time with solution. The presence of both positively charged and neutral forms of conducting polymer in the membrane results in high stability of potential readings in time. Optical signal generation is related to increase of positive charge amount in the membrane and coupled decrease in the oxidation state of the polymer, in the absence of redox potential change, resulting in increased emission, similarly as observed for solution containing dispersed POT nanoparticles [].

Under electrochemical trigger conditions herein proposed dual function ion-selective membrane allows in situ transduction of electrochemical responses of ion-selective electrodes to emission change signals [,]. The electrochemical trigger applied results in a redox process of the transducer, inducing ion exchange between the membrane and the solution, ultimately leading to change of its emission intensity. It is shown that electrochemical signals recorded for ion-selective electrodes operating under voltammetric/ coulometric conditions correlate with emission intensity changes recorded in the same experiments. Moreover, the proposed optical readout offers extended linear response range compared to electrochemical signals recorded in voltammetric or coulometric mode.

Polymeric Optode-Based Platforms with an Integrated Color Acale: Exploring Perspectives for Calibration-Free Analysis

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The need for *in situ* monitoring, high throughput and autonomy lead to the fact that the periodic calibration of existing sensor systems becomes burdensome. In the case of polymeric optodes, however, photodegradation, and/or leaching of active components from the sensing phase and corresponding gradual shift and/or decrease of the measured signal, renders calibration necessary [1, 2]. There is a lack of a general approach to developing calibration-free sensing devices. To date, a number of scientific groups have attempted to create calibration-free optical sensors, but the proposed solutions are complex in design and have limited potential for use in rapid sample analysis and continuous monitoring.

In this work, we report on a new approach to obtaining calibration-free arrays consisting of chromoionophore-based ion-selective optodes. Theoretical modeling of the response of optode sensors of various composition showed the feasibility of creating a color scale of an analytical signal built into the array, by using optodes containing only chromoionophore (C_T) and various amount of an ionic additive (R_T) in the sensor. The response of such optode sensors remains constant over a wide range of solution compositions (Fig. 1). This makes it possible to determine the concentration of an analyte in a solution by comparing the analytical signal of the indicator optode with the readings of the internal reference elements. This approach was tested experimentally and the influence of the composition of the sensor phase (the nature of the indicator and ionic additive), as well as the processes of coextraction, indicator leaching and chemical degradation on the possibility of creating a calibration-free array, were considered. The concept has been verified in model samples with the conventional ionophore-based optode and the developed built-in signal scale (Fig. 1C).

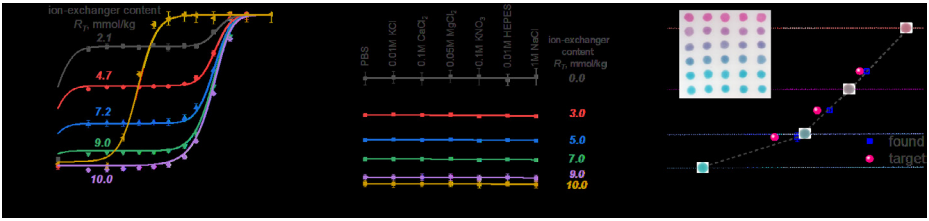

Fig. 1. A: pH response curves for the optodes with reduced amount of ion-exchanger (symbols – experimental data, lines – model data); B – the constancy of the color scale signal in solutions of different composition; C – determination of Na^+ in model samples with the developed integrated color scale.

Acknowledgment

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Hyper-Polarizing Organic Phase Nanosensors for Polycation/Polyanion Detection

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Recently we have reported on a novel strategy to quantify polyanions (heparin, a polyanionic saccharide used as an anticoagulant) and polycations (protamine, a polycationic heparin antiode) in undiluted blood plasma using optical nanoparticle-based sensors. This was based on the discovery of strong polarization of solvatochromic dye in organic phase by dinonylnaphthalenesulfonate ion ($DNNS^-$, an established ligand of protamine). [1] This resulted in an improved selectivity where even arginine, the main repeating amino acid moiety of protamine, cannot induce optical signal transduction, in contrast to the classical ion-exchange type that shows significant cross-reactivity to arginine. However, the reason and mechanisms have not been clear so far.

The first half of this contribution shows data to answer the two challenging questions: which process does the protamine experience in the course of signal transduction, surface adsorption onto or bulk extraction [2] into the nanoparticles? Why was the selectivity dramatically improved compared to the classical ion-exchange type sensor? The results indicate that bulk extraction is the dominant process in the event of optical signal transduction, and the outright absence of arginine signal is due to the modest complexation of arginine and $DNNS^-$ (Fig.1), not due to the extraction selectivity.

Those results suggest that other types of optical reporters may be polarized as well. Our investigation revealed that quantum dots (Qdots) and carbon quantum dots (Cdots) can be strongly polarized, seemingly in the absence of ionic paring, which indicates universality of this phenomenon. The Qdot/Cdot-based sensors were designed and applied to the direct detection of both polycations and polyanions (Fig. 2), which the solvatochromic dye strategy failed to achieve. This demonstrates the high potential to quantify biologically relevant polyanions (DNA, RNA) and to monitor the degree of protein phosphorylation.

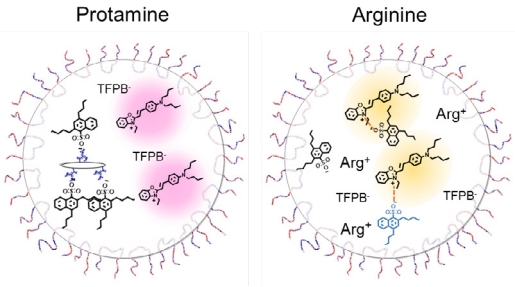

Fig.1. Optical signal of the sensor in the presence of protamine and arginin

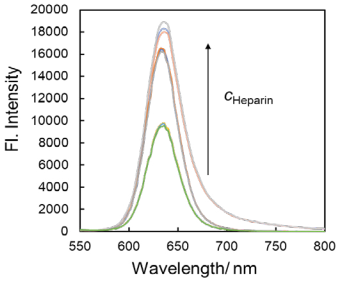

Fig.2. Heparin response signal of the Qdot-based sensor.

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Challenges in Development of Novel Chemical Sensors for Non-Steroidal Anti-Inflammatory Drugs (NSAIDs): Comparison of Optical and Potentiometric Transductions

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Non-steroidal anti-inflammatory drugs (NSAIDs) are widely employed to reduce inflammation, fever and other symptoms of colds and flu, to relieve the headache, muscular and other long - term courses of pain [1]. NSDAIDs such as ketoprofen (KF) and naproxen (NS) are the two most widely used, and have found in many pharmaceutical compositions both for adults and children. In pharmacological compositions KF and NS, belonging to the group of propionic acids, are often introduced in a form of more soluble salts, Figure 1.

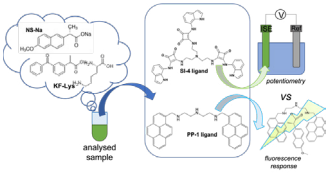

Figure 1. The sketch of potentiometric vs optical sensing comparison for NSAIDs assessment. On figure: KF-Lys- ketoprofen lysine salt and NS-Na –naproxen sodium salt chemical structures respectively; SI-4 and PP-1 – examples of tested acyclic squaramide and bis-pyrene-substituted polypyridine ligand structures respectively.

Due to the growing consumption, especially during the last two-years COVID-19 pandemic period, the anti-inflammatory painkillers became emerging environmental pollutants. Moreover, NSAIDs are toxic for biota and able to accumulate thus causing serious environmental damages [2]. The detection of NSAIDs and their careful concentrations screening in pharmaceutical compositions, and in environment is, hence, an important and challenging analytical task. Previously several types of analytical procedures have been proposed for the analysis of NSAIDs, including chromatography [3], spectrophotometry [4] and chemical sensors [5-7]. In this work, the performance of potentiometric and optical all-solid-state sensors recently developed by our group for KF-Lys and NS-Na assessment will be compared. Several acyclic squaramide receptors [8] were exploited as membranoactive components for potentiometric ion-selective electrodes with PVC solvent polymeric membranes, while the open-chain polypyridine ligands bearing pyrene fluorogenic groups able to signal selective analyte binding through the enhancement of the emission [9] were used for KF and NS optical sensors development. The importance of a proper transduction method selection for sensors detection limit lowering and selectivity enhancement, as far as the selectivity tuning by the accurate choice of sensing material composition, the selection of the appropriate solid substrate support and the overall design of sensing devices will be discussed. Finally, the applications of the developed optical and potentiometric sensors for NSAIDs assessment in real samples will be illustrated.

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Cubosome Ion-Selective Nanooptodes

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Optodes, with a dye and/or selective ionophore embedded in the lipophilic matrix, allow optical insight into ion concentration changes. These probes, similarly to other polymeric ion-selective sensors, require also the presence of a plasticizer–toxic organic liquids as the constituent. The presence of a plasticizer, and in consequence risk of its release to the sample, is clearly a bottleneck of application in a real analytical scenario.

We have reported a new generation of biocompatible optical ion-selective sensors benefiting from phytantriol cubosomes or hexosomes – nanostructural lipid liquid phase, that not only do not require presence of a plasticizer, but also are generally biocompatitible. Another advantage of proposed sensors is the ability to influence the sensor performance due to the change of the structure of the nanooptodes. Cubosomes consist of two interpenetrating, noncontacting aqueous channels that are surrounded by a lipid bilayer arranged in a thermodynamically favorable periodic 3D structure. Hexosomes, consist of closed reverse micellar cylinders that are arranged in a 2D hexagonal lattice that allows only the nanoparticle surface to be in contact with the sample. Thus adjusting the internal arrangement of channels by a temperature trigger, affects accessibility of the bulk probe for the analyte and ultimately enables tuning the sensitivity of the sensor.

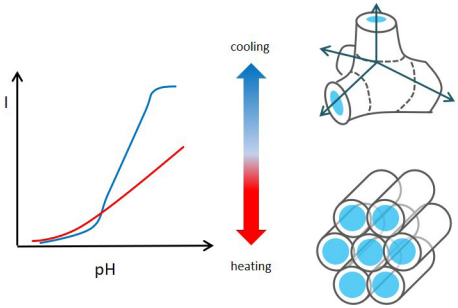

Fig.1. Scheme of the change of the sensor response pattern due to the phase transition of prepared nanostructures.

Thus, cubosome or hexosome optodes are highly promising emerging alternatives to conventional polymeric based optical nanoprobes.

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Halogen Bonding Ionophore for Potentiometric Iodide Sensing

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Iodide (I^-) is an essential micronutrient for the production of thyroid hormones and the regulation of metabolism and growth. Hence, it is commonly added to food for fortification and used as a urinary biomarker for thyroid problems. I^- concentrations are conventionally determined by HPLC or ICPMS, which offer high accuracy and precision, albeit at high cost and operation complexity. In contrast, ion-selective electrodes (ISE) are highly promising for in-field testing due to their portability, ease of use, wide dynamic range and low cost [1].

Halogen bonding (XB) is a supramolecular interaction between an electron-deficient polarisable heavy halogen atom (I/Br) and a Lewis base, and has been utilised in anion recognition and sensing. Herein, we report the first example of a XB tripodal ionophore (**XB1**) which is selective for the I^- anion [2]. Solution-phase NMR titrations of ionophore **XB1** and its H-analog with I^- demonstrated the dominant influence of XB interactions on binding.

The optimal iodide-selective electrodes formulated with ionophore **XB1** exhibited near-Nernstian response (–51.9 mV per decade) with a large dynamic range (10^{-1} to 10^{-6} M). Notably, anti-Hofmeister selectivity for I^- over thiocyanate was observed in comparison to an ISE without the ionophore. This work demonstrates the potential to harness XB as a viable supramolecular interaction for the potentiometric sensing of anions.

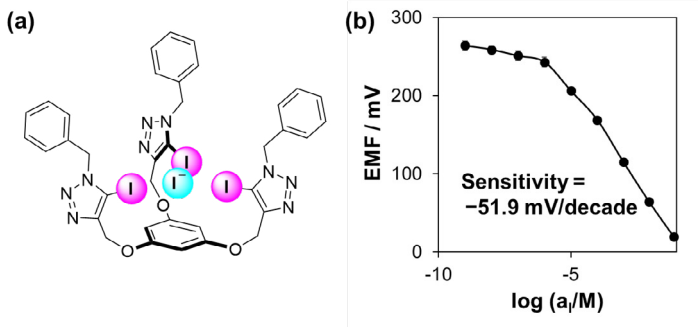

Figure 1. (a) Structure of ionophore **XB1** and proposed pocket for I^- binding; (b) response of the optimal iodide-selective electrode formulated with **XB1** towards I^- activity

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Abstracts of poster presentations: Monday

Amperometric Readout of Ion-Selective Electrode Potential Changes

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A new current-based readout of potentiometric responses of all-solid-state ion-selective electrode (ISE), used as a reference electrode [1,2], recorded at Ag|AgCl electrode is proposed (Fig. A). In this system changes of the potential of a typical calcium or potassium ion-selective electrode with poly(3-octylthiophene) [3] transducer layer determine the current recorded at Ag|AgCl electrode operating in the chronoamperometric mode. For Ag|AgCl electrode even small changes in potential of ISE cause a relatively large change in the current, therefore introducing of the proposed setup allows amplification of the recorded analytical signal, resulting in higher sensitivity.

The current recorded at Ag|AgCl electrode was linearly dependent on concentration of Ca^{2+} or K^+ ions with sensitivity ca. 27 μA/mmol dm^{-3} or 9 μA/mmol dm^{-3}, respectively (Figure B, C).

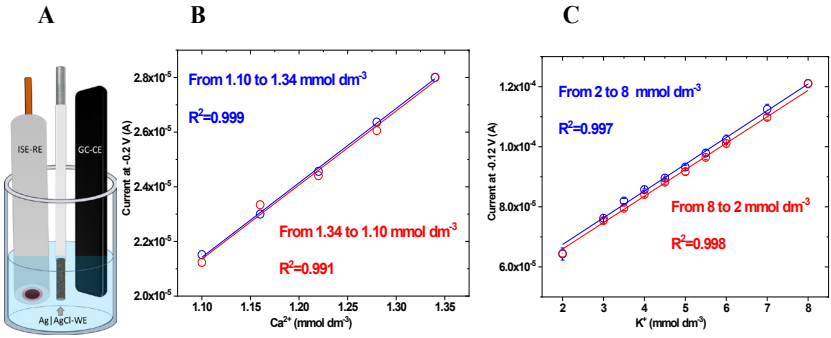

Figure A Scheme of electrochemical cell with Ag|AgCl wire, ISE and glassy carbon plate as the working, reference and counter electrode, respectively. Current recorded at Ag|AgCl electrode for various concentrations of Ca^{2+} (**B**) or K^+ (**C**) ion in the presence of 10^{-2} mol dm^{-3} NaCl.

Moreover, the proposed arrangement allows measurements in the range of physiological concentrations of Ca^{2+} or K^+ ions in the model solution of a physiological fluid (Fig. B, C) in the presence of interfering and a bovine serum albumin.

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Self-powered Potentiometric Sensor Based on Optical Signal Transduction with Liquid Crystal Display

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Self-powered sensors are attractive because they are environmentally friendly and allow for sensor miniaturization. However, developing self-powered potentiometric sensors is still quite challenging because only limited energy can be harvest by this measurement principle.A new type of self-powered ion-selective potentiometric sensor is illustrated here that requires very little power. For the first time, the potential of a glass pH electrode of very high impedance (130 M Ω) is directly read out optically. This is accomplished by a liquid crystal display (LCD) as the electrochromic transduction principle. The LCD gives a significant change of transmission upon applying an external voltage within a certain range. The transmission process requires a very small charge on the order of 100 pC to be transferred across the membrane owing to its low capacitance of ca. 50 pF.

For the LCD to be turned on, the cell voltage needs to be boosted by the addition of additional Zn/Zn^{2+} elements placed in series. Also, the LCD is found to give a time-dependent absorbance decrease, which may be due to reversible radical reactions at the underlying ITO electrode as a constant voltage is applied.[1] This is mitigated by adding a high resistance element in series to attenuate the associated decay, capturing the optical signal for 4 s and then short-circuiting the LCD for at least 5 s. This results in repeatable recovery of the LCD absorbance for an optical readout of adequate precision. The absorbance is found to decrease with increasing pH, which can be used to measure any sample of interest.

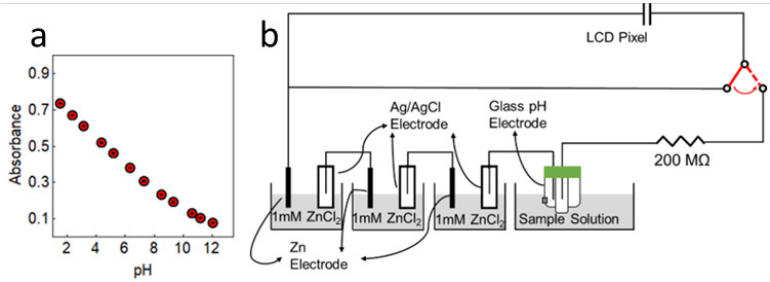

Figure 1. A) Direct LCD absorbance response to pH from a glass electrode. B) Scheme of the sensor circuit.

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Class-Selective Molecularly Imprinted Polymer-Based Sensor for Macrolide Antibiotics Detection

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Macrolides constitute a group of antibiotics that are widely used in human medicine due to their potency against most gram-positive and some gram-negative bacteria. Several reports illustrate the use of MIPs for determination of different individual macrolides including erythromycin (Ery), clarithromycin (Clary) or azithromycin (Azi) [1-2]. However, MIP for simultaneous selective recognition of these molecules are quite scarce. With the increasing usage of macrolides, their persistence and discovery in environmental water, although detrimental, is not unexpected hence, the continued inclusion of macrolides especially erythromycin, clarithromycin and azithromycin in the EU watchlist of environmental pollutants. Consequently, there is a growing need for the design of MIPs with macrolide group-selective capabilities having the potential for routine monitoring of macrolides in water. For this purpose, the focus of our current study is to design electrochemically synthesized MIP film on portable and cost-effective sensor platforms such as screen-printed electrodes.

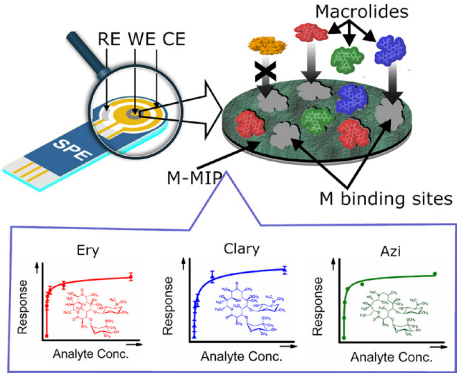

Fig. 1. Schematic representation of macrolide MIP based sensor.

This report demonstrates the possibility of preparing a portable MIP based SPE sensor for simultaneous electrochemical determination of Ery, Clary and Azi as representative macrolide antibiotics (Fig.1). For this purpose, a combination of covalent and non-covalent (semi-covalent) imprinting approaches were harnessed to afford specific recognition as well as high adsorption capacity. Optimization of the prepared MIP based sensor demonstrates its potential suitability for the required routine monitoring of these molecules in environmental water.

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Characterization of a Hydrophobic PEDOT Derivative Using eQCM

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Conducting polymers have been studied with great interest over the last decades, owing to their unique ion-to-electron transduction properties and the improved electrochemical performance they often offer. Some of the most common materials used in the field of electrochemistry include poly(3-octylthiophene) (POT), polypyrrole (PPy), poly(aniline) (PANI) and poly(3,4-ethylenedioxythiophene) (PEDOT). Although the choice of the polymer is a compromise of many aspects, the use of certain PEDOT derivatives can be advantageous, in view of the reported drawbacks manifested by other polymers, such as the light sensitivity of POT [1]. PEDOT is prepared via electrochemical polymerization from a solution of the monomer and a supporting electrolyte. The resulting polymer is positively charged and incorporates doping ions from the electrolyte, which act as charge compensators [2]. As of today, several studies have examined the influence of the supporting electrolyte on the electrochemical and mechanical properties of the resulting film [3,4]. As evidenced [5], the structure of the film and the level of water uptake can significantly influence the potential stability of sensors based on the non-hydrophobic form of PEDOT. Its hydrophobic derivatives, including PEDOT-C_{14}, have not been studied to such an extent. The enhanced hydrophobicity of PEDOT-C_{14} makes it particularly suitable for use as an ion-to-electrode transducer in potentiometry [6].

In the present study, we examine how different supporting electrolytes with doping ions of varying size and properties (e.g. lipophilicity), change the physicochemical properties of the film during the electropolymerization of EDOT-C_{14}. Using eQCM, we monitor changes on the polymer structure (mass growth, viscoelasticity) and electrochemical behavior (capacitance, ion-exchange properties). By comparing different PEDOT materials used as ion-to-electrode transducers, we show how the shift to a hydrophobic derivative can help us adjust and predict their electrochemical behavior with greater accuracy. This knowledge is valuable for building stable potentiometric sensors, as well as novel voltammetric sensors with refined charge- and ion-transfer properties.

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Electrochemical Studies of Ionophore-Analyte Interaction at a Liquid/Liquid Interface

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The organoboron compounds are widely used as selective receptors for the determination of carbohydrates [1], catechols [2] and some anions [3]. A number of methods utilizing organoboron compounds as recognition elements has been developed for the determination of fluoride anion [4]. However, fluoride recognition by organic receptors mostly takes place in an organic solvent, which greatly limits the scope of their use. Bearing in mind that fluoride is an important water pollutant, the basic problem is to find a receptor soluble in an organic solvent, which could interact with fluoride anion in the aqueous solution.

The most known analytical technique for the determination of ions, which takes advantage of the selective interactions between the receptor and the analyte is potentiometry using ion-selective polymer membrane electrodes. This method has many advantages, but also suffers from some limitations, including solubility of ionophores in the membrane and necessity to add additional substances, such as plasticizer and lipophilic salt, which can interact with the analyte and/or interfering species. Time to prepare and test different combinations of the membrane composition is also considerable. A method which allows for the same mechanism of recognition as the one encountered in ion-selective potentiometric sensors but could eliminate many aforementioned problems inherent to the composition of the membrane matrix is the electrochemistry at the interface between two immiscible electrolyte solutions (ITIES). This liquid–liquid interface allows transfer of ions from one phase to the other. The transport can be driven by change of potential of a pair of electrodes present in each phase. In this way amperometry at a liquid-liquid interface allows to screen ionophores in a much faster and more reliable manner.

A standard electrochemical cell used in this technique requires high amounts of solutions (around 10 mL of each phase). In this situation, we constructed a miniaturized version of the four electrode cell for measurements at the liquid/liquid interface , which allows to perform the study of ionophores using only 200-300 μL of the organic phase.

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Development of Conductive MIP Blends for Heptanal Vapor Detection.

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Molecularly imprinted polymers (MIPs) are synthetic materials that contain binding sites for selectively rebinding the target analyte. [1] Herein, conductive MIP blends serve as a receptor layer on quartz crystal microbalance (QCM) sensors as well as chemiresistors. QCM is a mass-sensitive sensor based on the piezoelectric properties of quartz. [2] Chemiresistors can detect volatile organic chemicals in the gas phase through thin conductive polymeric films. [3]

An acrylamide-based MIP was blended with the conductive polymer poly(3-hexylthiophene-2,5-diyl) (P3HT). QCMs coated with conductive MIP blends can detect heptanal in gas phase in a range of 250-1000 ppm (Figure 1A). Blending the MIP with P3HT increases the average sensor response for 1000 ppm heptanal from 5 to 8 Hz/10 nm polymer layer compared to pure MIP. Selectivity tests with other volatile organic compounds suggest selectivity towards the desired analyte. The blends also proved suitable for use in a chemiresistor (Figure 1B). On average 1000 ppm heptanal lead to a reversible shift of 0.5% in the sensor response.

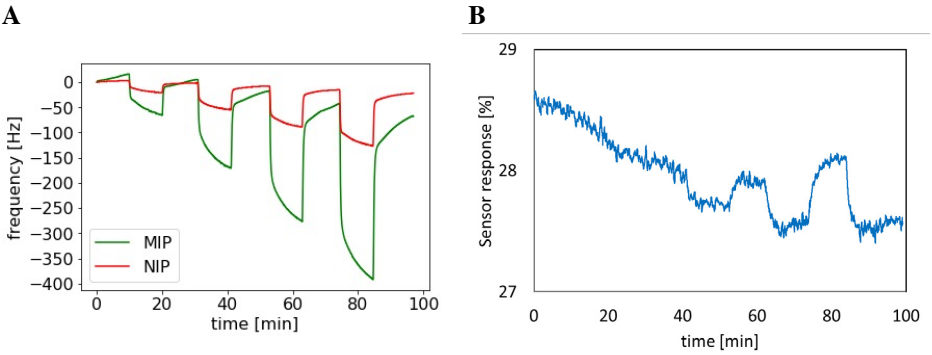

Figure 1: Examples of a QCM (A) and chemiresistor (B) measurement of different heptanal concentrations with MIP blends.

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Self-Plasticised Transducer Material based on 3,4-Ethylenedioxythiophene for Increased Stability in Ion-Selective Electrodes

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Solid-contact ion-selective electrodes have gained significant interest over the last few years, offering less maintenance and miniaturisation possibilities compared to classical liquid-contact ion-selective electrodes. This breakthrough was mainly supported by an increasing number of new available materials to be used as ion-to-electron transducer. Amongst carbon-based nanomaterials, conductive redox polymers, such as poly(3-octylthiophene) (POT), polyaniline (PANI) and poly(3,4-ethylenedioxythiophene) (PEDOT) have also been introduced and further developed.

Due to its ability to perform efficiently in combination with cation and anion selective membranes, PEDOT has become a transducer of choice. To enhance the lipophilicity of the resulting films, EDOT monomers have been modified with long alkyne chains and successfully used in ion-selective electrodes [1]. Owing to a simple synthetic pathway, a wide variety of EDOT derivatives can be synthesised and have been reported in biosensors, conducting hydrogels and many others [2].

We present here the novel synthesis of a 3,4-ethylenedioxythiophene derivative with a triazole analogue of phthalate. It is believed that grafting a plasticiser-like sidechain to the initial EDOT molecule will generate smoother polymer films upon electropolymerisation. The shared plasticised nature of the ion-selective membrane and the transducer layer should improve mechanical adhesion and reduce undesired underlying drifts at the interface.

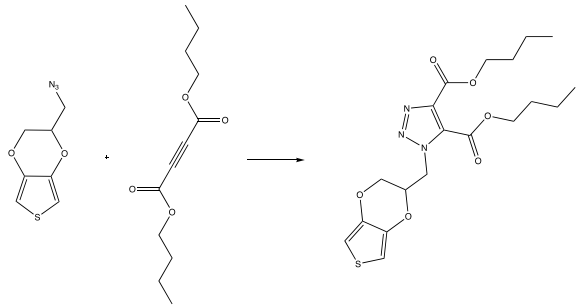

Figure 1: Huisgen 1,3-dipolar cycloaddition to synthesise a 3,4-ethylenedioxythiophene derivative with a triazole analogue of phthalate.

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Single-Use Potassium Sensor for Blood Potassium Home Monitoring

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Patients suffering from chronic kidney disease or chronic heart failure need close monitoring of their blood potassium values. We developed a potassium sensor in a test strip design for daily potassium monitoring at the patients´ homes using a blood drop accessed by finger-pricking [1]. The prototype of this sensor is based on single-use potassium test strips that use a potentiometric potassium selective electrode containing a hemolysis detection system for safe use by the patients themselves. The required accuracy is ensured by a customized microfluidic design that enables a user-friendly single-step calibration. Roll-to-roll technologies shall be used for low-cost fabrication of the microfluidic system [2].

The K^+ sensor is based on a screen-printed carbon working electrode functionalized with a PVC based potassium sensitive membrane. A screen-printed Ag/AgCl electrode is used as reference electrode for the measurements.

As hemolysis is a critical factor for measuring blood potassium from capillary blood samples accessed by finger-pricking a second sensor for hemolysis detection is integrated into the system. The sensor assesses free hemoglobin concentration in human plasma obtained passively by a plasma separation membrane located at the sample inlet of the test device.

Figure1: left: K^+ sensor accuracy; middle: prototype device; right: hemolysis detection

Acknowledgments

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Potentiometric Detection of Reactive Oxygen Species (ROS) by Metalized Polyporphyrine Layer Coated by Non-Biofouling Film

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One of the problems during joint replacement is complications connected with the post-surgery infections. Infection is caused by bacteria that form biofilms on surfaces and is referred to as biofilm-related infections (BRIs). It is also worth noting that due to the bioresponse, strong pathophysiological changes in the microclimate of an affected surface occur (decrease in pH, formation of different ROS, depletion of Fe ions and increase in the concentration of Ca ions) [1]. All these divergences could be used for the indirect detection of inflammation if corresponding in vivo sensors were available. Therefore, we constructed the potentiometric sensor of ROS as a thin layer, which enables the detection of inflammation at an early stage. The sensor consists of a conductive polymer layer based on polythiophene with an incorporated porphyrin-metal complex that potentiometrically detects the presence of ROS (H_2O_2 and ClO^- ions). This sensor is covalently coated with a nonbiofouling layer of poly(2-methyl-2-oxazoline), which works as a bio-compatibilizer and prevents the sorption of proteins and other biomacromolecules naturally occurring in organisms, which could interfere with the ROS signal. It was shown that potentiometric sensor shows a rapid response to hydrogen peroxide, does not experience interference with bovine serum albumin as a model serum protein when sensing ROS, is able to fully reversibly detect ROS with a linear response within a very wide range of biologically relevant concentrations (from 0.05 μM to 10 μM).

Acknowledgment

The authors acknowledged the Czech Health Research Council (NU20-06-00424) and Czech Science Foundation (grant 21-01090S).

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Development of Solid Polyvinyl Alcohol-Glycerol-NaCl Contact Gel for Electric Biopotential Sensing Applications

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Geometrically defined solid polyvinyl alcohol-glycerol-NaCl (PVA-G-NaCl) gel electrolyte [1] was prepared by gel casting in 3D printed moulds. Impedance measurements have been carried out to characterize gel conductivity as prepared and also in prolonged storage conditions. The aged gels never fully dried out, they kept their flexibility and conductivity; even though a two- to threefold resistance increase was experienced after 25 days, it still remained well below 1 kΩ. The interface between the differently produced Ag/AgCl electrode surfaces and the hydrogel has been also investigated by means of impedance measurements. It was proved that the PVA-G-NaCl / Ag interface possesses lower impedance compared to the resistance of the bulk gel. Electrode-skin impedance spectroscopy has been also performed and signal quality factor has been determined in view of biopotential sensing applications.

As summary, the PVA-G-NaCl hydrogel cylinders and the corresponding electrode systems possess high conductivity, excellent mechanical properties, and are less susceptible to dehydration than their water-based counterparts.

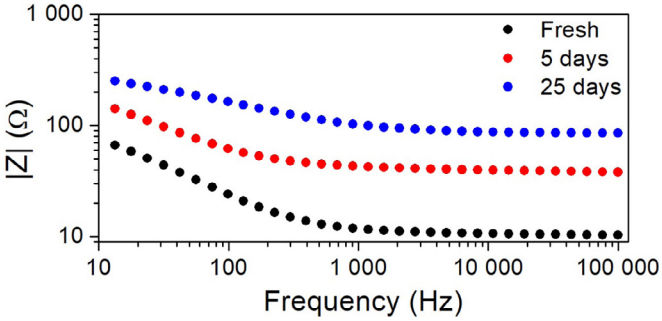

Figure 1. Results of the impedance measurements of bulk gels: effect of aging on resistance which is considered to be the limiting value of the impedance in the high frequency range.

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Sensorial Approach for Mao B Activity Detection

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Morbus Parkinson is one of the most frequent disorders of the central nervous system. Parkinson's disease (PD) is characterized by a dopamine deficiency in the brain and by intracellular accumulation of α synuclein [1]. There is no cure for this disease, but a symptomatic treatment. The monoamine oxidase B (Mao B) is involved in the metabolism of dopamine. Hence, it is an important target for the treatment of the disease [2] and Mao B inhibitors are applied as part of therapeutic methods. Direct measurements of enzyme activity could help to improve the individual treatment, particularly with respect to the duration of a successful medication. This gives the background for the investigations performed here.

A selective capturing of Mao B from a liquid sample has been used as the first step. For this purpose, antibody-modified cellulose beads are used. Different antibody clones have been tested which have to be able to specifically capture Mao B while at the same time not inhibiting the enzyme activity. Advantageously here one can not only enrich the enzyme but also eliminate potential interfering substances from the real sample.

After the capturing step the enzyme activity has been analyzed with benzylamine as substrate. Mao B converts it to benzaldehyde, NH_3 and H_2O_2. Hydrogen peroxide production is then detected at Prussian blue-modífied carbon electrodes in an amperometric mode within a flow system. Here, conditions have to be provided that the substrate and all other reaction products do not interfere with the specific hydrogen peroxide detection. This is crucial because high substrate concentrations are necessary for full enzyme activity analysis [3].

Amperometric measurements with the captured enzyme over a period of 30 min show a linear increase of the detected hydrogen peroxide concentration. The produced reaction product can already be quantified after 10 min of enzyme action. The time-dependent signal change depends clearly on the activity of the captured enzyme, demonstrating that Mao B activity can be followed by the established method and that the sensitivity allows the detection e.g. in a blood sample.

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Filtration Efficiencies of Crossflow Type Microfilters for E. Coli Separation

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Crossflow filtration is a pressure-controlled separation method, for size dependent segregation of fine particles, microorganism, spores or even micelles. It is preferred technology in the food industry to remove bacteria [1] or in healthcare for high-throughput plasma filtration. In contrast to dead-end filtration processes, in case of crossflow separation the filter surface is parallel to the liquid flow, significantly reducing the possibility of clogging. The particle separation in these crossflow sytems is based on the pore size, although the filtration process is sensitive to the flow rate, transmembrane pressure, membrane resistance, layer resistance, and particle size distribution in the suspension.

Crossflow microfluidic systems with parallel filter structure were parameterized and designed to optimize the separation efficiency and the amount of the target in the filtrate. For preliminary tests multi-disperse fluorescent beads (with 2μm, 6μm, 16μm diameters) were used as a model representing the particles and cells in urine. The target E.coli was initially modelled with 1.97μm diameter polystyrene beads before applying GFP-labeled E. coli bacteria. The trajectories of the fluorescent beads and GFP-E.colis, the developing compaction layer on the filter's surface and consequently the degree of target loss were characterised by fluorescent microscopy. The efficiency of the filtration was determined by particle counting by Bürker chamber and by Luna-II imaging based cell counter also. The columnar and weir type filter architectures were compared: the latter was considered more advanced.

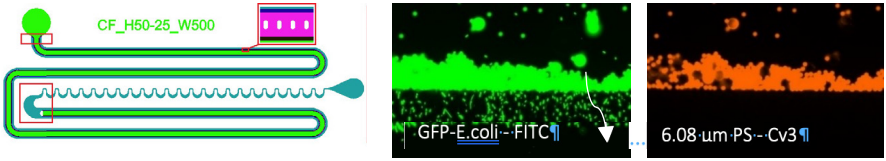

Figure 1. The developed crossflow filter architecture (left) and the structure of the compaction layer during filtration (right)

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Blocking Effect of Protein M on the Antibody-Antigen Interactions

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It was shown that Protein M acts as a universal antibody-binding protein with high affinity to the variable region of the κ and λ light chains and it blocks antibody-antigen union. [1] Therefore it could be used as a universal reagent for characterizing antibody modified surfaces and for competitive assays. The aim of the present research was to characterize explore these opportunities by using Protein M (MG281) and understand its binding behavior. For this purpose, we used surface plasmon resonance imagining (SPRi), which allows high throughput, label-free kinetic analysis, and simultaneous characterization of immobilized antibodies to the planar gold surface of SPRi biochips.

Antibodies binding antigens with various molecular weight from 8.5 kDa to 190 kDa and even virus-liked particles (VLP) (proBNP cTnI Spike Protein RBD CRP IgE SARS-CoC-2 VLP) were selected for the study. The equilibrium dissociation rate constants (K_D) of the antibody-antigen and antibody-Protein M interactions were determined, but no correlation was found regarding the blocking effect of the antibodies by Protein M towards antigen binding. However, the inhibition of antigen binding was found to be related to the size of the antigen. No significant blocking effect was induced by Protein M for small antigens (8-125 kDa). However, more than 40 % decrease in the antigen-antibody binding was detected for IgE-specific antibody, when preceding incubation with Protein M was applied. In addition, the binding of SARS-CoV-2 VLP to the selective antibody was fully inhibited by Protein M.

Based on our results, we presume that the antibody-antigen interaction is blocked sterically by Protein M and this can be exploited later in immunoassays.

Acknowledgment

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Microfluidic Device for Single Cell Trapping and Viability Testing

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In vitro testing of cell populations or individual cells in artificial systems that model their real environment is highly prospective from a biomedical and environmental point of view. Specially designed microfluidic systems allow the development of such a controllable chemical environment that is comparable to the size of cells. The application of such Organ-on-chip devices, which integrate sensing functions, can be a significant step in the research of pharmaceutical agents, but also in facilitating the spread of personalized medicine. [1] Cell trapping and fluorescent dying are powerful tools that enable the investigation of cell viability and proliferation in microfluidic structures. [2]

A microfluidic system capable of trapping cells individually was created and the viability of yeast cells was investigated. The microfluidic chip had narrowing channels with the dimensions compatible with the size of cells and being capable of trapping yeast cells (diameter 5-10 μm). The optimal concentration of cell suspension was determined to ensure the trapping of individual cells in the traps, thus conducting single-cell tests. After the cells were trapped a fungicide solution (50 mg/l Penconazole - $C_{13}H_{15}C_{l2}N_3$ - Syngenta TOPAS 100 EC) was injected into the fluidic channels and then the dead cells were dyed with propidium iodide fluorescent dye. Concentration dependent physiological effect on fungi was observed by fluorescent microscopy and compared to the results of optical spectroscopy. The established microfluidic system has been proved to be capable of trapping individual cells and observing their physiological processes in artificial chemical environment.

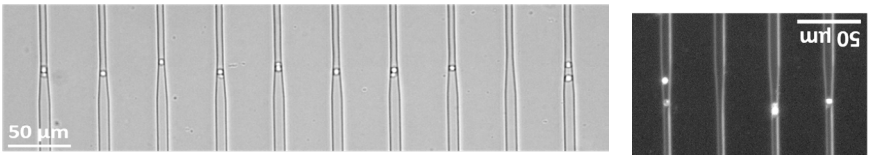

Figure 1. Captured cells in microfluidic systems (left) and dyed with propidium iodide in fungicide chemical environment (right).

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Platereader Compatible Microfluidic Chambers for Fluorescent Spectroscopy

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Commonly used methods to measure drug concentrations require specific instruments and high volumes of samples. Several active pharmaceutical ingredients (APIs) or their impurities possess fluorescent properties [1] and taking this advantage, plate readers or benchtop spectrophotometers are applicable for screening them. By designing the adequate, instrument compatible microfluidic structure and optimizing its parameters, the aim is to create a tool with significantly reduced sample volumes and appropriate sensitivity.

To reduce the sample volume a specifically designed plate reader compatible microfluidic chambers were designed and fabricated by soft lithography. Spectral fluorescent properties of the well known Alexa Fluor 350 dye were screened by Tecan Spark Plate Reader in both microplate and the manufactured microfluidic chip. For increased sensitivity, the geometric parameters of the microfluidic structure were optimized by modifying the chamber diameter and depth. The sample volume dependence of signal intensity was tested and advanced sensitivity was achieved by applying well-designed microfluidic chambers compared to other microplate based spectrophotometric methods. The plate reader compatible microfluidic chip could be appropriate to determine the concentration of APIs having fluorescent characteristics.

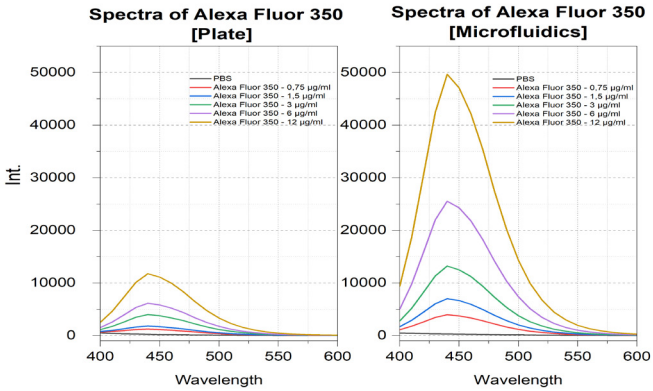

Figure 1. Fluorescent spectra of Alexa Fluor 350 dye measured in conventional plate and in microfluidic chip using 20 μl sample volume and 5 different concentrations (0,75-12 μg/ml).

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Biomimetic Strategies for the Electrochemical Detection of Quorum Sensing Molecules in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is one of the most opportunistic and common Gram-negative bacteria, with a high ability to develop antimicrobial resistance and to form biofilm, leading to severe healthcare-associated infections (HAI). In order to form the biofilm and produce the virulence factors, the bacteria communicate with each other through a communication system called quorum sensing (QS). *P. aeruginosa* cells produce hormone-like substances called autoinducers (AI), like N-3-oxo-dodecanoyl-homoserine lactone (3-O-C_{12}-HSL) and butanoyl-homoserine lactone (C_4-HSL) [1]. The detection of these molecules with highly sensitive and selective biomimetic sensors, using molecularly imprinted polymers (MIPs) or aptamers, would facilitate the rapid identification of HAI.

In this study, we developed sensitive and specific electrochemical sensors for the detection of AI molecules, using MIPs or aptamers. The MIPs were obtained by electropolymerization using cyclic voltammetry (CV). Several electropolymerizable monomers (3-aminophenylboronic acid, 4-aminobenzoic acid, 4,4'-dihydroxybenzophenone and methylene green) and several electrode surfaces (glassy carbon electrode (GCE), carbon paste, graphite felt and screen printed electrodes (SPE)) were tested in order to choose the most suitable conditions. The modified electrodes were characterized by CV and differential pulse voltammetry (DPV) using $[Fe(CN)_6]^{3-/4-}$ as a redox probe. The composition of the polymerization mixture, the electropolymerization method, the template extraction and the rebinding process were also optimized.

The developed aptasensors employed SPE, modified with Au nanoparticles to facilitate the immobilization of the aptamers, functionalized with thiol groups. A deposition step of 2-mercaptoethanol was applied to eliminate nonspecific interactions at the Au surface. All the analysis steps (the aptamer immobilization, the AI incubation, the readout method) were optimized in order to determine the optimum conditions for AI detection. The modified electrodes were characterized using different electrochemical techniques and surface plasmon resonance.

The developed MIP-based and aptamer-based sensors showed good selectivity and sensitivity for detecting QS molecules in *P. aeruginosa*.

Acknowledgements

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Quantification of Target Protein Biomarkers in Complex Media via Faradaic Shotgun Tagging

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Quantification of protein biomarkers constitutes a major clinical diagnostic tool for the detection of diseases and the monitoring of patient pre-symptomatic health like cardiovascular diseases or cancer. [1, 2] Electrochemical assays are considered to possess a high potential for the development of widely accessible, i.e. cost-effective and scalable, alternatives to the currently available standards. Although various techniques for the simultaneous quantification of proteins in complex samples have been proposed, currently available commercial assays still have limitations regarding sensitivity, analysis time and multiplexability. [3] Most commonly, these assays rely on a sandwich-type approach to enable simultaneous quantification, whereby an antibody on surface recruits the antigen, while a secondary labelled antibody enables quantification of surface-confined biomarkers. These approaches present multiple drawbacks including the necessity for two antibodies binding to different epitopes (or at least sites) on the protein of interest as well as generation of differently labelled-probes and above that often are associated with considerable time and cost factors as well as demand for highly trained personnel.

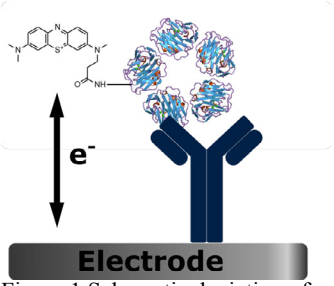

Figure 1 Schematic depiction of electrochemical quantification of directly redox-tagged biomarker at antibody-functionalized electrode.

Within the presented work we propose the direct non-specific chemical tagging of complex protein samples, with subsequent specific capturing and read-out of proteins of interest at an antibody-functionalized surface to omit the potentially laborious and costly development of specific labelled-antibody tags. In particular, methylene blue tags are utilized to demonstrate the simultaneous voltammetric detection of two cardiac biomarkers (CRP and cTnI) at two separately antibody-functionalized electrodes from the same sample solution containing various interfering species. The detection format can be microfluidically integrated in supporting an automatable quantification of both markers down to pg-mL levels from 25 µL of diluted human serum within 15 minutes. [4]

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Screen-Printed DNA-Based Sensors for Detection of the Prostate Cancer Biomarker miR-21 – a Feasibility Study

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The miR-21 was reported as one of the potential microRNA (miRNA) biomarkers to be used as diagnostics tool for the detection of prostate cancer. Statistically relevant over-expression in comparison with healthy controls has been reported for this marker for urine samples of prostate cancer patients. [1]

In the present feasibility study, we are investigating the use of electrochemical detection based on screen printed electrodes and DNA-based hybridization recognition of the miRNA strand of interest. The results of the present work will be the basis for an evaluation of the present technology for the development of a non-invasive, simple to use, cost-effective and rapid point of need diagnostic system.

This preliminary study is based on a DNA probe, designed analogously as similar literature reported probes used for the detection of nucleic acids of similar length (e.g. [2]). miR-21 was spiked in buffer solutions of different concentrations of NaCl, mimicking the range found in urine.

The selective binding of miR-21 to the DNA probe induces its conformational change, which displaces the electrochemical marker methylene blue. The signal is detected by square wave voltammetry. At a frequency of 15 Hz the sensors displays signal-on behaviour with a maximum signal gain of 96.0% ± 5.7% and an estimated dissociation constant (KD) of 137.7 ± 4.4 nM (n=4). The useful dynamic range (defined as the range from 10 to 90% of the maximum signal change) is from 55 nM to 343 nM with a limit of detection (LOD) of 31 nM (n=4).

This preliminary study will be followed by an extensive study aimed at the test and optimization of further parameters related to the target application in view, such as pH sensitivity, shelf life, stability of the biomarker in solution, etc. The analytical optimization will be followed by evaluating performance in real matrix, i.e. urine.

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A Modified Hummers Soft Oxidative Method for Functionalization of CNTs: Preparation, Characterization and Potential Application for Selective Determination of Norepinephrine

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Functionalized carbon nanotubes, CNTs-(COOH), of excess oxygen containing functional groups were prepared using a modified Hummers method. The method is based on oxidizing pristine carbon nanotubes (P-CNTs) by moderately concentrated sulfuric acid and potassium permanganate at low temperature to avoid possible CNTs fragmentation and to preserve the aspect dimensional ratio. The resulting compatible materials were characterized by scanning electron microscopy (SEM), energy dispersive X-ray (EDX), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS) and electrochemical method including cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Fabrication of a sensitive sensor was much reliable by directly casting of CNTs-(COOH) onto the surface of a glassy carbon electrode, CNTs-(COOH)-GCE, rather than the tradition electrochemically reduced deposition method, ER-CNTs-(COOH)-GCE, which ultimately misplaces large number of surface oxides. The functionalized surface materials exhibited an exceptional electrochemical activity on norepinephrine (NOR) oxidation in the presence of high concentrations of electrochemical active interference species such as ascorbic acid (AA) and uric acid (UA). The proposed sensor passed excellently the selectivity test and lowered the detection limit of NOR (DL3σ, NOR) into 0.035 μM (6 ppb). In addition, the sensor was applied successfully for detection of NOR in plasma blood sample with tolerable recovery percentages.

In situ Silver Nanoparticle Coating of Virions for Quantification at Single Virus Level

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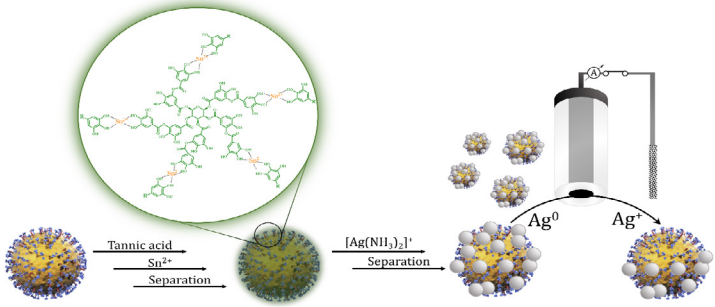
In situ labelling and encapsulation of biological entities may provide a versatile approach to modulate their functionality and facilitate their detection at single particle level. [1]

We introduced a novel virus metallization approach based on in situ coating of viruses in solution with silver nanoparticles (AgNP) in a two-step synthetic process, based on the surface activation with a tannic acid – Sn(II) coordination complex, which subsequently induces silver ion (I) reduction. [2] The metalic coating on the virus surface opens the opportunity for electrochemical quantification of the AgNP-tagged viruses by nano-impact electrochemistry on a microelectrode with single particle sensitivity, i.e. enable the detection of particles otherwise undetectable. We showed that the silver coating of the virus particles impacting the electrode can be oxidized to produce distinct current peaks the frequency of which show a linear correlation with the virus count. The proof of the concept was done with inactivated Influenza A (H3N2) viruses resulting in their quantitation down to the femtomolar concentrations (ca. 5×10^7 particles mL^{-1}) using 50 s counting sequences.



Schematic workflow of the *in situ* modification of virions for amperometric nano-impact measurements.

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Registration of Small Changes of Calcium Ion Concentration by the Constant Potential Coulometric Method

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Clinical analysis requires to control the ionized calcium in blood. The range of Ca^{2+} in human blood is 1.16 – 1.32 mM, therefore the sensitivity of potentiometric measurements with ion-selective electrodes (ISEs) is insufficient. A promising alternative is the constant potential coulometry [1]. The data on the determination of 0.1% changes of the Ca ion concentration in blood model solutions and in blood serum by this method will be presented. Since the changes are much smaller than the initial value, the charge is linearly dependent on the analyte concentration (not only logarithm, see Figure 1), which is convenient for analytical applications [2]. Features will be discussed of measurements with an electronic capacitor in series with the electrode like propose elsewhere [3], and of the analysis using the charge curve fitting [4]. It will be shown how inaccuracy of the potential setting affects the shape of the coulometric signal.

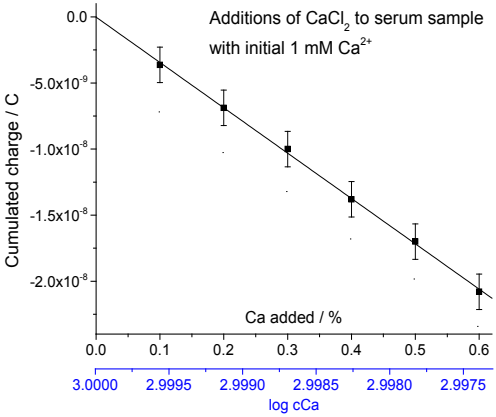

Figure 1. Calibration plot with charge values after additions of $CaCl_2$ to serum sample.

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Voltammetric Determination of Low Concentrations of Growth Stimulators in Plant Material Using Boron-Doped Diamond Electrode

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Pesticides are often used in the cultivation of fruits and vegetables to reduce production losses, increase the quality of the produce and prolong the necessary quality and shelf life of these commodities after harvest [1]. In most cases, these substances are stable, bioaccumulative and thus contaminate the components of the environment [2]. In particular, consumers of these products [3-4] and useful insects [6] are at risk. Often applied substances for these purposes include daminozide, paclobutrazole, mefentrifluconazole, etc.

The voltammetric determination of these substances is performed using electrochemical oxidation on BDDE electrode at a very positive potential. BDDE was chosen due to its excellent electrochemical properties, including a wide available potential window (3 V), low background current, low current noise, good chemical resistance, resistance to passivation, and high hardness [5]. From the plant matrix, ethanol extract of the analyte was isolated using 1 g AmberliteR IRC120 H ion-exchange resin in H+cycle. The capture was carried out at laboratory temperature for 1 h with stirring at 700 rpm. After that, the ionex was separated and subsequently extracted with 5 ml of 1 mol L^{-1} NaOH at 70 °C under stirring at 500 rpm for 20 min. The resulting solution was subjected to voltammetric analysis.

Acknowledgement

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Highly Stable Biosensors with a Diffusion-Limiting Membrane for Noninvasive Monitoring of Hypoxia

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Lactate is a well-known marker of tissue hypoxia. In sport medicine its concentration is considered as an index of the athlete's fitness. Continuous monitoring of lactate level is of most interest. It is obvious that blood analysis is not suitable for this purpose. While sweat appears to be one of the most promising liquids: it is a non-invasive liquid, which spontaneously releases during exercise, moreover there is evidence of a correlation between variation rates of sweat and blood lactate concentrations [1].

Currently, research groups around the world are developing wearable devices for non-invasive continuous monitoring of lactate. Among them, the most attractive and widely used are sweat analysis devices based on lactate electrochemical biosensors. The main task in the analysis of undiluted sweat is to develop a (bio)sensor capable of measuring high concentrations of lactate (about 10 times higher than in blood): notoriously, lactate level in sweat ranges from 4 mM to 60 mM and increase to 100 mM after exercise. Besides, to operate in undiluted sweat, the lactate sensitive electrode should possess high operational stability. The addition of a diffusion-limiting membrane seems to be one of the most promising ways to increase the upper limit of detection and stability of biosensors.

We propose a novel Fe-Ni hexacyanoferrates-based lactate biosensor for online noninvasive hypoxia monitoring. Additional perfluorosulfonated ionomer (Nafion analogue) layer on top of the enzyme containing membrane acts as a diffusion-limiting layer. This makes it possible to achieve a linear calibration range over the entire range of sweat lactate concentrations (1 – 100 mM). Moreover, the operational stability of the developed biosensor is really high: no current drop was observed for 5 hours in 20 mM of lactate, which is definitely enough for continuous analysis of sweat during long training. In conclusion, the applicability of elaborated biosensors for hypoxia monitoring during workout via analysis of undiluted sweat collected from working and latent muscles was shown.

Acknowledgments

Financial support through Russian Science Foundation (grant # 19-13-00131) is greatly acknowledged.

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New Analytical Strategy for Electrochemical Sensing of Tetracycline Based on rGO-ZnO Modified Glassy Carbon Electrode

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Since their discovery, tetracyclines (TCs) are one of the most commonly used antibiotics on a global scale [1]. Due to extensive activity towards a wide range of gram-positive and gram-negative bacteria, TCs were frequently used in the treatment and prophylaxis of a wide range of human infections including respiratory infections, malaria and dermatologic diseases, as well as in veterinary medicine to prevent animal diseases and promote animal growth [1, 2]. Studies have demonstrated that after medication more than 70% of the TCs are excreted and released into the environment [3]. Consequently, TCs were detected in surface waters, or accumulated with organic manure in soil [3]. Accordingly, scientists have been inspired to develop effective analytical methods for the determination of TCs in various real samples.

In the present work merits of rGO and ZnO were combined to synthesize a nanocomposite rGO-ZnO as a modifier for GCE. CV experiments revealed that rGO-ZnO as a modifier enhanced the oxidation of tetracycline (TC) and increased current intensity with respect to GCE. The synthesized modifier was characterised by utilizing SEM, EDS, AFM and FTIR analysis. Afterward optimisation of chemical and instrumental parameters of the SW voltammetric analysis, prepared electrochemical sensor rGO-ZnO-GCE was applied for voltammetric determination of TC in water and human urine samples with good recoveries.

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Multilayer Graphene Sheet as a Highly Sensitive Nitrite Sensor

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Increasing levels of nitrate (NO_2^-) in ground and surface water have been detected due to the expansion of nitrogenous fertilizers, disposal of animal farms waste, and usage as food preservatives. Due to its toxic nature, recommended levels of NO_2^- in drinking water are below 3 mg/L [1]. Carbon-based electrodes are widely used for the detection of NO_2^- due to their ability to oxidize NO_2^- into NO_3^-, however, they are usually modified with other metal/metal oxides for a higher sensitivity [2]. In this work, we used a pristine multilayer graphene sheet (MGS, Graphene Supermarket) as the sensitive electrode (SE) for NO_2^- detection. The cyclic voltammetry (CV) and differential pulse voltammetry (DPV) studies (Fig. 1a-b) demonstrated that the fabricated SE was able to detect a wide range of the analyte (10 – 1000 μM). Furthermore, the sensor showed good reproducibility and a high sensitivity of 81.22 ± 1.8 mMμAcm^{-2}, comparable to other modified carbon based NO_2^- sensors [2]. From all of the carbon allotropes, graphene is an excellent choice for NO_2^- sensing due to its high conductivity, specific surface area, and tensile strength. Due to these unique properties, the unmodified MGS network provides an enhanced electron-transfer environment (shown in Fig. 1d), showing a performance comparable to metal-carbon hybrid WEs. Furthermore, the use of MGS facilitates future integration with thick/thin film based electrodes for the fabrication of a low cost, portable NO_2^- detection system.

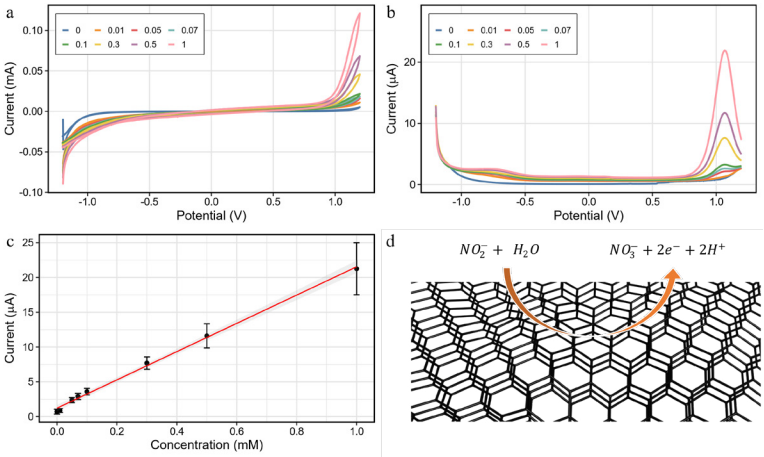

Figure 1. CV (a) and DPV (b) studies of the MGS based NO_2^- SE with varying concentrations of $NaNO_2$ (10 to 1000 μM) in 0.1 M PBS pH 7. (c) The corresponding calibration curve for DPV current response vs. concentration of NO_2^-. (d) Schematic representation of the sensing mechanism using multilayer graphene.

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Innovative Sensor and Analytical Approach for *In Situ* Sequential Quantification of Bioavailable As(III) and As(V) Species in Aquatic Systems

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Arsenic (As) from natural and anthropic sources is a trace element of environmental concern because of its carcinogen properties, its tendency to bioaccumulate, and its trophic transfer through the food chain. The inorganic oxyanions As(III) and As(V) are predominant in aquatic systems [1]. As (III) is more toxic than As(V) and the inorganic As species are more toxic than most of the organic forms [1]. Therefore, the development of analytical sensing devices and methods that allow for the *in situ* quantification of As(III) and As(V) is required to adequately assess and manage their potential threat to water ecosystems and human health. Recently we developed newly designed on-chip gel-integrated microelectrodes (GIME). They consist of renewable nanofilaments (AuNF) electroplated on an array of interconnected iridium-based microdiscs and covered with a gel acting as an efficient antifouling membrane. Incorporated in an advanced submersible probe (called TracMetal), they allow for the first time the direct *in situ* voltammetric quantification of the inorganic arsenite species available for bio-uptake, the so-called dynamic As(III) fraction ($As(III)_{dyn}$) [2].

We report here on the development and field evaluation of an innovative analytical approach for the quantification of the dynamic fractions of arsenite ($As(III)_{dyn}$), arsenate ($As(V)_{dyn}$) and total inorganic As ($As(III)_{dyn}$+$As(V)_{dyn}$) at natural pH by Square Wave Anodic Stripping Voltammetry on the nanofilament arrays mentioned above. To do so, direct quantification $As(III)_{dyn}$ is first performed, immediately followed by the quantification of As_{dyn} after manganese (Mn)-catalyzed reduction of As(V) to As(III) [3]. $As(V)_{dyn}$ is obtained by subtraction. The optimizations of the analytical protocols regarding the preparation of the AuNF-GIME and the sequential method for the detection of $As(III)_{dyn}$ and $As(V)_{dyn}$ at natural pH will be presented. Field evaluation, validation and application were successfully achieved using the TracMetal and an adapted flow-injection set-up for the controlled *in situ* Mn(II) addition and mixing with the natural sample. The performance and accuracy of the proposed approach to record short-term spatial and temporal variation of the targeted inorganic arsenic species will be demonstrated by selected examples.

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Hydrogen Sulfide and Wine Fermentation

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Hydrogen sulfide (H_2S) is a volatile, toxic, sulfur-containing compound that can appear in fermented foods. Its scent is reminiscent of a rotten egg. Its presence in beers, ciders and wines is clearly the result of a fermentation defect. The human olfactory sense is extremely sensitive to hydrogen sulfide. Its ppt level can be detected organoleptically. It can mask the fruity aromas of wine even in the smallest concentration. Hydrogen sulfide containing wine is not suitable for human consumption Sensitive method applicable for detecting H_2S during fermentation could help avoiding defect in wine-making.

Recently in our work a novel method of high sensitivity has been developed for measuring H_2S. It employs silver working electrode that surface is coated with a thin, basic reagent film containing $Ag(CN)_2^-$ complex. The electrode is built in an air gap cell and potentiometric detection is used for the measurement. The sample solution of basic pH is introduced into the air gap cell. Upon acid addition the liberated gaseous H_2S passing through the air gap enters the basic reagent film and interacts with the $Ag(CN)_2^-$ complex. The change of the electrode potential is the analytical signal. The applicability of this method for assessing H_2S production during wine fermentation has been proved. Impinger with zinc-acetate containing capture solution was used for sampling.

The method, its analytical properties, and results achieved employing it in model fermentation experiments will be presented in the Conference.

Using electrochemical methods, hydrogen sulfide can be quantified during fermentation. Appropriate interventions can prevent the accumulation of this undesirable compound in the wine.

Abstracts of poster presentations: Tuesday

Numerical Simulation of Polymeric Reference Electrodes Based on Organic Electrolytes: Developing a Basic Concept Into a Predictive Instrument

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Solid-state reference electrodes (REs) are an essential part of all-solid-state potentiometric devices for efficient and robust analysis. To avoid a conventional liquid-junction in REs, organic electrolytes are commonly introduced into the polymeric electrode membranes [1]. It is generally accepted that the partition of the ions of such an electrolyte between the aqueous and polymeric phases imposes a stable potential at the phase boundary [2]. Although this basic concept has been known for decades, there are currently few quantitative tools to control and predict the properties of potential REs, since the relationship between phase boundary potential and RE membrane composition has not been comprehensively established thus far.

In this work, we propose a theoretical approach to the explanation and quantification of the boundary potential stabilization phenomenon for the electrodes modified with organic electrolytes (Q^+B^-). It was obtained that the efficiency of stabilization depends on the closeness of the partition coefficients of the Q^+ and B^- ions and on the overall QB load in the membrane (Fig. 1A, B). The developed model predicts that the small additions of a traditional ion-exchanger enhance the performance of the QB-based REs. For some specific cases, the optimal concentrations of QB and ion-exchanger in the polymeric phase are suggested to provide stable electrode potential. The model results are verified experimentally with poly(vinyl chloride) membranes containing both ion-exchanger and ETH500 (QB) in ten various ratios (Fig. 1C). A good agreement between the measured electrode response and the theoretical results is observed in a broad range of solution concentrations.

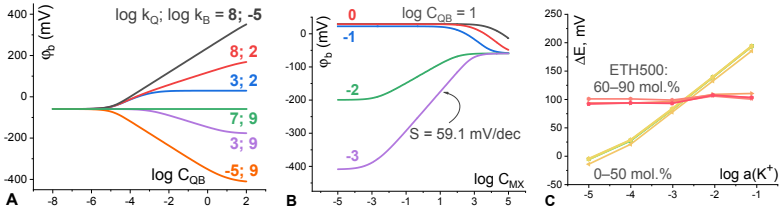

Fig. 1. A: phase boundary potential at various Q^+ and B^- ionic partition coefficients vs. QB concentration in the membrane; B: phase boundary potential vs. concentration of the aqueous electrolyte (MX) at different QB concentrations in the membrane; C: potentiometric K^+-response of the membranes containing KT*p*ClPB and ETH500.

Acknowledgment

This work was funded by the Russian Science Foundation, project number 20-73-10033.

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Variation of the ISE Resistance: Can We Use it Practically?

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The bulk resistance of ISE membranes increases along with a decrease of the solution concentration. We explain this in view of heterogeneity of the membranes consisting of a continuous organic phase and a dispersed aqueous phase (water droplets): lipophilic ions are confined to the organic phase of the membrane, and must circumvent the droplets [1]. On the other hand, the driving force for water uptake is the osmotic pressure of solution. Therefore, the membrane resistance is expected to be determined by the total concentration of ionic species in the solution, or by the ionic strength, rather than by the concentration of the primary ion. The data confirming these expectations will be presented (Figure 1). The sensitivity of the resistance to the ionic strength is high only in diluted samples. However, it will be shown that the non-uniform distribution of water in membranes [2] allows increasing the sensitivity by use of thin membranes (Figure 2).

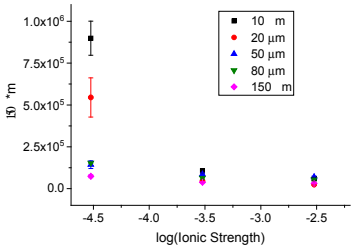

Figure 1. Resistivity of membranes with thickness of 400 µm.

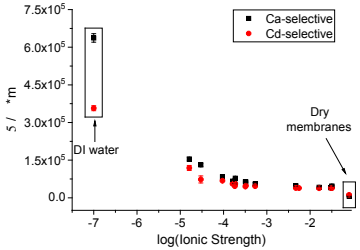

Figure 2. Resistivity of Cd^{2+} membranes with different thicknesses.

From the practical point of view, it opens an opportunity for measurements both of the activity of an analyte (in the potentiometric mode) and its concentration (using the value of the ionic strength estimated from the resistance) with the same sensor.

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Mapping the Electric Field with a Micro Reference-Electrode as SECM Tip

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The electric field present in charged systems has an undesired effect on spatial ion activity mapping with the SECM. Several methods has been worked out by our research group to cancel its effect and to get the true ion activity distribution above corroding systems [1, 2].

In this poster presentation the authors argue that the electric field is a valuable analytical parameter that can be used to investigate corrosion reactions. Using an microsized reference electrode the potential distribution can be mapped, and from that the electric field can be determined by derivation with respect to spatial coordinate. This can give information about the rate of the corrosion reaction as a function of spatial coordinates, and whether a certain location of the sample surface is anodic or cathodic (or neither).

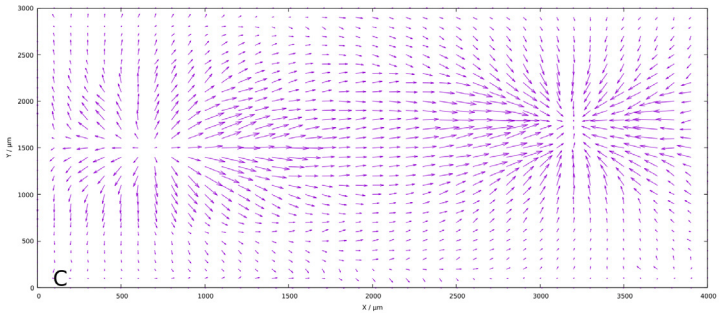

Figure 1. The electric field above a pair of electrodes polarized to 2 V with respect to each other.

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Microfluidic Test Strips Integrating Printed Electrochemical Sensors and Wireless Communication for Monitoring of Potassium in Whole Blood

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Close monitoring of the potassium concentration in blood is essential for patients suffering from conditions such as cardiac or renal insufficiency. Therefore, measurement of potassium in capillary whole blood samples should be enabled by means of inexpensive, rapid disposable tests, which patients ideally can perform themselves [1]. The concept of such smart test strips comprises printed electrochemical sensors, a microfluidic system for sample delivery and preparation, a semiconductor microchip for electrical signal evaluation and a printed antenna for wireless transmission of the measurement data. Enabling high throughput and low-cost manufacturing will be crucial to allow for successful application of such self- or home testing devices. Recently roll-to-roll technologies have been demonstrated as highly promising methods for manufacturing of polymer based microfluidic detection systems [2].

We present a polymer film-based sensor stripe system, comprising a capillary force driven microfluidic system for separation of plasma from whole blood and sequential delivery of sample and calibrator fluid to screen printed ion-selective potassium sensors. The fluidic structures consist of specific microstructures to assist extraction and transport of blood plasma from porous separation membranes that are directly generated in polymer films by a roll-to-roll extrusion coating technique [3]. These are integrated with films carrying printed solid contact ion-selective electrodes (ISE), antenna structures and circuitry for evaluation and wireless transmission of measurement values.

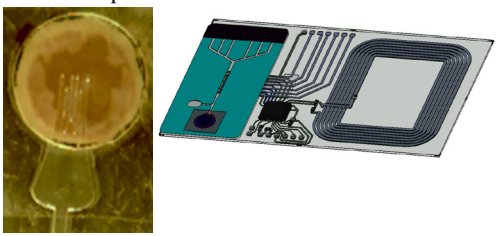

Figure 1: (left) Plasma extraction structure,
(right) scheme of microfluidics, printed K^+-ISE & circuity

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Addressing the Challenge of Preparing Solid-Contact Ion-Selective Electrodes with Excellent Batch-To-Batch E^0 Reproducibility

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Replacing the conventional liquid contacts with solid contact (SC) materials in ion-selective electrodes (ISEs) based on polymeric sensing membranes received attention practically from their inception. Such all-solid-state constructions largely facilitates the miniaturization and manufacturing of such electrodes beside opening new application due to their improved robustness. However, with the emergence of wearable and single use ions sensors the requirements with respect of potential stability that implies the lack of susceptibility to various environmental conditions got supplemented with the need for adjustable and reproducible E^0 values. This prove to be far to be straightforward even with the state of the art of solid-contact materials, which include conducting polymers [1] and large surface area nanomaterials (e.g. graphene, carbon nanotubes) with high double layer capacitance. We approached this problem by implementing solid contact nanomaterials functionalized with redox molecules having a well-defined and reversible electrochemical redox behavior [2]. We expected a more precise control over the E^0 of the respective solid contact ISEs by adjusting the ratio of the oxidized and reduced forms during the electrode fabrication. Indeed, by covalently grafting redox molecules on multi-walled carbon nanotubes (MWCNTs) we could combine the advantages of large double layer capacitance nanomaterials and redox couples in terms of potential stability. The choice of the redox molecule proved to be essential in terms of potential stability. Here we show that by judicious choice of a stable redox molecule with high oxidation potential we could reach extraordinary potential reproducibility of the relevant SCISEs even for fabrication batches spanning over a ca. 1 year period. Thus, this approach might represent the ultimate solution for the fabrication of 'calibration-free', single-use, disposable SCISEs.

Acknowledgment

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The Development and Characterization of Inkjet-Printed Solid-State Ag/AgCl Reference Electrodes

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Miniaturized solid-state reference electrodes (SSRE) with a stable potential are needed to enable novel application scenarios of potentiometric sensing. Inkjet printing has recently been highlighted as a prospective technology for the development of such electrodes in a high-throughput and cost-effective manner [1]. We present here the first steps in the development of inkjet-printed Ag/AgCl SSREs. Two different electrode designs were tested: First, a disk silver electrode was chemically chlorinated using iron(III) chloride and surface coated with a poly(vinyl butyral) (PVB) reference membrane [1] – RE1. Second, a reference Ag/AgCl paste was formulated based on AgCl reduction in a PVB solution [2] – RE2, which enabled development on non-silver (glassy carbon) disk electrodes. An innovative intense pulsed light (IPL) photoreduction technique was applied for AgCl reduction in the paste. The conditions of chemical chlorination and IPL photoreduction parameters were optimized, as well as the required volumes of the reference membranes and conditioning times. The electrodes were characterized by cyclic voltammetry and electrochemical impedance spectroscopy. The response of the electrodes in aqueous solutions of various salts, including KCl, $NaNO_3$, NaCl, and $CaCl_2$ of different concentrations was examined, as well as the electrodes' response to pH changes. In all cases, an acceptable voltage response was achieved, and both types of electrodes were stable over a wide pH range. Further on, comparative characterization of a commercial ion-selective electrode with prepared SSREs against a conventional (internal electrolyte based) double junction Ag/AgCl electrode was performed, yielding similar sub-Nernstian slopes for all systems. Finally, the optimized reference membranes were transferred onto planar electrodes fabricated by inkjet printing of silver and graphene inks and characterized correspondingly.

Acknowledgment

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Development and Research of Ionic Liquid-Based Reference Electrodes with Polymeric Membranes

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Miniaturization is an important trend in analytical chemistry, allowing fast and cost-effective analysis of small samples. In the case of miniaturized and especially planar potentiometric sensors, there is no space for liquid electrolyte, which is why the concept of "All-Solid State Electrodes" appeared. A great deal of research was devoted to the development of All-Solid State ion selective electrodes selective towards various ions [1], however there is still not enough study on planar reference electrodes.

In our work, reference electrodes with polyurethane membranes containing ionic liquids have been developed [2]. Particularly, the influence of physicochemical properties of ionic liquids on the performance of polymeric membrane reference electrodes was examined. Other membrane modifications' effect on electrode potential stability have also been checked, including addition of plasticiser or plasticisers polarity. It was noticed that potential stability of prepared electrodes strongly depends on the differences in partition coefficient values between cation and anion of the ionic liquid, as suggested throughout literature [3]. It was also shown that the amount of the ionic liquid in the polymeric membrane does not have significant influence on the performance of resulting reference electrodes. Moreover, results demonstrate that the addition of the plasticizer to the polyurethane membrane is not necessary to prepare reference electrodes that show sufficiently stable electromotive force values in solutions of various ion concentrations.

Membranes of optimal composition were used to prepare All-Solid-State reference electrodes on glassy carbon disc electrodes and screen-printed transducers, which exhibited satisfying potential stability. This kind of membrane all-solid state electrodes may find application in miniature electrochemical sensors for clinical measurements [4].

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Miniaturized Solid-State Ionophore-Based Microelectrode for Ion Transfer Voltammetry: Solution to Stability Problems

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Liquid based capillary microelectrodes have been well explored for ion transfer at the interface between two immiscible electrolyte solutions and many attempts to design ion-selective microelectrodes emerged based on it.[1] However, there has been a gap in producing durable and stable solid-state ion-selective microelectrodes for voltammetric ion sensing applications.[2] Some designs could be applied to a potentiometric readout, but a voltammetric control would be more attractive for numerous applications.[3] Here we show promising results with thin film K-selective microelectrode of 10 μm diameter interrogated by cyclic square wave voltammetry (CSWV). Using PEDOT-C_{14} as transducing layer and polyurethane as membrane matrix prevents the loss of ion sensing components. The calibration curve gave a super-Nernstian slope with lower detection limit of $10^{-4.5}$ M KCl, which was better than with cyclic voltammetry owing to the capacity character of PEDOT-C_{14} that results in a large baseline that masks the weaker ion transfer signal. The logarithmic selectivity coefficients over sodium and calcium are found as -4.2 and -12.7 respectively, which meets the requirement for physiological measurements. Importantly, the difficulty of developing voltammetric thin film solid-state microelectrodes was found coincidently, as the water layer deteriorated the stability and reproducibility more seriously when the electrode dimensions were reduced to the micrometer scale. As a rougher transducer layer was electrodeposited on the gold surface, the performance of the microelectrode would deteriorate, as confirmed with AFM morphology studies. The results may be able to help researchers develop versatile applications in the future.

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3D-Drawn Supports for Ion-Selective Electrodes

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A new concept of easy to make, potentially disposable potentiometric sensors is presented [1]. A thermoprocessable carbon black-loaded, electronically conducting, polylactide polymer composite was used to prepare substrate electrodes of user's defined shape/arrangement applying a 3D pen in a hot melt process (scheme of preparation 3D-drawn substrate electrode is shown in Fig A). Covering of the carbon black-loaded polylactide 3D-drawn substrate electrode with PVC-based ion-selective membrane cocktail results in spontaneous formation of a zip-lock structure with a large contact area.

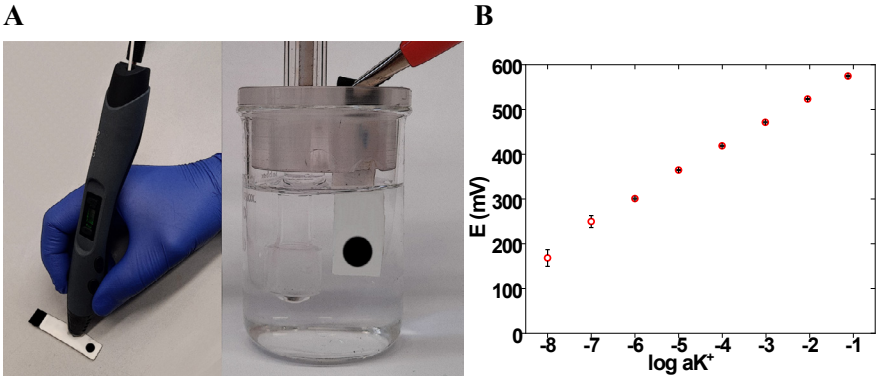

Figure **A** Schemes of preparation 3D-drawn substrate electrode and electrochemical cell with 3D-ISE electrode and reference electrode. **B** Potentiometric responses of 3D-K^+-ISE electrode (within one day reproducibility) error bars show ± SD for n = 6 calibrations performed during one day. Inset: between days reproducibility error bars show ± SD for n = 6 calibrations performed during five days.

Thus, obtained ion-selective electrodes offer sensors of excellent performance, including potential stability expressed by SD of the mean value of potential recorded equal to ±1.0 mV (n = 6) within one day (Fig. B) and ±1.5 mV (n = 6) between five days (Fig. B, inset). The approach offers also high device-to-device potential reproducibility: SD of mean value of E^0 equal to ±1.5 mV (n = 5).

The preparation of sensor substrates does not require a specialized setup or conditions allowing preparation under limited resource conditions. The herein proposed novel approach can be easily extended to be applicable for other ion-selective sensor application modes.

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Triple Pulse Control of a Potentiometric Biosensor For Immuno-Enzymatic Assay Application Using The Choline/Choline Oxidase Pair

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A new potentiometric immuno-enzymatic sensor principle is evaluated for the detection of antigen-antibody interactions at the cation responsive membrane surface without the need to add the enzyme substrate, which acts as the ion marker in the backside inner solution. This is accomplished by a triple pulse protocol to control the delivery of the enzyme substrate from the inner solution to the sample side using chronopotentiometry [1]. First, a zero current measurement (I) gives a stable open circuit potential (OCP) of the system. The second step of the protocol consists of a controlled anodic current pulse (II) of defined magnitude and duration (= 1μA and = 1.5 s, in this case), which leads to a flux () of enzyme substrate (choline, Ch^+) through the ion-selective membrane based on the Fick's first law:

$$i = nFAJ_{Ch^+}(t) \tag{1}$$

The anodic pulse is followed by a zero current measurement pulse (III). During this pulse, the previously extracted ions are consumed by the enzyme (choline oxidase, ChOx) after a competitive immuno-enzymatic assay, resulting in different OCP measurements since the ion-selective membrane is responding to the Ch^+ concentration (. At steady-state, the flux across the membrane () is equal to the contribution from the flux across the aqueous () diffusion layer in the sample side (is the diffusion coefficient and the diffusion layer thickness of different phases) in addition to the enzyme reaction (is the catalytic constant and the Michaelis-Menten constant). This process is described by the combined equation considering Fick's law of diffusion and the Michaelis-Menten kinetics for the ChOx enzyme.

$$c^{aq}_{Ch^+,(0)} + \frac{i\delta^m}{nFAD^m_{Ch^+}} = -\left\{ D^{aq}_{Ch^+} \frac{\left(c^{aq,*}_{Ch^+} - c^{aq}_{Ch^+}(\delta^m)\right)}{\delta^{aq}} + \frac{c^{aq}_{ChOx,1} k_{cat}}{K_m + c^{aq}_{Ch^+,1}} \right\} \tag{2}$$

In the competitive immunoassay, the unlabeled analyte (antigen) competes with the ChOx-labeled antigen for a limited amount of antibodies on the membrane surface. At equilibrium, the amount of bound ChOx labeled antigen-antibody complex is inversely proportional to the concentration of antigen from the sample, resulting in different OCP (III) measurements [2]. The ion-selective membranes are surface-modified with an antibody using click chemistry between alkyne-PEG-NHS molecules and azide groups from the modified poly(vinyl chloride) of the membrane. The antibodies are covalently immobilized based on the interaction between NHS ester-activated and primary amines from the antibody structure [3]. The surface modification is characterized by electrochemical impedance spectroscopy, fluorescence microscopy, and contact angle measurements.

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Scanning Electrochemical Microscopy (SECM) Study of the Regeneration of the Passivation Surface Oxide Layer of $TiAl_6V_4$ Alloy Printed by WAAM Technology

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Additive manufacturing that also called 3D printing is a rapidly developing technique. It has essential advantages over traditional ways of making products of specific shapes and size. It is particularly so when certain metal objects are fabricated. In certain 3D printing technologies used for producing metal items the cooling rate of the newly formed layers is much higher than in conventional casting. It can be as high as 10^3-10^5 K/s. In addition the anisotropy of thermal gradient during fusion and solidification can result in anisotropic microstructure and mechanic stress. It can result in substantial differences between certain properties of the 3D printed and that of the traditionally made ones.

Owing to its high specific strength and excellent corrosion resistance TiAl6V4 alloy made with conventional technology is a broad scale used material. It is applied in aerospace industry as well as in making implants for biomedical application.

Recently in our work a 3D printer has been constructed that employs a wire and arc additive manufacturing technology. It can be used for printing TiAl6V4 alloy items. It is hoped that the 3D printing technology worked out will be appropriate for producing biomedical implants of personalized shape and size. High corrosion resistance is one of the most important properties that a biomedical implant must have.

In our recent work the corrosion resistance of 3D printed TiAl6V4 alloy samples have been studied using scanning electrochemical microscopy (SECM). The action and stability of the spontaneously forming TiO_2 protective layer have been investigated. Furthermore the formation rate of it has been measured and self-healing kinetics studied. In the Conference the employed SECM method, as well as the results obtained will be presented.

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Enabling High Resolution Ocean Nutrient Measurements Through Novel Soft Sensors: Innovation in Printed ISE Arrays for Integration with Physics-Informed Data Science

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Coastal water quality is a critical driver of the economy (e.g., wild fisheries, aquaculture), is important for recreation and provision of ecosystem services, and is known to be heavily impacted by both point source (e.g., stormwater, treated wastewater) and non-point source (e.g., fertilizer) nutrient loads – yet measuring nutrients in marine settings remains significantly constrained by the cost and limitations of commercial sensors and instruments. Managing land-source driven eutrophication requires source identification and localization, while reducing the nutrient footprint of activities like aquaculture demands new systems that can, for instance, be self-cleaning. An improved ability to measure nutrients in seawater affordably, in real time, and at high spatial resolution is critical in achieving these goals.

In response this work maps a path forward for operationalizing ISEs for use in this high salinity environment by using a soft sensor approach: combining a low-power multi-sensor array with chemistry-informed machine learning [1-2]. Two innovations are leveraged here. First, use of a combination of screen, ink jet, and electrohydrodynamic jet printing enables miniaturization, allowing (1) creation of redundant and parametrically varied multi-ISE arrays at relatively low cost (e.g., see example of screen-printed array base layer in Figure 1) and (2) printing of mixed sensor type arrays (e.g., also including amperometric or voltammetric approaches). Second, the number and type of sensors in the array is co-designed with the machine learning algorithms to be used for data fusion, which takes advantage of the strengths, and mitigates weaknesses (e.g., known interferences), of individual sensors. By further explicitly embedding *a priori* chemical knowledge into the algorithms it is possible to improve trustworthiness of the results.

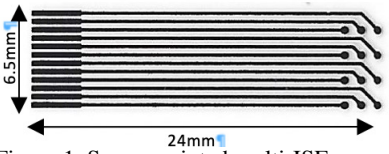

Figure 1. Screen-printed multi-ISE array base layer (this work, 2021-22).

This presentation will describe progress in the microfabrication of sensor arrays using printed electronics techniques, including material and method selection and leveraging existing literature to design and select ISE membrane chemistries. Proof of concept work will be presented on pH while the overall project aims to holistically characterize charged constituents of seawaters and specifically quantify nitrate at environmentally relevant levels.

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Hyperpolarized Solvatochromic Nanosensors Embedded in Agarose Gel Towards Heparin Sensing in Blood

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Heparin is a polyanionic anticoagulant extracted from natural sources and used in numerous surgical procedures. Its typical concentration in the blood steam ranges from 0.1 to 5 IU/mL and varies with time due to its clearance from the body. Too low of a heparin concentration increases clotting risk, whereas excessive levels can induced uncontrolled bleeding. Thus, it is crucial to be capable of quickly measuring heparin levels. The effects of heparin may be reversed via polyionic binding with protamine, an arginine-rich protein that can be used as an antidote in the case of heparin overdose.

The current gold standard of heparin determination is the anti-Xa assay. Since it is a fluorescence-based method, it requires significant sample processing times as it cannot be performed in whole blood. The demand for a more suitable analysis tool encouraged the development of more convenient heparin quantification methods.

Meyerhoff and coworkers achieved pioneering work on protamine and heparin detection since 1994 with ion selective electrodes [1] and optical sensors [2]. Our group recently developed emulsion-based particles sensitive towards protamine containing a solvatochromic dye as signal transducer [3]. The nano-optodes showed a significant absorbance shift in the presence of protamine, no pH cross response and were successfully used to quantify heparin levels in hospital patients' plasma. However, this approach still required sample treatment since it was unsuitable for whole blood detection owing to its high background absorbance.

Agarose gels have been previously used to embed optodes for ion detection [4]. Our protamine sensors were embedded in agarose gel, poured in commercially available polystyrene cuvettes and pictures were taken after protamine addition on top of the gel. The nanosensors showed the desired absorbance shift in presence of protamine when embedded in the gel. Here, however, the mixing between protamine and the nanoparticles was limited by protamine diffusion into the gel. Previous works using embedded nanosensors to detect cations utilized a distance-based readout. Such approach could not be applied to protamine or heparin sensing because physiological concentrations were much smaller. Thus, an intensity-based quantification method was developed here with spectral unmixing, allowing us to monitor the signal change induced by protamine diffusion over time. This method gave promising results for both protamine and heparin quantification in whole blood samples.

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Electrospun Polymeric Fibers Potassium Optical Sensors

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Ion-selective optical probes, allow access to information about analyte level in the samples. Among different format of receptor used, electrospun fibers are attractive alternative [1,2]. Nanofibers combine physical properties of macroscopic sensors with advances of nanostructural probes – large and tunable surface area to volume ratio. Advantageously nanofibers probes do not require presence of stabilizers as in the case of nanoparticles.

The aim of this work was to obtain electrospun fibers optodes using mixture of polymers: poly(vinyl chloride) and polycaprolactone. The optodes are characterized by good wettability, sensitivity and high selectivity to potassium ions, and have a wide linear response in the range of 10^{-6} to 10^{-3} M KCl.

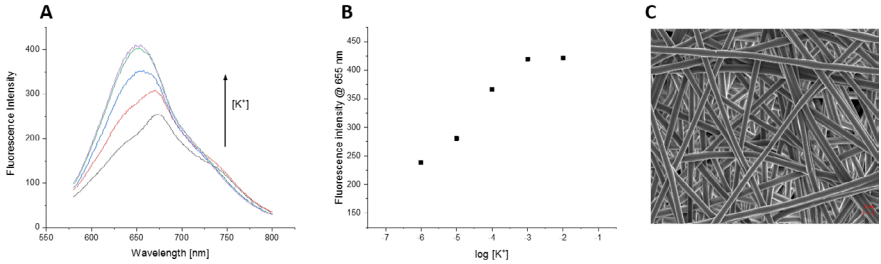

Figure 1 A) Fluorescence spectra at varying concentrations of KCl (10^{-6}-10^{-2} M), B) Dependence of fluorescence intensity on the logarithm of the concentration of K^+ ions, C) SEM image (scale bar 1 μm) of PCL/PVC nanofiber's mat.

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Dye Deaggregation Optical Ion-Selective Sensing Towards Ibuprofen Sensor

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Classical optical ion-selective sensors are important analytical tools, however their application is limited to analytes for which selective ionophores have been developed. The aim of this work was to investigate possibility of extending ion-selective optical sensors idea to other analytes, as alternative optical signal generation principle de-aggregation of a dye, typically leading to increase of optical signal, induced selectively by analyte was proposed [1].

As model system ibuprofen sensor was studied using solvatochromic dye rhodamine B octadecyl ester (RBOE).

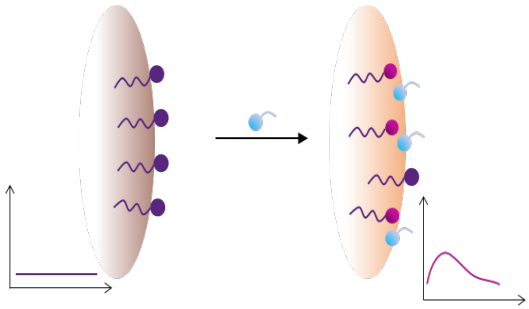

Fig. 1 Schematic representation of the idea of sensor operation: (/) RBOE respectively quenched and active in emission mode, ( ) ibuprofen; the organic phase is marked in color.

RBOE dye in aqueous solution is forming nanoprecipitate of significantly quenched emission, however interactions with ibuprofen results in reorganization of the dye structure that leads to a change in the environment of the chromophore group, what is observed as increase of emission (Fig. 1). This process is affected by the presence of other molecules in solution/ nanoprecipitate and occurs mostly at the interface of the lipophilic nanostructure with aqueous sample. The mechanism of observed interactions leading to emission change was elucidated. Under optimized conditions a linear dependence of the emission intensity on changes in the analyte concentration in the concentration range from $10^{-4,3}$ to 10^{-2} M, in turn on mode was obtained.

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Colourimetric and Fluoreometric pH Sensing with Polydiacetylene Nanofiber Mat

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Nanofibers mats with two types of polydiacetylenes (PDAs): 10, 12–tricosadiynoic acid (TCDA) and 10,12-pentacosadiynoic acid (PCDA) are introduced for colorimetric and fluorimetric pH sensing. PDAs belong to the group of conjugative polymers for which the absorption and fluorescence emission depend on the external stimuli, e.g. high pH [1], high temperature, organic solvents.

The fiber mats were prepared by electrospinning method from a mixture of supporting polymers: polyvinyl chloride (PVC) and polycaprolactone (PCl) with PDA monomers. The obtained mats during photopolymerization with UV lamp (254 nm) change the colour from white to dark blue. The morphology of both fiber mats is similar (SEM images in Fig. A), the average diameters of fibers was estimated as equal to 225 and 250 nm for TCDA and PCDA, respectively.

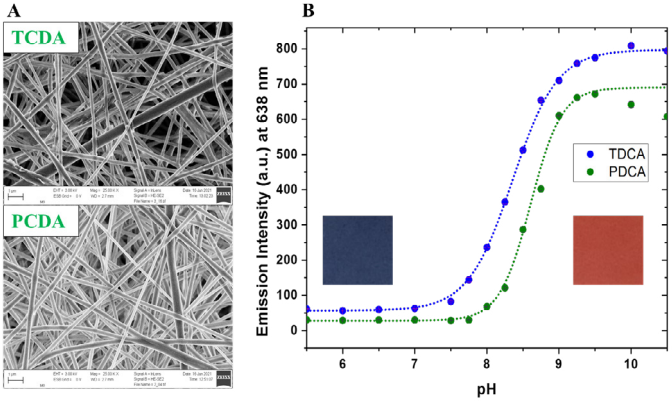

Figure **A** SEM images of fiber mats with PDA. **B** The dependence of emission intensity on the pH.

As the pH increases, the color of the fiber mat changes from blue to red (Fig. B). The result obtained by computer image analysis performed in Image J showed a sigmoidal increase in the intensity of red and a decrease in the intensity of blue color with increasing pH. A similar sigmoidal response is observed for the dependence of the emission intensity on the pH (Fig. B). Changes in the recorded signal occur above pH 7.5 or 8 for TCDA and PCDA, respectively. The results obtained with both methods are consistent with each other.

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Controlled Surface Modification of Fluorescent Latex Nanoparticles with Antibodies

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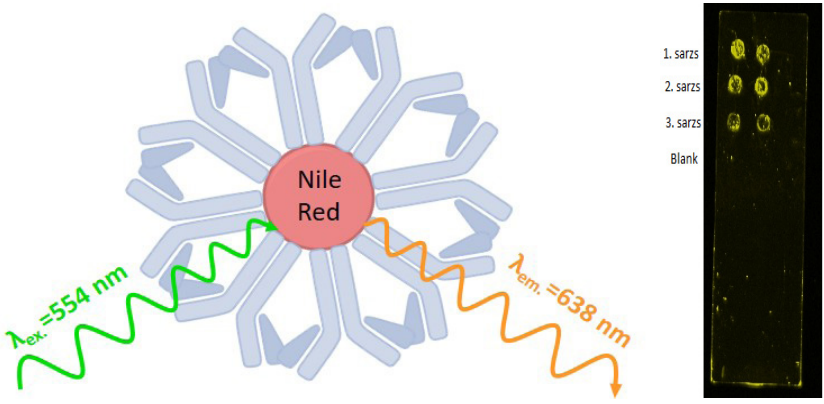
The use of fluorescent nanoparticles in diagnostic tests is becoming increasingly popular[1] due to the high signal amplification that results in a large improvement in the detection limit. The signal amplification is due to the high concentration of the fluorescent dye molecule loaded into the nanoparticles. Therefore, greater sensitivity can be achieved in lateral flow assays than with the routinely used gold nanoparticle conjugates.

In my research, I aimed at efficient and aggregation-minimized modification of latex nanoparticles containing Nile red dye ($\lambda_{ex.}$ = 554 nm, $\lambda_{em.}$ = 638 nm) with antibodies to be used as diagnostic reagent. I worked on the control of the surface concentration of antibodies and on the development and optimization of the appropriate recovery. In my work I used several approaches: passive adsorption, covalent immobilization and use of coordination polymer.

The success of the modification and the degree of aggregation were determined by dynamic light scattering (DLS), the antibody surface concentration by fluorescence measurements (Plate Reader). The applicability of the antibody-modified particles was further tested in immunoassays with a fluorescence imaging system. We found that the modification of nanoparticles by passive adsorption resulted in very low surface coverage, while the immobilization through coordination polymers was difficult to control. The highest control and adequate surface concentration was obtained aby using EDC/NHS coupling.



(a) (b)

Figure 1. a) fluorescence of antibody modified nanoparticle b) Fluorescence image of fluorescent nanoparticle-labeled affinity assay

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Carbonate Optical Nanosensors: New Mechanisms and Their Advantages

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In Situ concentration profile mapping of carbonate (CO_3^{2-}), bicarbonate (HCO_3^-) and dissolved carbon dioxide (CO_2) in natural waters (rivers, lakes etc.) as a function of depth is highly relevant for environmental analysis and might serve as a source of insight into the evolution mechanisms of such complex ecosystems. Numerous solutions for both potentiometric and optical sensing of carbonate and carbon dioxide in aqueous and gaseous samples have been proposed over the years [1], yet such practical tools for spatially resolved analysis as emulsion-based nanooptodes have generally been overlooked.

We are here describing carbonate-selective nanosensors based on either a chromoionophore or a solvatochromic dye as an optical reporter. The sensing appears to predominantly take place on the surface of the nanoparticle and is subject to an influence from lipophilic cations present in the sample transferring into the sensor phase. This is successfully used to enhance sensor performance by adding a second dye into the sample with the subsequent use of a ratiometric readout (Fig. 1). We note that sample alteration is unfavorable for routine *in situ* applications of carbonate-selective nanoparticles. A thorough mechanistic study is carried out, the dual solvatochromic dye approach is expanded and the nature of the interactions between the dyes is investigated.

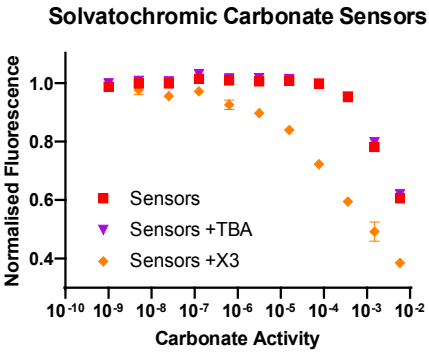

Figure 1. Solvatochromic carbonate sensor response modulated by the addition of a second dye, X3, to the sample solution.

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Environmental Monitoring (Water and Air) Using PiSENS, a Low-Cost, Portable, Raspberry-Pi based Colourimeter

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Here we are presenting a portable Raspberry-Pi based colourimeter. The optical signal provided, colour-change in this case, can be picked up by the camera of the portable device (PiSESNS) and processed automatically. To do so, we have written a fit-for-purpose code using the Python programming language. In addition, due to the versatility of the device, we have built two modes of PiSENS using materials from a DIY shop and pieces created with a 3D printer.

The first one, called PiSENS-A, was set up for continuous measurement of NO_2 in air. This system is based on the Saltzman reaction by which the absorbance solution changes colour because of its interaction with the gas. The device allows data acquisition in a continuous way, this is with high temporal resolution. Due to its portability and low-cost construction, a high spatial resolution can be achieved as well. Successful data collection has been got for periods of 24 hours and 48 hours. The R^2 that we got for 7 points calibration was 0.944, with a LOD of 7x10-5 ppm. The precision as relative standard deviation percentage was 2.8% (RSD%), and 6.4% was the standard error percentage (SE%).

The second one, PiSENS-O, was set up for one-shot measurements for the determination of specific analytes in water. In this case, ionophore-based polymer membranes with optical signals, optodes, are used for the analysis. As proof of concept, we successfully measured sodium (Na^+) and nitrate (NO_3^-) in water. The results show a good adjustment between the theoretical sigmoidal curve and the calibration points. For both ions, the average accuracy measured as SE% was 7.4%, and the precision as RSD% was between 2-12.5% and 5-27% for Na^+ and NO_3^- respectively.

The simplicity of the device in addition to its low construction and maintenance cost provide PiSENS with a lot of potential for its application in rural and remote locations. Particularly, where expensive equipment is not affordable or cost-effective. Furthermore, the data communication capabilities of Rasberry Pi such as WiFi and Bluetooth would allow the integration of multiple devices to create large networks that could assist with live decision-making or the creation of high precision models.

Use of Anti-CRISPR Protein AcrIIA4 as a Capture Ligand for CRISPR/Cas9 Detection

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Gene therapy has long held promise to correct a variety of human diseases and defects. Discovery of the Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR), the mechanism of the CRISPR-based prokaryotic adaptive immune system (CRISPR-associated system, Cas), and its repurposing into a potent gene editing tool has revolutionized the field of molecular biology and generated excitement for new and improved gene therapies. Additionally, the simplicity and flexibility of the CRISPR/Cas9 site-specific nuclease system has led to its widespread use in many biological research areas including development of model cell lines, discovering mechanisms of disease, identifying disease targets, development of transgene animals and plants, and transcriptional modulation [1].

New applications of CRISPR/Cas9 are vastly outpacing detection and quantification of gene-editing reagents. Detection of the CRISPR/Cas9 ribonucleoprotein (RNP) within biological samples is critical for assessing gene-editing reagent delivery efficiency, retention, persistence, and distribution within living organisms. Conventional detection methods are effective, yet the expense and lack of scalability for antibody-based affinity reagents limit these techniques for clinical and/or field settings. This necessitates the development of low cost, scalable CRISPR/Cas9 RNP affinity reagents as alternatives or augments to antibodies.

Herein, we report the development of the Streptococcus pyogenes anti-CRISPR/Cas9 protein, AcrIIA4, as a novel affinity reagent. An engineered cysteine linker enables covalent immobilization of AcrIIA4 onto glassy carbon electrodes functionalized via aryl diazonium chemistry for detection of CRISPR/Cas9 RNP by electrochemical, fluorescent, and colorimetric methods [2]. Electrochemical measurements achieve a detection of 280 pM RNP in reaction buffer and 8 nM RNP in biologically representative conditions. Our results demonstrate the ability of anti-CRISPR proteins to serve as robust, specific, flexible, and economical recognition elements in biosensing/quantification devices for CRISPR/Cas9 RNP.

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Assessment of Food Contaminants by Functional Acid Electrochemical Sensors

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Common food contaminants include pathogens, toxins, pesticides, veterinary drugs and illegal additives, while common allergens are mainly proteins. Inspectors or consumers can identify whether the food has been contaminated and whether the products contain allergens by using detection methods to analyze specific contaminants and allergens

Food contaminants represent possible threats for humans and animals as severe food safety hazards. Prolonged exposure to contaminated food often leads to chronic diseases such as cancer, kidney or liver failure, immunosuppression, or genotoxicity. Aflatoxins are naturally produced by strains of fungi species *Aspergillus,* being one of the most critical and poisonous food contaminants worldwide. Food induces different hypersensitivity reactions in allergic people when humans are exposed to harmful allergens. Generally, patients with food allergies undergo prophylactic practices as there is no medical treatment [1].

Since the high percentage of contaminated food products makes it impossible sometimes their detection using traditional methods, it is imperative to develop fast, accurate and easy-to-use analytical methods to enable safe food products and good practices policies.

This poster presents a strategy for the reduction and prevention of the presence of toxins and allergens in food products by enabling an electrochemical multiplexed approach based on DNA strands. The affinity reaction between the DNA strands and targets causes conformational and structural changes in the recognition layer, which can be assessed by electrochemical approaches. Possible applications on food samples will be presented.

Acknowledgements

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Development and Comparison of Hydrogen Peroxide Electrochemical Gas Sensors

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Fast and simple hydrogen peroxide (H_2O_2) detection still attracts great attention due to its important role and omnipresence in numerous fields, such as environmental and clinical research and monitoring, food safety, chemical and pharmaceutical industries, and biological and medical sciences. Various methods have been developed for the determination of H_2O_2, such as electrochemical methods [1], high-performance liquid chromatography, titrimetry, spectroscopy, chemiluminescence, and others [2, 3]. Excellent sensitivity and selectivity can be undeniably achieved by most of these techniques; however, expensive instrumentation, restricted portability, and often relatively complex sample preparation pose a challenge that still needs to be addressed. Furthermore, the detection of gaseous H_2O_2 brings additional complications connected to the sensor's sensitivity, the inherent instability of H_2O_2, and complex sampling protocols. Electrochemistry offers several attractive advantages from this aspect, such as portable and inexpensive instrumentation, simple or even no sample pretreatment, direct on-site detection followed immediately after analyte sampling/accumulation, excellent sensitivity, and numerous possibilities for various electrochemical sensing schemes, together with sensor miniaturization.

In this work, we will present the preparation and characterization of novel sensors for sensitive on-site detection of gaseous H_2O_2. We will discuss their advantages and drawbacks together with potential application scenarios for indoor/outdoor H_2O_2 monitoring. Finally, we will demonstrate the operation of such sensors in the real environment.

Acknoweldgments

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A Modified Hummers Soft Oxidative Method for Functionalization of CNTs: Preparation, Characterization and Potential Application for Selective Determination of Norepinephrine

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Functionalized carbon nanotubes, CNTs-(COOH), of excess oxygen containing functional groups were prepared using a modified Hummers method. The method is based on oxidizing pristine carbon nanotubes (P-CNTs) by moderately concentrated sulfuric acid and potassium permanganate at low temperature to avoid possible CNTs fragmentation and to preserve the aspect dimensional ratio. The resulting compatible materials were characterized by scanning electron microscopy (SEM), energy dispersive X-ray (EDX), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS) and electrochemical method including cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Fabrication of a sensitive sensor was much reliable by directly casting of CNTs-(COOH) onto the surface of a glassy carbon electrode, CNTs-(COOH)-GCE, rather than the tradition electrochemically reduced deposition method, ER-CNTs-(COOH)-GCE, which ultimately misplaces large number of surface oxides. The functionalized surface materials exhibited an exceptional electrochemical activity on norepinephrine (NOR) oxidation in the presence of high concentrations of electrochemical active interference species such as ascorbic acid (AA) and uric acid (UA). The proposed sensor passed excellently the selectivity test and lowered the detection limit of NOR (DL3σ, NOR) into 0.035 μM (6 ppb). In addition, the sensor was applied successfully for detection of NOR in plasma blood sample with tolerable recovery percentages.

Comparison of Mesityl Oxide and Methyl Isobutyl Ketone as Solvent for Preparation of Organic Modifying Layers on Electrodes

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In a recent work, we found that mesityl oxide has favorable properties in electropolymerization reactions of aromatics compared with methyl isobutyl ketone. The only difference between these solvents is the carbon-carbon double bond found in mesityl oxide which makes it capable of participating in a copolymerization process and so altering the properties of formed polymers. This difference was obvious in the electrochemical investigations as in methyl isobutyl ketone the usual deactivation process could be observed. Platinum electrode was a significantly more appropriate template for the films than glassy carbon as on the latter formation of a compact layer took place. The organic deposits formed from phenylphenols increased the sensitivity towards voltammetric detection of 4-chlorophenol and 4-methoxyphenol which could be improved by incorporating into the film a calixarene having biphenyl groups. The electrochemical copolymerization process (Figure 1.) showed an unusual behavior as instead of continuous deactivation the increase of peak currents could be observed by repeating the scans.

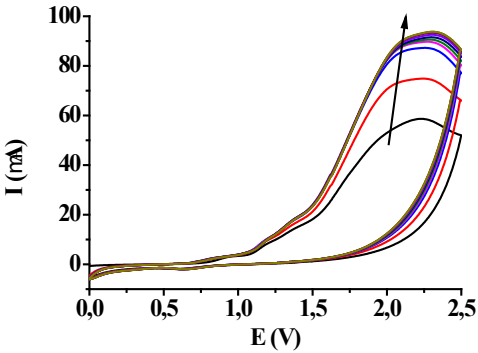

Figure 1. Electrochemical copolymerization of 2-phenylphenol and a calixarene having biphenyl groups in mesityl oxide.

Electrochemical Detection of Ergosterol as an Indicator of Fungal Contamination of Foodstuff

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Ergosterol (provitamine D2, ergosta-5,7,22-trien-3β-ol) is a fungal-specific membrane sterol with structure similar to cholesterol [1,2]. Some fungi are common contaminants of cereals, nuts, dried and other foods, which leads not only to a deterioration of food quality, but especially due to the production of secondary metabolites (mycotoxins), to the risk of health poisoning [3]. In connection to ergosterol, it has been found that there is a correlation between the amount of produced ergosterol and the growth of a given fungus [4,5].

Therefore, ergosterol could be used as biochemical marker of fungal contamination of food raw materials or products. Our aim was to implement and optimize square-wave voltammetric method for detection of ergosterol as simple screening method instead of time-consuming microbiological methods or instrumentally more complex HPLC.

Three different working electrodes, concretely glassy carbon electrode (GCE), common boron doped diamond electrode (BDDE) and screen-printed boron doped diamond electrode (SPE-BDDE) were used and compared in terms of calibration range and sensitivity. Conditions of electrochemical detection were optimized. Finally, implemented method was tested for the analysis of the real samples represented by fungal mycelium and grain which confirmed the possibility of ergosterol detection.

Acknowledgments

This work was supported by the European Union project CZ.02.1.01/0.0/0.0/17_048/0007421 and project of The Czech Science Foundation No. 20-01589S.

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Voltammetric Determination of βCarotene in Vegetable and Pharmaceutical Samples

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A new voltammetric approach for the determination of β-carotene has been developed. Contrary to already presented electroanalytical methods, the proposed setup utilizes a less harmful organic solvent and a non-mercury electrode. The respective procedure is based on direct extraction of the analyte from the sample with acetone containing 0.1 mol L^{-1} $LiClO_4$ and subsequent anodic oxidation of β-carotene at a gold electrode using square wave voltammetry in the same medium. Gold electrode exhibited better repeatability of consecutive measurements than in the case of glassy carbon or platinum electrode and was therefore selected as an electrode material of choice. After optimization of experimental parameters, the method can be characterized by a linear range from 6.0×10^{-6} to 5.9×10^{-4} mol L^{-1} with regression equation $I_p^a = 0.0184c - 0.1631$ and correlation coefficient $R^2 = 0.9998$, limits of detection and quantification LOD = 1.6×10^{-6} mol L^{-1} and LOQ = 5.4×10^{-6} mol L^{-1}, respectively. The applicability of the method was tested in the analysis of vegetables (raw carrots and sweet potatoes) and two pharmaceutical formulations containing β-carotene. The results were in good agreement with those obtained by the reference spectrophotometric assay. Due to simple instrumentation, including sample preparation, the voltammetric method for the determination of β-carotene can be recommended as a quick screening assay in food and pharmaceutical analysis [1].

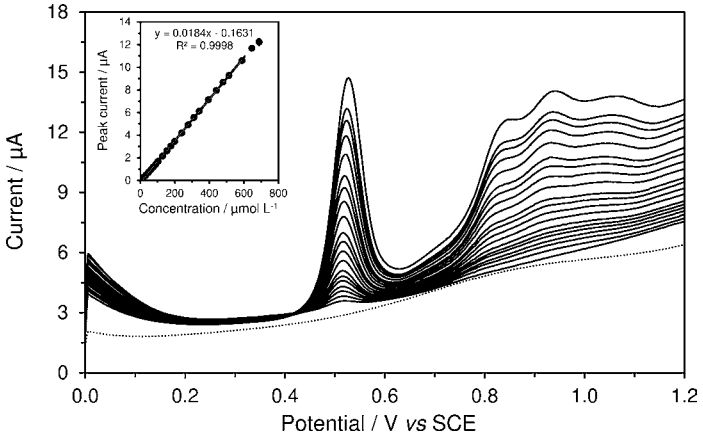

Figure 1. Square wave voltammograms of 16 – 590 µmol L^{-1} β-carotene at the gold electrode in pure acetone containing 0.1 mol L^{-1} $LiClO_4$ with corresponding calibration line (inset).

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Graphene Oxide Film Improves Stability of Meldola Blue Mediator Layer on Glassy Carbon Electrode

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Recently we proposed a novel method for measuring antioxidant activity in different samples. The method employs thin redox mediator film immobilized on glassy carbon electrode (GCE) and uses chronopotentiometric detection. In a short controlled potential step the film is brought to its oxidized state. Upon exposing the electrode to reducing samples, the redox potential changes. The initial slope of the electrode potential – time function is used for assessing the antioxidant activity. Chemically modified GCE with Meldola Blue $(MB)^+$(N,N dimetyl-7-amino-1,2-benzophenoxazinium ion) mediator layer was used in the measurement[1].

In our recent work, reduced graphene oxide linked layer was employed to increase the stability of the MB layer and the applicability of the method was tested for measuring different antioxidant molecules.

Our most recent results showing the improvements in electrode stability and improving the applicability of the novel method will be presented in the meeting.

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Electroanalysis of Neurotransmitter Mixtures Using a Paper-Based E-Tongue System

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Paper-based sensors became so popular in the last two decades that reproducible, low-cost, disposable electrodes can be easily made in any laboratory using published protocols[1]. The aim of this work is the simultaneous detection of dopamine and serotonin, which is not trivial as due to similar structure, they are detected at overlapping potentials. Another hurdle is the polymerization of dopamine at physiological pH, which results in sensitivity loss and deactivation of the sensor surface. For this reason we chose to use disposable, low-cost, one time use sensors made of paper for this proof-of concept study.

We have recently tested different carbon-based electrode modifications[2] and noted that the signals of dopamine and serotonin could not be fully separated from the interfering species such as epinephrine and norepinephrine. However, the differences in the position of the signals on each modification suggested it might be possible to develop a multi-electrode system coupled with multivariate data analysis, e.g. with partial least squares (PLS).

We have screened several electrode modifications, which were described in the literature as able to shift the signals of the selected neurotransmitters, including ionic liquids and surfactants. The final sensor array is composed of carbon nanotube and graphite electrodes modified with ionic liquids or graphene oxide). Data from analysis of single neurotransmitters and their mixtures is used to construct a database to train the algorithm, which is in turn applied to quantify dopamine and serotonin in test samples. Thanks to the multivariate analysis simultaneous detection of both compounds is possible even though neither modification allowed to fully separate signals of the tested species.

Acknowledgment

Authors would like to thank The National Centre for Research and Development, Poland, for funding under LIDER/38/0138/L-9/17/NCBR/2018 grant and National Science Centre, Poland for NCN Sonata 2020/39/D/ST4/02256. EWN would like to also thank the Foundation for Polish Science (FNP), from which she was supported through START programme.

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Abstracts of poster presentations: Thursday

Solvent Effects in Electrochemical Anion Sensing

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Sensing anionic species in competitive aqueous media remains a prominent challenge to long-term applications across a multitude of fields.[2.3] Despite this, fundamental studies of receptors capable of sensing anions in increasingly polar/competitive solvent systems are rare and are often restricted to less polar organic solvents due to solubility limitations and/or negligible responses due to the competitive nature of the solvent media. Herein, the comprehensive investigation and comparison of the electrochemical anion sensing performance of novel halogen bonding (XB) and hydrogen bonding (HB) bis-ferrocene-(iodo)triazole receptors, in a range of increasingly competitive aqueous organic solvent systems (ACN/H_2O), is presented. In solution, the XB sensor notably outperforms the HB analogue, with significant anion recognition induced cathodic voltammetric responses of the ferrocene/ferrocenium redox couple, persisting even in highly competitive solvent media of 20% water content. Of note, is that the response to halides showed a markedly lower sensitivity to increasing water content associated with a unique halide selectivity at unprecedented levels of solvent polarity. In contrast, the HB sensor generally displayed a preference towards oxoanions. Immobilisation of the XB/HB sensors onto a gold surface not only enabled sensing in even more competitive solvent media (up to 30% water content), but is importantly associated with a significant surface-enhancement effect for both XB/HB receptive films in all solvent systems. Interestingly, at the surface a reversal in sensing behaviour was seen with the surface-bound HB sensor outperforming the XB analogue.

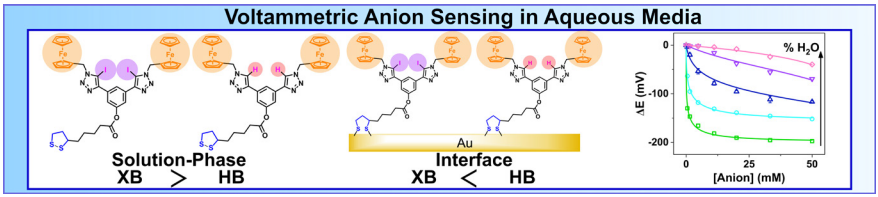

Figure 1. Schematic depiction of the solution-phase XB/HB receptors (left), and when surface-immobilised (centre). The graph on the right depicts the response isotherms to HSO_4^- in increasingly competitive aqueous organic media.

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Response Patterns of Optical Sensors Containing Lipophilic Electrolytes

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Ion-selective optical sensors are highly promising analytical tools due to ease of signal acquisition and use, a broad variety of analytes, and high selectivity. However, due to their response mechanism, they have a fundamental flaw: their response depends on the activity of two ions simultaneously. It was shown in [1] that for realizing single ion sensing within certain extra-thermodynamic assumptions, it is necessary to stabilize the Galvani potential difference between the membrane and the aqueous phase, similarly to liquid junction-free polymeric reference electrodes. For the latter, this is commonly achieved by adding a moderately lipophilic organic electrolyte (MLOE) to the membrane, the distribution of which between the phases stabilizes the interfacial potential [2]. However, the dependence of the optical response on the nature of organic electrolyte and other optode components has not been systematically studied.

In this contribution, we propose a model to predict the response of chromoionophore-based I^+/H^+-selective optical sensors doped with various lipophilic electrolytes. The simulated pH-response is independent of cations over a wide range of I^+ concentrations (Fig. 1A). This was confirmed experimentally with optodes containing salts of various lipophilicity, which showed little to no cationic response compared to a conventional optode (Fig. 1B), while retaining a pronounced pH-function (Fig. 1C). It has been shown both numerically and experimentally that with an increase in the lipophilicity of the organic cation, the response shifts to a more acidic domain, while an increase in the lipophilicity of the anion leads to the opposite effect. The nature of the chromoionophore and ionophore influences the ion-exchange constant and, respectively, the response range of the MLOE-based optode in a conventional manner.

The observed patterns allow adjusting the response range for the target object of analysis. The described concept makes it possible to assess individual cation activities using conventional optodes by normalizing their readings to pH values obtained with the developed cation-independent pH optode.

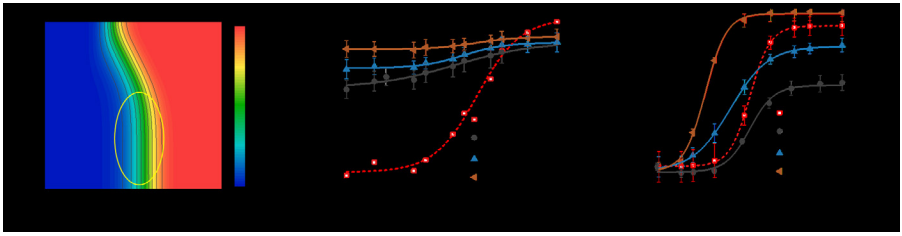

Figure 1. A: simulated optical response of a sensor containing lipophilic electrolyte. B, C: Na- and pH-response of optodes containing organic salts.

Acknowledgment

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Potentiometric Determination of Synthetic Cathinones

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Synthetic cathinones are new psychoactive substances (NPSs) with amphetamine-like effects and currently pose problems in society. Therefore, it is necessary to develop new, fast, sensitive, and inexpensive methods for the large-scale screening of NPSs. "Forensic electrochemistry" is a relatively new field and voltammetric analysis is the main applicable technique. The aim of this study is to demonstrate the possibilities of the potentiometric sensors based on ion-selective membranes (ISMs) to detect the chosen synthetic cathinones. The potentiometric selectivity of neutral carriers (calix[4]arene and dibenzo-18-crown-6-ether, DB18C6) and the cation exchanger (sodium tetraphenylborate, TPBNa) as an active component of ISMs was evaluated toward two structural analogues of cathinone, namely mephedrone (4-MMC) and clephedrone (4-CMC) in model and saliva samples. The main potentiometric parameters were verified in the model samples. The experimental ISMs exhibited the potentiometric response in the concentration range from 7.7 x 10^{-5} up to 1.4 x 10^{-2} mol.l^{-1}. The values of the potentiometric coefficient selectivity () were as follows: –1.1 for TPBNa, –0.9 for DB18C6 and –0.8 for calix[4]arene. Furthermore, potentiometric detection of the above-mentioned cathinones was carried out in saliva samples spiked to a concentration of 7.6 x 10^{-5} and 5.6 x 10^{-4} mol.l^{-1}. The results of the potentiometric determination are in agreement with those obtained by HPLC/MS-MS analysis (Fig. 1).

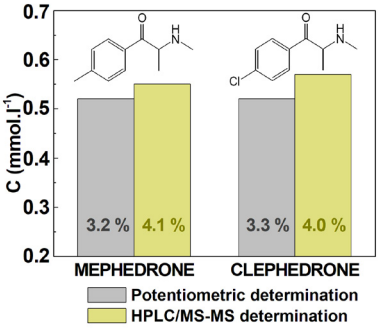

Figure 1. Comparison of potentiometric (gray) and chromatographic (yellow) determination of 5.6 x 10^{-4} mol.l^{-1} synthetic cathinones in saliva.

Acknowledgments

This work was supported by a specific University research grant (Ministry of Education, Youth and Sports of the Czech Republic UCT Prague, CZ, 402850055). M. Kuchař acknowledge the support from the project "Express and portative detection of prohibited substances using innovative techniques: flexible and chiral SERS, selective surface extraction, artificial neural networks" (MVO VJ01010065).

An Electrochemical Approach to Quantification of Volatile Organic Solvents Dispersed in Solution – Towards Bipolar Electrode Sensors

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A novel approach to electrochemical determination of volatile organic compounds (VOCs) suspended in a solution is proposed [1]. A tailor designed hydrophobic polyvinylidene fluoride (PVDF) membrane modified with polyoctylthiophene (POT) was used in Swagelok®-type cell. The approach developed benefits from high affinity of VOCs towards the applied hydrophobic polymer membrane. As a result, on the surface of PVDF membrane, a nanometer thin layer of lipophilic liquid is formed, this liquid contains dispersed POT. In the presence of external polarization applied to the collector electrodes oxidation / reduction of POT can occur (Fig.1 left) in a contactless manner, typical for a bipolar electrode system. This effect leads to change of electrical parameters of the system: capacitance and resistance dependent on amount of VOCs present in the aqueous phase (Fig.1, right).

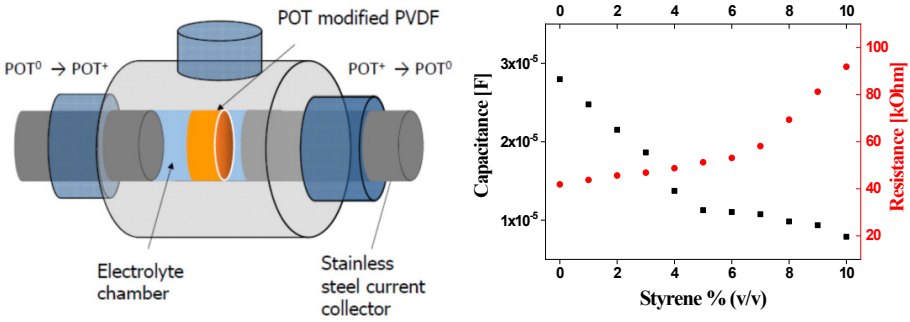

Figure 1 . Scheme of Swagelok®-type cell used as model VOCs – styrene- electrochemical sensor and change of (■) capacitance and (●) resistance of system with POT modified PVDF membrane in response to increase of styrene contents in aqueous sample

Formation of water immiscible liquid layer on the hydrophobic polymer dipped in the aqueous phase is expected to be spontaneous and fast, thus offers significant advantages compared to other system where VOCs diffusion/absorption inside the polymer occurs.

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Solid-Contact Potentiometric Cell for pH Sensing with Symmetry

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The fragility and high resistance of the pH glass electrode are limitations to its miniaturization. From a first glance these problems can be easily overcome by adopting solid-contact electrodes with polymeric sensing membranes. But this solution also has its own drawbacks that have to be addressed. A conventional potentiometric cell composed of a pH glass electrode possess an intrinsic symmetry owing to two Ag/AgCl elements present in its design. This symmetric arrangement allows to keep the potential values stable and reproducible [1]. Meanwhile a solid-contact potentiometric cell is asymmetric (Figure 1a) which makes it prone to potential drifts owing to uncontrollable factors such as temperature fluctuation. Additionally, a solid-contact potentiometric cell demonstrates a significant shift of a zero point in comparison with the standardized value of pH 7.0 for pH glass electrodes.

One way to re-establish symmetry in a solid-contact potentiometric cell is to use a reference electrode that is an identical solid-contact electrode immersed into a solution with a constant background of an ion of interest (Figure 1b) [2]. This approach was successfully tested for solid-contact nitrate-selective electrodes [2]. A limitation of that setup is the necessity to add a high concentration of an equitransferent electrolyte to a reference electrode mixture to reduce junction potential values.

Here we propose a more universal approach where the reference element is represented by a solid-contact electrode and Ag/AgCl element immersed into a solution containing an ion of interest and chloride ion. The Ag/AgCl element in its turn is connected to a single-junction reference electrode immersed in a sample solution (Figure 1c). By implementing this design, a zero point of the pH sensing system with solid-contact ion-selective electrodes is successfully shifted to pH 7.0. The reproducibility of the zero point is confirmed at different temperatures in the range from +5 to +25 ºC. The new symmetric solid-contact potentiometric cell demonstrates comparable long term potential drifts to combination pH glass electrodes.

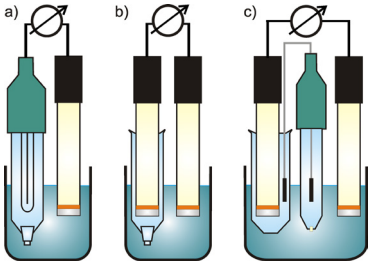

Figure 1. a) Traditional potentiometric cell with solid-contact electrode, b) and c) proposed symmetric solid-contact potentiometric cells for best stability.

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Potentiometric Detection of pH and Fe Ions Changes Associated with the Bacterial Infection

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Implant-associated infections are the result of bacteria adhesion to an implant surface and subsequent biofilm formation at the implantation site. The most common microorganisms identified in surgical site infections were: gram-positive cocci (S. aureus, enterococci, streptococci) and gram-negative bacilli (E. coli, Proteus, and Enterobacter species). After bacterial infestation and bacterial attachment on the protein coated surfaces process of biofilm formation starts. Changes in pH are associated with bacterial infection. While the intracellular pH of bacteria is close to neutrality and remains almost constant in order to preserve the metabolic capacity and cellular integrity, the extracellular pH changes [1]. Chronic wound pH ranges between 7.15 and 8.9 while pH of and acute infection is significantly lower [2].

Bacterial pathogens face a problem because free iron is not available since it is bound to heme or by circulating proteins such as transferrin or lactoferrin [3]. Pathogens use different strategies to obtain iron from the host, via the production of extracellular Fe3+-chelating molecules termed siderophores (either their own or produced by other microorganisms), the uptake of heme, and the uptake of Fe2+ (Feo system) [4]. A single pathogen can adapt its iron-uptake strategy in response to the type of infection (acute or chronic) and the availability or lack of ferrous iron [5].

That is why it is decided to design the systems of sensors based on modified polyaniline coated with a protected non-biofouling layer, which will simultaneously detect the change in pH and depletion of Fe ions

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A 2D pH/O_2 Dual Optical Sensor Based on Sol-Gel Technology

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Previous studies in our group have shown ratiometric pH optical sensors with both fluorescence NPs and pH-sensitive dye in sol-gel to deliver reliable sensor result and a long product shelf-life. [1] Based on this, we present our recent work on a dual optical sensor for both oxygen and pH. The material relies on a combination of an oxygen-sensitive platinum porphyrin-ketone complex (PtOEPK) embedded into polystyrene nanoparticles and a triangulenium pH indicator (DAOTA) in an organically modified sol-gel (ORMOSIL) matrix. To avoid spectral crosstalk, two indicator probes are separated by a sequential two-step deposition. The optode was shown to have high stability, fast response, and independent response to pH and O_2.

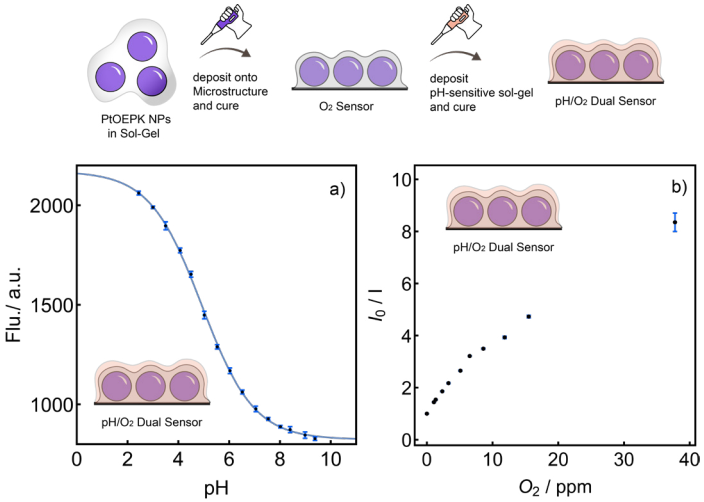

Figure 1. Illustration of preparation and construction of pH/O_2 dual sensor (top). a) pH calibration curve for the pH/O_2 dual optical sensor (pK_a = 4.92). b) Stern-Volmer plot of the pH/O_2 dual optical sensor at different concentrations of DO. Error bar: standard deviations from five replicates.

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Comprehensive Analysis of Mixing Processes Within Microdroplets

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Development and application of compact models of tissues or cell-populations can support the comprehension of physiological processes or disease-mechanisms in cell level and accelerate pharmaceutical drug agent experiments. These cell-size models can be established in specific microfluidic systems capable to manipulate the chemical environment of the cell population, or a single cell in a microreactor. Specific two phase microfluidic systems were developed to generate stable suspensions of droplets forming a closed chemical environment of a single cell. However, understanding and prognosticating hydrodynamic processes on the microscale are quite challenging, thus deeper characterization of the behaviour of the fluids in such confinements is crucial.

The mixing processes in microscale confinements were characterised in droplets generated in microfludic environment and by in-silico Finite Element Modeling (FEM) also. Specific microfluidic systems have been designed to be applicable for parallel fluid injection and droplet generation and manufactured by soft lithography in Polydimethylsiloxane (PDMS) polymer. Water and fluorescently dyed water injected as dispersed phase forming quasi monodispers droplets in a silicon oil based continuous phase. The mixing process evolving in closed containment were analysed in a serpentine type channel system to identify the factors affecting the hydrodynamic behaviour of the droplet – such as Dean vortexes [1] or the channel geometry [2],[3]. Droplets were analysed by fluorescent microscopy using high-speed imaging. The mixing effects in closed volume have also been studied by finite element modelling, using available 2D and 3D standard methods in Comsol Multiphysics. The influence of the rotating oil rind, during the movement of the droplets in the turns was determined in successive parametric sweep simulations.

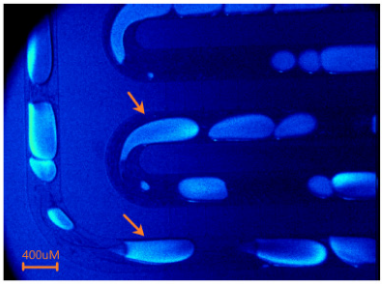

Figure 1. Dean vortexes in the generated microdroplets.

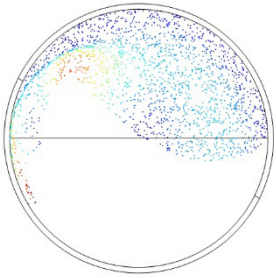

Figure 2. Evolution of mixing process within a microdroplet is visualised by 2D finite element simulation.

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Hydrophilic Nanoporous Membranes as Non-Ion-Selective Potentiometric Sensors for Measuring Total Ion Concentrations

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While inherently the focus of ion-selective electrode research is on the development of ionophores and membranes for highly selective ion sensing, "ideally" non-selective membranes may also have important applications. In fact, conventional ion-exchanger –based plasticized polymer-based membranes, are unsuitable for this purpose due to their inherent, hydrophobicity-induced selectivity following the Hofmeister series. This problem was largely overcame by using hydrophilic ion exchanger membranes, either with membranes consisting of chemically modified straight through nanopores[1] or more general electrodialysis membranes. In terms of applications the use for quantitation of highly abundant hydrophilic ions[2], polyions,[3] and ion chromatography detectors were reported.[4]

Here we report the preparation and analytical application of hydrophilic, cationic and anionic permselective nanoporous membranes with potentiometric responses approaching ideal non-selectivity. Ion-exchangers with terminal thiol groups were self-assembled onto the inner walls of gold nanopores fabricated from polycarbonate filter membranes following a method developed for making solid-state ion channels[5]. The permselectivity of these nanoporous membranes was investigated in detail as function of the chemical modification and nanopore diameter with special emphasis on eliminating ion-selectivity. Most importantly, such membranes are shown to be extremely useful for wearable sensing applications.

Acknowledgment

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Solid Phase Synthesis and Optimization of Clickable Molecularly Imprinted Nanoparticles for QCM Sensor Applications

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Solid phase synthesis of nanoscale molecularly imprinted polymers (nanoMIPs) represents a promising approach when it comes to applying MIPs as recognition units in sensing. Comprising homogeneous binding sites and low non-specific binding, nanoMIPs have performed comparable to natural antibodies in ELISA, SPR and QCM-based assays [1–3]. However, limitations regarding achievable sensitivity are set by the low change in mass (QCM) or refractive index (SPR) upon adsorption of the polymer material. Conjugation with metallic nanoparticles of higher (optical) density such as gold could strongly increase the achievable limit of detection.

The present project aims at synthesizing nanoMIPs with inbuilt azide- or alkyne groups, allowing for easy conjugation to other functional components via click chemistry. First experiments focused on the optimization of poly(N-isopropylacrylamide) based, vancomycin (VM)-imprinted nanoparticles. Incubating varying amounts of the nanoMIPs with VM lead to a concentration-dependent decrease of the VM fluorescence intensity (F1 A), while the non-imprinted reference NPs did not cause any quenching (F1 B). This indicates nanoMIP-VM interaction attributable to imprinting, rather than unspecific binding to the polymer material. NanoMIPs of varying hydrodynamic radius were injected onto a VM-functionalized QCM surface. The largest frequency response was observed for particles smaller than 50 nm (F1 C). Lastly, the monomer propargyl acrylate was integrated into the optimized polymerization procedure to obtain clickable nanoMIPs. First conjugation experiments with cyanin-5 azide indicate the presence of accessible alkyne-groups on the nanoMIPs surface (F1 D).

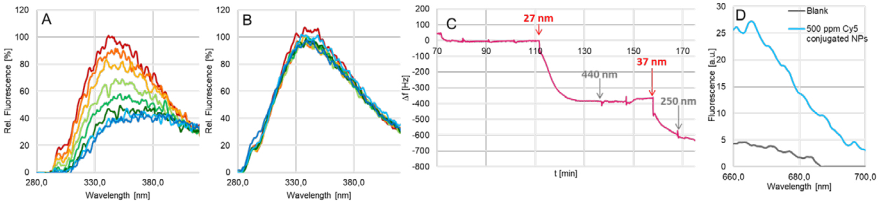

Figure 1: Fluorescence of 250 ppm VM and 0-250 (red to blue) ppm of A) nanoMIPs B) non-imprinted NPs. C) Response of QCM to 500 ppm nanoMIPs of varying size D) Fluorescence of Cy5-conjugated clickable nanoMIPs and solvent blank.

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Metal Modified Paper-Based Microfluidic Substrates for Potentiometric Determination of Lead Ions in Complex Environmental Samples

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Potentiometric determination of lead ions using Pb^{2+}-ion selective electrodes (ISEs) is hindered when coupled with unmodified microfluidic sampling paper-based substrates. In such instance, the super-Nernstian response of the electrode is believed to be caused by interactions between lead ions and functional groups on paper-based substrates. In this work, various metal modified instead of unmodified paper-based substrates were used in controlling properties of the paper substrates in relation to their interactions with primary ion. Different thicknesses of gold, platinum and palladium were sputtered onto paper substrates, followed by their use in potentiometric measurements with Pb^{2+}-ISEs. The results revealed that the thickness of the metal layers on paper substrates influenced the potentiometric response of the electrode. Out of all samples investigated, paper-based substrates coated on both sides with 38 nm gold layers were found the most suitable in controlling the super-Nernstian response of ISEs [1]. Additionally, a durability study was conducted where the lifetime of Pb^{2+}-ISEs was investigated when used with modified paper-based substrates and when used directly on complex environmental samples with high solid-to-liquid content. It was found that for complex environmental samples the use of modified paper-based substrates almost doubles the lifetime of the electrode. The data was also used to conduct a detailed life cycle assessment for model screen printed potentiometric sensors with and without metal modified paper-based solution sampling substrates and results indicated that the environmental impact per potentiometric measurement of Pb^{2+}-ISE was lower in sensors utilizing metal modified paper-based solution sampling substrates.

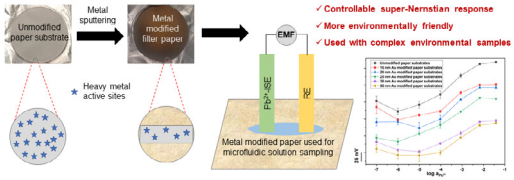

Figure 1. Schematic for the use of metal modified paper-based substrates coupled with Pb^{2+}-ISE.

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Impedimetric Electronic Tongue for the Detection of Marine Toxins

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Most of coastal countries are affected by out-of-control proliferation of microalgae which can biosynthesize phytotoxins, such as paralytic shellfish toxins (PSTs). PSTs can be accumulated in bivalve mollusks, representing a healthcare concern. In order to avoid commercialization of contaminated bivalves, monitoring programs were established in the EU [1]. Several assays and biosensors have been proposed for PSTs' detection. However, most of them are antibody-based which requires animal host for their production, cold chain of storage and entails batch to batch variations [2]. Thus, development of alternative sensing methodologies not involving use of antibodies for PSTs' detection is of practical interest.

The purpose of this work is the implementation of PST transforming enzyme – carbamoylase in an impedimetric test for rapid simultaneous detection of several carabamate and N-sulfocarbamoyl PSTs. Combination of electrochemical impedance spectroscopy using metal electrode and carbamoylase-based assay was applied to harness changes in the enzyme adsorption to the electrode surface during enzymatic reaction as an analytical signal. After optimization, the developed impedimetric assay could quantify both toxins with the detection limit of 0.1 μM (Fig.1), which allows detection of these toxins at the concentration levels observed in bivalves with PST toxicity close to the regulatory limit.

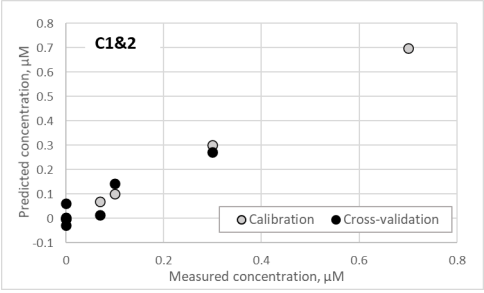

Figure 1. Predicted vs. measured concentration plot for C1&2.

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Development of Impedimetric SARS-CoV-2 Sensor on Screen-Printed Carbon Electrodes Modified with Gelatin

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The coronavirus pandemic brought to light the need for rapid, point-of-care sensors that can detect the initial stages of the infection and stop the dangerous spreading of the virus. The detection of COVID-19 can be done through molecular or serological tests; the first detecting the virus or part of it, the second detecting the antibodies produced in response to the viral infection [1]. In this work, a simple impedimetric sensor for SARS-CoV-2 spike protein (S1), a heavily glycosylated protein residing in the envelope that encapsulates the virus [2], was developed. The sensor was fabricated by site-directed immobilization of IgM antibodies onto the surface of screen-printed carbon electrodes (SPCE) modified with gelatin from porcine skin. Gelatin is a naturally occurring polymer recently introduced for the development of biosensors due to its biocompatibility and non-immunogenic properties [3]. Electrochemical impedance spectroscopy (EIS) measurements with the assistance of a hexacyanoferrate redox probe ($[Fe(CN)_6]^{3-/4-}$) were used to characterize the surface modification steps and the analytical performance. The optimized immunosensor detected S1 in phosphate-buffered saline (pH=7.4) and artificial nasopharyngeal fluid, with limits of detection of 14.7 and 13.4 μmol L^{-1}, respectively.

Acknowledgments

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Electrochemical Platforms for Allergens Aptasensing

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Ara H1 is one of the major peanut allergens. It is considered one of the most life-threatening food allergens since it triggers the highest frequency of severe and fatal reactions, even in trace amounts. Thus, it is crucial to develop fast, accurate and easy-to-use analytical methods for the determination of Ara H1 allergen from food products to aid diet compliance within sensitive subjects [1].

Lysozyme is an enzyme found in multiple organisms that plays various roles. One of the most important relies on its intrinsic antibacterial activity, also called the body's natural antibiotic. Despite its proven utility, lysozyme can potentially trigger allergic reactions in sensitive individuals; thus the need for continuous monitoring of lysozyme in products like wine or egg white is of high importance [2].

Electrochemical aptasensors have a high specificity thanks to the affinity reaction between the ssDNA receptor and the analyte, even in complex matrices. However, key steps in their development are represented by the immobilisation of the ssDNA probe and the signal generation [3]. Therefore, platforms with overall improved electrochemical features that can immobilise the ssDNA probes in a tailored manner to enable the optimum signal generation are of high demand.

Two different platforms for the electrochemical sensitive aptasensing of Ara H1 and Lysozyme allergens will be presented. For Ara H1 detection, a platform based on graphene oxide as carboxylic groups donnors and metallic nanoparticles was developed. Further, the conjugation of a polymer matrix (poly-L-lysine) with gold nanostructures was exploited for the ultra-sensitive detection of lysozyme [4]. Both platforms were developed at disposable screen-printed carbon electrodes (SPCE) to obtain effective biosensors that could be potentially applied for on-site analysis. The two developed platforms were functionalised with the specific ssDNA strands and optimised for the detection and quantification of Ara H1 and lysozyme, respectively. Applications on food/drinks samples will be presented.

Acknowledgments

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Chitosan-Based Glucose Biosensors with a High Linear Range

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The reliable sensors for express blood glucose analysis are always of great demand. It is impossible to verify the result or calibrate personal devices immediately before the measurement because test strips are disposable. Enzymes used in biosensors are naturally unstable. Since the analytical signal of the biosensor is directly related to the activity of the enzyme, there is a high probability of obtaining false results. The solution to this problem is changing of limiting stage of the process by creating diffusion barrier for the mediator. For such purpose an additional polymeric membranes over the sensitive enzyme layer of biosensor can be used [1]. However it is complicated and expensive for mass production.

In this work we proposed a membrane-forming mixture, which is drop-casted on an electrode surface in a single step and contains an enzyme, mediator, and polyelectrolyte. We`ve demonstrated the linear dependence of amperometric biosensor response on the concentration of glucose up to 30-50 mM (Figure 1).

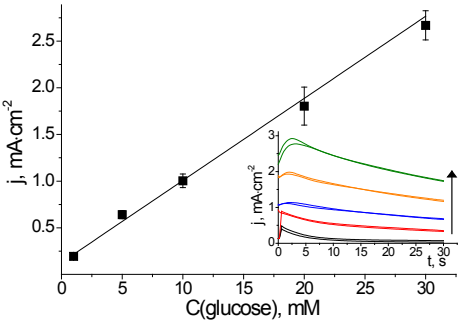

Figure 1. Calibration graph and chronoamperometric response of glucose biosensors.

Wide linear range proves the diffusion controlled process, because this dependence is hyperbolic in accordance with the enzymatic kinetics. In case of diffusion limited process the enzyme inactivation should not lead to significant changes in sensor sensitivity. The thermal stability tests were performed under 60°C: 50% of the initial biosensor response is preserved after 3 hours of thermal loads. The stability tests during long-term storage within 1 month at room temperature have shown that created biosensors retains 85-90% of the initial sensitivity. The developed electrochemical test strips were successfully used for the detection of glucose in undiluted biological fluids (blood serum and whole blood). It was shown that the test strips are suitable for high-precision glucose detection in hypo/ hyperglycemic conditions.

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The financial support of the Russian Science Foundation (Grant No. 22-23-00545) is greatly acknowledged.

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Contributing to the Diagnosis of Colorectal Cancer by Rapid Amperometric Immunosensing of Exosomal CD147 Protein

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Naturally secreted nanometer-sized exosomes are important cell-cell orchestrators in different processes, such as the regulation of the immune response [1], and metastasis or tumor recurrence. The exploration of the extensive and dynamic biological information nested in these vesicles has proven extremely useful to decipher the course, stage, and response to a given treatment applied at a precise point of a specific disease, these vesicles now being considered as potential early 'bio-meters' for clinical applications. Moreover, their presence in almost all body fluids with high stability facilitates their use as valuable markers in minimally invasive liquid biopsies [2].

Cluster of differentiation 147 (CD147), also called extracellular matrix metalloproteinase inducer (EMMPRIN), is a cell surface glycoprotein implicated in essential cellular processes as tumor invasion and progression [3]. As it is also differentially expressed in exosomes isolated from a wide variety of cancer subtypes, including colorectal cancer (CRC) [4], CD147 stands as an attractive biomarker for the early diagnosis and prognosis of cancer.

Looking for viable methodologies for early cancer diagnosis, of vital importance to reduce cancer deaths, a sensitive electrochemical immuno-strategy for the rapid detection of exosomal CD147 will be presented, based on the selective capture of the target protein by specific anti-CD147 antibodies covalently coupled onto the surface of magnetic microparticles and its recognition by a biotinylated secondary antibody and labelling with a streptavidin-horseradish peroxidase conjugate. Amperometry at a constant potential (*vs*. an Ag pseudo-reference electrode) is proposed to monitor the presence of the CD147 target using screen-printed carbon electrodes as transducers and the H_2O_2/hydroquinone (HQ) system. The sandwich-based immunoplatform applicability is demonstrated through the successful detection and quantification of CD147 in both intact exosomes and exosomes' lysates previously isolated from five CRC cell lines with different metastatic capacity. The obtained results concordance with those provided by gold standard ELISA and Western Blot demonstrates the reliability and practical utility of the developed biosensor as an effective tool to improve the diagnosis and management of CRC patients through the interrogation of one of the most recently explored exosomal protein biomarkers.

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Binary MoS_2 Hybrid Nanocarriers for Efficient Immunosensing of Cancer and Autoinmune Diseases Candidate Biomarkers

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Clinical laboratories and health services are influenced by a Society that increasingly demands more personalized and higher quality care. In biomedical science, among many other growing fields, the detection of clinical biomarkers in biological samples is crucial for early-stage and reliable screening of diseases, thus allowing for more efficient treatments that improve patient statistics and quality of life and reduce side effects. Electrochemical immunosensors are of particular interest for this purpose. These devices have undergone impressive development in recent years due to attractive surface chemistries and the design and modification of new nanomaterials that have proven to provide them with analytical characteristics in terms of simplicity, sensitivity, selectivity compatible with their clinical application even for multiplexed and point-of-care determinations [1].

In this communication we will summarize the most relevant aspects of the first immunosensing platform described for the simultaneous determination of two important immunity and cancer related cytokines: BAFF (B cell activation factor) and APRIL (a proliferation-induced ligand) [2]. Both immune platforms involve sandwich-type immunoassays, performed on the surface of screen-printed carbon electrodes electrochemically grafted with *p*-aminobenzoic acid and pioneeringly involving MoS_2 nanozymes/multi-walled carbon nanotubes (MoS_2/MWCNTs) hybrid nanostructures as nanocarriers of the detector antibodies and HRP molecules for signal amplification, and amperometric transduction using the H_2O_2/hydroquinone system.

Results will be discussed confirming that the bioplatform offers suitable analytical and operational characteristics for the direct and reliable determination of target endogenous biomarkers in complex samples such as human serum and tissue extracts from healthy, cancer diagnosed and autoimmune patients, simply by placing a drop of the diluted biofluid or a small amount of tissue extract on the electrode surface and with simple and short duration (1-2 h) protocols.

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Application of Multiharmonic QCM to Monitor the Interaction of the Nanowires Modified by DNA Aptamers with Cytochrome c Adsorbed at the Supported Lipid Membranes

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Cytochrome c (cyt c) is the main initiator for cell apoptosis. This predetermines cyt c to become useful biomarker for evaluation of chemotherapy efficacy. Cyt c is associated with the surface of mitochondrial membranes. In order to detect cyt c we designed the supported lipid membranes formed at the hydrophobic surface of 1-dodecanethiol chemisorbed at the gold surface of quartz crystal transducer. The lipid monolayer has been formed by fusion of small unilamellar liposomes composed of the mixed phospholipids: L-α-Phosphatidylcholin (lecithin) and Dimyristoyl phosphatidylglycerol (DMPG) [1] that provide negatively surface charge onto which the cyt c has been adsorbed by electrostatic interactions. To monitor the kinetics of the formation of the lipid membranes and cyt c adsorption, the multiharmonic quartz crystal microbalance with dissipation (QCM-D) has been used. In order to amplify the detection of cyt c we used gold nanowires modified by DNA aptamers specific to cyt c [2]. The formation of the lipid monolayer as well as adsorption of cyt c has been accompanied by decrease of resonant frequency and an increase of dissipation. Similarly, addition of nanowires modified by aptamers resulted in further changes of these values. The method allowed detection of sub nanomolar concentration of cyt c and to evaluate the changes of viscoelastic properties of the biolayers using Voinova-Voigt model [3].

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A New Concept for the Development of Aptamers with Outstanding Affinity for Virus Recognition

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Aptamers are small single-stranded artificial oligonucleotides (DNA or RNA) folded into secondary structures, that have a remarkable binding ability to selected epitopes of the target molecules [1], even for larger particles, e.g. for viruses [2]. In clinical practice, antibodies are used in protein-based viral assays, but due to the excellent target selectivity, binding affinity and thermal stability of aptamers, increasing attention is being paid to their potential application in point-of-care testing. However, aptamers require further improvement in some cases, e.g. when their affinity is not sufficiently high and the concentration of the target is very low. Therefore, we explored the possibility of post-selection chemical modification of aptamers to increase their affinity. Here we are going to show the proof of principle for virus targets, through the design of aptamer-based receptors that can recognize the virus directly without destruction. For this purpose we used aptamers that we previously selected and characterized for binding the G-protein of Respiratory Syncytial Virus (RSV) [2] [3]. The new concept that we developed led to the synthesis of a new class of chemically modified aptamers with significantly increased affinity for virus recognition.

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A New Solid-State Thiabendazole-Selective Sensor Based on Functionalized MWCNT

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Thiabendazole (TBZ) is commonly used benzimidazole fungicide so its residues can be found in fruits and vegetables [1]. Considering TBZ potential toxicity, its concentration should be monitored to prevent the exposure to TBZ through the diet [2]. For that purpose, ion-selective electrode (ISE) could be convenient due to its simplicity [3]. In order to get better properties, ISE membrane can be modified with multi-walled carbon nanotubes (MWCNT) considering their unique properties [4].

The new solid-state TBZ-selective sensor, with liquid type of membrane, was prepared using MWCNT functionalized with a sulfate group and TBZ ion as a sensor material and dibutyl sebacate as a plasticizer. The direct potentiometry was used as a method for its characterization (response characteristics, dynamic response, influence of pH, selectivity). The applicability of newly developed sensor for TBZ determination was tested using fruit samples as the analytes. The standard addition method was used for confirmation of sensor accuracy and investigation of the possible matrix component interferences.

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An Electrochemical Sensor for Determination of Spermine Using Modified Carbon Paste Electrode with ZnONPs And MWCNTs: Practical Application for Biological Samples

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Polyamines (PAs) represent a small group of organic polycationic alkylamines that occur in all organisms which comprise putrescine, spermidine and spermine [1]. These molecules are known to play an essential role in many biological activities including cell growth and proliferation, protein and nucleic acid synthesis, apoptosis, the regulation of immune response, and antioxidant activity [2]. The normal physiological status of the organism implies tight regulation of PAs cell level by complex mechanisms, while increased intracellular PAs concentration has been associated with various types of cancers and chronic diseases [3]. Hence, analytical methodologies developed towards monitoring PAs levels in biological fluids can be used as an important diagnostic marker in various types of cancer.

In this work, an electroanalytical protocol for the selective determination of spermine is presented. The properties of ZnONPs as a nanomaterial are consolidated with MWCNTs to design ZnONP-MWCNT-CPE as a modified sensor that demonstrated upgraded analytical performance compared to bare CPE. CV, SWV and EIS were utilized to explore the electrochemical performance of the designed sensor. The modified sensor exhibited enviable analytical performances such as low LOD (0.30 μmol/dm^3) and a wide linear range (1-100 $_{\mu mol/dm^3}$) with high reproducibility and selectivity of measurements. Eventually, the herein presented protocol demonstrated excellent practical performance during the determination of spermine content in diluted human urine samples.

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Electrochemical Properties of Screen-Printed Sensors with Boron-Doped Diamond Working Electrode for Point-Of-Care Testing

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In current electroanalytical chemistry, the intensive development of smart, real-time, and point-of-care testing sensors represents one of the main research directions. The novel screen-printed sensor with a boron-doped diamond working electrode (SP/BDDE), fabricated employing a large-area linear antenna microwave chemical deposition vapor system (LA-MWCVD), presented in this manuscript, belongs to this area. It combines the advantages of printed sensors [1, 2] such as tailored design, low cost, and easy mass production, thanks to which it can be used as a disposable sensor, with the excellent electrochemical properties of BDDE [3, 4] including a wide available potential window, low background current, low current noise, good chemical resistance, and resistance to passivation predetermine this sensor for successful application in modern electrochemistry.

In this work, the newly prepared SP/BDDEs were characterized by scanning electron microscopy (SEM) and Raman spectroscopy. Their electrochemical properties were investigated using cyclic voltammetry of commonly used inner sphere ($[Fe(CN)_6]^{4-/3-}$) and outer sphere ($[Ru(NH_3)_6]^{2+/3+}$) redox markers. The applicability of new sensors was tested by analysis of several bioactive substances important in the field of environmental protection or human health. The tested SP/BDDEs were found to provide very good electrochemical properties and suitability for electroanalysis. It was confirmed that the intra-electrode repeatability of the recorded current signals is very good, indicating excellent sensor stability. Moreover, excellent inter-electrode repeatability was proven also in the case of signals recorded by individual SP/BDDE tested, which allows their use as disposable sensors.

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Voltammetric Determination of *Diclofenac* and *Flufenamic Acid* at Surfactant-Modified Carbon Paste Electrodes in Model Samples Purified by Adsorption onto Impregnated Carbonaceous Sorbents

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Currently, traces of drugs are being found not only in technological water streams produced by pharmaceutical industry but even in natural water and groundwater [1-3]. The occurrence of drugs in the environment shows that the biological stage of the wastewater treatment plant is not able of complete removal of these pollutants. Hence, it is necessary to monitor such pollutants --- including the titled drugs --- in the environment (especially in water) by applying effective methods for removal of such substances from the polluted water [1].

In this respect, of continuing interest are non-steroidal anti-inflammatory drugs (NSAID) which include *Diclofenac* (Dcf; Fig. 1). In Czech Republic, it is contained in more than twenty commercially marketed preparations [1] and, therefore, Dcf is very frequent and also problematic pollutant in natural waters. In 2018, the concentration of Dcf was determined in Elbe river up to 50 ng/L [2]. Another NSAID is polyfluorinated *Flufenamic acid* (Ffa). Although this drug is less common, it is structurally and in function very close to Dcf (Fig. 2).

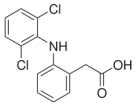

Figure 1 Diclofenac

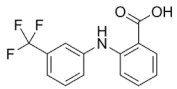

Figure 2 Flufenamic acid

This contribution deals with possibilities and limitations of monitoring and determination of both drugs in one sample. The experimental work follows our previous study of voltammetric determination of Dcf at carbon paste electrodes (CPE) [3]. However, the main aim of current study is comparison of voltammetric determination of both Dcf and Ffa at a CPE modified *in situ* with cetyltrimethylammonium bromide (CTAB). Applicability of the already developed purification procedure was examined on a set of model samples polluted with Dcf or Ffa before and after treatment with carbonaceous adsorbent *Biochar* impregnated with ionic liquids. Besides this, adsorption capacity of this material for both Dcf and Ffa was compared and its further improvement examined when using various ionic liquids.

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Towards Multiplexed Neurochemical Sensing *via* Aptamer-Functionalized Nanopipettes

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Understanding how the brain processes information is one of the grand challenges of today; novel measurement strategies are required to improve our basic knowledge of neurological function and diseases. In addition to electrical signaling through action potentials, chemical signaling *via* neurotransmitters is at the core of neuronal communication. To measure neurochemical signaling dynamically in complex environments (*e.g., in vitro* neuronal cultures and *ex vivo* brain tissue), aptamer-functionalized nanopipettes are being developed. We couple neurotransmitter-binding aptamers inside glass nanopipettes using robust surface chemistry. The nanopipettes have ~10 nm pore openings, which facilitates recordings of neurochemical flux with nanoscale spatial resolution. Upon target-specific binding, the aptamers undergo a significant confirmational change, leading to a rearrangement of the highly negative DNA backbone, and enabling detection of a change in current [1]. The nanoscale dimensions and specificity of these sensors allows for unprecedented precision, enabling the possibility of measurement in highly localized brain regions. To enable robust measurements of neurochemical flux in complex environments with high amounts of interferents, it is crucial to self-reference the specific sensor with a control sensor [2]. We functionalize the reference sensor with a DNA sequence containing the same DNA bases as the specific aptamer, but in a scrambled order, hindering target recognition. The reference sensor will observe the same environmental changes with the same chemical signature as the specific sensor, with minimal response to the neurotransmitter of interest. We have explored different techniques to multiplex reference and sensing DNA-modified nanopipettes. In parallel, we are broadening the repertoire of neurochemicals we can detect to enable multiplexing with different targets, e.*g.,* dopamine, serotonin and glutamate, in addition to the control.

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Electrocatalytic Properties of Prussian Blue Based Nanozymes: Towards Advanced (Bio)Sensors

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Catalytically synthesized Prussian Blue nanoparticles are considered as the most advantageous peroxidase mimic for (bio)sensing purposes. The catalytic synthesis consists in reducing ferric ferricyanide, $Fe[Fe(CN)_6]$, with H_2O_2. This procedure is analogous to the electrocatalytic oxidation of H_2O_2, which results in highest growth rate in the most catalytically potent structures and thus, leads to formation of PBNPs in their highest activity. As a result, noted nanozymes beat natural HRP in terms of turnover number by up to 4 orders of magnitude. Moreover, they are catalytically specific (exhibiting no O_2-utilizing oxidase activity) are highly active in neutral media (7.0-7.4), making them prominent candidates for analyses in biological matrices [1].

Electrocatalytic properties of the PBNPs are also notable. Simple drop-casting of PBNPs colloidal solution followed by annealing results in ready-to-use H_2O_2 electrochemical sensors. Sensitivity of noted sensors reaches 0.85 $A \cdot M^{-1} \cdot cm^{-2}$, which is higher than that of PB film based sensors by 30% and allows detecting submicromolar H_2O_2 concentrations [2]. Achieved sensitivity of 0.85 $A \cdot M^{-1} \cdot cm^{-2}$ can be further increased with carbon black nanoparticles (CBNPs), resulting in record sensitivity of 1.15 $A \cdot M^{-1} \cdot cm^{-2}$, almost doubling the sensitivity of PB film based sensors.

Noted PBNPs-CBNPs mixture can be co-immobilized with oxidase enzymes, such as glucose and lactate oxidases. The aforementioned drop-casting approach can be used for the enzyme-nanozyme mixture for one-step manufacturing of biosensors. Such biosensors are advantageous over conventional sensors produced upon layer-by-layer immobilization. This approach results in one order of magnitude higher sensitivity and record transducer/biosensor sensitivity ratio of approximately 0.3 [3].

The PBNP based lactate biosensors were tested in a novel method of impulse chronoamperometry. Instead of working in a three-electrode scheme at a constant potential of 0V, it is possible to short-circuit the working electrode and the Ag|AgCl reference electrode, which automatically sets the required potential of 0V. This allows using PB based sensors in galvanic cell mode, which does not require a potentiostat to function. By alternatingly closing or opening the short circuit we can generate an impulse of quasi-stationary current. This lead to a drastic increase in sensitivity up to 7.8±0.2 $A \cdot M^{-1} \cdot cm^{-2}$, which is up to two orders of magnitude higher than that of the same sensors in stationary mode utilizing a potentiostat. The overall signal/background ratio is up to 3.7 times higher than in stationary mode.

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