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OPENING REMARKS

Dear participants, dear students and teachers,

On behalf of Faculty of Science at the University of Zagreb, it is a great honor to welcome you to the 23rd International Symposium and Summer School on Bioanalysis.

This year's symposium provides a dynamic platform for the exchange of knowledge, ideas, and recent research findings through a carefully curated program of oral and poster presentations. By showcasing a wide range of innovative approaches and analytical techniques, the event aims to foster scientific dialogue and inspire new collaborations across institutions.

The Book of Abstracts captures the breadth and depth of contributions presented at this event. Each abstract reflects the dedication and expertise of its authors and contributes to our shared mission of expanding the frontiers of bioanalytical science.

We hope that the discussions sparked by these presentations will lead not only to fruitful academic discourse, but also to meaningful partnerships that will shape the future of research in this vital area.

Welcome to a week of learning, sharing, and connecting.

Prof. Nives Galić, Chair of Organizing Committee

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LECTURES

STUDY OF SOLUBILITY-PERMEABILITY INTERPLAY

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Most novel active pharmaceutical ingredients have low water solubility, therefore, solubility enhancing methods are applied, such as particle size reducing, inclusion complex formation etc.[1] However, bioavailability is influenced not only by solubility but also by permeability. To achieve proper bioavailability, a molecule must first dissolve in the gastrointestinal tract, enabling its absorption and ensuring sufficient concentration at the target site to achieve an adequate receptor response.[2,3] Since passive diffusion is the main absorption pathway, the molecule must exist in its neutral or "transport form" to permeate through the membranes. Therefore, it is important to investigate the effect of different solubility enhancing excipients on the permeability of the materials. During our research, we investigated the effect of 9 different types of excipients (surfactants, fillers, polymers, cyclodextrins) on solubility and permeability, using 3 model compounds with different acid-base characteristics.

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CHARACTERIZATION OF ANTIFUNGAL SURFACTANTS SYNTHESIZED BY THE ANTARCTIC *BACILLUS SUBTILIS* WA_51 STRAIN

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One of the greatest challenges in public health is the growing phenomenon of drug resistance to commonly used antimicrobial drugs. To address this issue, extensive research is being conducted to discover new bioactive substances with applications not only in medicine and veterinary medicine but also in the food industry to ensure the microbiological safety of food. Consequently, scientists are increasingly focusing on biosurfactants produced by various microorganisms, as some are known to exhibit biological activity, including antibacterial and antifungal properties. *Bacillus subtilis* strains produce a wide range of bioactive cyclic lipopeptides with potential antibacterial and antifungal effects.

The main goal of the research was to identify the composition of biosurfactants produced by antarctic *Bacillus subtilis* ANT_WA51. previously isolated from a temporary Antarctic water body located on King George Island (62°14'4.76'' S, 58°28'22.29'' W).

The effect of different media on lipopeptide production was evaluated using four formulations: M9 supplemented with glycerol, glucose, or molasses, and LB medium. The impact of incubation time on surfactant concentration was also assessed. Cultures were incubated at 25°C with shaking at 130 rpm for 7 days, with measurements taken every 24 hours. LC-MS/MS and LC-HRMS were used to identify the lipopeptides in such solutions. The inhibitory effect of biosurfactant towards fungal growth was studied using plate diffusion technique. A comparison of media compositions revealed that the best media for surfactant production were M9 with glucose and LB. In all media, surfactin homologues were detected after 48 hours, while fengycins appeared after 36 hours, with their levels remaining stable in the following days. The structures of the detected surfactants were proposed based on the acquired HR-MS and MS/MS spectra, as well as relevant literature.[1] LC–MS analysis of the extracts revealed several pseudomolecular ion peaks corresponding to fengycin homologues, observed at retention times ranging from 12 to 15 minutes. In contrast, retention times between 23 and 36 minutes were characteristic of surfactin homologues.

Additionally, the antifungal activity of the produced compounds was tested. Among the tested species, *Fusarium oxysporum* was found to be the most sensitive.

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SPECIES AND CULTIVAR DIFFERENTIATION FOR FOOD AUTHENTICATION - DEVELOPMENT OF ASSAYS BASED ON PCR AND HIGH-RESOLUTION MELTING

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Economically motivated food adulteration remains a global issue. One of the common practices is the replacement of higher-value species or cultivars with cheaper ones.

DNA-based methods play an increasing role in species and cultivar differentiation. A common approach is the analysis of evolutionary conserved DNA regions, so-called DNA barcodes.[1] More precisely, both the 5´ end and the 3´ end of the DNA barcode should be conserved, allowing for amplifying the DNA barcode with one single primer pair in all species/cultivars of interest. However, in between these conserved parts serving as primer binding sites, the DNA sequence should be variable, enabling species/cultivar differentiation. In many cases, DNA barcodes selected for food authentication contain single nucleotide polymorphisms (SNPs) or microsatellites (simple sequence repeats, SSRs).

In case the DNA barcode is amplified with a PCR mix containing an intercalating dye such as EvaGreen, the amplicons can subsequently be subjected to high-resolution melting (HRM).[2] By slowly increasing the temperature, the amplicons are dissociated (melted) into the two single strands and fluorescence decreases. For data evaluation, the (normalized) fluorescence signal is plotted against temperature. The melting behaviour of the amplicons depends on various parameters, including their length and the ratio of guanine and cytosine to adenine and thymine.

The development and optimization of assays based on PCR and HRM will be discussed on several examples, including the differentiation between 1) edible insect species approved in the EU[3]; 2) bilberry and blueberry; and 3) wine varieties from North Macedonia.

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ANALYSIS OF FLUID MARKERS FOR PHYSICAL EXERCISE

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Finding a simple non-invasive method to determine various markers after exercise is of prime importance for research in athletes. One of the main indicators that changes during intense training is the level of creatinine in body fluids. Creatinine is a by-product of the breakdown of creatine phosphate in muscles. Creatinine, which is synthesized in the liver, is essential for its synthesis. Its concentration in the blood is a major indicator of the condition of the kidneys - it reflects their filtration ability. Since the excretion of creatinine is at a constant flow rate (constant speed), it can be used to judge the efficiency of the kidneys to excrete other waste products. Creatinine levels increase with dehydration, increased meat consumption, muscle tissue damage, as well as the intake of certain medications (for example, antibiotics from the aminoglycoside group - gentamicin; some diuretics, H2 blockers, and others). During intensive physical exercises, creatinine increases. Increased creatinine levels are present in athletes who take supplements containing larger amounts of creatine. This occurs because physical exercise causes increased muscle breakdown and turnover, releasing creatinine (a waste product of creatine phosphate metabolism in muscles) into the bloodstream. The mechanism involves both acute and chronic effects: during intense exercise, rapid energy utilization in muscles breaks down creatine phosphate, producing creatinine; additionally, exercise-induced muscle damage releases more creatinine into circulation.

We present a simplified method for the determination of creatinine levels in blood and urine, which is based on colorimetric measurement. A kinetically modified Jaffé method is used with the application of a ready-to-use liquid reagent. Plasma and urine from Wistar rats, a control and experimental group with altered renal function, were used. The study was conducted with an ELISA reader. The advantage of the method we used is miniaturization (it works in quantities of 20 microliters), automation (the ability to conduct a large number of measurements at the same time) and accuracy. In the future, a methodology for determining creatinine in saliva will also be developed.

The application of non-invasive methods for characterizing the changing parameters as a result of physical exercises in athletes will allow for a more complete understanding of the mechanisms of metabolism under these conditions, and will also make the method more accessible for wider use. Reducing the volumes of the single sample will allow us to study a large number of parameters in it.

REMOVAL OF APOLAR COMPOUNDS FROM AQUATIC ENVIRONMENT USING POLYDIMETHYLSILOXANE SPONGE

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The polydimethylsiloxane (PDMS) is a soft, flexible silicone polymer which has an inert, hydrophobic and biocompatible nature, chemical and UV light resistance. The applications of PDMS range from contact lenses and shampoos to food additives (antifoaming agent) and lubricants due to its low manufacturing costs. Choi et al[1] proposed a simple procedure to make a sponge-like porous PDMS, which provides a structure with large specific surface area. In their work, sugar granules (sugar cube) served as a mold and were dissolved after the PDMS had cured. They proved that the PDMS sponge efficiently absorbs oils and organic solvents, and the sponge could be reused through simple squeezing. Our study highlights the effectiveness of PDMS sponges in removing organic UV filter compounds, such as octinoxate, from aqueous environments. The sponges were produced using straightforward templates composed of hydrophilic fused or compressed particles (sugar or NaCl) with an average size of approximately 0.4 mm. These sponges were fully regenerable, allowing for the complete removal of octinoxate without any measurable change in their adsorption efficiency or dry mass. Thanks to their simple fabrication, ease of handling, buoyancy, and reusability, PDMS sponges offer a sustainable, low-maintenance alternative to conventional filtration techniques for eliminating octinoxate and possibly other apolar components from surface and recreational waters.

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DOPING IN SPORT: A NEVERENDING STORY

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The high prevalence of doping in professional sport reflects a complex interplay between scientific progress, regulatory frameworks, cultural accomodation, and ethical ambiguity. This interdisciplinary analysis traces the trajectory of anti-doping initiatives from early 20th century to the establishment of international coordinated institutions such as the World Anti-Doping Agency (WADA). The fight against doping is marked by a constant race between scientific innovation in detection methods and the sophisticated techniques used to evade them.

Despite notable progress in analytical capabilities, ranging from high-resolution mass spectrometry (MS) to longitudinal monitoring via the athlete biological passport, doping remains a "moving target," continually reshaped by pharmaceutical innovation and clandestine practices. The cornerstone of modern anti-doping efforts lies in robust bioanalytical approaches capable of detecting exogenous substances and physiological anomalies at trace concentrations. Techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), and advanced metabolomic profiling have enhanced the sensitivity and specificity of testing protocols.

Yet, the development of detection methods must contend with the ingenuity of doping practices, which often evolve more rapidly. This dynamic gives dopers a tactical advantage, as new compounds and masking agents are frequently introduced before corresponding detection methods can be validated and implemented. Beyond the laboratory, psychological and sociocultural factors contribute to the persistence of doping. Athletes often cite systemic pressures, financial gains, and normalization within performance-driven environments as justifications, with moral disengagement and peer influence further reinforcing the behavior, particularly in strength- and endurance-based sports.

Doping in sport is not only about the mere rule-breaking; it is a reflection of deeper societal expectations, institutional complicity, and evolving concepts of fairness, morality, health, and performance. Combating it requires a multidisciplinary response that integrates cutting-edge bioanalytical science with education, policy reform, and ethical engagement, aiming to ensure that detection keeps pace with deception; however, given the complexities of human nature, doping will probably never be entirely eradicated.

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NOT JUST GROWING CRYSTALS: CHALLENGES AND INSIGHTS FROM STRUCTURAL STUDIES OF MACROMOLECULES

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High-resolution structural biology techniques, especially single crystal X-ray crystallography, are often seen as separate from mainstream bioanalysis – but in reality, they offer uniquely powerful insights into biomolecular function, interactions, drug binding, and conformational dynamics that are central to many bioanalytical applications. I will present case studies from our work on *Helicobacter pylori* proteins, [1–3] plant seryl-tRNA synthetase,[4–5] the human Kelch domain with peptide partners,[6] and DNA–drug complexes. Each of these systems presented typical bioanalytical challenges: maintaining molecular stability and homogeneity, struggling with conformational heterogeneity, and achieving interpretable data from imperfect samples. We addressed these issues through integrated strategies combining sequence bioanalysis, recombinant expression optimization, co-crystallization with ligands to stabilize flexible regions, dynamic light scattering, additive screening, microseeding and microfluidics. These approaches enabled high-resolution insights into molecular recognition and function – even in systems that initially resisted crystallization. Our experiences highlight how structure-based workflows can complement other analytical techniques in biology. How these structural insights complement classical bioanalytical methods and provide practical strategies for integrating crystallography into protein function discovery, protein–ligand profiling, and drug design will be discussed.

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ADVENTURES AROUND THE STRUCTURE AND FUNCTION OF A PROTEIN - THE TRANSFERRIN

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Transferrin is a serum glycoprotein of molecular mass of 80 000 with the property of reversibly binding ferric ions (forming a salmon-like-colour-complex in solution), thus acting as a reservoir and transport protein for physiological iron. The two-lobe structure[1,2,3] has an unusual conformational stability, which is strongly related to the complexation of the two metal-ions. [4,5] The complex is formed only in the presence of synergistic anions. Human transferrin has an isoelectric point in the range 5.3–6.1, the variation in pI presumably caused by the varying carbohydrate and iron content.[6-8] The mapping of the stereoselective interaction sites on the protein surface prosseses strong discrimination of chiral substances[9-13], while the molecular recognition has also an importancy.[14-16] The specific properties of the synergistic anions increase the importancy of the internalization of iron-anion-transferrin complexes trough the cell membrane.[17-19]

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EXPLORING HOST:GUEST INTERACTIONS IN DRUG–MACROCYCLE SYSTEMS USING MASS SPECTROMETRY

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Understanding non-covalent molecular interactions in supramolecular systems is fundamental to their practical applications. In particular, detailed analytical characterization of macrocyclic host molecules such as cyclodextrins (CDs) is essential for selecting the most suitable CD derivative for a given guest molecule. However, comprehensive characterization of CD inclusion complexes is a complex task, requiring the integration of multiple analytical techniques and a holistic interpretation of their results. There is increasing interest in developing efficient and reliable analytical methods for elucidating drug–CD host:guest interactions. Among these, mass spectrometry (MS) has emerged as a particularly powerful tool. When coupled with soft ionization methods such as electrospray ionization, MS enables the investigation of drug–CD inclusion complexes in the gas phase. This approach provides valuable insights into non-covalent ion formation, complex stoichiometry, structural characteristics, gas-phase reactivity, and thermochemical properties.[1] Moreover, tandem MS experiments have been successfully employed to assess the relative stabilities of gas-phase conformers, offering potential correlations to their behavior in solution. In this work, we present our recent results on the mass spectrometric characterization of inclusion complexes formed between various drugs—such as praziquantel[2], nabumetone[3], loratadine[4], and cinnarizine—and β-cyclodextrin and its derivatives (randomly methylated β-CD, hydroxypropyl-β-CD, and sulfobutylether-β-CD sodium salt). We also report our latest findings on the formation of host:guest complexes between nabumetone or naproxen and cucurbit[7]uril.[5] Beyond gas-phase structural studies, our research extends to evaluating the stability of drugs in potential CD-based pharmaceutical formulations with enhanced properties. In this context, liquid chromatography coupled with high-resolution mass spectrometry serves as a powerful technique for the sensitive and definitive identification of degradation products formed during forced degradation studies and long-term or accelerated stability testing.

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APPLICATION OF CAPILLARY ELECTROPHORESIS IN CONTROLLED DRUG RELEASE STUDIES

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HPLC is considered the method of choice for monitoring drug release from polymer nanocarriers and other nanomedicines. Nevertheless, polymer carriers usually cannot be injected into the HPLC system as they can irreversibly adsorb to the stationary phase or other components of the system, causing deterioration of separation efficiency or even column clogging. A liquid-liquid or solid-phase extraction step is thus necessary. On the contrary, the open-tubular columns used in capillary electrophoresis can tolerate more problematic sample matrices, and eventually adsorbed polymers can be washed out using a strong base, acid, or organic solvent. In this work, we report on a successful application of capillary electrophoresis to the separation and determination of hydrophilic drugs released from polymer carriers.

We have developed a method for monitoring the release of 5-aminolevulinic acid and its hexyl ester from a hydrophilic N-(2-hydroxypropyl)methacrylamide-based copolymer. The separation was performed in 1M formic acid using capacitively coupled contactless conductivity detection, as the analytes do not exhibit significant UV absorption. Using Tris cation as an internal standard and flushing the capillary with 1M NaOH, water, and 1M HCOOH before each analysis provided very good linearity and repeatability. The application of pressure to the inlet end of the capillary helped to stabilize the baseline and shorten the separation time to 5 minutes. The total analysis time, including the flushing procedure, was 14 minutes.

The second method developed within this work for monitoring the release of another hydrophilic drug, acetylsalicylic acid, from the same polymer carrier used direct UV detection. In this case, the background electrolyte was 20 mM sodium tetraborate, allowing an efficient separation of acetylsalicylic acid, salicylic acid, and salicyl hydrazide from the polymer carrier. Benzenesulfonate was used as an internal standard. Separation was completed within 4 minutes. Together with the flushing procedure, the analysis took 9 minutes. Excellent linearity and repeatability were reached without any sample treatment steps.

The last method developed served for monitoring the release of the 14-amino-acid peptide $(KLAKLAK)_2$, abbreviated KLAK. This peptide was supposed to be cleaved off the polymeric nanocarrier by the Cathepsin B enzyme. A method using 1M HCOOH as a background electrolyte, combining contactless conductivity detection and direct UV detection at 200 nm, was used for the separation and monitoring of potential products of enzymatic cleavage and other components of the reaction mixture. In this case, our method has shown that the enzymatic cleavage was not limited to the desired bond but provided various fragments of the peptide and the linker connecting the peptide to the polymer.

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ASSESSING MICROBIAL CONTRIBUTIONS TO METABOLITE VARIATION IN MICROBIOME-METABOLOME ASSOCIATION STUDIES

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Microbial communities exert a significant influence on their hosts, frequently generating a diverse array of metabolites that interact with various host pathways. In humans, metabolites derived from microbiomes have been identified as contributing factors to a broad spectrum of diseases. Microbiome-metabolome association studies, which involve a comprehensive analysis of both species composition and metabolite concentrations across various microbial community samples, followed by an examination of correlations between microbial taxa and metabolites is crucial step in advancing our understanding and manipulation of microbiome metabolism.[1] Broadly, gut metabolite profiles are jointly derived from diet, modified human metabolites and microbially derived compounds that shape the microbiota–host interactions.[2]

In our study, we searched for associations between microbiome composition and their metabolites in type 2 diabetes (T2DM) and rheumatoid arthritis (RA) affected patients, using the Human Microbial Metabolome Database (MiMeDB). Human and bacterial metabolites were assigned and the main phyla that are responsible for different metabolite production. Differences in microbiome related metabolome in different pathologies were defined.

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ENGINEERING THERMOSTABLE METAGENOMIC ESTERASE FOR POLYLACTIC ACID BIODEGRADATION

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The escalating issue of plastic pollution demands sustainable and efficient solutions, including the enzymatic breakdown of synthetic polymers such as polylactic acid (PLA). In this work, we identified a previously uncharacterized PLA-degrading esterase, MGY, from a metagenomic dataset.[1] Although catalytically active, MGY's industrial application was hindered by low solubility, limited expression yield, and poor thermal stability. To overcome these limitations, we employed ancestral sequence reconstruction (ASR)[2] to generate three MGY variants with inferred ancestral sequences.

All ASR-derived variants maintained robust PLA-degrading activity, with one variant also showing catalytic activity against polycaprolactone (PCL).[3] These ancestral enzymes demonstrated significantly improved solubility, enhanced expression levels, and increased thermostability. Differential scanning calorimetry (DSC) confirmed these improvements, with one variant achieving a melting temperature (Tm) of 84 °C—the highest reported for any PLA-degrading enzyme to date.

Molecular dynamics simulations indicated notable flexibility in the N-terminal region of all enzyme variants. To probe its functional role, we engineered N-terminal truncation mutants (ΔN) by removing 16 residues. Furthermore, the dynamic analysis guided the application of limited proteolysis to generate crystallizable proteins for X-ray crystallography.

These findings highlight the power of ancestral sequence reconstruction and molecular dynamics simulations in optimizing enzyme performance and offer valuable guidelines for defining crystallization conditions required for structural characterization. Our engineered MGY variants represent promising candidates for sustainable plastic recycling and broader biotechnological applications.

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MICROCHIP ELECTROPHORESIS IN BIOANALYSIS: RECENT INNOVATIONS AND METHODOLOGICAL ADVANCES

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Microchip electrophoresis (MCE) is a powerful miniaturized separation technique that offers several advantages, including high separation efficiency, rapid analysis, ease of automation, low reagent consumption, reduced waste generation, and minimal operating costs. When performed on microchips with coupled channels, MCE becomes a versatile platform capable of on-line sample pretreatment, two-dimensional separations, and compatibility with a wide range of detection techniques. This multifunctionality makes MCE highly suitable for the analysis of complex biological, food, and pharmaceutical samples.

Several practical applications illustrate the analytical potential of MCE in bioanalysis. For instance, a microanalytical method combining isotachophoresis (ITP) and zone electrophoresis (ZE) with conductivity detection was developed for the quantification of nitrite and nitrate in cerebrospinal fluid, aiding the diagnosis of neurological disorders.[1] In another application, ZE coupled with a photometric detector enabled the determination of carminic acid, a natural red dye, in food and pharmaceutical products.[2] ZE combined with conductivity detection was used for the determination of carnitines, endogenous non-protein amino acids, in milk products.[3] Furthermore, an MCE-based ITP method integrated with surface-enhanced Raman spectroscopy (SERS) facilitated the detection of synthetic dyes in pharmaceutical samples.[4] Universal ITP methods with conductivity detection were developed for the determination of active pharmaceutical ingredients and counterions, amlodipine besylate and perindopril erbumine, in cardiovascular drugs.[5] Additionally, ITP followed by ion mobility spectrometry was employed for the determination of carboxylic acids in various biological samples, yielding high repeatability and accuracy.[6] These examples underscore the significant analytical capabilities of MCE, demonstrating its effectiveness for rapid, precise and integrated bioanalysis.

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PROTEOMICS IN THE AMINOACYL-tRNA SYNTHETASE RESEARCH

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Shotgun proteomics based on nanoLC-HRAM MS/MS provides unique insight in the cellular proteome composition, dynamics and responses in a global and unbiased manner. We have applied the shotgun proteomics to the study of aminoacyl-tRNA synthetases (aaRS), enzymes essential in protein biosynthesis, as they supply the ribosome with its substrates - aminoacylated tRNA. AaRS play a pivotal role in maintaining the accuracy of protein biosynthesis, since erroneous tRNA aminoacylation leads to inaccurate protein biosynthesis, i. e. mistranslation of the genetic message. The focus of our research is the fidelity of aminoacyl-tRNA synthesis, consequences of protein mistranslation, and bacterial response to antibiotics targeting aaRS.

The (in)accuracy of protein biosynthesis and consequences of mistranslation were studied on isoleucyl-tRNA synthetase (IleRS) in bacterium *Escherichia coli*. It was shown that *E. coli* IleRS quite efficiently mischarges valine and norvaline (Nva) to cognate $tRNA^{Ile}$.[1] Thus IleRS evolved separate proofreading domain for hydrolysis of mischarged tRNA. We have designed a genetically engineered *E. coli* strain with editing-deficient IleRS to study the consequences of Ile to Val and Ile to Nva mistranslation *in vivo*.[2] *E. coli* strain with editing-deficient IleRS accumulated and tolerated substantial levels of mistranslation, up to 20 %, as measured proteome-wide by LC-MS/MS. Proteomic analysis showed upregulation of major chaperones, disaggregase ClpB and proteases under mistranslating conditions, indicative of proteotoxic stress. Consequently, increased protein aggregation was observed and protein aggregates formed *In vivo* were further enriched in mistranslated polypeptides.

IleRS is the target of mupirocin, a natural antibiotic in clinical use. Curiously, bacterium *Priestia megaterium* possesses two genes for IleRS, one typical for bacteria (type I IleRS), and the second one homologous to eukaryotic IleRS (type II IleRS). Biochemical and *in vivo* characterization has shown that the second, type II IleRS is resistant to mupirocin inhibition.[3] Proteomic analysis revealed that type I IleRS is constitutively expressed and thus functions as housekeeping IleRS, while type II IleRS expression is induced upon mupirocin treatment, endowing *P. megaterium* with mupirocin resistance. In closely related *Bacillus thuringiensis* multiple aaRS genes are duplicated, so *B. thuringiensis* was tested for resistance to various antibiotics targeting some of the duplicated aaRS (IleRS, ProRS, ThrRS and TrpRS). Although not all aaRS duplications conferred antibiotic resistance to *B. thuringiensis*, proteomic analyses revealed unanticipated changes to amino acid biosynthesis pathways upon treatment with antibiotics targeting aaRS.

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PEPTIDE DRUGS: CHEMICAL STRATEGIES TO IMPROVE THEIR THERAPEUTIC EFFICACY, ANALYTICAL CHARACTERIZATION AND PHARMACOLOGICAL SCREENING

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Therapeutic peptides represent a distinct class of biopharmaceuticals characterized by high target specificity and structural flexibility, offering unique advantages over small molecule drugs and biologics.

To date, over 80 therapeutic peptide drugs have been commercialized worldwide, while many more are in preclinical and clinical trials. These peptide pharmaceuticals have found widespread application in the treatment of diabetes, cardiovascular diseases, neurodegenerative disorders, oncology, infectious diseases and vaccine development. However, their clinical application is limited by factors such as short residence time in the bloodstream, rapid clearance, inadequate cellular uptake and high structural flexibility.

By using appropriate chemical methods for peptide modification, it is possible to regulate important physicochemical factors such as charge, hydrophobicity, conformation, amphiphilicity and sequence that affect the physicochemical properties and biological activity of peptides. These techniques can overcome the inherent shortcomings of peptides and improve their pharmacokinetic properties and biological activity, and promote continuous progress in the field of research.

Based on data obtained from our collaborative research, this presentation covers the main physicochemical factors and significant chemical modification strategies that influence the properties and biological activity of peptides as pharmaceuticals.

This presentation also discusses our achievements in the development of biologically active peptides, providing guidance for the design and optimization of their synthesis, characterization, and biological screening.

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RECONSTRUCTING ENVIRONMENTAL CHANGES THROUGH ELEMENTAL AND ISOTOPIC ANALYSIS OF HONEY

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Honey is an excellent indicator of environmental conditions, and its analysis holds significant value from both environmental and food safety perspectives. Its composition is influenced by various factors, including the quality of the foraging area, geographic location, nectar-producing plant species, climatic conditions, handling and storage practices. A unique characteristic of honey is that, although the concentration of volatile compounds, aromas, and enzymes decreases over time—and its flavor, color, and scent may transform—it fundamentally does not spoil, unlike most other food products. Mineral components remain chemically stable even during long-term storage, and honey's virtually unlimited shelf life makes it suitable for both short- and long-term environmental monitoring.

Between 2016 and 2025, we collected over 1.000 honey samples, the majority of which originated from Hungary. Using modern atomic spectrometry techniques, we analyzed the presence and temporal variation of macro- and microelements, toxic metals, and their correlation with anthropogenic emissions. In addition to determining the mineral content we analyzed botanical and geographical relationships between the honey samples and their collection sites, as well as the associated nectar sources.

Our research also included the analysis of a unique honey archive with nearly 100 samples collected between 1959 and 2020, covering various nectar-producing plant species and regions across Hungary. This long-term dataset revealed that the concentration of certain inorganic elements correlates with total precipitation in May, likely linked to the vegetative period of black locust (*Robinia pseudoacacia*) and its late-May blooming. Both acacia and sunflower honeys showed a decreasing trend in lead concentration over time. This multi-decade sample series also enabled us to be among the first to study, over such a long time span, how handling and storage practices affect honey's long-term shelf life.

Our research is unique in the field of apicultural product analysis. We found that honey is not only a sensitive indicator of current environmental conditions but also preserves valuable information about past environments. Therefore, it can be effectively used to trace both natural and anthropogenic processes in nectar-producing ecosystems.

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INNOVATIVE APPROACHES IN PHYTOCHEMICAL ANALYSIS: THE ROLE OF DEEP EUTECTIC SOLVENTS AND MASS SPECTROMETRY

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Phytochemicals, particularly polyphenols, are vital bioactive compounds widely used in traditional medicine, nutraceuticals, and pharmaceuticals due to their broad spectrum of biological activities. Efficient extraction and accurate characterization of these compounds are essential for maximizing their therapeutic potential.

Traditional extraction methods often rely on organic solvents mixed with inorganic or organic acids, which are not are eco-friendly. For this reason, deep eutectic solvents (DESs) and especialy their biologically derived counterparts, natural deep eutectic solvents (NADESs), have gained significant attention as sustainable and green alternatives.

These solvents are formed by combining hydrogen bond donors and acceptors, resulting in systems with tunable physicochemical properties, high solubilizing power, low toxicity, and excellent biodegradability. NADESs, composed of natural metabolites such as sugars, amino acids, and organic acids, are especially attractive for the extraction of polyphenols due to their biocompatibility and non-toxic nature. They facilitate the selective extraction of various polyphenol classes – such as flavonoids, phenolic acids, tannins, and anthocyanins – while minimizing degradation and enhancing yield. Parameters such as water content, temperature, and solvent composition can be optimized to further improve extraction efficiency and compound stability.

Coupling DES- and NADES-based extraction with advanced analytical techniques – particularly liquid chromatography coupled with mass spectrometry (LC-MS/MS or LC-DAD-MS) has significantly advanced phytochemical analysis. These methods enable high-resolution, sensitive, and accurate identification and quantification of secondary metabolites in complex plant matrices.

The presentation will discuss the results of characterizing bioactive secondary metabolites from various phenolic compound groups and highlight specific insights gained from the use of tandem mass spectrometry in their identification and quantification. Key findings will underline the effectiveness of NADESs in polyphenol extraction and the critical role of MS-based techniques in revealing structural details and enhancing analytical precision.

THE CHALLENGE TO ANALYZE NEW PSYCHOACTIVE SUBSTANCES

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In 2025, the European drug market is still undergoing constant changes due to the emergence of New Psychoactive Substances (NPS), as annually around 50 new substances have been registered by the European Union Drugs Agency (EUDA) in recent years, whereas a total of 1000 NPS have been reported to circumvent law. Among them, more than 100 synthetic cathinones, amphetamines and ketamines play an important role. They possess a chiral center, leading to the existence of two enantiomers with presumably different pharmacological properties. For them, little is known about the distinct effect of the enantiomers. Misleadingly, they are often traded as “bath salts” or "room odorizers".

The aim of this study is to provide an overview of both achiral and chiral separation methods by high performance separation techniques, such as HPLC, gas chromatography and capillary electrophoresis for the separation of NPS. Hundreds of samples were purchased by ghost shopping or seized by Austrian police.

For example, commercially available chiral HPLC columns mainly based on polysaccharide basis were tested and compared for their enantiomer separation ability of cathinone derivatives. All measurements were carried out under isocratic conditions, and *intraday* and *interday* repeatability tests were performed. Furthermore, chiral capillaries for gas chromatography were shown to be suitable for enantioseparation of novel amphetamine derivatives, which are being sold as recreational drugs. However, in this case, samples have to be derivatized prior to measurement.

In the recent decades, capillary electrophoresis turned out to be a powerful alternative to chromatographic approaches. Low electrolyte and sample consumption are advantages to be highlighted for this technique. For separation of enantiomers, simply chiral additives can be added to the buffered electrolyte. For the enantioseparation of NPS, cyclodextrin derivatives were used as chiral selectors successfully. Many of the cathinones or amphetamines misused for drug consumption possess different substitution patterns at the phenyl ring being available as positional isomers. The aforementioned separation methods were also applicable to differ between o-, m- and para substituted compounds.

METHOD DEVELOPMENT AND APPLICATION FOR STUDYING PROANTHOCYANIDIN PROFILES OF RED WINES

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Polyphenols are widespread secondary metabolites found in plants and food sources such as fruits, herbs, chocolate, and red wine. They have antioxidant and nutraceutical properties and contribute to color and taste. In red wine, polyphenols, especially flavan-3-ols, play a key role in sensory perception. Condensed tannins, or proanthocyanidins (PAs), are oligomers and polymers of flavan-3-ols that significantly influence wine quality and can form red pigments under acidic, oxidative conditions. Procyanidins, which are condensed tannins composed of flavan-3-ol subunits, play a key role in wine astringency, bitterness, and color stabilization. Due to their polymeric nature, direct analysis is challenging. Therefore, a depolymerization approach such as acid-catalyzed cleavage in the presence of nucleophiles is usually employed to break down the procyanidin polymers into their constituent monomers and extension units. This approach enables detailed profiling of the procyanidin composition, including the average degree of polymerization (aDP) and the proportion of terminal versus extension units. Such data are essential for understanding the sensory characteristics of wine and how they evolve during vinification and aging.

In this study, a depolymerization strategy using phloroglucinolysis was applied to wines from the Vranec and Merlot grape varieties to characterize their proanthocyanidin profiles. Two sample preparation procedures using solid-phase extraction and precipitation with methanol were tested and compared. The latter was then used for assay of procyanidins in twenty-four experimental wine samples obtained under different vinification conditions. The depolymerization approach enabled the breakdown of proanthocyanidin polymers into monomeric and extension units, providing insight into their composition and allowing the estimation of the mean degree of polymerization (mDP). Flavan-3-ols were quantified using spectrophotometry and reversed-phase HPLC-DAD, while HPLC-ESI-MS/MS was employed for identification of flavan-3-ol monomers and phloroglucinol adducts. The results demonstrated that longer maceration time and higher SO_2 concentrations led to wines with significantly increased levels of proanthocyanidins, flavan-3-ols, and total polyphenols, highlighting the impact of vinification parameters on the polyphenolic composition and potential sensory attributes of red wine.

USE OF FULLERENE-, OCTADECYL-, AND TRIACONTHYL SILICA FOR SOLID PHASE EXTRACTION FOR IMPROVED PROTEOMIC ANALYSIS

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Proteomics is defined as the study of protein (quantitative and qualitative) expression, localization (within organelles and compartments), structure, modifications, function, and interactions. This enables the mapping and understanding of biological pathways, signal transduction mechanisms, networks, tracking the pathomechanism of diseases, and identifying biomarkers and potential drug targets.

Post-translational modifications can provide insight into protein activity (e.g., regulation through phosphorylation) or possibly the patient's condition (e.g., haemoglobin glycation).

In addition to the dynamic changes in the proteome, the large number of proteins, their different sizes, and their wide-ranging amounts pose a great challenge for analysts. Since the samples under study are usually very complex mixtures, containing many components in large quantities. Significant biological changes are often caused by very small concentration changes or structural modifications; therefore, it is important to get rid of constituents that are not of interest, and at the same time, components of interest should be enriched.

The application of C60 silica as an SPE material to silicas with different pore sizes with other RPs (C18-, C30-silica) has been implemented based on recovery studies and SPE fractionations of unmodified and glycated HSA and fibrinogen. The findings may lie in the basis of a feasible SPE method followed by mass spectrometry to be used for the investigation of polar constituents of complex biological samples

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THE MULTIFACETED ROLE OF IONIZING RADIATION: CHALLENGES AND PERSPECTIVES IN ENVIRONMENTAL PROTECTION

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Wastewater pollution poses a significant threat to the environment and human health, as it contains various pollutants that are difficult to remove using conventional methods. Pharmaceuticals are among the main pollutants whose improper disposal can affect ecosystems and water quality. The use of ionizing radiation as one of the advanced oxidation processes for the degradation of pharmaceuticals in water represents an effective and sustainable method for the removal of pollutants from aquatic systems. These processes are increasingly being researched as they are able to efficiently remove even persistent pharmaceutical substances that are not easily degradable by conventional water treatment methods. In this presentation, the results of degradation of different groups of pharmaceuticals (α-blockers and anti-inflammatory drugs) using ionizing radiation will be presented as one of the environmentally friendly and effective methods for their removal.[1,2] The efficiency of degradation will be presented under different irradiation doses, pH values, in the presence of different inorganic salts and different radical scavengers. In addition, the presentation will include results to evaluate the toxicity of the degradation products in different bacterial strains as well as *in silico* toxicity predictions to assess potential environmental and health risks after radiation treatment.

The second part of the presentation will focus on the application of ionizing radiation in the synthesis of bacterial nanocellulose (BNC) using alternative carbon sources from biowaste. BNC is a natural biopolymer with unique structural and functional properties that make it a valuable component for various technological applications. However, large-scale production of BNC is limited by low yields and the high cost of conventional growth media. Preliminary results on the biosynthesis of BNC using environmentally friendly feedstock will be presented, aiming to promote sustainability and reduce biowaste through the application of radiation technology.

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IN VITRO AND *IN VIVO* ANALYSIS OF INDOCYANINE GREEN AND ITS USE IN HEPATOBILIARY SURGERY

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Indocyanine Green (ICG) is a near-infrared fluorescent dye extensively used in medical diagnostics. Initially approved for cardiac and hepatic function evaluation, ICG has become increasingly relevant in image-guided surgery, especially in hepatobiliary procedures. ICG binds tightly to plasma proteins and is exclusively cleared by the liver, making it a useful indicator of hepatic function and perfusion. Its fluorescence is excited at around 780 nm and emits in the near-infrared range (~820–840 nm), enabling real-time imaging of vascular and tissue structures. In hepatobiliary surgery, ICG is used for anatomical segment delineation, tumor margin visualization, and assessment of hepatic reserve in cirrhotic patients. In clinical settings, ICG is administered to patients with hepatic cirrhosis undergoing resection, and intraoperative fluorescence imaging facilitate clear identification of hepatic segments and tumor borders.[1]

Various techniques are available for the qualitative and quantitative analysis of ICG *in vitro* and *in vivo*. UV-Vis spectrophotometry is widely used for its simplicity and cost-effectiveness, suitable for aqueous solutions. However, its sensitivity in biological matrices is limited. For higher sensitivity and specificity, HPLC - often with UV or fluorescence detection - is preferred, allowing precise quantification in plasma or serum after sample preparation. LC-MS, though less common due to complexity and cost, enables simultaneous detection of ICG and its metabolites with superior sensitivity. NIR fluorescence imaging systems are increasingly used for real-time, non-invasive visualization of ICG *in vivo*, aiding in assessing tissue perfusion and tumor margins during surgery. Other advanced methods, such as capillary electrophoresis and photoacoustic imaging, support research applications. Together, these methods form a comprehensive toolkit for studying ICG's pharmacokinetics and biodistribution.[2]

In our study, we analyzed pure ICG substance and ICG in plasma samples using UV-Vis spectrophotometry (λ = 781 nm) and high-performance liquid chromatography (HPLC). The calibration curves were linear in the tested concentration range, and recovery studies confirmed methods accuracy.

ICG represents a versatile tool for both diagnostic and surgical applications. Our analytical results validate the precision of spectrophotometric and HPLC methods for ICG determination. Further research should explore individualized dosing strategies and expand applications in minimally invasive procedures.

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SHORT ORAL PRESENTATIONS

NAVIGATING THE METHODOLOGICAL AND ANALYTICAL COMPLEXITIES OF ICP-MS FOR ELEMENTAL IMPURITY ANALYSIS IN PHARMACEUTICALS

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Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is the method of choice for trace-level quantification of elemental impurities in pharmaceutical products due to its exceptional sensitivity and multi-element capability. However, its application in pharmaceutical analysis presents a series of complex challenges across methodological, analytical, and regulatory domains.

Matrix effects remain a significant methodological concern, particularly given the chemical diversity of pharmaceutical formulations—including solid and parenteral dosage forms—which often contain excipients, buffers, and organic constituents that can suppress or enhance ionization efficiency. Strategies such as matrix-matched calibration and the use of internal standards are routinely employed to address these effects, though they add procedural complexity and may not fully account for all interferences.

From an analytical standpoint, isobaric and polyatomic interferences continue to compromise specificity, particularly in low-concentration regimes. For example, the overlap between ^{40}Ca and ^{40}K exemplifies a common challenge in trace analysis. Advanced instrumental solutions such as high-resolution sector field ICP-MS and collision/reaction cell technologies (CRC) improve selectivity, though at the cost of increased method development time and instrumentation demands.

Contamination control is another critical factor, especially when operating near regulatory limits defined by guidelines such as ICH Q3D. Reagent purity, laboratory environment, and sample handling protocols must be rigorously managed to prevent artifactual signals and ensure data integrity.

Ultimately, robust implementation of ICP-MS for elemental impurity analysis in pharmaceuticals requires a multidisciplinary approach, integrating analytical rigor with an in-depth understanding of matrix behavior, instrumental capabilities, and compliance requirements. Continued methodological innovation and harmonization with evolving regulatory standards will be essential to advance the reliability and applicability of ICP-MS in this context.

EVALUATION OF SYNERGISTIC DRUG COMBINATIONS FOR LOCAL GLIOBLASTOMA TREATMENT

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Glioblastoma (GBM), the most frequently diagnosed and aggressive form of primary malignant tumor of the central nervous system, continues to lack a definitive cure, with resistance-driven relapses being nearly inevitable.[1–3] Multi-drug combination therapies offer a powerful tool to address GBM's complexity, heterogeneity, and resistance to standard chemotherapy by engaging distinct yet potentially synergistic biological mechanisms, ultimately aiming at improving therapeutic outcomes while minimizing systemic toxicity.[4,5] Locally implantable polymeric drug reservoirs employed for sustained release can further enhance treatment effectiveness by simultaneously overcoming systemic limitations and synchronizing pharmacokinetics to maintain synergistic ratios, if existent.[4] This study explored alternative local therapeutic strategies for GBM by repurposing antiepileptic drugs with antitumor potential and combining them with the JAK/STAT3 inhibitor ruxolitinib (RUX). The cytotoxic effects of valproic acid (VPA), oxcarbazepine (OXC), and gabapentin were investigated on A172 and U251 GBM cells. VPA and OXC showed a notable reduction in cell viability, prompting further evaluation with RUX in 3D multicellular tumorspheres (MCTS), which revealed suboptimal combination effects compared to single agents. A factorial experimental design for cell viability data and subsequent Bliss synergy analysis revealed synergism exclusively for RUX + VPA on A172 cells. Despite their interaction being additive, GBM cells displayed higher sensitivity to RUX + OXC, suggesting potential therapeutic benefits. The most effective drug ratios were further determined in 3D MCTS using live/dead cell fluorescent staining. The variability in response across different GBM cell types underscores the need for personalized therapy based on the specific molecular profile of each tumor.

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BETWEEN WHAT WE KNOW AND WHAT WE WONDER: MECHANOCHEMICAL SYNTHESIS OF MOLECULARLY IMPRINTED POLYMERS

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Upon the first successful proof-of-concept, solvent-free, mechanochemically-assisted synthesis of molecularly imprinted polymers described by our group,[1] it became obvious that through a better understanding of the underlying reaction mechanisms and of their correlations with common process variables a series of novel opportunities may be explored and the experimental constrains related to the conventional solution-based non-covalent MIP synthesis may be circumvented.

Using a data-driven experimental design on a model template molecule, we sought to progressively reveal currently unresolved correlations by identifying hidden patterns and dependencies that impact key performance metrics such as yield, imprinting factor, chemoselectivity, rebinding efficiency, and kinetics.

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HIGH-AFFINITY APTAMERS FOR VANCOMYCIN MONITORING IN CLINICAL SAMPLES BY MAGNETIC BEADS-BASED SELEX

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The emergence of antibiotic-resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) has become a critical global health concern, particularly in intensive care unit settings. Vancomycin, a glycopeptide antibiotic, remains the gold standard first-line treatment for severe infections caused by MRSA. Given its narrow therapeutic window, therapeutic drug monitoring is highly required to ensure efficacy while minimizing toxicity.[1] Aptamers are single-stranded DNA or RNA oligonucleotides, selected *in vitro* by systematic evolution of ligands by exponential enrichment (SELEX) technique, exhibiting high affinity towards specific target molecules. Offering antibody-like recognition properties with greater stability, aptamers hold significant potential as biorecognition elements in personalized medicine.[2] This study presents the selection of a novel DNA aptamer targeting vancomycin by magnetic beads-based SELEX. The selection strategy envisioned the introduction of negative and counter-selection steps to promote the enrichment of aptamers capable of distinguishing vancomycin from other structural analogs after only seven rounds. Affinity characterization of the resulting aptamer candidates by surface plasmon resonance yielded dissociation constants in the nanomolar range. Computational modeling, including molecular docking and dynamics simulations, was employed to predict the three-dimensional structure of the aptamer and its complex with vancomycin. Furthermore, a disposable electrochemical aptasensor was developed using screen-printed gold electrodes to enable rapid vancomycin quantification from serum samples. Differential pulse voltammetry was employed as detection method, providing results within 30 minutes. The aptasensor was clinically validated using serum from hospitalized patients receiving vancomycin treatment and showed comparable performance to the gold standard particle-enhanced turbidimetric inhibition immunoassay. Given its rapid response time and high selectivity, the proposed aptasensor offers a promising tool for personalized vancomycin treatment, potentially improving clinical outcomes while minimizing resistance and toxicity.

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USING NATURAL DEEP EUTECTIC SOLVENTS FOR EXTRACTION OF POLYPHENOLS FROM *STACHYS* SPECIES

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The genus *Stachys* L. (Laminaceae) is considered a valuable source of bioactive phenolic compounds with great pharmacological potential. This research focuses on the use of eco-friendly natural deep eutectic solvents (NADES) for a selective extraction of polyphenols from *Stachys iva* Griseb.

Fourteen NADES were synthesized and characterized by pH, density, conductivity, refractive index and surface tension. The density of all solutions was higher than that of water at 22 °C, which is why they are classified as hydrophilic. The measured conductivity values are generally higher than those previously published (2.65–22.2 mS cm^{-1}). Also, the solvents have a higher surface tension (40.64–81.69 mN m^{-1}) compared to the most commonly used solvent for extraction of polyphenols – methanol (32.55 mN m^{-1}). An analysis of the infrared spectra of the individual components and the resulting solutions, recorded by the FT-IR method, was also performed. The main characteristic observed in these spectra was the shift of the band originating from the –OH vibration (~3200 cm^{-1}) towards higher wavenumber values (~3300–3400 cm^{-1}). This indicates the formation of new intermolecular hydrogen bonds are formed.

The synthesized solvents were applied for the extraction of polyphenolic compounds from the plant *Stachys iva* Griseb. In general, solvents based on choline chloride-organic acid (eg. acetic – ChA, lactic – ChL, citric – ChC) were more efficient. Moderate surface tension and low pH were identified as the most significant factors for obtaining greater extraction yields. Optimal extraction conditions (time, solvent volume, temperature and water content), using a Box-Behnken design, were determined: 40 min, 10.25 mL, 59.9 °C and 20 % water.

The findings highlight the critical role of physicochemical properties in optimizing extraction efficiency, supporting the potential of NADES as sustainable alternatives to conventional solvents.

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A NOVEL METHOD FOR CHARACTERISATION OF IONIC PROPERTIES OF MIXED-MODE STATIONARY PHASES

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Mixed-mode chromatography is a promising analytical method for complex mixture separation.[1] However, the unavailability of ligand information of many commercially sold columns, such as the p*K*a value of its functional group, hinders its full application potential.[2] We propose a novel method for ionic interaction evaluation, designed for stationary phase characterisation. The method uses buffer blending by pumps to produce a range of mobile phase pH within the liquid chromatography system. The applicability of the method was utilized for ionic property elucidation of weak anion-exchange and weak cation-exchange mixed-mode stationary phases. The interaction mechanism of these mixed mode columns was investigated using pump blended mobile phases in the 2.5–8.6 pH range. The retention response of strong acid/base analytes to the dissociation/protonation of the ionic groups present on the stationary phase was observed. This method presents an efficient alternative for stationary phase/analyte p*K*a value determination.

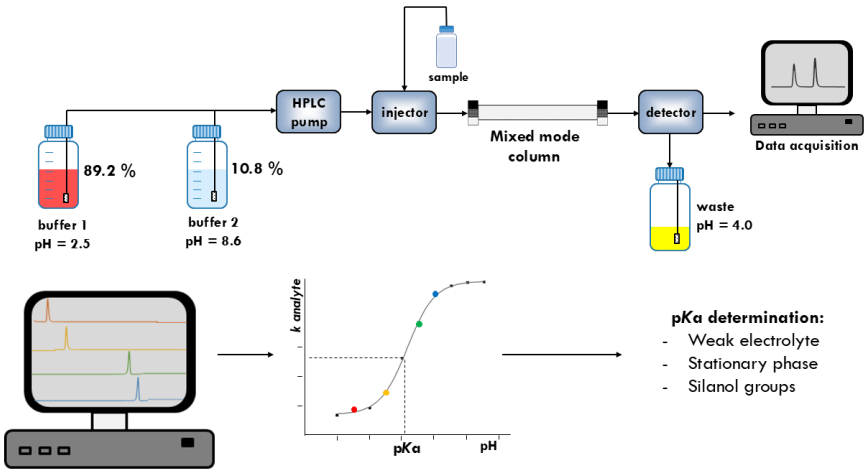

Figure 1. Buffer blending method by the pumps and its utilisation for p*K*a determination

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BIOLOGICAL, BIOCHEMICAL AND CHEMICAL OSCILLATORS

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Periodic phenomena and spatial organizations can be often observed at all levels of matter organization, from molecules, cells, to a whole population. Their period time varies from miliseconds to years. These phenomena occur in chemical, biochemical, biological, geological, ecological systems as well. There are two conditions to set up oscillations in chemical systems:

1. The thermodynamic condition is that the system must be far from equilibrium
2. The kinetic condition is that in the mechanism of oscillators must be at least one nonlinear step (autocatalysis, autoinhibition).

In spite of the main differences between the chemical and biological oscillators, the kinetic models based on the chemical oscillators' mechanism still can explain some of the oscillatory behavior of the biological systems.

In biological systems we can observe periodic phenomena, such as the glicolytic oscillations, the periodic variation of intracellular Ca^{2+} level, the mitotic oscillations, the spatial organization of the Dictyostellium, the membrane oscillators, and many others.

One of the most important and interesting oscillators are those in which the pH-value changes periodically between two levels (pH-oscillators). In these systems the concentration of dissociated form of some ionizable drugs increase and decrease periodically, so it may be possible to realize a periodic drug-delivery through a membrane.

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ELECTROCHEMICALLY CONTROLLED RELEASE FROM A PEDOT-FUNCTIONALIZED MICROGEL ON AN ELECTRODE SURFACE

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Microgels are 3D, crosslinked hydrophilic polymer networks capable of absorbing large volumes of water, typically forming spherical structures with dimensions in the colloidal range. A subset of these materials, termed "smart" microgels, can undergo significant, reversible volume changes in response to external stimuli. Temperature- and pH-responsive systems are the most thoroughly investigated to date; however, growing attention is now focused on electroactive and electroresponsive hydrogels.[1]

In this study, a hybrid microgel was synthesized from a copolymer of *N*-isopropylacrylamide and acrylic acid, incorporating the conductive polymer PEDOT (poly(3,4-ethylenedioxythiophene)) as dispersed particles. Crosslinking was achieved using a cystine-based derivative (BISS) bearing disulfide (-S–S-) linkages, which also enabled chemisorption-based surface modification of a gold electrode. The presence of negatively charged carboxyl groups within the polymer network facilitated the electrostatic incorporation of positively charged active substances. Upon applying a suitable electrochemical potential, oxidation of PEDOT introduced positive charges into the network. The resulting electrostatic competition triggered the release of the model substance into the surrounding solution. Release was monitored using UV-Vis spectroscopy. This hybrid microgel system shows strong potential as a drug delivery platform, particularly for drug-eluting implants requiring precise, electrochemically controlled release profiles.

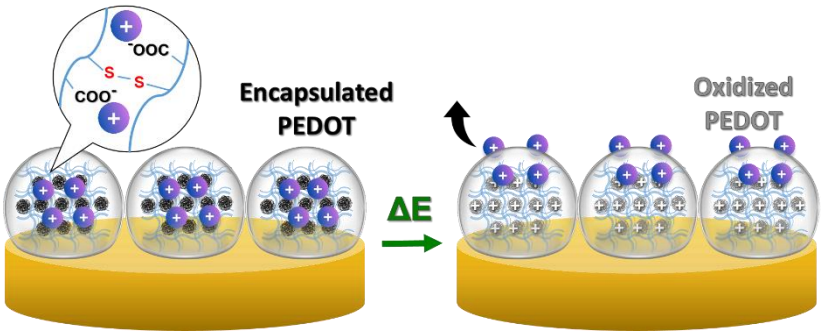

Figure 1. Scheme of the electrochemically controlled release of the active substance from an electroactive microgel monolayer on the electrode surface.

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FROM RESIN TO RECOGNITION: EXPLORING 3D TECHNOLOGY IN THE FABRICATION OF MOLECULARLY IMPRINTED POLYMER-BASED SENSORS

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The increasing presence of pharmaceuticals, pesticides, and other active substances in our environment—particularly through food production and agriculture—raises serious concerns. Among them, the excessive and irresponsible use of antibiotics stands out, especially when these compounds find their way into the food chain or natural ecosystems. While conventional analytical methods are available for detecting such contaminants, there's a growing need for alternatives that not only match these methods in sensitivity and accuracy but are also simpler, more affordable, and portable—enabling on-site applications. To achieve these desirable outcomes, the incorporation of selective recognition elements is frequently essential. In this context, molecularly imprinted polymers (MIPs) offer substantial advantages, as they are capable of selectively binding target analytes and thus play a crucial role in sensor development.[1] The primary objective of this study was to explore a novel approach for the fabrication of MIPs using 3D printing technology and commercially available synthetic resins. This strategy aims to pave the way for a faster, more cost-efficient, and precise method of producing MIP-based sensors.

For the fabrication of MIP films, we employed a 3D printer based on Digital Light Processing (DLP) technology, which allows the precise printing of sufficiently thin polymeric films. Commercially available synthetic resins served as the polymer matrix, with their composition modified using porogenic solvents to optimize performance. The template molecule used in this study was the antibiotic moxifloxacin (MOX), selected due to its intrinsic fluorescence, which facilitated monitoring of each step of the polymerization process. Fluorescence measurements of the template molecule were conducted using a Desaga CD 60 photodensitometer.

In order to produce a functional MIP-based sensor, both the parameters of the 3D printing process and the resin composition had to be carefully optimized to yield a sufficiently porous and stable MIP film. Filter paper was chosen as the substrate due to its favorable surface properties, supporting the adhesion and stability of the thin polymer film. The sensor demonstrated the ability to detect MOX at relevant concentrations in both water and milk samples. This study presents a promising step toward low-cost, rapid, and sensitive sensor development—using methods accessible even outside the traditional lab setting.

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STABILITY STUDIES OF SEMI-PREPARATIVE FRACTIONATED CATHINONE DERIVATIVE ENANTIOMERS USING HPLC-UV

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In addition to well-known traditional synthetic illicit drugs like cocaine, amphetamines, and heroin, an increasing number of new psychoactive substances (NPS) have appeared on the drug market. Cathinones represent a prominent class within these NPS, and specific types of these molecules contain a stereogenic center, resulting in the possible presence of two enantiomers with potentially distinct biological activities.[1] The objective of this study was the evaluation of the stability of pure enantiomers of cathinone derivatives and to monitor and compare any racemization depending on storage conditions and solvent environment. For this study, these substances must be provided on a multi-milligram scale. A Phenomenex Lux i-Cellulose-5® 5µm 250 × 10 mm column containing cellulose tris(3,5-dichlorophenylcarbamate) as a chiral selector serves to collect pure enantiomeric fractions. The method was operated in normal phase mode under isocratic conditions. After the isolation, the collected substances were converted to their HCl salts. Following further optimization, it was possible to collect 16 pure enantiomers out of 8 racemic cathinone derivatives. Moreover, the stability of the enantiomeric compounds was assessed over six months, considering both their solid salt form and their dissolved state in water or methanol. Samples were stored under three different temperature conditions: room temperature (20 °C), refrigerator (4 °C) and freezer (-20 °C). Qualitative experiments were carried out to determine the purity of the isolated enantiomers using high-performance liquid chromatography.[2] Stability tests revealed that factors such as aqueous conditions, solution pH, temperature, the chemical structure of the cathinone derivative, sunlight, and atmospheric oxygen all influence stability. Therefore, long-term storage of cathinone derivative enantiomers is best carried out in solid form under deep freeze conditions or in a slightly acidified solvent, protected from air and light.

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GUT MICROBIOME STRUCTURE AND METABOLIC ACTIVITY IN TYPE 2 DIABETIC PATIENTS

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Diabetes is a worldwide health issue problem. The number of cases has increased significantly over the past three decades. Today we count 350 million more patients than in 1980.[1] The disease can be described by the malfunction of releasing and secreting insulin by pancreatic beta cells, and also with the lacking response from adipose tissue and/or liver cells to it.[2] The gut microbiome can be influenced by age, diet, living location, health status or the way of birth as well. In the case of diabetic and prediabetic patients, the gut microbiome is significantly altered. In T2DM patients the abundance of the Proteobacteria phyla has significantly increased or decreased, and the Bacteroidetes phyla has been significantly decreased compared to the prediabetic control group.[3] The alternation of gut microbiome can lead to differences in metabolite profile (bile acids, branched-chain amino acids, short-chain fatty acids, and lipopolysaccharides).[4]

In our study, we searched for associations between the insulin and metformin-treated patients' microbiome composition and their metabolites. At the phylum level, we observed some differences in the case of abundance of Verrucomicrobia, Firmicutes, and Bacteroidetes. Following the principal component analysis, we established that two groups of metabolites were highlighted: fatty acids and their derivatives, as well as monosaccharides and sugars.

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DEVELOPMENT OF A DUAL APTASENSOR FOR THE DETECTION OF INFLAMMATORY CYTOKINES IN BIOLOGICAL FLUIDS

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Cytokines are signaling biomolecules that play a crucial role in cell proliferation, immune responses, inflammation, and various cancer-related processes. Owing to their pivotal functions, cytokines are valuable biomarkers for diagnosing various medical conditions and monitoring responses to pharmacological treatments.[1,2] This study focused on developing a tailored platform for the simultaneous and specific electrochemical detection of Interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) in biological fluids, with potential applications in both biomedical research and clinical diagnostics.

The aptasensor was fabricated based on in-lab printed electrochemical cells. To enhance detection sensitivity, the working electrodes were functionalized with Au and Pt nanoparticles. Two specific aptamers, each functionalized with a distinct redox label, were employed to ensure high specificity in detecting the target cytokines. Each modification step was validated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), while the actual detection was carried out using CV.

The dual aptasensor was thoroughly evaluated in terms of its analytical performance, confirming its capability for the selective and simultaneous detection of IL-6 and TNF-α. The optimized sensor platform was applied to the analysis of real biological samples, specifically saliva and sweat collected from both patients and healthy individuals. To validate the obtained results, the same samples were tested using an ELISA technique, and the results were statistically analyzed.

Overall, the developed sensor demonstrated effective, specific, and simultaneous electrochemical detection of IL-6 and TNF-α, emphasizing its potential for use in medical diagnostics.

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SPECTROSCOPIC AND THEORETICAL STUDY OF EDA COMPLEXES BETWEEN ANILINE DERIVATIVES AND TCNE

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Among the key types of intermolecular interactions are electron donor–acceptor (EDA) interactions between planar π-conjugated molecules. In such systems, one molecule acts as the electron donor - e.g., benzene, aniline, naphthalene and their derivatives - while the other functions as the electron acceptor, such as tetracyanoethylene (TCNE).

According to Coppola,[1] in recent years the scientific community has made significant efforts to develop new, sustainable light-harvesting materials in order to replace fossil fuels and rare-earth metals. Charge transfer (CT) processes play a central role in these systems, as they are highly versatile and can be applied in fields such as optoelectronics, solar energy conversion, and nonlinear optics.

This study presents a UV-VIS spectrophotometric analysis of CT complexes formed between TCNE and various aniline derivatives: ortho-chloroaniline, o-toluidine, and aniline. The experimental findings were compared with theoretical results obtained using the Gaussian 16 [2] software packages on wb97xd/6-311++g(2d,2p) level.

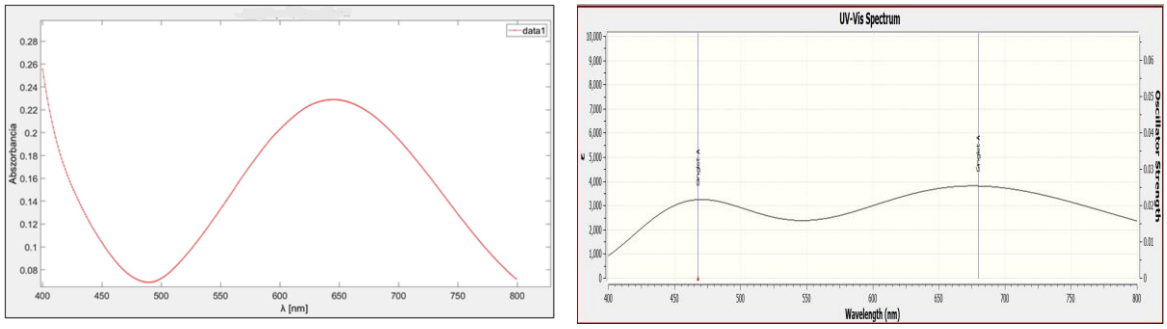

Figure 1 The experimental and calculated wb97xd/6-311++g(2d,2p) absorption spectra of the CT complex of o-toluidine-TCNE in Solvent CH_2Cl_2

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SUB/SUPERCRITICAL FLUID CHROMATOGRAPHY FOR SYNTHETIC CANNABINOIDS

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Sub/supercritical fluid chromatography (SFC) is a suitable separation technique for both chiral and achiral applications. The possibility of increasing the mobile phase polarity by adding an organic modifier or low amount of water opens the possibility of analyzing simultaneously compounds with a broad range of polarities.[1] One of the biggest and fastest-growing novel psychoactive substance (NPS) classes comprises the synthetic cannabinoids.[2] The group of synthetic cannabinoids contains chiral and also achiral molecules.

In this work we utilized SFC and Torus diol column for separation of a set of eight achiral synthetic cannabinoids. The effects of organic modifier type and its amount in the mobile phase, separation temperature and gradient type on retention and separation were evaluated. SFC with polysaccharide-based column also demonstrated high separation potential for a set of eleven chiral cannabinoids available either as racemates or pure enantiomers.

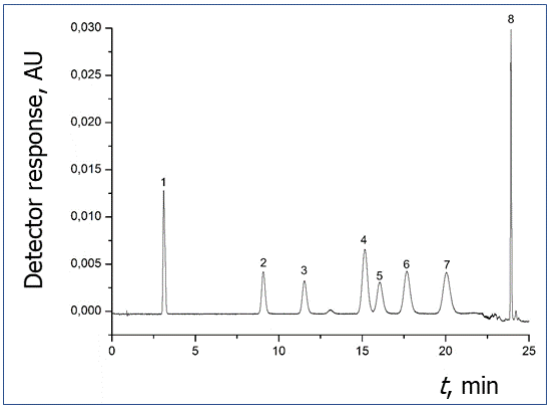

Figure 1. Chromatogram of separation of a set of 8 synthetic cannabinoids. SFC conditions: Torus diol column, CO_2/dimethylcarbonate (*v*/*v*), gradient elution, temperature 35 °C, ABPR: 2000 psi, 2 mL min^{-1}, 300 nm. 1: UR-144, 2: 5F-AKB48, 3: 5C-AKB48, 4: JWH-144, 5: AM-694, 6: AM-2201, 7: MAM-2201, 8: 5F-MN-24.

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SPENT COFFEE GROUNDS AS BIOSORBENTS AND A SOURCE OF ANTIOXIDANTS

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In the face of new trends related to bio-waste management, recycling food and agricultural waste is becoming increasingly important. Spent coffee grounds (SCG) are a waste with a potentially wide range of uses.

Biosorbents are sorbents based on biological material, which can be used in two main applications: removal of environmental pollutants, and sample pre-concentration. Biosorbents exhibit several advantages over inorganic sorbents, such as low cost and no creation of additional waste. A wide variety of biosorbents have been proposed, ranging from living organisms to plant pollen.[1] Different types of physical and chemical pre-treatment can be applied to a biosorbent to increase its sorption capacity. SCG have been used as biosorbents for several potentially toxic metal ions, with and without pre-treatment. Their chemical composition allows for effective metal ion complexation to occur due to the presence of carbonyl, carboxyl, hydroxide, sulfur- and nitrogen-containing groups. Some of the most important parameters affecting sorption are: pH, temperature, amount of used coffee grounds, time of contact and presence of other ions.[2]

SCG contain high levels of compounds with antioxidant properties. Therefore, potential commercial applications of this waste also include: cosmetics, food and pharmaceutical industries. The conducted studies used oil produced from SCG by a Polish start-up, two commercially available oils pressed from roasted coffee beans and dry coffee grounds. Water-ethanol extracts were analyzed for polyphenol content, flavonoid content and total antioxidant capacity using five spectrophotometric methods. SCG extracts showed higher antioxidant properties than oils. The studies also estimated the relative content of different flavonoid classes in tested samples.

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STUDY OF THE EFFECT OF PROBIOTIC BACTERIA ON THE METABOLISM OF ANTIOXIDANTS PRESENT IN FOOD PRODUCTS

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The aim of this study was to test whether selected probiotic bacteria have an effect on the metabolism of selected antioxidants present in food products. Caffeine, resveratrol, theobromine and theophylline were chosen as model compounds, and they are found in coffee, red wine and chocolate, as well as melatonin.

Epidemiological studies show a strong correlation between a diet rich in plant-based foods and a reduction in the incidence of cardiovascular disease, diabetes and cancer. The occurrence of these diseases is increasingly attributed to external factors such as free radicals. These compounds may be responsible for oxidative stress, i.e. an imbalance between free radicals and antioxidants. Oxidative stress, can lead to damage to cell structures, lipid oxidation or changes in enzyme structure and function. The positive effect of plant-based foods is due to the presence of natural antioxidants in them, which, by reacting with free radicals, counteract many undesirable reactions.

However, the effect of antioxidants on organisms depends on their bioavailability and the form in which they are delivered to cells. Therefore, it is extremely important to supply them to organisms in an adequate diet and to study the metabolism of these compounds in particular under the influence of selected probiotic bacteria.

Four different strains of probiotic bacteria were used in the experimental part, these were: *Lactiplantibacillus plantarum 299v, Limosilactobacillus reuteri DSM 17938, Lacticaseibacillus rhamnosus GG (ATCC 53103) and Lacticaseibacillus rhamnosus Lcr35*.

As a first step, the antioxidant activity of the compounds tested was determined and compared. One of the most commonly used methods for this purpose is the DPPH$^{\bullet}$ (2,2diphenyl-1-picrylhydrazyl) radical method. Of the compounds tested, resveratrol showed the highest ability to neutralise DPPH$^{\bullet}$ and caffeine the lowest. The series of antioxidant properties varied as follows caffeine < theophylline < melatonin < theobromine <<resveratrol.

In a second step, analysis was carried out using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) of post-culture extracts of the bacterium + antioxidant. Control samples consisted of solutions of pure analytes in the medium in which the bacteria were grown and the inoculum itself. The tests were performed 24 hours after the bacteria had been incubated with the analytes.

Analysis of the HPLC-MS/MS results showed that all bacterial strains cause the degradation of melatonin and resveratrol, while for caffeine, theobromine and theophylline, no significant effect of microbial action was observed. For melatonin and resveratrol, studies were also carried out on the kinetics of their degradation over 24 h

In conclusion, the effect of probiotic bacteria on the degradation of selected analytes is not fully correlated with their antioxidant properties of the model compounds studied.

DETECTION OF CHLORAMPHENICOL USING A NOVEL CATIONIC COVALENT ORGANIC FRAMEWORK

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Cationic covalent organic frameworks (cCOFs) are crystalline porous polymers well known for their permanent porosity and highly ordered structures, making them suitable for biomedical applications [1]. Chloramphenicol (CAP) is a key antibiotic with broad-spectrum antibacterial properties used to treat severe infections when other antibiotics may not be effective. This study presents an electrochemical method for detecting CAP in human and animal medical products using carbon screen-printed electrodes (C-SPE) modified with cCOF to enhance sensitivity and specificity.

C-SPE were modified with a suspension made of chitosan, cCOF I, and ethanol. CAP was preconcentrated on the modified surface and detected using cyclic voltammetry (CV) in Britton-Robinson buffer at pH 6 (**Figure 1**). The detection method was utilized for CAP in different pharmaceutical products for human and animal use. The antimicrobial activity of the cCOFs was assessed against 7 pathogenic bacterial strains.

The developed detection platform demonstrated a broad detection range, a low limit of detection, high specificity, and good recovery percentages for CAP in pharmaceutical products.

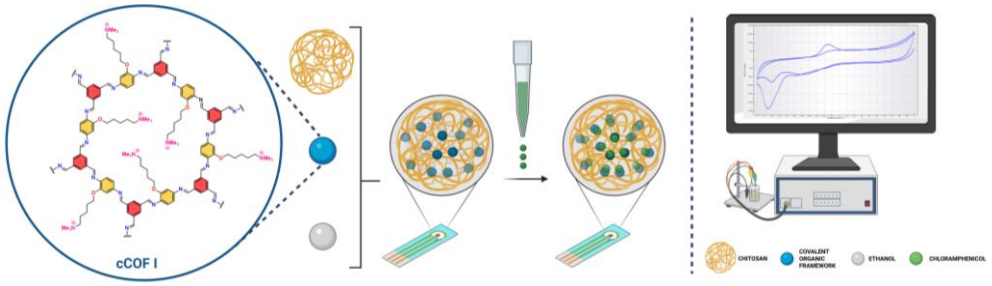

Figure 1. cCOF **I**-Based Electrochemical Sensor for CAP detection.

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MGMT ENHANCER METHYLATION IN MELANOMA BRAIN METASTASES: ASSOCIATION WITH MGMT EXPRESSION, GENETIC VARIANTS, AND CLINICAL PARAMETERS COMPARED TO GLIOBLASTOMA

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Melanoma is one of the the primary cancers in adults that show the strongest potentials to metastasize to the brain.[1] Treatment modalities for melanoma brain metastases include temozolomide (TMZ),[2] an alkylating agent also used as part of the standard therapy for glioblastoma. In glioblastoma, high activity of the tumour suppressor protein O^6-methylguanine-DNA methyltransferase (MGMT) has been shown to counteract the therapeutic effects of TMZ. Since methylation of the *MGMT p*romoter has widely been found to inversely correlate with MGMT protein expression, it has been established as a predictive biomarker for the responsiveness of glioblastoma patients to TMZ therapy. However, this inverse correlation is not given for all glioblastoma patients.[3, 4] In a previous study, we showed that in addition to *MGMT* promoter methylation, also methylation of *MGMT* enhancers is associated with MGMT protein expression, and clinical parameters in glioblastoma.[5]

Even though glioblastoma and melanoma brain metastases arise from different primary tumour sites, both are developing within the unique microenvironment of the brain and may therefore share similar epigenetic and microenvironmental characteristics. This study aimed at determining DNA methylation status in the *MGMT* promoter as well as in five intergenic and four intragenic *MGMT* enhancers in melanoma brain metastases and compare the findings with those previously obtained in glioblastoma.

Genomic DNA from primary cell line samples, derived from patients with melanoma brain metastases, was subjected to bisulfite conversion, regions of interest were amplified by polymerase chain reaction and methylation levels were determined by pyrosequencing.

While some associations of *MGMT* enhancer methylation with MGMT protein expression, *MGMT* promoter methylation and clinical parameters were found for both melanoma brain metastases and glioblastoma, distinct differences were also identified.

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ADVANCED ELECTROCHEMICAL SENSING PLATFORMS FOR NON-INVASIVE CORTISOL MONITORING

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Cortisol (COR) is an endogenous hormone with multiple roles in numerous physiological and pathological processes. Fluctuations of COR levels in biological fluids can be observed in multiple diseases, such as inflammatory conditions like long-COVID syndrome. Thus, accurate and selective detection of COR could play a crucial role in diagnosing and monitoring these. Electrochemical techniques present notable benefits for analyzing biomarkers in biological samples, including low cost, high sensitivity and specificity, potential for miniaturization, and suitability for in situ analysis.

The aim of this work was to develop in-lab printed electrochemical platforms for the sensitive and selective detection of COR. Two different platforms were developed, namely (*i*) a flexible electrode modified with Ni nanoparticles for the direct electrochemical detection of COR and (*ii*) an aptsensing platform with improved selectivity and sensitivity for the indirect electrochemical detection of COR.

In the case of direct detection, the surface of in-lab produced electrodes was functionalized with Ni nanoparticles via chronoamperometry. The influence of the pH and the scan rate on the detection of COR was assessed, and the method was optimized. The detection of COR was performed via cyclic voltammetry in a concentration range between 75 ng mL^{-1} to 0,1 mg mL^{-1}, with a limit of detection (LOD) of 25 ng mL^{-1}.

Taking into account the need for higher sensitivity and selectivity, a second electrochemical method was developed. In this case, the surface of in-lab printed electrodes was modified with Au and Pt nanoparticles and an aptamer specific for COR was bound to the surface via Au-S bonds. Each step in the development of the platform was characterized by CV and electrochemical impedance spectroscopy. Cortisol detection was carried out using CV, and the aptasensor demonstrated a linear detection range from 0.5 to 100 nM, with an LOD of 0.1 nM. Furthermore, the aptasensor was utilized to analyze saliva and sweat samples from both long-COVID patients and healthy individuals and the results were compared with those obtained using standard control methods (ECLIA and HPLC).

In conclusion, two different platforms for the detection of COR were successfully fabricated, paving the way for the development of wearable sensors for COR monitoring.

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EVALUATION OF MICROBIOLOGICAL AND PHYSICO-CHEMICAL CONTAMINANTS IN VALEA BOROȘ SPRINGS

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This study investigated the contamination levels of spring waters located along the Boros stream in Valea Boroș, Hargita County, Romania. The research included physico-chemical and hygienic microbiological analyses. Water samples from various springs exhibited a wide temperature range (3.0–10.5 °C), influenced by the depth of the water source, the degree of canopy cover and sunlight exposure. All sources had slightly basic pH values. Turbidity values exceeded permissible thresholds in two springs, likely due to recent anthropogenic activities, such as logging, rather than meteorological conditions. Ammonia and nitrate concentrations remained within acceptable drinking water limits in all samples.

Microbiological evaluations revealed that the water quality was not suitable for human consumption. Coliform bacteria were detected in all samples, except one, with *E. coli* present in most of them. Additionally, fecal Enterococci and *Clostridium spp.* were identified in two samples each. Further identification using MALDI-TOF-MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) confirmed the presence of fecal and opportunistic pathogenic bacteria (e.g., *E. coli, Enterobacter cloacae*), often resistant to one or more antibiotics. These findings substantiate the evidence of fecal contamination from human or animal sources, underscoring the public health risks associated with consumption of these waters.

EFFECT OF ETHANOL ON THE FORMATION AND STRUCTURE OF COCHLEATE DRUG DELIVERY SYSTEMS

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Background: Cochleate membrane rolls are promising drug delivery systems with favorable biopharmaceutical properties. However, their application is limited by suboptimal particle size distribution and the frequent presence of non-cochleate by-products formed during preparation.

Objectives: This study aims to investigate the effect of ethanol on the formation and structural–nanomechanical properties of cochleates.

Methods: Cochleates were prepared from unilamellar dioleoyl-phosphatidylserine liposomes (d = 100 nm) and from phospholipid solutions in alcohol by adding $CaCl_2$. The samples contained 0–100 vol% ethanol. Structural and nanomechanical characterization of the particles was performed using atomic force microscopy (AFM) imaging and force spectroscopy. In addition to ethanol content, the effect of the initial lipid concentration was also examined.

Results: The introduction of calcium resulted in the formation of lipid aggregates measuring a few micrometers in diameter. In the absence of ethanol, these aggregates contained not only cochleates but also multilamellar vesicles (MLVs) and other lipid structures. The ratio of these forms depended on the lipid concentration: MLVs dominated at higher concentrations, while cochleates increasingly appeared at lower concentrations. In the presence of ethanol (≥50 %), the proportion of cochleates significantly increased within the aggregates. When ethanol concentration exceeded 70 %, particle length decreased from 2–3 µm to 300–500 nm. However, at high ethanol concentrations (80–100 %), the boundaries of the particles became increasingly blurred with longer incubation times. AFM force spectroscopy revealed nanomechanical signatures consistent with cochleate structures, including identifiable, component-specific force responses.

Conclusions: Ethanol has a dual, concentration-dependent effect on cochleate nanoparticle formation: it significantly promotes their formation and reduces particle length. The likely mechanism is that ethanol loosens the structure of calcium-bound bilayers, facilitating the formation of the membrane roll structure. Notably, reducing particle crowding alone also promotes cochleate formation, presumably by decreasing the spatial hindrance of the MLV-to-cochleate transition. Our findings may pave the way for the formulation of cochleate drug delivery systems with optimal particle size distribution.

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TWO TRANSLATIONAL START CODONS ARE USED FOR SYNTHESIS OF ENDOLYSIN GP24 FROM BACTERIOPHAGE BFK20

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Endolysins are lytic enzymes used by bacteriophages to break down the host cell wall during the final stage of the lytic cycle.[1] The gp24 endolysin from corynephage BFK20 is encoded by the *orf24* gene (813 bp), has a modular structure with an N-terminal catalytic domain and a C-terminal cell wall-binding domain.[2]

In this study, we investigated whether the ATG codon at position 565–567 in *orf24*, corresponding to methionine at position 189 in protein, acts as a secondary translation start site. Using site-directed mutagenesis, we generated the gp24M189I mutant, in which methionine 189 was replaced by isoleucine.[3] Expression of the wild-type-like gp24N-His in *Escherichia coli* produced two protein products with predicted molecular weights of 32.2 and 9.3 kDa, while the gp24M189I mutant yielded only the full-length 32.2 kDa protein.

Western blot analysis confirmed that the truncated binding domain was produced only in the wild-type-like sample and **was not detected in the mutant sample**.[4] The truncated domain, lacking a His-tag, was co-purified with gp24N-His, likely due to their interaction. These results demonstrate that endolysin gp24 utilizes a secondary translation start site to generate an N-terminally truncated cell wall-binding domain, a mechanism also observed in other phage endolysins.[5,6] Our findings provide novel insights into the translational regulation of phage endolysins and the functional diversity arising from alternative translation initiation.

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SIMULTANEOUS BACTERIA DETECTION – OPTIMISING THE ELECTROCHEMICAL METHODS USING AI-BASED APPROACHES

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The antimicrobial resistance represents an increasing concern for the healthcare systems, as more and more bacterial strains are not susceptible to the available antibiotherapy. One of the solutions considered by the health and security organisations is represented by the rapid detection of the pathogens, hence the need to develop fast and efficient detection methods. Electrochemical analysis can be adapted for the detection of microorganisms, either for the whole cell identification, or for measuring redox active molecules, such as virulence factors (specific bacterial metabolites). Combining the advantages of electrochemical techniques and the potential of siderophores as markers for infections, portable sensors can be developed for the rapid and point of care diagnostic.[1] In order to improve the parameters of the sensors, the issue of complex biological matrices and possible interferences should be addressed. Chemometrics and machine learning aalgoriths have been used to study the electrochemical behaviour of three bacterial markers (aerobactin, enterobactin and pyocianin) and to evaluate the possibility of simoultanous electrochemical detection in clinical samples[2] By using this novel approach, the fast detection of bacteria could be possible, involving an electrochemical unmodified sensor and the assistance of AI for improving the accuracy and diminishing the interferences from the biological matrices, with impact in healthcare.

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DETERMINATION OF COLCHICINE-SERIC PROTEIN INTERACTIONS BY SPECTRAL METHODS

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In the present work we investigate the effect of colchicine, originating from autumn crocus (Colchicum autumnale) on serum proteins using spectral methods such as fluorescence, UV-VIS and LC-MS/MS while taking into account the toxicological importance of this alkaloid. This plant of the Colchicaceae family, recognized for its content of colchicine, a toxic alcaloid with therapeutic importance. Autumn crocus is studied for its multiple applications in medicine, especially in the treatment of gout and inflammatory diseases. The objectives of this work are to identify and characterize colchicine from Colchicum autumnale and to investigate the interaction between colchicine and proteins.[1,2]

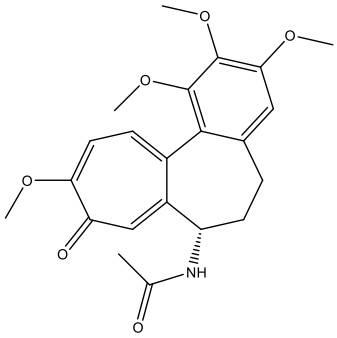

Figure 1. Chemical structure of colchicine

Following the experiments performed, the presence of colchicine in the extracts obtained from *Colchicum autumnale* was confirmed and significant spectral changes were observed in the interaction between colchicine and proteins, indicating the formation of complexes.

The interaction of colchicine with serum proteins can be efficiently monitored through spectral methods, which provide important data for understanding the mechanisms of action. The main reason for the pros and contras for the therapeutic use of colchicine is the interaction of colchicine at the cellular level. Toxicokinetic studies on colchicine have established that the activity of colchicine is related to its ability to bind to microtubules.[3]

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INTRAOPERATIVE NEUROCYTOLOGY OF BRAIN TUMORS

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Intraoperative examinations still pose a major challenge for neuropathologists today; often, serious decisions that may even determine the surgical strategy must be made based on minimal, suboptimal native samples.

Cytological preparations, touch prints and smears can be important supplements to frozen sections. By using several types of rapid staining, experienced pathologists can obtain a lot of additional information from smears, including cytomorphology, matrix, the nature and extent of the vascular network of the tumor. These informations can help to rule out or confirm malignancy, as well as to distinguish between primary and metastatic tumors, especially lymphoma.

The aim of our presentation is to demonstrate the value and applicability of neurocytology, while also drawing attention to its limitations. It is particularly important to emphasize that neurocytological preparations are of fundamental importance in the differential diagnosis of small, stereotactic biopsies and in the differential diagnosis of lymphomas, but they can also provide useful and reassuring additional information for all intraoperative histomorphological diagnoses.

POSTER PRESENTATIONS

A COMPARATIVE STUDY OF CARBON FARMING AND CONVENTIONAL SYSTEMS IN CORN AND SUNFLOWER CULTIVATION: CASE STUDY IN NORTH MACEDONIA

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Precise carbon determination transforms carbon farming from an empirical concept into a scientifically-driven, economically viable, and policy-relevant practice. It enables farmers and researchers to quantify soil health improvements and climate benefits, unlock new revenue via carbon markets, and foster adaptive management and sound policymaking rooted in hard data. In doing so, it advances the broader goal of sustainable, climate-smart agriculture—anchoring the study's comparative findings in real-world impact and long-term viability.

This study presents a comparative evaluation of carbon farming and conventional agricultural systems in the cultivation of corn (*Zea mays L.*) and sunflower (*Helianthus annuus L.*), focusing on their effects on soil carbon, nitrogen dynamics and other soil characteristics over a two-year period (2024–2025). Soil samples were collected at the beginning, midpoint, and end of the study to assess total organic carbon (TOC) and total nitrogen (TN) under each management system. The results demonstrated a consistent and significant increase in both carbon and nitrogen content in soils managed under carbon farming practices compared to those under conventional management. In corn plots, carbon farming led to a progressive accumulation of TOC and TN, attributed to organic matter inputs, minimal soil disturbance, and enhanced microbial activity. Sunflower plots also showed increased TOC and TN, although with a delayed response, likely due to the crop's higher nutrient demand and biomass turnover. In contrast, conventional systems showed stagnant or declining trends in TOC and TN, underscoring the limitations of intensive tillage and synthetic input dependence in maintaining long-term soil fertility. The findings highlight the potential of carbon farming as a viable strategy for enhancing soil health, increasing nutrient retention, and contributing to climate-smart agricultural practices in cereal and oilseed production systems.

BIOMARKERS AND METHODS ON ANALYSING THE EFFECT OF PRIMING ON CULTURE PLANTS

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Plant priming is a physiological and molecular process through which a plant, after exposure to a mild stress or stimulus, enhances its ability to respond more effectively and rapidly to future abiotic or biotic stresses. Abiotic stress refers to environmental factors such as drought, salinity, extreme temperatures, and nutrient imbalances that negatively affect plant growth and productivity.[1] Priming activates the plant's defence mechanisms, improves water and nutrient uptake, and enhances the activity of antioxidant enzymes.

Selecting appropriate biomarkers is a significant challenge, as it requires a strategic approach based on the research objectives, plant species, and the specific type of abiotic stress. Biomarkers can be classified as molecular, biochemical, or physiological indicators. Molecular biomarkers include stress-responsive genes, transcription factors, which show altered expression patterns in primed plants. Biochemical markers encompass antioxidant enzymes, osmoprotectants like proline or glycine betaine, and hormonal shifts associated with stress adaptation. Physiological biomarkers include changes in chlorophyll content, electrolyte leakage, stomatal conductance, and growth recovery rates under repeated stress exposure.[2]

For detecting genetic biomarkers, qRT-PCR is commonly used, while biochemical markers—such as amino acids (e.g., proline) and plant growth hormones (e.g., abscisic acid, salicylic acid, jasmonic acid, auxin)—are typically measured using LC-MS/MS, GC-MS, or ELISA immunoassays. The upregulation of heat shock protein synthesis in primed plants contributes to the formation of stress memory, which can be evaluated through a combined analysis of hormone level shifts (via LC-MS/MS) and DNA methylation profiling.[3]

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CRYSTALLIZATION OF TERNARY COPPER(II) COMPOUNDS WITH dsDNA: SCREENING AND OPTIMIZATION

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The discovery of cisplatin as an antitumor agent and its application in clinical practice paved the way for a whole new field of cancer treatment research.[1] A class of compounds, called ternary metal compounds, where the central metal ion is coordinated by two ligands, possess similar properties. Coordination compounds containing copper(II) as central ion coordinated by an amino acid and a heterocyclic ligand are the primary focus of this research.[2] Four copper(II) ternary compounds were synthesized and used for crystallization runs with a B-DNA dodecamer (PDB code: 1BNA)[3]: **1** – $[Cu(Gly)(H_2O)(phen)][Cu(Gly)(SO_4)(phen)]\cdot 5H_2O$, **2** – $\{[Cu(\mu\text{-L-hSer})(H_2O)(bpy)]_2SO_4\cdot 2H_2O\}_n$, **3** – $[Cu(\text{L-Phe})(H_2O)(bpy)][Cu(\text{L-Phe})(SO_4)(bpy)]\cdot 8H_2O$, **4** – $\{[Cu(\mu\text{-L-hSer})(H_2O)(phen)][Cu(\mu\text{-L-hSer})(phen)]SO_4\cdot 6H_2O\}_n$. Crystallization runs were screened against commercially available crystallization solutions using the Oryx 8 robot. The isolated crystals were evaluated by the single crystal X-ray diffraction at Elettra synchrotron (XRD2, Trieste, Italy). Compound **3** was found to yield the highest quality crystals diffracting to the resolution of 1.8 Å. Screening showed that crystals most abundantly grow in conditions with lower salt concentrations while PEG concetration and pH do not significantly affect crystal growth. Crystal growth was favoured at lower temperatures and at higher ternary compound to dsDNA ratio (2:1). New trigonal and tetragonal crystal forms of the dsDNA dodecamer were obtained in addition of ternary compounds **1**–**4**. These results highlight the need for further investigation into the nature of interaction between dsDNA and compund **1**–**4**.

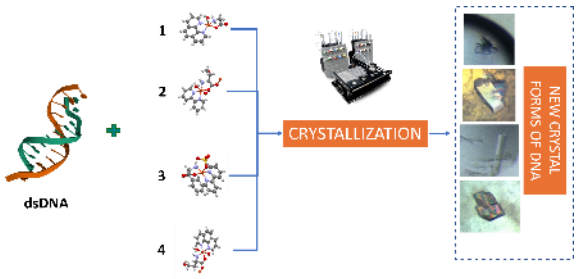

Figure 1. Crystallization of dsDNA with four different ternary copper(II) compounds (**1**–**4**).

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CAPABILITIES AND LIMITATIONS OF TOTAL REFLECTION X-RAY FLUORESCENCE SPECTROMETRY (TXRF) TECHNIQUE FOR ELEMENTAL ANALYSIS OF DERMOCOSMETIC FORMULATIONS

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Everyday use of dermocosmetic products by consumers of all ages leads to frequent and prolonged exposure to the ingredients, which may increase the risk of adverse health effects, especially in the presence of hazardous impurities such as potentially toxic elements (PTEs). PTEs can be introduced into dermocosmetic formulations through various routes, including contaminated raw materials, manufacturing processes and packaging components. The presence of PTEs in dermocosmetic products affects not only the safety of dermocosmetic products, but also their stability and efficacy. Therefore, routine elemental analysis of cosmetic products is urgently needed to ensure compliance with established safety standards and regulatory requirements as those outlined in the European Union Cosmetics Regulation (EC) No 1223/2009.

As modern analytical approaches require the use of more environmentally friendly sample preparation methods and techniques, total reflection X-ray fluorescence spectrometry (TXRF) is increasingly used for elemental analysis of various samples due to its speed, cost-effectiveness and environmental sustainability.[1,2] The aim of the present study was to develop and optimize the TXRF technique for the multi-elemental analysis of prepared topical formulations (gels, emulsions, emulgels and creams). Two different sample treatment methods were used for TXRF analysis, including a microwave digestion and a more environmentally friendly suspension preparation method. The results were compared with those obtained by analysing digested dermocosmetic formulations using inductively coupled plasma mass spectroscopy (ICP-MS). In addition, the different preparation methods were critically evaluated in terms of their compliance with the concepts of white and green analytical chemistry using the RGB algorithm. The study showed the promising potential of the TXRF technique for elemental analysis of dermocosmetic formulations. It also provided an assessment of the possible strengths, critical parameters and limitations of the TXRF technique and emphasised its potential for wider application in the quality control of dermocosmetic products.

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ARE INTERNET-ACQUIRED ANTIBIOTICS SAFE? CAPILLARY ELECTROPHORETIC AND LIQUID CHROMATOGRAPHIC ANALYSIS OF AMOXICILLIN CAPSULES

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The World Health Organization (WHO) has identified three categories of counterfeit medicines: substandard, unregistered/unlicensed and falsified medical products. A 2017 study found that antibiotics and antivirals are the second most commonly counterfeited drugs, after antimalarials. β-lactam antibiotics, especially amoxicillin, are frequently adulterated.[1] Amoxicillin is a broad-spectrum semisynthetic penicillin effective against Gram-positive and Gram-negative cocci.

Amoxicillin capsules labeled to contain 250 mg of active ingredient were acquired online from India and arrived in a cardboard box without a patient information leaflet. These were compared with a Romanian-authorized medicinal product. Organoleptic examination was first performed. The internet-sourced sample did not match the description in the Pharmacopoeia, consisting of a yellow, grainy powder. Uniformity of dosage units was assessed based on weight variation. The internet sample showed a relative standard deviation (RSD) of 3.4 %, whereas the Romanian samples showed a more uniform distribution (RSD = 2.58 %). Both samples were analyzed using capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). In CE, a 25 mM borax solution was used as the background electrolyte. A short, uncoated fused capillary (33 cm length, 75 µm inside diameter) maintained at 25 °C was used with short-end injection (-50 mbar × 2 s), for fast screening of the products. The applied voltage was -12 kV and the detection was at 210 nm. Electropherograms indicated substandard amoxicillin content in the internet sample, the active substance content was about 70 %, prompting further impurity profiling using HPLC. The HPLC analysis followed the United States Pharmacopeia monograph for Amoxicillin Capsules, using a Zorbax Eclipse XDB-C8 column (4.6 × 150 mm) maintained at 40 °C. The mobile phase consisted of 0.05 M phosphate buffer (pH = 5.0), delivered with a flow rate of 2 mL min^{-1}, under gradient conditions. Detection was carried out at 230 nm with an injection volume of 10 µL. The internet-purchased sample contained approximately 13 % total impurities, exceeding the USP limit of 7 %, while the authorized product contained less than 3 %. The present study underlines once again the dangers of internet-acquired medicines, especially antibiotics.

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INNOVATIVE TECHNOLOGY FOR TEMJANIKA WINE PRODUCTION WITH HONEY ADDITION BEFORE FERMENTATION

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In this study, addition of honey before fermentation (20 and 40 g L^{-1} added honey) of Temjanika white grapes was performed in order to study the influence of the honey on the wine quality. Fast and accurate analytical technique, Fourier-transform infrared spectroscopy (FT-IR), was used to determine the chemical composition of wines produced with honey and compared with the control wine (produced without addition of honey). Basic parameters such as alcohol, density, glycerol, pH, total acidity, total sugars, individual carbohydrates (glucose, fructose and saccharose), individual organic acids (tartaric lactic, malic, citric and acetic) as well as total phenolic content and total antioxidant activity have been determined. Results showed that wine fermented with 20 g L^{-1} honey added before fermentation presented highest content of almost all parameters, with exception of antioxidant activity, which concentration was slightly highest in the controlled wine. Concerning organic acids, tartaric acid was the dominant organic acid in wines, as was expected, followed by malic and citric acid. In general, wines presented satisfactory values for alcohol, pH, total acidity, glycerol and acetic acid, confirming that the wines are stable, with satisfied quality.

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FT-IR ANALYSIS OF SMEDEREVKA WINES PRODUCED WITH HONEY ADDITION BEFORE FERMENTATION

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Smederevka is a Balkan variety grown in Macedonia, Serbia and Bulgaria, as well as other parts of the Balkan. It is a leading grape variety for production of white wine in Macedonia, widely spired in the Tikveš wine region, with total area of 5 389 ha. Wines are fruity, with aromas of citrus peel and green apple. Since this variety is very popular in Macedonia and winemaking techique is well developed and applicable, in this study we aimed to produced a new wine stayle of Smederevka, adding honey in the must before starting the alcoholic fermentation. Honey was added in a dose of 20 and 40 g L^{-1} in order to study the physico-chemical composition of wines produced with honey and compare them with the wine produced without honey addition. Fourier-transform infrared spectroscopy (FT-IR) was applied for analysis of alcohol, density, pH, total acids, total reducing sugars, glucerol, acetic acid, fructose, glucose, saccharose, tartaric acid, malic acid, lactic acid, total polyphenolic content (TPC) and total antioxidant acitvity (TAA). It was concluded that wine fermented with 40 g L^{-1} honey added before fermentation presented highest content of alcohol, total sugars, glycerol, acetic acid, fructose, glucose, TPC and TAA. Tartaric acid was the dominant organic acid in wines, followed by malic and citric acid. Lactic acid was not detected which means that malolactic fermentation did not start spontaneously in the wines. Wines presented satisfactory values for alcohol, pH, total acidity and acetic acid, confirming the quality and stability of the wines.

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FORMULATION DEVELOPMENT OF CATIONIC LIPOSOMES WITH SORAFENIB

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Hepatocellular carcinoma (HCC) is in the top five causes of death associated with cancer, highlighting the urgent need for improved treatment options.[1] Conventional chemotherapy is often limited by notable disadvantages, such as poor efficacy and significant side effects, which can be ameliorated through nanotechnology-based drug delivery systems.[2] This study aims to develop and evaluate a nanosystem for targeted drug delivery of sorafenib (SOR), the first-line chemotherapeutic agent for advanced HCC, to HepG2 cells, serving as a model of HCC. To this end, we have prepared cationic PEGylated liposomes through the thin-film hydration method, followed by extrusion to achieve liposomes sized under 200 nm. To remove the unbound SOR, several liposome purification strategies have been employed – dialysis and centrifugation. Liposomes were characterized in terms of size and polydispersity index (PDI) using a Nano Zetasizer device, before and after reducing their size through 800 nm and 200 nm size membranes, respectively. The SOR concentration (μg mL^{-1}) was determined through UV-Vis spectrophotometry at λ = 266 nm. Preliminary *in vitro* cell viability assays were conducted on HepG2 cells using the alamarBlue assay, demonstrating low toxicity for the unloaded liposomes and a concentration-dependent increase in cell death for SOR-loaded formulations. The formulation study showed a correlation between the liposome characteristics and the formulations parameters. The optimal liposome formulation will further be functionalized with a HepG2 specific aptamer, to enhance the cell-targeting properties and improve the nanosystem's efficacy and specificity. These developments aim to mitigate the disadvantages of SOR treatments, potentially offering a more effective and less toxic therapeutic strategy for HCC patients.

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DETERMINATION OF MELAMINE IN MILK PRODUCTS BY COUPLING ISOTACHOPHORESIS WITH ZONE ELECTROPHORESIS ON THE MICROCHIP WITH CONDUCTIVITY DETECTION

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Melamine, a nitrogen-rich industrial compound (1,3,5-triazine-2,4,6-triamine) is attained central attention due to melamine-contaminated product scandals. It is usually involved in the synthesis of melamine-formaldehyde resins and used in making products such as adhesives, furniture material, food packaging and food containers. In the food industry, melamine is illegally added to animal feeds or food products to enrich the protein content even though it is seriously prohibited to the addition of food products in markets. Therefore, quantification of melamine products is highly significant due to their health issues and even death. This is apparently evident in first reported issue in USA and in China. [1] Due to these incidents and many health scandals around the world, higher exposure melamine content have created global concern in milk products. According to the US Food and Drug Administration, the European Commission and authorities in other countries and regions have set 1.0 mg kg^{-1} for infant formulas and 2.5 mg kg^{-1} for other milk products as a standard limit for content of melamine in various milk products.[2] Although the melamine content is still a major concern in many countries worldwide, determination of melamine by analytical techniques is growing concern.

Analytical methods such as chromatographic methods are employed for the analysis of melamine content.[1] However, as the world is rapidly adapting to green analytical techniques in every industry due to faster analysis and low sample and reagents, miniaturized analytical methods like microchip electrophoresis with conductivity detection are well suitable for the analysis of melamine content in infant milk samples. In our study, online combination of isotachophoresis (ITP) with zone electrophoresis (ZE) performed on a microchip was used for the analysis of infant formula samples. The samples were first deproteinated, filtered through a centrifugal filter, diluted and analyzed using ITP-ZE method with discrete spacers. The presence of discrete spacers in the analyzed samples made it possible to define a narrow ITP mobility interval in which melamine migrated without unwanted interferents. The addition of triethylenetetramine to the background electrolyte suppressed the adsorption of sample compounds onto the inner walls of the microchip channel, and methylhydroxyethylcellulose was added to the electrolytes to suppress the electroosmotic flow on the microchip. The ITP-ZE analyses were performed in cationic mode of separation at pH 3.2. Thus, the developed ITP-ZE method can be suitably used in food quality control as a screening method for the analysis of milk products.

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A DNA-BINDING COPPER(II) TERNARY COMPOUNDS WITH PROMISING ANTITUMOR POTENTIAL

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Copper(II) ternary compounds have emerged as promising candidates in anticancer drug development due to their redox activity, structural adaptability, and favorable biocompatibility. These compounds are known to engage in DNA binding through intercalative and groove-binding modes, potentially disrupting the double-helical structure and mimicking the activity of artificial nucleases.[1] In this study, the DNA-binding properties of a copper(II) ternary compound $\{[Cu(\mu\text{-L-hser})(H_2O)(bpy)]_2SO_4{\cdot}2H_2O\}_n$ (**1**) containing bipyridine and homoserine ligands were investigated using UV-VIS absorption spectroscopy and fluorimetry. UV-VIS data revealed red shifts and hyperchromic effects, suggesting interactions between the ternary copper(II) compound **1** and the double-stranded DNA molecule (**dsDNA**). Fluorescence titration experiments were conducted using GelRed, a sensitive, stable, and environmentally safer fluorescent probe that intercalates into DNA, serving as a non-toxic alternative to traditional dyes such as ethidium bromide.[2] Upon titration with the compound **1**, fluorescence quenching of the GelRed-**dsDNA** complex was observed indicating the external groove-binding interaction[2]. The calculated binding constants for both techniques are three orders of magnitude lower than that observed for classical intercalators such as ethidium bromide[3] supporting moderate DNA-binding of ternary copper(II) compound **1**. These findings support the potential of ternary copper(II) compounds as viable scaffolds for anticancer drug design. The spectroscopic evidence provided here lays the groundwork for further biochemical and biological evaluations of these compounds.

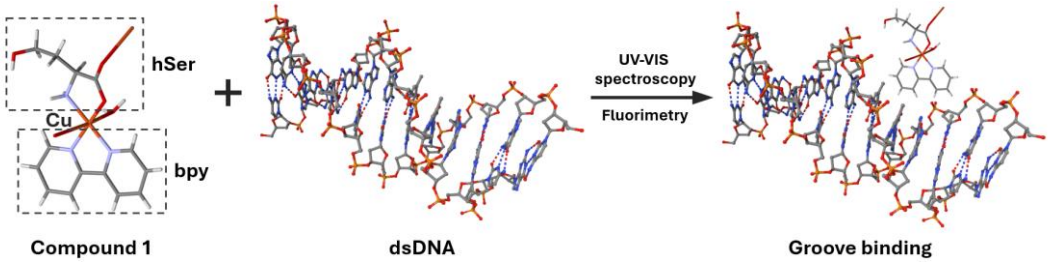

Figure 1. Schematic illustration of interaction studies between the ternary copper(II) compound **1** and double-stranded DNA (**dsDNA**).

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CONTENT EVALUATION AND ANTITUMOR ACTION OF A WATER-ETHANOL EXTRACT FROM FRUITING BODIES OF AMANITA MUSCARIA

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The fly agaric (*Amanita muscaria* (L.Fr.) Hook), also known as fly amanita and mukhomor among others, is a fungus with a cosmopolitan distribution among the boreal regions of Asia, Europe and North America, being found growing in a mycorrhizal relationship with various tree species. Its fruiting bodies are widely considered to be inedible and even poisonous due to the presence of the neuroactive alkaloids ibotenic acid (IBO) and muscimol (MUS), compounds which are known to produce an unpleasant physiological syndrome known as 'pantherina-muscaria' syndrome. In spite of that, in recent years, there have been documented cases of consumption of different *A. muscaria* preparations by patients suffering from various illnesses, including cancer, to no apparent ill effect. In our study, we aim to evaluate a number of water-ethanol *A. muscaria* extracts' effectiveness against several lung cancer cell lines *in vitro*, as well as to measure said extracts' IBO and MUS content via HPLC, capillary zone electrophoresis with contactless conductivity detection and UHPLC-MS/MS. We have also evaluated the content of a known fungal anticancer compound, ergosterol (ERG) in the extracts in question via HPLC. Our results show that the *A. muscaria* fruiting body water-ethanol extract shows significant cytotoxic activity against the lung cancer cell lines A549 and H1299 *in vitro*, as well as that it contains very small quantities of the compounds IBO, MUS and ERG.[1] We have also includex a preliminary study of the effect of the *A. muscaria* extract on cell replication via an ethynyl 5'-deoxyuridine inclusion assay, which demonstrates its cytostatic effect in addition to its cytotoxicity.

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PROTEIN RESTORATION OF CATARACT-ASSOCIATED P23T HγD-CRYSTALLIN SOLUBILITY BY VITAMIN C

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The human lens is a unique optical structure composed of 90 % highly concentrated crystallin proteins (~400 mg mL^{-1}) that must maintain stability and solubility throughout life. **Cataract**, characterized by lens opacification with light scattering and decreased visual acuity, presents two distinct forms with different pathological mechanisms. Age-related cataract is rare in young and middle-aged adults but increases sharply after age 50, resulting from progressive protein damage accumulation. In contrast, congenital cataract manifests at birth or shortly after, caused by specific genetic mutations.[1]

The **P23T** (Pro23Thr) mutation in human γD-crystallin represents a paradigmatic example of congenital cataract. This single amino acid substitution causes dramatic loss of protein solubility without significantly affecting stability or three-dimensional structure. Despite advances in current surgical techniques for cataract treatment, identifying specific **inhibitors of γD-crystallin protein aggregation** remains a major therapeutic goal.[2]

The assessment of vitamin C influence on mutant protein solubility was performed by measuring turbidity at 405 nm with variable vitamin C concentrations (2–20 mM) during 37 °C incubation. The fluorescence study analyzed the inhibitor-protein aggregation complex at various inhibitor concentrations, determining complex stability by calculating the dissociation constant from collected data.

NMR data showed that **vitamin C** interacts with **HγD-P23T** amorphous aggregates, restoring them to their soluble monomeric form, with spectroscopic measurements confirming the efficacy of vitamin C in dissolving these protein aggregates. Human γD-crystallin protein was obtained through fermentation using *E. coli* BL21, and purification was achieved through ion-exchange and size-exclusion chromatography.[3]

This in vitro study demonstrates vitamin C's potential for cataract treatment and expands possibilities for identifying new inhibitors through understanding molecular aggregation mechanisms.

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DNA METHYLATION ANALYSIS OF MGMT SILENCERS IN GLIOBLASTOMA

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In glioblastoma multiforme (GBM), methylation of the O6-methylguanine-DNA methyltransferase (MGMT) promoter region shows an inverse association with MGMT gene expression. As the MGMT protein counteracts the cytotoxic effects of the alkylating agent temozolomide (TMZ), promoter methylation of the MGMT gene can be used as a predictive marker for TMZ sensitivity in GBM patients.[1,2]

Typically, patients with a methylated MGMT promoter respond better to temozolomide-based therapy than those without this epigenetic alteration, although exceptions exist.[1]

Previous studies performed by our research group have explored whether DNA methylation in regulatory elements beyond the MGMT promoter—specifically in enhancer regions—contributes to MGMT gene regulation. These studies revealed associations between methylation levels in defined enhancer regions, MGMT promoter methylation, MGMT protein expression, and overall patient survival.[3]

The present study focuses on potential associations between DNA methylation in silencers and MGMT gene expression. Therefore, a set of six PCR- and pyrosequencing-based assays targeting distinct MGMT silencer regions has so far been developed and applied to 38 glioblastoma samples. These assays currently cover 25 CpG sites located in both intergenic and intragenic silencer elements. Results analyzed so far suggest that methylation levels at specific CpGs within a silencer region proximal to the MGMT promoter are inversely associated with MGMT gene expression. This finding highlights the potential relevance of silencer methylation affecting MGMT activity and underscores the complexity of its epigenetic regulation in GBM.

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DESIGN AND EVALUATION OF BORONIC ACID-BASED MOLECULARY IMPRINTED POLYMERS FOR SUSTAINED DOXIFLURIDINE RELEASE FOR LOCAL GLIOBLASTOMA TREATMENT

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Glioblastoma (GBM) is one of the most aggressive and lethal forms of primary brain tumor. Even after surgical resection, recurrence is frequent and often more aggressive, due to the tumor cells' ability to infiltrate healthy brain tissue.[1] Considering the high expression of thymidine phosphorylase in GBM cells and its absence or trace presence in healty brain tissue,[2] 5-deoxy-5'-fluorouridine (DFU), a prodrug converted into its active 5-fluorouracil form by this enzyme,[3] emerges as a potential therapeutic agent for local GBM therapy.

The aim of this study was to explore molecularly imprinted polymer (MIP)-based drug reservoirs for the sustained release of DFU. Covalent imprinting was selected as a strategy to obtain high affinity binding cavities by leveraging DFU's vicinal diol groups for ester bond formation with boronic acid-based functional monomers. [4] The most straightforward approach consisted in the synthesis of three distinct MIPs with varying crosslinking agents (N,N'-methylene-bis-acrylamide, pentaerythritol triacrylate, bis[2-(methacryloyloxy)-ethyl] phosphate), but-3-enylboronic and itaconic acids as functional monomers, and ethanol as porogenic solvent. Drug loading assessment by high-performance liquid chromatography revealed suboptimal loading (19, 16, 10 µg mg^{-1} polymer), suggesting relatively weak template-monomer interaction.

Consequently, a more rational approach for MIP development was adopted by investigating the degree of interaction between DFU and several functional monomers via isothermal titration calorimetry (ITC) in both aqueous and non-aqueous media. The affinity ITC screening results prompted a reassessment of the optimal MIP synthesis conditions to maximize the imprinting factor and loading capacity.

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ENHANCING NETWORKING AND PUBLICATION ACTIVITY AMONG CEEPUS PARTNERS

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The Teaching and Learning Bioanalysis network, established in 1998 within the Central European Exchange Program for University Studies (CEEPUS), has been a true success. In the years 2025-26 alone, 21 academic units from 10 countries participated. Mutual visits and interactive programs of university instructors and students are on the rise, Summer Schools have been welcomed with interest, and there is anticipation of our intensive hands-on Course. However, currently little is known about the evidence-backed outcome of the program.

As a decision has recently been made about the continuation and renewal of the CEEPUS program, it is highly timely to contemplate about its future so that we may enhance its focus beyond teaching and learning activities, towards achieving research, development and innovation outcomes that can be quantified in scientometric (publications, patents) and grant-support measures. PubMed and Google Scholar searches with the key word “CIII-RO-0010” (ID number of our grant) resulted in 6 and 49 hits, respectively, whereas a search with the key word “CEEPUS” gave 59 and 49600 hits, respectively. Thus, there is room to grow.

We recommend enhancing our efforts to generate publications between participating units. For example, a presentation given at the Summer School may form the basis for a joint publication. Furthermore, we may use the occasion of the upcoming Summer School for brainstorming about enhancing creative collaboration among our research groups. After all, brainstorming is known to be the best tool for group idea generation.[1] There is strong evidence that international collaboration leading to high citations has global impact and a home-country effect.[2] We should strive to intentionally create opportunities for new connections, to foster scholarship projects, explore intriguing questions, and engage in educational research with like-minded colleagues.[3] By sharing practical research, publication and leadership tips among members, our Network is destined to achieve even greater success.

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ANTIOXIDANT CAPACITY AND TOTAL POLYPHENOL CONTENT OF SELECTED SLOVAK MEADS

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Honey harvesting was one of the first agricultural activities, therefore mead production appeared relatively early in human history.[1] The honey used affects the taste and color of the final product. Other spices and fruit juices can also be added to mead to enhance the flavors and aromas of this beverage.[2] Most tests developed to determine antioxidant capacity are based on the neutralization of free radicals by antioxidants present in the sample. The DPPH (1,1-diphenyl-2-picrylhydrazyl) test is one of the most commonly used methods to determine antioxidant capacity.[3] The Folin-Ciocalteu test is often used to determine the polyphenol content of food samples, where a reagent containing phosphomolybdic or phosphotungstic acid reacts to produce a blue chromophore that is measured spectrophotometrically at λ = 765 nm [4]. Our study evaluated homemade craft and commercially available meads, highlighting the differences caused by production methods and the quality of ingredients. The antioxidant capacity of selected meads was determined using the DPPH method and the values found ranged between 33.10 and 5.06 mg TE/100 ml. Total polyphenol content was also determined spectrofotometrically using the Folin-Ciocalteu method and the values found ranged between 17.28 and 4.05 mg GAE/100 ml. The results indicate a higher antioxidant capacity and polyphenol content in craft meads, especially in barrique types.

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DEVELOPMENT OF MICROCHIP ISOTACHOPHORESIS METHOD FOR DETERMINATION OF PHARMACEUTICAL MACROCOMPONENTS IN CARDIOVASCULAR DRUGS

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Pharmaceutical analysis is important for assessing the safety and effectiveness of a pharmaceutical product for the patient. The validation of an analytical method for the determination of a drug substance must meet the criteria set out in the ICH guideline, ensuring a thorough evaluation of accuracy, precision, linearity, concentration range, and specificity.[1]

This study focused on the use of microchip isotachophoresis (μITP) for the analysis of macrocomponents (active pharmaceutical ingredients and counterions) present in cardiovascular drugs marketed in salt form, amlodipine besylate and perindopril erbumine.[2] μITP separations were performed on a poly(methyl methacrylate) microchip with integrated conductivity detection (IonChip™ 3.0; Merck, Darmstadt, Germany) in a hydrodynamically closed separation system with suppressed electroosmotic flow in cationic or anionic mode, depending on the analyte.

The developed μITP methods were evaluated using the AGREE software, based on the twelve principles of green analytical chemistry,[3] and achieved a score of 0.81. Linearity was evaluated in the range of 70 to 130% of the nominal concentration of the analyte in the presence and absence of matrix. The ratio of the slopes of the calibration curves was in the range of 98.8 to 100.9 % for all analytes and the correlation coefficient was at least 0.9993. The intra-day and inter-day precision, expressed as the relative standard deviation (RSD) of the response factor of the analyte, was below 0.9 %. The accuracy, reported as a percent recovery, was evaluated by analyzing standard samples prepared in the presence of matrix at three concentration levels and ranged from 98.3 to 101.6 %.

The suitability of the proposed μITP methods for the determination of pharmaceutical macrocomponents in cardiovascular drugs was confirmed by the analysis of commercially available formulations containing amlodipine besylate or perindopril erbumine. The relative error of determination of the content of macrocomponents in formulations was below 1.8 %.

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QUANTIFICATION OF HYDROXYL RADICALS ON BDD ELECTRODES

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This diploma thesis contributes to a larger project focused on improving wastewater treatment through electrochemical degradation. A key approach is oxidative degradation, an eco-friendly method that breaks down pharmaceutical contaminants using highly reactive hydroxyl radicals (•OH) generated via water oxidation on boron-doped diamond electrodes (BDDEs). These radicals convert pollutants into simpler compounds or fully mineralize them to CO_2 and H_2O.

This study aims to optimize •OH radical production by determining the ideal doping level (500–8000 ppm) and applied potential for BDDEs. Hydroxyl radical generation is monitored using the radical sond technique, which employs terephthalic acid (TA) as a selective probe. TA reacts specifically with •OH to form 2-hydroxyterephthalic acid (HTA), quantifiable by HPLC-UV.

To assess the actual contribution of •OH radicals, quenching experiments using scavengers such as mannitol will be conducted. The •OH concentrations determined via radical sond will be compared with degradation rates observed during quenching experiments, providing validation and insight into process efficiency.

These findings will support the development of optimized, efficient electrochemical methods for wastewater treatment.

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SHRINKABLE POLYSTYRENE DECORATED WITH GOLD NANOSTARS AS SURFACE-ENHANCED RAMAN SPECTROSCOPY BIOSENSOR FOR GLUCOSE DETECTION

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The increasing number of diabetes mellitus cases requires the development of simple, rapid, sensitive and selective analytical methods for glucose detection. This study presents the facile fabrication of a Surface-enhanced Raman spectroscopy (SERS) based glucose sensor using polystyrene sheets decorated with gold nanostars (AuNSs) as SERS substrate. [1]

AuNSs were synthesized through an one-pot seedless protocol in aqueous solution using tetrachloroauric(III) acid, silver nitrate and ascorbic acid as reducing agent. [2] The colloidal AuNSs were centrifuged and concentrated. Then, a given volume of AuNSs concentrate was placed on a polystyrene disk and heated on a hot plate until full contraction to generate a continuous AuNSs layer. The solid SERS substrate was functionalized with 4-mercaptophenylboronic acid (4MPBA), used as Raman probe and selective glucose receptor[3], and exposed to glucose at different physiologically relevant concentrations. The effect of various interferences on the SERS signal was tested, as well as the reproducibility and stability of the SERS signal over time. SERS data was collected using a Raman spectrometer and a laser with a wavelength of 785 nm (Figure 1).

The sensor demonstrated quantification capabilities in glucose detection, revealing a linear relationship between glucose concentration and SERS intensity of 4MPBA, as well as high selectivity for the analyte of interest.

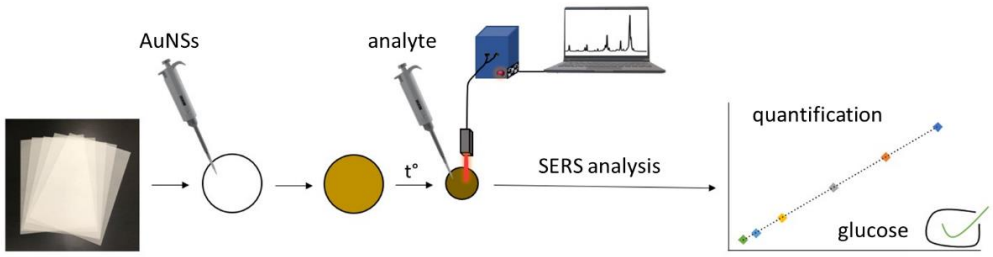

Figure 1. Schematic representation of the SERS biosensor fabrication and glucose detection.

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BREAKING THE MOLD: A NEW ROUTE TO FEBUXOSTAT-IMPRINTED POLYMERS

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Building upon our group's pioneering work on solvent-free, mechanochemically-assisted synthesis of molecularly imprinted polymers (MIPs) for atenolol, we report the successful application of this method to febuxostat, a xanthine oxidase inhibitor used to treat hyperuricemia. This solvent-free approach, employing liquid-assisted grinding (LAG), offers an environmentally benign and potentially higher-efficiency synthetic strategy.[1]

A standardized planetary ball mill was used to systematically optimize the mechanochemical synthesis of febuxostat-imprinted polymers. Parameters investigated included milling time, vial type, milling media, co-milling agents, LAG solvent volume, monomer-to-template ratio, atmosphere, initiators, and catalytic solvents. Template rebinding capacity, a direct measure of binding affinity, was used to assess the impact of each parameter. The selectivity of mechanochemically synthesized MIPs was compared to those prepared using conventional liquid-phase methods.

Results demonstrated that the mechanochemical approach yielded MIPs with superior febuxostat binding capacity, reduced waste generation, and simplified processing, establishing it as a promising, environmentally friendly, and efficient alternative for high-performance MIP synthesis.

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RISK ASSESSMENT OF EXPOSURE TO TRIHALOMETHANES AND HALOACETIC ACIDS IN THERMAL WATER POOLS

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Disinfection of swimming pool water with chlorine is essential for preventing the spread of infectious diseases. However, it may lead to the formation of disinfection by-products (DBPs), such as trihalomethanes (THMs) and haloacetic acids (HAAs), which may pose health risks.[1] Due to their specific chemical composition, thermal waters are particularly prone to the formation of these compounds. Previous studies have shown that THM and HAA concentrations in swimming pools often exceed recommended levels, while research focusing specifically on thermal pools and their characteristics remains limited.[2]

The aim of the planned study is to assess the exposure risk associated with THMs and HAAs in selected indoor thermal pools in Croatia. Monthly sampling of water and air will be conducted over a 12-month period at six locations. Water samples will be analyzed for physicochemical and microbiological parameters in accordance with current regulations, along with additional determination of unregulated HAAs. THM concentrations will be measured in the surrounding air as well as in water samples. Based on the results, a quantitative risk assessment for ingestion, inhalation, and dermal exposure pathways will be conducted. The study will also explore the potential correlation between water mineralization and DBP formation.

This research is expected to contribute to a better understanding of thermal pool water safety and serve as a scientific basis for improving regulations and management practices in public swimming pools, particularly those using thermal water.

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ANTIFUNGAL ACTIVITY OF SELECTED *N*-SUBSTITUTED IMIDAZOLE AND BENZIMIDAZOLE CATIONIC SURFACE ACTIVE IONIC LIQUIDS

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Modern society has an utmost need for new antimicrobials that provide effective protection against pathogens but have limited stability in the environment. Therefore, it is crucial to develop a new generation of quaternary ammonium salts (QAS). N-substituted imidazole and benzimidazole cationic surface active ionic liquids were synthesized by neat reactions or one-pot synthesis. The influence of the carbon chain length of substitution at the N3 position on the antimicrobial activities was investigated. The antifungal activities of all compounds were studied, and their potent antifungal properties were demonstrated. In antifungal activity tests towards four foodborne mycotoxigenic fungi, *A. flavus*, *A. ochraceus*, *F. graminearum*, and *F. verticillioides*, imidazole derivatives were proven to possess a very high activity in terms of growth inhibition, depending on the fungi investigated. Among the tested compounds, 3-hexadecyl-1-vinyl-1*H*-imidazol-3-ium bromide exhibited the strongest antifungal effect against Candida species, while compounds 1–5 demonstrated notable activity against filamentous fungi, including Fusarium, Aspergillus, and Penicillium species. In addition to their antifungal properties, several compounds are known from our prior studies to possess antibacterial effects, particularly against E. coli and S. aureus, suggesting potential for broad-spectrum antimicrobial application.

Table 1. Antifungal activity of the quaternary ammonium salts (1-7) on molds at a wavelength of 450 nm (the concentrations of the compounds are 10 μg mL^{-1}).

Compounds	*Fussarium spp*	*Alternaria spp*	*Aspergillus spp*	*Penicillium spp*
1	-11 %	100 %	-3 %	-5 %
2	-12 %	85 %	-6 %	-8 %
3	-11 %	94 %	-4 %	-6 %
4	-12 %	89 %	4 %	-7 %
5	-12 %	86 %	-3 %	-8 %
6	-4 %	66 %	60 %	47 %
7	-6 %	75 %	77 %	117 %

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DETECTION OF APPLE IN COMMERCIAL JUICES AND SMOOTHIES USING PCR AND HIGH-RESOLUTION MELTING ANALYSIS

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Apple is not only suitable for balancing the taste of other fruits due to its soft, neutral taste and natural sweetness, but is also widely used in mixed juices and smoothies because it is easy to grow and process and is inexpensive. However, the act of using relatively inexpensive apple juice as the main ingredient in mixed fruit drinks without clearly indicating this is called “applejuicification”.[1]

To address this issue, a selective apple DNA detection method using PCR-based high-resolution melting (HRM) analysis was developed to verify the presence of unlabeled apple in commercial juice and smoothie products. First, various DNA extraction methods were compared and optimized for apple juice. A comparison of commercial DNA extraction kits showed that the extraction efficiency of the DNeasy mericon Food Kit (Qiagen) was superior to that of the NucleoSpin Plant II kit (Macherey-Nagel). In addition, centrifugation conditions (time, sample amount, speed) and the concentration of polyvinylpyrrolidone (PVP) to minimize the inhibitory effects of polyphenols were optimized. The primer pair Hi02f12 was used for the analysis, whose amplicons showed a melting peak characteristic for apple DNA, independent of the apple variety.[2][3] The sample set included 12 commercially available apple juices, six blended juices containing apple, eight smoothies, and three types of model smoothies (a red smoothie based on strawberry, blueberry, and raspberry; a yellow smoothie with pineapple, mango, and peach; and a green smoothie containing avocado, kiwi, and banana).

Apple DNA was selectively detected in most samples, and detection was possible down to an apple content of about 1.4 % in the model smoothies. In addition, when the model smoothies were heat-treated at 85 °C for 5, 10, or 15 minutes and compared with untreated samples, DNA degradation increased with heating time and detection sensitivity tended to decrease, but apple DNA was still detectable even after heat treatment. This suggests that apple DNA can be detected in commercial smoothie products.

On the other hand, DNA extraction was difficult from clear juices and some smoothies that appeared to be based on clear juices. In these samples, DNA concentration was very low, and even freeze-drying of the products failed to improve detectability. Although apple DNA detection was limited in some clear juices, PCR-based HRM analysis showed high sensitivity and selectivity in most processed beverages.

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FAST DETERMINATION OF FUROCOUMARINS USING CAPILLARY ELECTROPHORESIS

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Furocoumarins are secondary metabolites of plants with an aromatic, tricyclic chemical structure, in which the furan ring is fused to a coumarin. They are commonly found in the *Apiaceae*, *Fabaceae*, and *Rutaceae* families.[1]

The objective of this study was to develop a simple and fast capillary electrophoretic method for the simultaneous screening of multiple furocoumarins (bergapten, isobergapten, isopimpinellin, imperatorin, and xanthotoxin). Since furanocoumarins are electrophoretically neutral molecules, their separation requires the use of pseudostationary phases (electrokinetic chromatography). The commonly used pseudostationary phases include surfactants above their critical micellar concentration (micellar electrokinetic chromatography (MEKC), microemulsions (microemulsion electrokinetic chromatography (MEEKC) and cyclodextrins (CD-EKC). After initial screening, a MEKC method has been developed using sodium cholate a pseudostationary phase and methanol as an organic modifier. The optimized method (10 mM sodium tetraborate supplied with 100 mM SDC and 15 % ethyl alcohol; capillary temperature: 25 °C; injection: -30 mbar x 2 sec; applied voltage: -20 kV (short-end, normal polarity mode); detection at 315 nm) enabled the baseline separation of the furanocoumarins under investigation in less than 3 minutes. The analytical performance of the developed method was verified regarding sensitivity (limit of detection - LOD, limit of quantification - LOQ), linearity, accuracy, and precision (repeatability and intra-day precision).

The developed method was applied for the analysis of the furocoumarin content in different parts (root, stems, leaves, fruits) of *Heracleum sphondylium* L., commonly known as hogweed, a plant used as an ingredient in several food supplements. The procedure allows the analysis of real samples after a quick extraction procedure, without an additional cleaning step. Furthermore, ten food supplements were investigated, in two of them the level was above the harmless dose, indicated by the European Food Safety Authority (EFSA).

In conclusion, this study demonstrated that MEKC is an effective and rapid tool for the screening of furocoumarins in herbal products and food supplements.

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VOLTAMMETRIC DETERMINATION OF TADALAFIL ON A CARBON COMPOSITE ELECTRODE

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The work deals with finding optimal electrochemical conditions for the determination of the drug tadalafil (TAD). These conditions were then used to determine the TAD content of the commercially available drug Tadalafil Teva 5 mg. The electrochemical techniques used for the determination were differential pulse voltammetry (DPV) and DC voltammetry (DCV). A working carbon composite electrode, an argentochloride reference electrode, and a platinum auxiliary electrode were used.

Britton-Robinson (BR) buffer at pH 4.0–ethanol (7:3) was determined as the optimal medium. The linear dependence was obtained over the range of concentration from 5×10^{-5} mol L^{-1} to 1×10^{-4} mol L^{-1} for both techniques used. The limit of quantification (LQ) and limit of detection (LD) for DPV were 3.1×10^{-5} mol L^{-1} and 4.0×10^{-6} mol L^{-1}, respectively. For DCV, LQ = 2.0×10^{-5} mol L^{-1} and LD = 6.0×10^{-6} mol L^{-1}.

UV–VIS spectrophotometry using the standard addition method was used as a comparative analytical method to the newly developed voltammetric methods to determine the TAD content of Tadalafil Teva 5 mg.

VOLTAMMETRIC DETERMINATION OF NIMESULIDE AT A POLISHED SILVER SOLID AMALGAM ELECTRODE

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This study focuses on investigating the electrochemical behavior of the substance nimesulide. The stability of the stock solution was monitored using UV-VIS spectrophotometry. A solid silver amalgam electrode with a polished surface was chosen as the working electrode, a silver chloride reference electrode (3M KCl), and a platinum auxiliary electrode were used.

For the techniques of DC voltammetry (DCV) and differential pulse voltammetry (DPV), optimal conditions for the determination of nimesulide were sought (optimal pH, range of regenerative potentials). The calibration dependence was measured in concentration ranges of 10^{-7}, 10^{-6}, and 10^{-5} mol L^{-1}. In the case of DCV, a linear calibration dependence was achieved in the concentration range of 1 – 100 µmol L^{-1} (with a limit of detection (L_D) ≈ 0.27 µmol L^{-1} and with a limit of quantification (L_Q) ≈ 0.91 µmol L^{-1}). In the case of DPV, linearity was achieved in the range of 4 – 100 µmol L^{-1} (L_D ≈ 0.60 µmol L^{-1} and L_Q ≈ 2.0 µmol L^{-1}).

The developed voltammetric methods were used to determine nimesulide in drinking and well water and in pharmaceutical forms of Nimesil 100 mg and Aulin 100 mg.

STABILITY AND STRUCTURAL ASPECTS OF ALL-AQUEOUS EMULSIONS STABILIZED BY INTERFACIAL ASSEMBLIES OF OPPOSITELY CHARGED NANOPARTICLES AND POLYELECTROLYTES

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Aqueous two-phase systems (ATPS) are formed by mixing aqueous solutions of two incompatible water-soluble polymers, such as polyethylene oxide (PEO) and dextran.[1] These systems are biocompatible and nontoxic, which makes them promising for applications ranging from cosmetics to 3D printing.[2,3] However, their low interfacial tension and thick interfacial region pose challenges in stabilizing emulsions prepared from them.

Previously, we demonstrated that emulsions formed from dextran and various PEO solutions could be stabilized using aggregates of negatively charged silica nanoparticles with adsorbed polydiallyldimethylammonium chloride (PDADMAC). The size of the PEO molecules and the composition of the stabilizing components significantly affect emulsion stability.[4]

In this study, we fabricated ATPS systems using one dextran sample (450 – 650 kDa) and three PEO samples (4, 20, and 100 kDa). Emulsions were also prepared using fluorescent dextran to study emulsion types and structures. We achieved stabilization using the same method as in the previous study. Our aim was to investigate the effect of polymer solution rheology and stabilizer concentration on emulsion kinetic stability. The results indicated that higher viscosity in the initial phases, as well as an increased PDADMAC/silica ratio and particle concentration, markedly improved the emulsion's kinetic stability.

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IN SILICO TOXICITY PROFILING OF DEGRADATION PRODUCTS IDENTIFIED FROM FORCED DEGRADATION OF CINNARIZINE

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Stress testing (forced degradation) is the main tool used to develop analytical methods, predict stability issues, and identify degradation products and pathways. Well-designed stress-testing studies can determine the susceptibility of a compound to hydrolysis, oxidation, photochemical degradation, and thermal degradation. Degradation products are unintended chemicals that may form during the manufacturing, transport, or storage of pharmaceutical products. These substances can compromise the effectiveness of the drug and, even in small quantities, may pose safety risks due to the potential to cause adverse effects in patients.

Cinnarizine (Cin) is a piperazine-based compound that exhibits antihistaminic, antiserotonergic, antidopaminergic, and calcium channel-blocking properties. It is commonly prescribed to treat nausea, vomiting, and vertigo associated with Meniere's disease and other vestibular conditions.[1] Administered orally, Cin has limited and slow bioavailability due to its weakly alkaline nature. It is only soluble in highly acidic environments (pH ≤ 1), and its solubility drops significantly at pH levels of 3 or above. This pH-dependent solubility contributes to considerable variability in absorption and poor bioavailability in patients.[2] However, forming inclusion complexes with cyclodextrins can greatly improve its bioavailability.

In this study, forced degradation experiments were conducted on Cin and its binary complexes with β-cyclodextrin (β-CD) and its hydroxypropylated derivative (HP-β-CD). The samples were exposed to hydrolytic conditions (acidic, neutral, and alkaline) and oxidative stress under elevated temperatures. These conditions led to the formation of several potential degradation products, particularly under acidic and oxidative environments. The identification and structural analysis of these degradation products were carried out using high-resolution mass spectrometry with an Agilent 6550 Series Accurate-Mass Quadrupole Time-of-Flight mass spectrometer, coupled to an Agilent 1290 Infinity II UHPLC system. The potential toxicity of the degradation products was predicted *in silico* using the Toxicity Estimation Software Tool (TEST), which applies Quantitative Structure-Activity Relationship (QSAR) models to estimate chemical toxicity.

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ECO-FRIENDLY APPROACH FOR ANTIBIOTIC DIRECT SENSING: A BIOCHAR-BASED PLATFORM FOR BIOMEDICAL AND ENVIRONMENTAL APPLICATIONS

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Antimicrobial resistance is a critical global problem due to inappropriate use of antibiotics both in humans and in animals, as resistant microorganisms are also transmitted through trophic chain. Surveillance of antibiotics use at all health levels and in food industries is mandatory, along with robust methods for assessing the presence or the levels of antimicrobial compounds in biological, environmental or food samples being an important modality for addressing the issue.[1] Electrochemical approaches represent a good strategy for analysis due to their main advantages, such as ease-of-use, fast response, low cost, high sensitivity and selectivity, stability, and repeatability. Modifying electrodes with biochar, a carbon-rich eco-friendly material, can improve the analytical performances for the detection of antibiotics, due to its electrocatalytic effect.[2] Thus, the main objective of the study was to develop a biochar-based approach for direct and sensitive detection of antibiotics in complex real samples.

The sensor was developed by embedding the biochar onto the surface of carbon-screen-printed electrodes via an electropolymerization-generated film. Biochar derived from spent coffee biomass was produced by pyrolysis at 850°C under an oxygen-free atmosphere and subsequently activated using KOH and CO_2, respectively. Biochar, as a solid powder, as well as the electrode surface functionalized with a biochar and polymer based composite film were characterized using microscopic, spectrometric, and electrochemical methods. Direct detection of the target was performed using differential pulse voltammetry (DPV) and solutions of various concentrations and different scan rate and pH values of the electrolytic media were evaluated in order to obtain the fingerprint of the antibiotic.

The highest current signal for the electrochemical transformation of the antibiotic was achieved in the presence of the polymeric composite film. The analytical performance of the sensor was thoroughly evaluated, and its selectivity for the antibiotic was tested in the presence of potential interfering compounds, simulating complex real samples.

The improved analytical performance of the optimized sensor highlights its strong potential as a foundation for developing decentralized and portable detection platforms, with promising applications in biomedical diagnostics, environmental monitoring, and food safety.

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MOVE AND LEARN - STUDENT AND TEACHERS MOBILITY IN VOCATIONAL EDUCATION: INTEGRATING LABORATORY PRACTICE, ICT AND INTERCULTURAL LEARNING

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As part of an Erasmus+ mobility project, ten students from Vladimira Preloga Natural Sciences School in Zagreb, Croatia (Chemical Technician programme), participated in practical training in microbiology laboratories at CETECAL and CESUR in Málaga, Spain. The main objective of the mobility was to enhance students' professional competencies through hands-on experience in real laboratory settings, while also fostering independence and adaptability in a new environment.
Throughout the programme, students—under the guidance of accompanying teachers-actively monitored and documented their progress using digital tools, thereby further developing their ICT skills. The mobility also included professional visits to relevant institutions such as chemical institutes, a local brewery, and a waste management facility. These visits provided students with broader insight into the application of microbiological and chemical knowledge across various sectors.
In addition, the programme emphasised language and cultural immersion, which promoted intercultural learning and social integration. The final evaluation showed a positive impact on the professional development of both students and teachers, increased motivation for learning, and improved readiness for collaboration in an international educational context.

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SELEX SELECTION OF APTAMERS FOR CANCER BIOMARKERS

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According to the 2022 GLOBOCAN report, hepatocellular carcinoma (HCC) ranks as the eighth most commonly diagnosed cancer and the third leading cause of cancer-related mortality globally, with the number of deaths expected to rise to 1.26 million by 2045.[1] Early detection is critical for improving prognosis and survival rates, yet current diagnostic methods remain limited in sensitivity and specificity, particularly during the early stages of the disease. Recent diagnostic strategies have increasingly emphasized the monitoring of specific serum biomarkers for HCC, such as Golgi protein-73 (GP-73), a transmembrane glycoprotein that is significantly overexpressed in HCC patients. GP-73 has demonstrated superior diagnostic accuracy and sensitivity in early-stage detection compared to alpha-fetoprotein, the conventional biomarker for HCC.[2] Aptamers which are short, single-stranded DNA or RNA molecules, offer a promising alternative to antibodies for biomarker detection due to their high binding affinity, specificity, chemical stability, and cost-effective synthesis. These molecules are generated through an *in vitro* selection process known as SELEX (Systematic Evolution of Ligands by EXponential enrichment), which involves iterative rounds of binding, separation, and amplification to enrich high-affinity sequences against a specific target, such as GP-73. To facilitate the selection and to enhance the efficiency, magnetic beads-based SELEX (MBs-SELEX) has been widely adopted.[3] While the ultimate goal is to develop an electrochemical aptasensor for early diagnosis to complement existing tools, potentially reducing mortality and healthcare costs, this poster presents our current progress in aptamer selection specific to GP-73.

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APPLICATION OF REAL-TIME PCR AND HIGH-RESOLUTION MELTING (HRM) FOR AUTHENTICATION OF FOREST HONEY

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The aim of this study was to investigate the potential of real-time PCR combined with high resolution melting (HRM) analysis for the authentication of forest honey available in Austria and other European countries. Our approach was based on amplifying a polymorphic region of honey bee (*Apis mellifera*) DNA by real-time PCR and distinguishing subspecies through differences in the melting behavior of the amplicons. According to literature, the presence of DNA from specific honey bee subspecies is associated with the geographic origin of honey.

DNA was extracted using the Dneasy mericon Food Kit (Qiagen), according to the manufacturer's instructions. As positive control, DNA was also extracted from honey bee specimens collected in Austria. Primer pairs for the amplification of honey bee DNA were selected from the literature and tested *in silico*. The BEE2 primer pair, designed by Honrado et al.[1], targets a region of the cytochrome oxidase I (COI) gene. This region contains single nucleotide polymorphisms (SNPs), as confirmed through BLAST analysis, and thus leads to the formation of subspecies-specific amplicons that can be differentiated by HRM.[1]

By designing a novel forward primer, a shorter amplicon was generated than reported in the literature, and thus the resolution of HRM could be improved. Pyrosequencing of the BEE2-derived amplicons enabled the assignment of specific melting profiles to the genotypes of distinct honey bee subspecies. The method has already been applied to 40 honey samples from Austria and other European countries.

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COMPATIBILITY EVALUATION OF THE LACTOSE WITH SOME ACTIVE PHARMACEUTICAL INGREDIENTS USING THERMAL AND SPECTROSCOPIC TECHNIQUES

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DSC and FT-IR techniques are essential in the preformulation stage, providing information on thermal stability and molecular interactions between excipients and active pharmaceutical ingredients (APIs). These methods allow for the anticipation of potential incompatibilities in pharmaceutical formulations.[1]

This study aims is to evaluate the compatibility between lactose (Lac) and four APIs (potassium aspartate, magnesium aspartate, potassium orotate, spironolactone) using thermal analysis (DSC) and FTIR spectroscopy, in order to identify possible physicochemical interactions.

Binary mixtures (1:1 *w*/*w*) of lactose monohydrate and each active substance were analyzed. DSC analyses were carried out on a TA Instruments DSC 250 (25 – 300 °C) with a heating rate of 10 °C min^{-1} in a nitrogen atmosphere. FT-IR spectra (4000 – 650 cm^{-1}) were recorded with a PerkinElmer instrument using the ATR technique.

DSC analysis revealed distinct melting behavior for each pure substances and lactose–API mixture. In the Lac/potassium aspartate system, two endothermic peaks appeared at 149.10 °C (ΔH = 101.74 J g^{-1}) and 160.81 °C (ΔH = 198.89 J g^{-1}), corresponding to lactose and polyaspartic acid. The Lac/magnesium aspartate mixture showed decomposition before melting. A single peak at 145.36 °C (ΔH = 63.02 J g^{-1}) in the Lac/potassium orotate system reflected lactose melting. The Lac/spironolactone mixture exhibited two effects: at 146.14 °C (ΔH = 126.16 J g^{-1}) for lactose and slightly shifted to 196.74 °C (ΔH = 23.38 J g^{-1}) for spironolactone, indicating interaction. FTIR analysis confirmed compatibility through key spectral changes. In the Lac/potassium aspartate mixture, the bands at 3286, 3192, and 3075 cm^{-1} (N–H, O–H) disappeared. For magnesium aspartate, bands at 3034 and 2643 cm^{-1} vanished, and COO^- shifted from 1571 to 1583 cm^{-1}, suggesting hydrogen bonding. In the Lac/potassium orotate system, the disappearance of bands at 3147, 3103, 3083, 1696 cm^{-1} and the appearance of a new one at 2390 cm^{-1} implied ion–dipole interactions. In the Lac/spironolactone mixture, characteristic bands shifted (e.g., from 1764 to 1737 cm^{-1}, 1436 to 1414 cm^{-1}, 1011 to 1030 cm^{-1}) and disappeared, indicated significant interactions, likely via hydrogen bonding or complex formation.

In conclusion, Lac shows good compatibility with potassium and magnesium aspartates, moderate interactions with potassium orotate, and strong structural interactions with spironolactone, which is the essential aspect for the safe and effective formulation of pharmaceutical medicines.

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METAL-ORGANIC FRAMEWORKS: A PROMISING MATERIALS IN BIOANALYSIS

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Coordination polymers, or metal-organic frameworks (MOFs), are gaining attention for their high porosity, surface area, and structural flexibility. In bioanalysis, MOFs are effective in sample preparation methods like SPE, D-μSPE, and SPME, enabling efficient trace analyte enrichment from complex biological samples. [1] Their tunable structures allow selective adsorption of small molecules such as antibiotics, hormones, and contaminants. These properties make MOFs highly valuable in modern bioanalytical applications.

Here we report the synthesis of four new coordination polymers using the rigid ligand 3,3',5,5'-tetrakis(4-carboxyphenyl)-2,2',4,4',6,6'-hexamethyl-1,1'-biphenyl (H_4L). Reactions of H_4L with $M(NO_3)_2$ (M = Co, Cd, Zn, Mn) in DMF or DAAM under solvothermal conditions at 80 °C yielded rigid, porous frameworks. Single-crystal X-ray diffraction revealed that deprotonated H_4L functions as a tetradentate ligand, coordinating with metal ions to form 3D networks (Fig. 1). High thermal stability was assessed by thermogravimetric analysis, showing an initial loss of crystallization solvent around 20 – 25 °C, followed by a stepwise collapse of the frameworks. Major decomposition, attributed to ligand degradation, occurred between ~400 and 600 °C. Additional characterization was performed using IR spectroscopy, luminescence measurements, and elemental analysis.

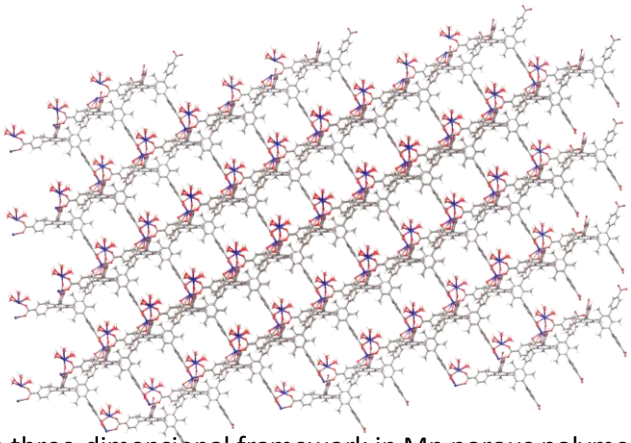

Figure 1. The three-dimensional framework in Mn porous polymer with H_4L.

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IMPACT OF BIOPRIMING WITH PHYTOHORMONE-PRODUCING BACTERIA ON MAIZE SEED GERMINATION: ANALYTICAL CHARACTERIZATION AND BIOEFFICACY ASSESSMENT

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The application of plant growth-promoting rhizobacteria (PGPR) represents a promising biotechnological approach to enhance crop tolerance to abiotic stress. In this study, salt- and heavy metal-tolerant bacterial strains were isolated from saline and contaminated soils and screened for their ability to produce key phytohormones (indole-3-acetic acid, gibberellic acid, and cytokinins).[1] The strains were taxonomically identified using MALDI-TOF mass spectrometry and 16S rDNA sequencing.[2] The quantitative analysis of phytohormone production was carried out using UV-Vis spectrophotometry, applying Salkowski's reagent for IAA, ethyl acetate extraction for gibberellins, and colorimetric estimation for cytokinins, each calibrated with appropriate standard curves.[3]

Four selected bacterial strains – *Pseudomonas koreensis* (2 strains), *Pseudomonas extremorientalis*, and *Bacillus megaterium* – were used in biopriming experiments on maize (*Zea mays*) seeds under controlled salt and cadmium stress conditions. Germination indices (e.g., GP, MGT, CVG, GRI)[4] were evaluated to assess the physiological impact. Results demonstrated significant strain-dependent variation in hormone production and germination enhancement, with the best-performing isolates improving germination rates even under 0.5 mM Cd or 5 g L^{-1} NaCl stress.

The combination of MALDI-TOF MS-based bacterial identification and UV-spectrophotometric hormone quantification provides a robust analytical platform to screen PGPR candidates for sustainable agriculture. This approach bridges microbial ecology, plant physiology, and bioanalytics, contributing to the development of eco-friendly biofertilizers and stress-mitigation strategies in crops.

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AROMATIC PROFILE OF CROATIAN STRAWBERRIES

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Aroma is one part of the properties list that complements the overall quality of a food product. Aroma depends not only on the type of food product, but also on the variety of the individual food product, as well as the degree of maturity and storage conditions. Each aroma profile contains volatile compounds belonging to the chemical classes esters, aldehydes, ketones, carboxylic acids, and alcohols. Strawberries contain compounds from all of the above chemical classes, plus terpenes, lactones and furans. Available literature gives an extensive aroma compounds list for strawberries and underlines 2,5-dimethyl-4-methoxy-3(2H)-furanone (DMMF or mesifurane) and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF or furaneol) as the most characteristic for this type of fruit.[1] Within our research, 15 strawberry samples grown in Croatia were analyzed using the GC-MS/SPME method to establish the most common components of the aroma profile and any mutual differences, with the ultimate goal of fingerprinting for different regions. The individual compounds were identified using NIST and Wiley libraries and the “area normalization" method. The aroma analysis is influenced by the method parameters (incubation time and temperature, column type) as well as sample preparation (sample mass and ionic strength). Samples are analyzed using 5 grams of sample in a 20 % NaCl water solution. Some compounds present in the analyzed samples are esters (ethyl hexanoate, octyl butanoate, octyl isovalerate and other), lactones (gamma decanolactone, delta decanolactone), furans, sesquiterpens (linalool, farnesol, cis-lanceol). Also present is 2-Furanmethanol (furfuryl alcohol) which is soluble in water, oxidizes to furfural and can be found in fresh strawberries, but is more often present in the jam production due to the high temperature effect.[2] The largest contribution to the total area of analyzed signals in individual samples is gamma-Decanolactone. The largest number of analytes was found in samples of which one is of unknown variety and location and the other originates from Opuzen. In general, it can be said that many compounds that make a desirable food aroma are present in Croatian strawberry samples. Aromas are rich in many esters, and compounds from the furan and lactone chemical classes. Carboxylic acids are also present. For fingerprinting by location or variety, an additional data set of analyzed metals, isotopes and polyphenols needs to be included in statistical analysis.

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CHARACTERIZATION OF POLYMERS ELEMENTAL COMPOSITION AND BIO-BASED CONTENT USING ICP-OES AND ^{14}C TECHNIQUES

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Plastics are one of the anthropogenic era's dominant materials, widely used in many areas of industry and life over the years due to their versatility and low costs. More than a third of the world's plastic production is used for packaging that comes into contact with food and the human body. Environmental concerns and the limited availability of fossil resources mean that plastics from renewable sources are becoming increasingly important. This study presents a combined analysis of partly fossil and partly bio-based polymers using accelerator mass spectrometry radioactivity (C-14) and inductively coupled plasma optical emission spectrometry techniques. Different types of polymer samples were analysed, including raw materials, commercially available final products and selected materials from recycling plants. Elemental analysis was used to determine the amounts of selected elements (Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cu, Cr, Fe, K, Li, Mg, Mn, Na, Ni, P, Pb, Pd, S, Sb, Sn, Sr, Ti, Zn). The measured concentration results give a good indication of the quantitative relationships of the additives used in the manufacturing processes and the importance of Ca. When the concentrations of lead and cadmium in the samples were compared with the limit values of the EU legislation, it was found that only one bin liner with a lead concentration of 294 mg kg^{-1} exceeded the limit value of 60 mg kg^{-1}. The bio-based carbon content of each fossil and bio-based polymer tested by our C-14 measurements was within the expected quantitative ranges. In the case of the PLA-based samples, we observed products that differed from the expected, 100 % bio-based value. By comparing the two analyses, it was concluded that despite the lack of a close relationship, a much more comprehensive picture of the polymers can be obtained by combining the two measurement techniques.

MOLECULARLY IMPRINTED POLYMER-BASED SENSOR FOR MOXIFLOXACIN DETECTION USING 3D PRINTING TECHNOLOGY

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Antimicrobial resistance (AMR) of pathogenic bacteria poses a significant global health threat, contributing substantially to morbidity and mortality rates.[1] The widespread use of antibiotics in both human and veterinary medicine including sub-therapeutic use in animal feed to promote growth and prevent disease has accelerated the emergence of resistant strains.[2] Moxifloxacin (MOX), a fourth-generation fluoroquinolone, is commonly used to treat a wide range of infections caused by Gram-positive and Gram-negative bacteria in both humans and animals.[3] As a result, the monitoring of antibiotic residues is essential to mitigate AMR development.

Optical sensors provide a promising alternative to conventional analytical techniques such as HPLC and GC-MS, offering high sensitivity and selectivity, rapid analysis, on-site applicability, and reduced costs. Among these, molecularly imprinted polymers (MIPs) are widely employed for their ability to create selective recognition sites for target analytes.[4]

Our study introduces, for the first time, a fluorimetric sensor for MOX detection based on MIPs fabricated via Digital Light Processing (DLP) 3D printing technology. A polymerization mixture consisting in the MOX template dispersed in commercial resins with the aid of a porogenic solvent blend (octanol, methanol) was subjected to DLP 3D printing on various porous substrates. Upon template removal with methanol:acetic acid (9:1), rebinding tests were performed in various sample matrices (i.e. water, whole and acid coagulated cow milk). Sensor development was monitored by measuring the fluorescence of MOX using scanning densitometry.

Optimized 3D printing-assisted polymerization conditions prone to mass production yielded a functional MIP sensing platform capable of detecting MOX in the range of 10^{-7} M to 10^{-5} M. Furthermore, it was successfully applied for the quick and accurate analysis of traces of MOX below and above accepted maximum residue limits (MRL) spiked in unprocessed milk samples, confirming its practical utility as a cost-effective and portable platform for antibiotic monitoring.

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MONITORING G-QUADRUPLEX FORMATION IN C-KIT1 AND C-KIT2 PROMOTER SEQUENCES USING SERS SPECTROSCOPY

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Surface-enhanced Raman scattering (SERS) spectroscopy is a powerful analytical technique that significantly amplifies Raman signal of molecules adsorbed on metal nanostructures, such as gold or silver nanoparticles. This enhancement enables the detection of very low concentrations of analytes, making SERS particularly valuable for bioanalytical applications and nucleic acids research.[1] G-quadruplexes are non-canonical DNA structures formed by guanine-rich sequences, often located in gene promoter and telomeric regions, that play roles in transcriptional regulation and potential therapeutic targeting.[2]

In this study, SERS was used to investigate formation of the G-quadruplexes of two guanine-rich sequences present in the promoter region of the human c-kit gene: c-kit1 and c-kit2. Citrate-stabilized silver nanoparticles were employed as SERS-active substrates. Spectra were recorded for both unfolded and folded (G-quadruplex) forms of the oligonucleotides. The folding was induced in the presence of potassium ions (50 mM), known to stabilize c-kit1 and c-kit2 G-quadruplex structures. [3]

Comparison of the SERS spectra before and after folding revealed characteristic vibrational changes associated with G-quadruplex formation. The distinctive SERS signatures, particularly the bands corresponding to breathing vibrations of adenine (~730 cm^{-1}) and guanine (~660 cm^{-1}) rings, allowed the detection of the molecule at concentration of 4×10^{-5} mol L^{-1}. Changes in the relative intensity of two these bands indicated structural changes from the unfolded oligonucleotide to the G-quadruplex form.[4] The results demonstrate that SERS can effectively monitor formation of the G-quadruplex in promoter sequences and may serve as a valuable tool for studying DNA secondary structures relevant to gene regulation and drug design.

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DETERMINATION OF B-GROUP VITAMINS AND CAFFEINE IN THE SAMPLES OF AN ECOLOGICAL, HEALTH-PROMOTING BEVERAGE USING LIQUID CHROMATOGRAPHY

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Accurate determination of vitamins and caffeine in food products is important due to the requirement to include information about their content on labels.[1] According to the European Parliament Regulation No. 1169/2011, when caffeine content of a product exceeds 150 mg L^{-1}, the concentration and a warning must be provided on the label.[2] Measurement of B vitamins in food products is constricted by their low natural content and sensitivity to light, temperature and pH.[3]

The objectives of this project were optimization of separation parameters of nine B-vitamins and caffeine using liquid chromatography and an attempt to determine these compounds in the samples of an ecological beverage, made from fermented bread waste with the addition of spent coffee ground extracts. The topic of the work is based on a research project Food2Good, focused on processing food waste using innovative technologies.

After optimizing the HPLC-DAD parameters, full separation of all ten compounds was achieved in less than eleven minutes. Caffeine was detected in the samples, but vitamins B were not. Due to the incomplete separation of the caffeine signal and the signal from an unknown matrix component, it was impossible to determine its content. Using LC-MS/MS vitamin B3 (0.81 – 2.32 mg L^{-1}) and caffeine (117.6 – 148.7 mg L^{-1}) were determined. The caffeine content of the samples did not exceed 150 mg L^{-1}, so a warning on the label is not essential. The content of the other vitamins was most likely below the detection limit. The low concentrations of analytes may have been caused by the degradation during beverage preparation.

Figure 1. Samples of analysed beverages.

Acknowledgements. This work has been supported by project Food2Good.

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ENZYME-ASSISTED EXTRACTION AND BIOANALYTICAL PROFILING OF POMEGRANATE PEEL AND SEEDS (*PUNICA GRANATUM* L.) FOR COSMECEUTICAL APPLICATIONS

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Pomegranate (*Punica granatum L.*) is a rich source of bioactive compounds with documented antioxidant, antimicrobial, and anti-aging effects. In this study, we have applied an enzyme-assisted extraction (EAE) using five hydrolytic enzyme preparations (cellulase, pectinase, lipase, proteinase, amylase) to separately obtain extracts from pomegranate peel and seeds (edible part). The goal was to evaluate their bio-functional properties relevant to cosmeceutical applications.

Both extracts were analyzed for total polyphenols, flavonoids, amino acids, and fatty acids. Their biological activity was assessed through antioxidant capacity (DPPH assay), inhibition of skin-related degrading enzymes (elastase, tyrosinase, collagenase), sun protection factor (SPF), and antimicrobial efficacy against *Propionium acnes*, *Staphylococcus aureus*, *Escherichia coli*, and *Staphylococcus epidermis*.

Results demonstrated that the peel extract exhibited significantly higher levels of polyphenols and flavonoids, along with superior antioxidant and enzyme-inhibitory activity compared to the seed extract. It also showed higher SPF values and moderate antimicrobial effects. The seed extract, while milder in biological activity, was rich in essential amino acids and fatty acids, supporting its potential as a nutraceutical ingredient.

These findings support the sustainable valorization of both pomegranate peel and seeds, particularly highlighting the peel as a promising candidate for natural cosmeceutical formulations targeting skin aging, pigmentation, and microbial protection.

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CHIRAL SEPARATION OF NEW KETAMINE DERIVATIVES AND DETERMINATION OF THE ENANTIOMER ELUTION ORDER USING HPLC-UV-ORD

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In recent years, ketamine has gained increasing polularity on the European and international drug market. Additionally, through small structural modifications, new ketamine derivates have emerged, which are sold as designer drugs.[1] Ketamine and some of its derivatives possess a chiral centre and therefore exist as two enantiomers. While differences in effects of S- and R-ketamine are well studied, this is not the case for ketamine derivatives. Therefore, development and adaptation of suitable enantioseparation methods for those compounds is important to deal with the problems of the constantly changing drug market. In this study on HPLC enantioseparation, four different polysaccharide-based chiral stationary phases were tested on 11 ketamine derivates using two different mobile phase compositions, N-hexane:isopropanol:diethylamine (95:5:0.1 *v/v/v*) and N-hexane:ethanol:diethylamine (95:5:0.1 *v/v/v*). A Lux® i-Amylose-3 column showed best results concerning number of separated compounds as well as chromatographic resolution. By applying different combinations of mobile and stationary phase, all 11 compounds were baseline separated. Furthermore, the enantiomer elution order (EEO) was determined by using an optical rotation detector. It was shown that the choice of stationary phase had a significant influence on the EEO and also the replacement of isopropanol with ethanol in the mobile phase led to an EEO reversal in certain cases.[2]

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DNA BARCODING AND HIGH-RESOLUTION MELTING (HRM) ANALYSIS OF NATIVE MACEDONIAN *STACHYS* SPECIES

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Accurate identification of plant species is essential for biodiversity conservation, phytochemical research, and the safe use of medicinal plants. The genus *Stachys* (Lamiaceae), known for its taxonomic complexity and significant morphological variability, presents challenges in species-level discrimination, particularly among closely related taxa. Over 300 Stachys species are recognized globally, many of which are used in traditional medicine and hold considerable pharmaceutical potential.

In this study, a rapid and reliable molecular approach combining DNA barcoding and high-resolution melting (HRM) analysis was applied to identify three morphologically similar species: *Stachys* recta, *Stachys iva* Griseb., and *Stachys horvaticii* Micevski. All species are native or endemic to Macedonia and were collected from natural habitats. Genomic DNA was extracted from dried material and assessed spectrophotometrically. The universality and discriminatory power of ITS region barcodes were evaluated using three designed primer pairs (P1, P2, P3). All primers successfully amplified the target regions, indicating their suitability for the analyzed *Stachys* species.

HRM analysis of the PCR amplicons revealed species-specific melting profiles, high genetic diversity, and notable polymorphism. Primer P1 showed the lowest discriminatory power, while P2 and P3 reliably differentiated all three species. Amplicon size differences were also confirmed through agarose gel electrophoresis. The results demonstrate that ITS-based markers have strong potential for the molecular identification of Stachys species.

Overall, the combined use of DNA barcoding and HRM analysis proved to be a robust and efficient method for species authentication, particularly useful in cases of morphological similarity or processed plant materials. This approach supports accurate taxonomic classification, which is critical for pharmacological research, conservation efforts, and quality control in herbal products.

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A STUDY OF THERAPEUTIC PROTEIN AGGREGATION USING CAPILLARY ELECTROPHORESIS

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The aggregation of therapeutic proteins is an undesirable phenomenon that may occur during drug manufacturing, storage, or handling by the patient. Various external factors such as formulation parameters (e.g., pH, ionic strength) and applied stresses (e.g., shear forces, temperature, primary packaging) can induce conformational changes in the proteins, thereby facilitating aggregate formation.[1]

Capillary gel electrophoresis (CGE) serves as an effective analytical technique for the separation and characterization of mass variants in biopharmaceutical products.

Exposure of the monoclonal antibody (mAb) to mechanical stress and elevated temperatures (50°C) resulted in the formation of fragments with molecular weights lower than that of the monomer; however, no aggregates were observed. Aggregation of the mAb was observed under UV light (360 nm), with the structure assumed to be a dimer.

Insulin was exposed to strongly (pH = 1.0) and mildly (pH = 4.0) acidic conditions, mechanical effect (vortex, 24 h), UV light irradiation (360 nm) and high temperature (50°C). In a strongly acidic medium, no aggregation was observed, as confirmed by CGE. Under these conditions, deamidation emerged as the predominant modification, as revealed by capillary zone electrophoresis (CZE), which detected deamidated charge variants. Under mildly acidic conditions (pH = 4.0), insulin showed increased aggregation, as CGE analysis identified covalent dimer and oligomers. A low extent of deamidation was also detected in the same sample by CZE. At pH 4.0, aggregation is the dominant process, as the formation of negatively charged deamidated groups is limited. As a result, the reduced electrostatic repulsion allows closer intermolecular interactions between insulin molecules, thereby facilitating aggregation. Elevated temperatures promoted insulin aggregation by enhancing protein denaturation and facilitating intermolecular interactions. Both mechanical agitation and UV exposure contributed to aggregation, however insulin exhibits a greater tendency to aggregate under UV light compared to mechanical stress.

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REMOVAL OF OCTINOXATE A UV-FILTER COMPOUND FROM AQUATIC ENVIRONMENT USING POLYDIMETHYLSILOXANE SPONGE

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The UV-filter materials can be either inorganic or organic compounds. The presence of organic UV-filter compounds used in sunscreen cosmetics in aquatic environments represents a growing environmental concern problem. Concentrations of octinoxate (one of the most commonly detected UV-filters in aquatic environments) measured ranging from ng L^{-1} to low µg L^{-1} levels, particularly in recreational areas or downstream of wastewater treatment plants.[1] Its presence raises ecological concerns due to potential endocrine-disrupting effects and developmental toxicity observed in aquatic organisms, including fish and invertebrates.[2]

In this work we demostrate the potential of PDMS sponges for removing organic UV filter compounds such as octinoxate from aqueous solutions. PDMS sponges were fabricated using simple templates: commercial sugar cube, fused sugar particles, pressed NaCl salt particles. The 10:1 mixture of PDMS oligomer and cross-linking agent was degassed before casting onto the templates.

Among the prepared sponges, those templated with sugar cubes or coarse salt exhibited the highest adsorption capacity, effectively adsorbing up to 0.6 % of their own mass in octinoxate. The PDMS sponges were fully regenerable, allowing complete removal of octinoxate without any detectable changes in their adsorption properties or dry weight. Due to their simple fabrication, ease of handling, ability to float, and reusability, PDMS sponges present an environmentally friendly and low-maintenance alternative to conventional filtration systems for the removal of octinoxate and potentially other UV filter compounds from environmental surface waters and recreational water bodies.

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A METHOD FOR DIRECT INJECTION ANALYSIS OF PROTEINS WITH HIGH MATRIX CONTENT: TAYLOR-ARIS DISPERSION ASSISTED MASS SPECTROMETRY

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A key technique for protein analysis is electrospray ionization mass spectrometry (ESI-MS). While direct infusion represents the quickest and simplest approach for sample introduction, numerous matrix components can interfere with the ionization process, often resulting in poor-quality spectra or even a complete lack of usable data.

Taylor-Aris dispersion occurs when a sample plug moves slowly through a narrow capillary, leading to symmetrical band broadening due to the radial diffusion of analytes across a pressure-driven parabolic velocity profile.[1] This dispersion is more pronounced for high molecular weight species with lower diffusion coefficients, producing broader peaks for proteins, while low molecular weight matrix components yield sharper peaks. As a result, a matrix-free zone forms at the front and rear of the sample plug, enabling clean protein spectra acquisition without relying on traditional separation techniques.[2]

Taylor-Aris Dispersion Assisted Mass Spectrometry can be especially useful for analyzing native proteins and protein complexes since it offers a rapid, online, partial buffer exchange. This allows samples to be present in any buffer (e.g., PBS) that be exchanged to ammonium acetate only seconds before ionization.[3]

The first demonstration of this method was carried out in a CE-MS system, since the standard dimensions of commercial capillary electrophoresis instruments align well with the optimal range for Taylor dispersion. Nonetheless, similar results can be easily achieved using a syringe pump or LC system.[4]

Determining the optimal conditions for these analyses can be complex, as both Taylor-Aris dispersion and ionization are influenced by multiple variables. Our results show that the expected taylograms under varying conditions can be accurately described using mathematical models. This allows for in-silico exploration of operational parameters for Taylor-Aris Dispersion Assisted Mass Spectrometry.[5]

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BIOACTIVE COMPOSITE FROM CHITOSAN AND FERVERFEW EXTRACT AND ITS IMPACT ON CANCER CELLS

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Feverfew (*Tanacetum parthenium* L.), a member of the Asteraceae family, is a ubiquitous perennial. Historically, maidenhair tansy was used to treat arthritis, asthma, constipation, dermatitis, earache, fever, headache, inflammatory conditions, insect bites, menstrual disorders, potential miscarriage, psoriasis, cramps, stomach pain, bloating, tinnitus, toothache, vertigo, and worm infestation. Feverfew has also been used as an abortifacient, as an insecticide, and to treat coughs and colds. Traditionally, the herb was used as an antipyretic, hence its common English name, feverfew.[1] Substances with medicinal potential, contained in the feverfew are, mainly from the classes of sesquiterpene lactones, flavonoids and essential oils.[1,2] This study is focused on the synthesis of composites consisting of chitosan and hydroalcocholic extract of dried feverfew aerial parts. The composites of chitosan and Tanacetum parthenium were synthesized and characterized using techniques such as powder X-ray diffraction, infrared spectroscopy, dynamic light scattering (DLS), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The extract composition was analyzed using combined column chromatography and UHPLC. The extract biological activity effectiveness was tested against human keratinocytes and human melanoma cells **(Figure 1)**.

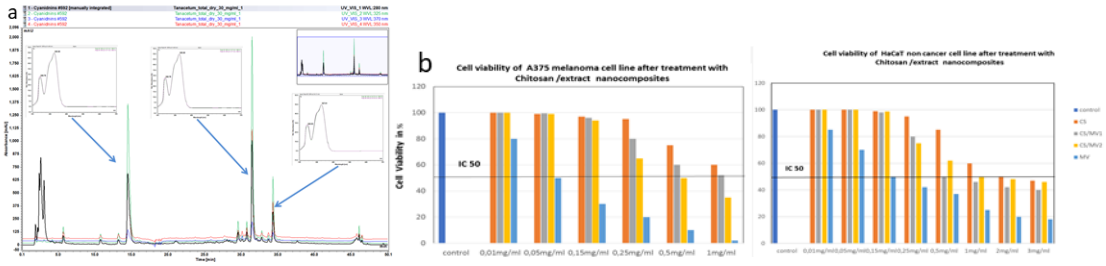

Figure 1. a – HPLC chromatogram of the feverfew extract; b – results from the MTT cytotoxicity assay.

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ELECTROPHORETIC AND CHROMATOGRAPHIC APPROACHES TO WINE POLYPHENOL ANALYSIS: ADVANCES IN PROFILING, QUALITY ASSESSMENT, AND AUTHENTICATION IS GOOD

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The polyphenolic matrix of wine, influenced by grape variety, environmental conditions, and vinification techniques, encompasses a structurally diverse array of compounds, ranging from simple phenolic acids to complex flavonoids and condensed tannins, that play a pivotal role in defining the wine's sensory profile, stability, and authenticity. Due to the structural variability of these substances, the exact characterization of these polyphenolic constituents necessitates advanced analytical strategies capable of capturing subtle compositional variations across different grape varieties, terroirs, and vinification practices. Among these, high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), in conjunction with UV-Vis, electrochemical, or mass spectrometric (MS) detection, are widely employed for the targeted quantification and untargeted profiling of key phenolic subclasses, including flavan-3-ols, flavonols, stilbenes, and phenolic acids.

Techniques such as capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) offer advantages for the efficient separation of low-molecular-weight phenolic compounds, while CE-MS enable the fingerprinting of oxidation-prone constituents and polymeric structures, especially relevant in wine aging studies. Conversely, HPLC-DAD and HPLC-MS remain the methods of choice for resolving co-eluting compounds in complex matrices and are instrumental in the discrimination of oenological practices such as maceration time, oak aging, or the use of exogenous tannins.

By integrating these high-resolution separation methods with robust detection systems, researchers should be able to construct highly discriminative polyphenolic profiles that reflect both endogenous grape composition and exogenous technological influences. Collectively, these analytical strategies offer a robust framework for tracing compositional variability, verifying authenticity, and supporting the development of chemometric models for quality assessment, provenance determination, and the identification of potential adulteration.

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VOLTAMMETRY IN A FLOW-THROUGH CELL USING SENSOR WITH BORON-DOPED DIAMOND ELECTRODE

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The flow-through electrochemical cell (FTEC) is an efficient tool for voltammetric analysis of liquid samples, providing repeatable and controlled measurement conditions. The design of the cell determines which electrodes can be used in them. Boron doped diamond (BDD), is an electrode material that is characterized by a wide range of applicable potentials, low background current, high chemical and mechanical resistance. FTECs are usually made of PEEK or PMMA. 3D printers represent a tool by which electrochemical cells can be produced at a relatively low cost.

In this work, the possibilities of using a 3D printed FTEC, manufactured at FEI STU in Bratislava, in anodic voltammetry, in the determination of acetaminophen (AAP) were investigated. The cell was constructed for a sensor with integrated BDD electrodes (working and auxiliary) and Ag/AgCl reference electrode on a ceramic plate (7 × 25 mm) (FEI STU). Voltammetry is usually performed with diffusive transport of the analyte to the electrode, i.e. without convection. After the measurement, the concentration of the analyte near the electrode is much lower than beyond the diffusion layer, therefore a smaller signal is obtained in subsequent measurements. Repeatability of the signal (current) can be ensured by restoring the analyte concentration in the diffusion layer by moving the solution or mixing between measurements. In a FTEC this movement is carried out by a peristaltic pump controlled by the voltametric analyser.

The reference electrode of the sensor does not have its own solution, therefore the influence of the chloride concentration in the analysed solution on the AAP signal was experimentally investigated. The low RSD value (1.9 %) of the AAP peak heights, in KCl solutions in the concentration range of 100 – 300 mmol L^{-1}, indicates a negligible effect of chloride concentration on the quantitation of AAP in the samples. The highest currents were obtained in acid electrolytes. Voltammetry of AAP at different scan rates confirmed, that the mass transport is diffusion controlled. The calibration dependence was linear over three decadic orders (0,003 – 2,0 mmol L^{-1}). Good repeatability of slopes of calibration lines, characterized by RSD = 2.5 %, calculated from 8 calibrations performed over 2.5 months, confirms the long-term stability of the BDD electrode. The LOD (0.4 µmol L^{-1}) was determined using SD of the peak height obtained in 29 repeated runs of AAP solution with a concentration of 1 µmol L^{-1}. The analysis of the pill samples was characterized by a 2,6 % error, required only minimal treatment prior to analysis (dissolution and appropriate dilution) and three replicate runs took approximately 1 min.

The results demonstrated the great potential of utilization of BDD sensor in a 3-D printed FTEC for the voltammetric determination of drugs with electrochemically active functional groups.

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INCOMING MOBILITY OF STUDENTS AND TEACHERS - STUDENT AND TEACHERS MOBILITY IN VOCATIONAL EDUCATION: INTEGRATING LABORATORY PRACTICE, ICT AND INTERCULTURAL LEARNING

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Within the Erasmus+ mobility programme, 12 students from Slovakia and 10 students from the Chemical Technician programme at Vladimir Prelog Natural Sciences School in Zagreb participated in a series of laboratory activities aimed at developing professional knowledge and skills in the school's laboratories. The work programme and manual were authored by the teachers presenting this poster.

The programme included experimental exercises such as biochemical oxygen demand (BOD) determination, biodiesel synthesis, protein molar mass determination, chromatographic techniques, and biodiesel viscosity measurement. Activities were conducted through research-based and group work, with continuous monitoring and evaluation of both students' and teachers' work using digital tools.

The mobility was enriched by organised professional visits to companies and institutions including TAPI (TEVA), the Zagreb Wastewater Treatment Plant, and the Biocentre, where students attended expert lectures and engaged in discussions with field researchers. Throughout all activities, students developed teamwork and presentation skills, culminating in the public presentation of their project results.

Special emphasis was placed on familiarising students with the language and culture of the host country, promoting intercultural learning and personal development. The project contributed to increased motivation for learning, the practical application of theoretical knowledge, and the development of students' independence and responsibility.

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PCR and high-resolution melting curve analysis for differentiating novel pear varieties

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Pears (*Pyrus spp.)* are of great economic and nutritional value and are consumed all over the world in unprocessed or processed form, for example as drinks or jams. They have health promoting properties, e.g. anti-inflammatory effects, and are a source of nutrients such as fibre and phytochemicals such as antioxidants.[1,2]

Breeders attempt to create new pear cultivars to improve fruit quality and fruit appearance, better storage and shelf life, and resistance to diseases and pests. The aim of this study was to investigate if novel pear cultivars with common paternal and/or maternal cultivar, can be distinguished by polymerase chain reaction (PCR) with subsequent high-resolution melting curve analysis (HRMA). For this purpose, 15 primer pairs targeting microsatellites (simple sequence repeats, SSRs) were tested. These primer pairs had previously been used in a master's thesis to differentiate old pear cultivars.[3,4,5]

This study included 17 new pear cultivars, obtained by crossing maternal cultivars Abata, Fetel, Conference, Williams Christ, Dita, or Dicolor with paternal cultivars Bunte Julibirne, Winterdechantsbirne, Forellenbirne, Dicolor, Carmen, Sanguinole, Fondante de Charneuses, Norma, Vereinsdechantsbirne, Winterdechantsbirne or unknown paternal cultivars.

DNA was extracted from pear leaves using the NucleoSpin Plant II- Kit (Macherey-Nagel). After analyzing the DNA extracts by PCR and HRMA, data was evaluated by comparing melting curve profiles and their derivatives obtained for the novel pear cultivars with those obtained for the maternal and paternal cultivars.

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WORKSHOPS

Crystallization of biological macromolecules using the ORYX8 robot

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This hands-on workshop will introduce the crystallization of biological macromolecules using the sitting drop vapor diffusion method, automated by the ORYX8 crystallization robot. Participants will learn how to set up crystallization experiments efficiently and reproducibly and will gain insight into the practical aspects of protein and/or nucleic acid crystallization.

The workshop will also demonstrate the use of seeding techniques as a powerful approach to improve crystal hit rates and optimize crystal growth. Participants will have the opportunity to observe or participate in the seeding process and understand its practical application in crystallization workflows.

Participants are welcome to bring their own samples of biological macromolecules (proteins, DNA, or small water-soluble molecules) for setting up crystallization trials.

Note: Samples must be aggregate-free and preferably at concentrations above 5 mg mL^{-1}.

Chiroptical properties of peptidomimetics: a TD-DFT study

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Computational chemistry combines numerical methods based on quantum mechanics (QM), molecular mechanics (MM), molecular dynamics (MD) and Monte Carlo (MC) simulations for the prediction of the structure, electronic and thermodynamic properties of chemical systems. Today, it enables researchers to explore the properties of new functional materials with remarkable accuracy and efficiency, often more cost-effective and rapidly than experimental approaches allow.

In our recent studies, we have explored the potential of the ferrocene chromophore as a circular dichroism probe for the assignment of the screw-sense preference in small ferrocene-based peptides.[1,2]

The accompanying workshop introduces theoretical background and computational methods used to simulate CD spectra. It covers the principles of circular dichroism, TD-DFT for excited-state calculations, and Boltzmann-weighted spectral averaging based on conformational populations. A case study on ferrocene peptidomimetics illustrates the complete workflow—from conformational search and optimization to TD-DFT calculation and spectrum generation—using widely adopted quantum chemistry tools. The workshop aims to provide participants with practical insight into the use of chiroptical simulations in modern molecular research.

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Analysis of sulfonamides by LC-QTOF working in simultaneus full scan and MS/MS mode

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Sulfonamides (SA) are one of the most widely used veterinary drugs for treatment of bacterial infections and promoting growth and productivity of livestock and poultry. Due to this, traces of SA can be found in foods of animal origin, such as meat, dairy products, eggs and honey, which can have negative effects on human health. Therefore, the development of analytical methods for their detection is of great interest.

This workshop will introduce the LC-QTOF-MS analysis of sulfonamides in honey by using All ions acquisition mode. In this technique high resolution accurate mass data is obtained by simultaneously acquiring low energy spectra, that contains predominately precursor ions, and high energy spectra that also contain fragment ions. Personal Compound Database and Libraries (PCDLs) enables identification of the compounds based on their molecular and fragment ions. This technique is a powerful tool in targeted and untargeted analysis that decreases method setup time, increases throughput and enables highly sensitive qualitative and quantitative analysis in a single analytical run.

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