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Content

The process of designing a biosimilar antibody

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The process of developing a biosimilar entail creating biological medicinal product that closely resemble licensed reference product. This process emphasizes replicating the original active substance, permitting minor variations that do not significantly affect clinical outcomes.

Unlike generic drugs, biosimilars are complex proteins, which may differ in structure and properties from the reference product due to the sophisticated manufacturing process involving the transfection of target cells and purification steps. The development of biosimilars requires extensive analytical testing, including evaluations of molecular structure, impurity profiling, and assessments of biological activity both *in vitro* and in human subjects. When developing antibodies, the goal is to verify a high degree of similarity, the structure, the biological activity rationality, in terms of immunogenicity effect.

Our research group managed to design a new biosimilar to Nivolumab, through designing different plasmids for co-transfection of CHO DG44 cells with both heavy and light chains of the antibody. After a rigorous monoclone selection, FPLC purification was carried out, then characterization of the product and verification of the glycosylation profile by CE-LIF.

Key words: Nivolumab, biosimilar, development

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Human carbonic anhydrase II – a new tool for asymmetric synthesis of enantiopure *anti*- and *syn*-1,2-diols

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Both *anti*- or *syn*-1,2-diols are important intermediates for a number of industrially relevant products, as the hydroxyl group is a relatively easily substitutable substituent.

Herein, we present an enzymatic method mediated by human carbonic anhydrase II in the presence of phenylsilane [1] for the production of (1*R*,2*S*)-1-phenyl-1,2-propanediol and (1*S*,2*S*)-1-phenyl-1,2-propanediol starting from 1-phenylpropane-1,2-dione. Developed detection methods that were used to identify and characterise the formed diols are described.

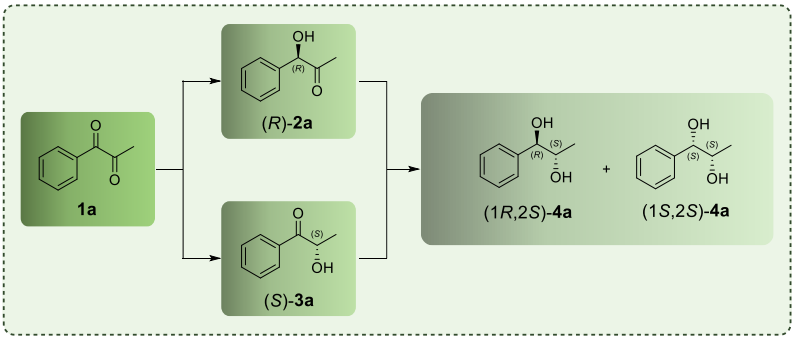

Figure 1. Enzymatic reduction of 1-phenylpropane-1,2-dione mediated by hCAII in the presence of $PhSiH_3$

Key words: Vicinal diols, Enzymatic reduction, Human carbonic anhydrase II, Chiral HPLC chromatography

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Alginate-Based Molecularly Imprinted Hydrogels for Glioblastoma Postoperative Therapy

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Local treatment strategies for glioblastoma multiforme (GBM) have emerged as safer and more effective alternatives to systemic chemotherapy, as they can bypass the blood-brain barrier, directly tackle residual GBM cells in the resection cavity, and subsequently diminish the risk of recurrence [1]. Among recent innovations for drug delivery are molecularly imprinted polymers (MIPs), synthetic materials that act as effective drug reservoirs due to their specific binding cavities that can provide tailored release kinetics, high stability, and protection against metabolic degradation. However, conventional biocompatible acrylic-based MIPs pose limitations due to their lack of biodegradability, which might restrict the total amount of available drug release and require secondary surgical intervention [2].

Alginate-based hydrogels show promise for molecular imprinting due to their biocompatibility and biodegradability [3,4], but their limited control over drug release and reduced loading capacity of hydrophobic drugs often require adjunct functional polymers, like the thermo-responsive poly(N-isopropylacrylamide) (PNIPAm) [5-7].

The study aimed to develop a MIP-based hydrogel for the controlled release of ruxolitinib (RUX), a JAK/STAT-3 signaling pathway inhibitor [2]. Comprising alginate and PNIPAm, the hydrogel was conceptualized for localized delivery in the post-resection cavity following GBM surgery.

Key words: glioblastoma, molecularly imprinted polymer, hydrogel

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Rapid analysis of Stanušina wines using a fourier-transform infrared spectroscopy

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Stanušina is an autochthonous Macedonian grape variety, grown mainly at the oldest Tikveš wine district. The main characteristic of this variety is its high endurance, especially on droughts and it ability to grow at vineyards with not very fertile soils. In this study, three Stanušina wines have been produced with addition of two doses of honey before fermentation (20 and 40 g/L added honey) and one control wine without addition of honey. A fourier-transform infrared spectroscopy (FT-IR) was applied for rapid and simultaneous determination of 14 parameters in Stanušina wines, including alcohol, density, glycerol, pH, total acidity, total sugars, individual carbohydrates (glucose, fructose and saccharose) and individual organic acids (tartaric lactic, malic, citric and acetic). Addition of 20 g/L honey before fermentation increased the content of almost all parameters, with exception of glucose and saccharose, which concentration was highest in the wine with highest amount of added honey. Tartaric acid was the dominant organic acid, followed by malic, citric and lactic acid. All wines presented satisfactory values for alcohol, pH, total acidity, glycerol and acetic acid, which confirm the quality and stability of the wines.

Key words: organic acids, carbohydrates, basic parameters, FT-IR, Stanušina wine

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This work was supported by the CEEPUS Network (SK-1516) BioScience, Food and Health, which is acknowledged.

Development and Application of Aptamer-Functionalized Electrochemical Sensors for the detection of Gram-positive and Gram-negative Bacteria

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The rapid and sensitive detection of infectious pathogens such as *Campylobacter jejuni* and *Staphylococcus aureus* is of major importance in the biomedical field. We aimed to develop two electrochemical sensors based on aptamers (APTs): the ONS-23 APT for *C. jejuni* cells and the PA#2/8 [S1-58] APT for *S. aureus* protein A (PrA) [1].

Carbon-based screen-printed electrodes (SPEs) decorated with Au nanoparticles and commercial Au SPEs were functionalized with the thiolated label-free APTs by multi-pulsed amperometry. The remaining unbound sites were blocked with 6-mercaptohexanol. Optimization was conducted to determine the optimal experimental conditions and the modifications after each step were monitored by differential pulse voltammetry and electrochemical impedance spectroscopy. Characterization of the surface was performed by atomic force microscopy and scanning electron microscopy. To assess the performance of the aptasensors, multiple dilutions of *C. jejuni* NCTC 11322 and *S. aureus* ATCC 25923 strains cultivated in selective media were tested.

Both aptasensors showed promising results for the detection of *C. jejuni* and protein A (as a target of *S. aureus*) and could be the starting point for the development of point-of-care diagnosis tools.

Key words: electrochemical sensors, aptamers, *Campylobacter jejuni*, *Staphylococcus aureus*

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Electrochemical detection of bacteria and biofilm

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Bacterial infections are associated with high mortality due to multidrug resistance and biofilm formation, necessitating early diagnosis. Bacteria can survive and develop through the biofilm in a variety of natural, clinical, and industrial environments [1]. Thus, it is crucial to develop new methods to identify and monitor biofilm formation. There are new detection methods targeting representative structures such as quorum sensing (QS) molecules. Gram negative bacteria produces various QS molecules, including 3-O-C_{12}-HSL, and PQS. cdGMP is an intracellular signaling molecule with increased importance in biofilm formation and its detection can provide important information regarding the presence of bacterial biofilm on different products [1].

In this study we successfully developed three electrochemical sensors to detect biofilm-associated molecules: 3-O-C_{12}-HSL, PQS, and cdGMP. For the detection of 3-O-C_{12}-HSL, a specific thiolated aptamer [2] was immobilized onto screen-printed electrodes modified with gold nanoparticles. The sensor was then incubated with the target molecules and the difference in the electrochemical signal was monitored. Each step was optimized and characterized using various electrochemical techniques.

For PQS and cdGMP detection, screen-printed electrodes modified with nanomaterials were used, due to their high conductivity and large surface area. The sensors were optimized in terms of electrode surface, electrolyte, and electrochemical techniques. All the developed sensors demonstrated wide detection ranges, high specificity, low limits of detection, and promising results in real sample analysis. These sensors serve as a foundation for the development of "Point-of-care" devices, enabling real-time monitoring of biofilm formation.

Key words: Biofilm detection, cdGMP, 3-O-C_{12}-HSL, PQS

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Aptamer selection for assisted treatment of hepatocellular carcinoma

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Hepatocellular carcinoma is one of the most common types of primary liver cancer and is characterized by rapid progression and poor survival rates. Current treatment options have limited efficacy against this malignancy, primarily because of difficulties in early detection and inherent resistance to conventional therapy [1]. Aptamer technology has been widely investigated in various biomedical fields for biomarker discovery, *in vitro* diagnosis, *in* vivo imaging, and targeted therapy. Their high affinity towards specific targets, along with their small size and non-immunogenicity, make them suitable for efficient tissue penetration and accumulation at tumor sites more efficiently than larger-sized protein antibodies [2].

The main objective of this project was to select a novel aptamer using magnetic-bead SELEX technology for glypican-3, an HCC biomarker found on the surface of hepatic tumor cells. First, the protein was immobilized on tosylactivated magnetic beads, after which multiple selection rounds were performed using different serum proteins as counter molecules to make the selection more stringent. The progress of aptamer selection was monitored by quantitative real-time PCR, melting curve analysis, and an enrichment assay. After selection, the resulting oligonucleotides were sequenced for primary structure determination and their affinities were evaluated by optical measurements.

The obtained aptamer will be further explored for the potential development of a targeted delivery system based on magnetic nanoparticles functionalized with an anti-angiogenic tyrosine kinase inhibitor drug, which can be guided to the tumour site using a magnetic field for both imaging and drug delivery purposes.

Key words: aptamer, hepatocellular carcinoma, SELEX technology, personalized therapy

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Species and Cultivar Differentiation by High Resolution Melting Analysis to Detect Food Adulteration

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Commercial foodstuffs must be safe and authentic to comply with national and international regulations, protecting human health and consumer interests. However, economically motivated food adulteration remains a global issue, with common practices including the replacement of higher-value species or cultivars with cheaper ones.

DNA-based methods, such as real-time polymerase chain reaction (PCR) and sequencing technologies, play an important role in species and cultivar differentiation. High resolution melting (HRM) is a cost-effective technology that exploits differences in the melting behavior of PCR products. The melting behavior depends on various parameters, including amplicon length and the ratio of guanine and cytosine to adenine and thymine. Single nucleotide polymorphisms (SNPs) and microsatellites are frequently used molecular markers for species and cultivar differentiation in food by HRM analysis.

This lecture will discuss the development and optimization of HRM assays for differentiating edible insect species approved in the EU; bilberry and blueberry; and wine varieties from North Macedonia. The potential and limitations of HRM analysis for food authentication will be addressed.

Key words: food authentication; species; cultivars; PCR; high resolution melting

Bottom-up Analysis of Commercial Protein Supplements Using CZE-MS

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Proteins are crucial biomolecules essential for human health and play a significant role in various bodily functions and can be supplemented with commercially available proteins. These protein supplements can be animal based and plant-based proteins. As the world population is rapidly growing demand for low cost and environmentally feasible high nutritional foods including alternative protein sources are growing. The most plant-based protein which contains all the essential amino acids for nutritional supplements are pea proteins which are rich in protein, starch, and fiber [1]. Bottom-up approach using capillary electrophoresis coupled with mass spectrometry play an important role in proteomics study and have a primary role in determining characteristics about protein samples, including quantitative and qualitative aspects as well as the identification and localization of post-translational modification [2]. In this research work we focus on the study of 8 different commercial pea proteins with bottom-up analysis. Using 1 M formic acid (pH= 1.8) as BGE, resulted in minimal adsorption and narrow peak shapes of the peptides in the digested sample, indicating excellent separation efficiency for the protein constituents in commercial pea samples.

More than 200 wide varieties of protein were identified and 18 common unique proteins were identified in all samples (mainly storage protein like vicilin, convicilin and legumins). Thus, the diversity of proteins present in commercial protein supplements may contain nutritional benefits and can be used as an alternative source as animal derived proteins for commercial supplements.

Key words: commercial pea protein extracts, bottom-up analysis, proteomics, capillary electrophoresis-mass spectrometry

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Optimizing Extraction and Purification Efficiency for GC-ECD Monitoring of Pesticides in Hair

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With increasing concerns about the impact of environmental pollution on human health, there is a growing need to develop effective biomonitoring methods for analysis of hair, blood or urine samples. Hair analysis, a non-invasive biomonitoring tool, allows researchers to assess long-term exposure to harmful chemicals such as heavy metals and organic pollutants effectively.

The aim of this study was to optimize the sample preparation procedure for analysing 11 organochlorine pesticides (OCPs) and 13 polychlorinated biphenyl pesticides (PCBs) in hair using GC-ECD, following the EPA TO-10A method for pesticide analysis in ambient air. Optimization included selecting the extraction solvent, refining the extraction technique and purifying of the extract simultaneously.

Five different extraction solvents were tested for efficiency: acetonitrile, hexane, dichloromethane, hexane/dichloromethane 1:1 (V/V) and hexane/dichloromethane 4:1 (V/V). The optimal recovery, averaging 96%, were achieved using the hexane/dichloromethane 1:1 (V/V) extraction mixture. Next, three extraction techniques were evaluated: orbital shaker, ultrasound-assisted extraction, and reflux extraction. Extraction using an orbital shaker at room temperature yielded the highest recovery rates (average 112.2%) and was selected as the most suitable method, unlike ultrasound-assisted extraction and reflux extraction, which likely led to analyte degradation or evaporation. Finally, deactivated alumina was chosen over PSA-dSPE for the purification phase, leading to more favorable recovery rates.

The optimized extraction and purification method was applied to analyze hair samples from individuals with varying levels of pesticide exposure: workers involved in cleaning up pesticide-contaminated areas, analysts handling pesticide samples, and individuals with no direct pesticide exposure.

This non-invasive method is effective for screening various pesticides and serves as a reliable basis for estimating population exposure to organochlorine pesticides.

Key words: pesticides, biomonitoring, hair, GC-ECD, PCBs, OCPs

Investigation of polar organic eluent mixtures in chiral HPLC

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With the growing number of enantiopure drugs marketed, it is increasingly crucial to accurately determine the less potent or toxic enantiomer, known as the distomer, at a level of 0.1% of the quantity of the eutomer. This is vital due to the different pharmacokinetic and pharmacodynamic behaviour of the enantiomers in our body. Enantioseparation methods are still developed on a 'trial-and-error' basis with screening several chiral stationary phases. Chiral HPLC methods until this day apply normal phase methods, which means toxic solvents such as hexane. In recent years, polar organic mode (POM) has been proven to be as effective as normal phase mode for chiral separations. In POM the eluent consists of different alcohols and acetonitrile or the mixture of these. These are less toxic, offer easier sample preparation and are better for preparative purposes.

The use of a neat solvent has the advantage of simplicity and easier method development. However, it can also be used as a mixture, where enantioseparation could be improved. Hysteresis is a physical phenomena where the state of a system is dependent on the history of the investigated system. In chiral HPLC it means that the retention factor and selectivity may be dependent on the eluent that the CSP was rinsed in before.

The aim of our research was to investigate the impact of polar organic eluent mixtures on various polysaccharide CSPs with focus on understanding hysteresis phenomena.

For this, HPLC measurements were performed using six different polysaccharide CSPs, namely Lux Amylose-1, Lux i-Amylose-1, Lux Amylose-2, Lux Cellulose-1, Lux Cellulose-2 and Chiralpak AS. The eluent composition was set from 100 V/V% of A eluent in 10 V/V% to 100 V/V% B eleunt and then the process was repeated backwards.

It should be noted that the use of eluent mixtures in POM can offer several advantages, such as better peak shape, faster elution, improved resolution and the ability to fine-tune the enantiomer elution order. Investigating structurally different compounds, we have found that the hysteresis phenomenon is common in amylose-based CSPs; however, negligible hysteresis was found in cellulose-based CSPs. This might be because the higher order structure of amylose may exist in different, stable conformations in various eluent mixtures. Adequate hysteresis can be found in ACN-alcohol and MeOH-IPA mixtures. In several cases EEO change was observed due to hysteresis. Our most recent investigation revealed

that an alteration in retention and selectivity can also be observed in reversed phase mode especially in MeOH-water mixtures.

Our investigations reveal that amylose-based chiral selectors likely exist in different stable conformational states, each with varying enantiorecognition potentials. The measurements conducted in these stable conformational states can pave the way for a novel approach to chiral method development.

Key words: chiral separation, HPLC, polysaccharide-type stationary phases, hysteresis

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Teaching and Learning Bioanalysis CEEPUS Network: Past, Present and Future

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CEEPUS (Central European Exchange Program for University Studies) is an international program, which promote the cooperation at high education level. Our Network entitled „Teaching and learning Bioanalysis" was founded in 1998 by Prof. Ferenc Kilár from University of Pécs. In the founding year the Network included only five partner Universities: University of Pécs, Hungary, Karl-Franzens-University of Graz, Graz, Austria, Medical University, Sofia, Bulgaria, Vienna University, Vienna, Austria and Comenius University, Bratislava, Slovak Republic. Since then several Universities joined our Network, and several left due to different reasons.

At this time the Teaching and Learning Bioanalysis CEEPUS Network have twenty one partner Institutions, from eleven different Central and East European Countries. During the twenty six years of activity of the Network a huge number of mobilities were completed and twenty two Summers Schools (including the actual one) were organized. As a result of research collaboration of the Partner Institutes, 229 scientific papers were published and 107 BSc, MSc and PhD theses were defended. As a recognition of the high level of scientific cooperation between the partners, the Network was awarded three times the Minister's Prize.

The future plans include the development of the partnership between the participant Universities, finding new topics for scientific collaboration, and involving as many young scientists in the Network's activity, as possible.

Key words: CEEPUS, Bioanalysis, Network

Development of microchip isotachophoresis method for analysis of pharmaceutical macrocomponents

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Pharmaceutical analysis is highly regulated worldwide to guarantee the safety and effectiveness of drugs for patients and those involved in clinical trials. This includes vigilant observation of process-related impurities, degradation products, and the active pharmaceutical ingredients in drugs. The validation process of the analytical method utilized in assay test must adhere to the criteria outlined in the ICH guidelines, ensuring that accuracy, precision, specificity, linearity, and concentration range are adequately addressed [1].

The main purpose of this study was to develop microchip isotachophoresis methods for the analysis of various pharmaceutical macrocomponents, i.e., active pharmaceutical ingredients (amlodipine, perindopril) and counterions (besylate, erbumine). The greenness of the developed methods was evaluated by AGREE software and a highly favourable green score was achieved. The developed methods met all the requirements of the validation process according to the ICH guideline. The applicability of the developed methods was demonstrated by the analysis of six commercially available pharmaceutical formulations containing amlodipine besylate and perindopril erbumine. The content of macrocomponents in tablet formulations was determined with a relative error below 1.8%.

Key words: Microchip isotachophoresis, Green analytical chemistry, Pharmaceutical analysis

Acknowledgements

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Steroid hormone responses to exercise - determination and analysis

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Physical exercise is a powerful stimulus for the endocrine system, modifying the plasma concentration of many hormones, including the steroid testosterone and cortisol. Testosterone is a major anabolic hormone, while cortisol produces generally catabolic effects. Thus, the testosterone/cortisol ratio (T/C) is considered a good indicator of anabolic/catabolic status in individuals. Evidence shows that plasma variation of testosterone and cortisol occurs in response to continuous aerobic exercise; i.e., during aerobic exercise, plasma concentrations of both hormones increase without modification of the T/C ratio and this increase is proportional to the intensity of physical exercise. Among other factors, the response of these hormones to exercise depends on the degree of training and hydration status of individuals [1].

Over the past four decades, there has been an increased emphasis on training young athletes, many of whom now train year-round [2]. At the same time, most research on endurance training responses involves elderly subjects, and considerably less is known about the learning capacity and development of younger individuals [3]. Therefore, research on training young athletes focused on achieving a high level of performance is necessary to maximize gains and enable young athletes to succeed in elite sports.

It is very important to have a sensitive and valid measurement of the stress associated with exercise testing, training, and competition. It was found that monitoring the levels of the hormone cortisol is a valid and reliable index of an athlete's stress linked to physical effort during, training and competition, Different body fluids have been for analysing cortisol, including serum, plasma, saliva, urinary free samples, collected both as overnight and 24 h collection periods. Currently, the main method for determining hormone concentration is in blood samples. This is an extremely reliable method, but in a number of cases its application faces limitations. The main problem is related to the fact that the parents of the athletes do not agree their children to give blood for research. Another obstacle is the phobia of a large number of people to blood and needles. This leads to more stress during the examination and often the subjects probably do not perform to the best of their ability in the test.

In a sports and exercise medicine setting the non-invasive nature of urine and saliva collection allows for personalised timing of sample collection, limited increases in stress hormone concentrations,

rapid sample collection and reduced risk of cross-contamination which does not require a specific professional for obtaining a sample (e.g. phlebotomist). Moreover, the added advantage of saliva and urine over blood collection can be attributed to the simplicity of the collection devices.

The current gold standard in cortisol analysis uses an enzyme-linked immunosorbent assay (ELISA). However, colorimetric reactions also have been studied [4] to determine cortisol concentrations. Colorimetric reactions can be used as a fast and simple qualitative and quantitative diagnostic tool for the detection of cortisol concentration in biofluids such as saliva, urine and sweat. The simplicity of such low-cost methods facilitates their widespread use and possibility of design and development of the low-cost wearable devices.

Key words: testosterone, cortisol, saliva, urine

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Drug/cyclodextrin supramolecular complexes with enhanced functionality

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To be pharmacologically active, all drugs must be soluble in water to some extent, and most of them should be lipophilic to penetrate biological membranes. According to the Biopharmaceutical Classification System, class II drugs are characterized by good permeability and poor solubility. One way of improving drug solubility in water is the formation of inclusion complexes with cyclodextrins (CD).

Cyclodextrins are cyclic oligomers composed of glucopyranose units that are connected through glycosidic a-1,4 bonds. Their structures resemble a truncated cone having a narrow rim, a wide rim, and a cavity. The hydrophilic exterior of the cavity makes cyclodextrins water-soluble, while the hydrophobic interior enables them to form inclusion (host-guest) complexes with various molecules. Besides naturally occurring β-CD having 7 glucopyranose units, hydroxypropyl-β-CD (HP-β-CD), sulfobutylether-β-CD (SBE-β-CD) and randomly methylated β-cyclodextrin (RM-β-CD) derivatives have gained pharmaceutical interest as they are also approved as pharmaceutical excipients.

Within the project DrugCD.com we aim to improve the solubility of some poorly soluble drugs that are administered orally, like praziquantel, loratadine, nabumetone, etc., by encapsulation into β-CD, and its derivatives. In this lecture, the recently published results on inclusion complexes of nabumetone with β-CD and its derivatives are presented, including (i) phase-solubility measurements in water and simulated biorelevant media (simulated gastric, pH 1.0; duodenal, pH 4.5 and intestinal media, pH 6.8), (ii) spectrofluorimetric titrations, (iii) isothermal microcalorimetric titrations, (iv) NMR spectroscopy analysis.

Key words: Poorly soluble drugs, Cyclodextrins, Inclusion complexes

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Direct Injection ESI-MS Analysis of Proteins with High Matrix Content: Utilizing Taylor–Aris Dispersion

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Recently we demonstrated the utility of the Taylor–Aris (TA) dispersion in avoiding serious interference issues commonly occurring in the electrospray ionization-mass spectrometric (ESI-MS) determination of therapeutic protein pharmaceuticals undergoing no pre-separation or sample purification [1]. It was also pointed out that the TA dispersion conditions and its analytical utilization for proteomics can be easily accomplished in a commercial CE-MS instrument. In the proposed Taylor–Aris dispersion-assisted mass spectrometry (TADA-MS) analysis 0.5 μL sample is injected into a 65 cm long 50 μm i.d. capillary and pumped with 1 bar toward the MS. The procedure is efficient for the direct injection analysis of components having low diffusion coefficients (proteins) that are present in complex matrices of small organic and inorganic compounds. In the lecture beside the main features of the TADA-MS we demonstrate the exceptional utility of TADA-MS in native protein analysis as well: (i) a dramatic improvement in detection sensitivity was found due to its ability to strongly reduce matrix interferences, (ii) more "native-like“ conditions can be used during analyses, (iii) the direct injection of non MS-compatible matrices is allowed into MS and (iv) a considerable simplification and economization of the workflow is ensured.

Key words: Mass spectrometry, Taylor-Aris dispersion, Proteins

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Optimization of purification parameters for biosimilar Nivolumab monoclonal antibody

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Advancements in recombinant DNA technology have significantly increased the importance of targeted therapeutic monoclonal antibodies (mAbs), making them central to the global biotechnological pharmaceutical industry. The production of these biosimilar products takes place in mammalian expression systems, predominantly in Chinese hamster ovary (CHO) cell cultures, with relatively high yields. The key step in biopharmaceutical development is efficiently recovering and purifying mAbs from the cell culture medium while maintaining product stability. Protein A affinity chromatography is a prevalent and primary chromatographic purification step in the downstream process of monoclonal antibodies.

The aim of our research is to optimise the purification of biosimilar Nivolumab antibodies, produced in CHO cell lines, using Protein A affinity chromatography. We seek to extract the Nivolumab protein from cell cultures with maximum efficiency and purity, ensuring both quality and quantity. This includes purification using Protein A affinity chromatography with binding and elution buffers of various compositions and pH values. We confirmed the antibody size with MALDI TOF-MS and SDS-PAGE separation. During the optimization of Protein A affinity chromatography purification steps, protein purification was conducted under eight different conditions, with variations in factors.

Our results confirm that protein extraction with a low pH citrate elution buffer yields sufficient purity. The structure of biosimilar Nivolumab monoclonal antibodies has been successfully characterised, confirming their presence and integrity.

Key words: Monoclonal antibody purification, Protein A affinity chromatography, biosimilar Nivolumab

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Mechanisms of Controlled Release of Active Substances from Hydrogel Materials Anchored on a Conductive Surface

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Hydrogels are 3D soft materials made of water-filled, cross-linked polymers. "Smart" hydrogels can significantly change their volume in response to external stimuli, depending on their polymer composition. Those sensitive to temperature and pH are extensively studied, particularly for drug delivery. Recently, interest in electroresponsive hydrogels for controlled drug release has increased [1].

Two approaches of electrochemically controlled release will be presented. One project features a thermosensitive thin hydrogel layer modified with β-cyclodextrins (βCD), obtained through electrochemically induced free radical polymerization. Electric pulses controlled the inclusion complex stability between βCD and a model substance (ferrocene (Fc) modified with Rhodamine B), allowing precise, stepped release of the substance via the oxidation potential of Fc [2]. Another project involves a pH-sensitive microgel with Fc groups, obtained by distillation polymerization and anchored to a gold electrode via chemisorption. Positively charged model substances were incorporated into the microgel with electrostatic interactions, and their release was controlled by applying an Fc oxidation potential, generating a positive charge that induced electrostatic repulsion and released the model substance into solution.

Key words: electrochemically controlled release system, smart hydrogels, drug carriers

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Application of metal nanoparticles in order to develop electrospun wound healing matrices

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In today's healthcare, combating multidrug-resistant bacteria is a significant challenge, particularly in treating large and slow-healing wounds like diabetic ulcers. As commercially available traditional antibiotics are not effective against such microorganisms, other types of antimicrobials are necessary [1,2]. Metal oxide nanoparticles (MeNPs) are promising candidates because of their potent antibacterial activity, coupled with lower susceptibility to resistance development. Despite their suspected superiority over traditional antibiotics, their cytotoxic effects make it challenging to apply them to living organisms [3].

The aim of this research is to create a wound dressing by incorporating MeNPs into a biocompatible and bidegradable polymer matrix prepared by electrospinning. A particular challenge is the need to work with a volatile organic solvent for electrostatic fiber formation, so that the nanoparticles should be synthesised and dispersed in such a medium.

First, polysuccinimide was synthethised and oleic acid modified magnetite nanoparticles (MNPs) were dispersed in the polymer solution, followed by ultrasound agitation. Then ZnONPs were synthethised in dimethyl sulfoxide, which was followed by a concentrating method (optimizing the precursors, reaction parameters and centrifugation), then dispersed it in the polymer solution. The NPs synthesis was proved with Dynamic Light Scattering (DLS) and UV-Vis Spectrophotometric measurements. In both cases electrospinning was used for fiber formation. The mesh was characterized by Scanning Electron Microscopy (SEM) and Fourier-transform Infrared Spectroscopy (FTIR).

In our research, we synthesized MNPs, ZnONPs and polysuccinimide (PSI). DLS experiments confirmed that the hydrodynamic diameter is approximately 200 nm of the ZnONPs. The presence of NPs was proved by UV-Vis. ZnONP solution was successfully concentrated by centrifugation. MNP solution was stabilized with oleic acid, and dispersed by ultrasonic bath in the polymer solution. Successful fiber formation by electrospinning was proved by SEM images. FTIR spectra of precursors, intermediates and final products was recorded.

Both NPs were successfully dispersed in the polymer solution, and PSI-based fibrous matrices were created. These materials can be potentially used to treat multidrug-resistant bacteria on open wounds. In the next phase of our research, we plan to perform antibacterial assays both on standard and isolated bacteria, and cytotoxicity assays on fibroblast cells. We plan to further characterize the

wound dressing using Transmission Electron Microscopy (TEM) and Small-angle X-ray Scattering (SAXS).

Key words: nanoparticles, nanotechnology, electrospinning, antibacterial

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Unveiling Deception: Exploring Cutting-Edge Approaches for Uncovering Adulteration in Dietary Supplements

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The widespread use of dietary supplements has become a global phenomenon, driven by the pursuit of wellness and the desire for optimal health. However, concerns regarding the integrity of these products have escalated due to rampant adulteration practices within the industry.

The adulteration of dietary supplements with illegal synthetic pharmaceuticals and their analogues presents a severe public health risk, as these products are often marketed to consumers as safe or natural alternatives to conventional medications. Adulteration not only compromises the efficacy of dietary supplements but also poses significant health risks to consumers.

The unauthorized inclusion of undeclared synthetic compounds in so-called "dietary supplements", can have serious health consequences. This deceptive practice aims to confer therapeutic effects beyond what the declared ingredients can achieve independently. Detecting such adulteration proves challenging as these illicit substances are not listed in the product's ingredients or disclosed to consumers. Despite the inherent dangers, consumers persist in purchasing and using these adulterated supplements, exacerbating the potential for significant harm to public health.

Statistically, the most frequently used dietary supplements are sexual enhancement, weight loss and sport performance enhancement supplements. Sexual enhancement supplements are often adulterated with phosphodiesterase type 5 enzyme (PDE5) inhibitors and their analogues. Weight loss supplements, on the other hand, frequently contain a varied assortment of compounds such as stimulants, laxatives, diuretics, or anorexiants. Meanwhile, sport performance enhancement supplements may be tainted with anabolic steroids and stimulants.

Dietary supplements are popular worldwide since they may be purchased without a prescription and are sold not only in pharmacies but also in a variety of outlets, as well as online. Internet sales of dietary supplements are a major source of counterfeit products, giving counterfeiters easy access to consumers and markets. The illicit trade in counterfeit supplements is highly lucrative, fuelled by sustained consumer demand and low production expenses.

Given the widespread occurrence and rising instances of this issue, the advancement of modern analytical methods for detecting counterfeit products stands as a prominent area within contemporary pharmaceutical research. Currently, a variety of methodologies are employed for identifying adulterants, with chromatographic and spectroscopic techniques prominently featured among them.

These approaches facilitate the separation, detection, identification, and in certain instances, quantification of undisclosed substances. Recent advancements in analytical chemistry, including high-resolution MS, NMR spectroscopy, and DNA barcoding, offer high precision and sensitivity in identifying adulterants. Hybrid approaches, such as LC-MS/MS or LC-NMR, make it possible to detect and identify adulterants quickly and easily. Yet, more traditional methods like TLC or HPLC are still useful in the detection of illicit compounds.

Despite these advancements, challenges persist, including the rapid evolution of adulteration techniques and the emergence of novel adulterants. Consequently, ongoing research endeavours focus on enhancing the versatility and adaptability of detection methodologies to keep pace with the dynamic landscape of adulteration.

Key words: dietary supplements, adulteration, analytical methods, authentication, consumer safety

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Isolation of pure NPS enantiomers by semipreparative HPLC-UV using a cellulose tris (3,5-dichlorophenyl-carbamate) column

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Besides widely recognized traditional synthetic illicit drugs like heroin, cocaine and amphetamines, numerous new psychoactive substances (NPS) have surfaced in the drug market. These compounds show comparable effects with slight alterations in their chemical composition to evade legal restrictions. Many of these altered molecules contain a stereogenic centre, meaning that two different enantiomeric forms with potentially different potencies and effects might be present. Over the last decade, more than 930 new psychoactive substances have been synthesized in clandestine laboratories, primarily located in the Far East and distributed worldwide through online platforms [1]. In order to investigate pharmacological or toxicological properties or to establish enantiomeric elution and migration orders of pure enantiomers of NPS, they have to be provided in a multi-milligram scale. Due to their novelty, the availability from official suppliers on the commercial market is limited.

The objective of the following study was to develop a semipreparative HPLC-UV method for isolation and characterization of NPS test compounds in a multi milligram scale. A commercially available Phenomenex Lux® 5µm 250 x 10 mm column containing the chiral selector cellulose tris(3,5-dichlorophenylcarbamate) served to collect pure enantiomeric fractions. After NPS enantiomer isolation, the collected substances were converted to their HCl salts and stored at -20°C. Furthermore, qualitative experiments were carried out to determine the purity of the isolated enantiomers using HPLC-UV [2].

Key words: chiral stationary phase, semipreparative high-performance liquid chromatography, new psychoactive substances

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Determination of total polyphenol content and antioxidant activity of selected Slovak meads

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Mead is an alcoholic beverage that has been used for thousands of years. Collecting honey was one of the first agricultural activities. Mead is made by fermenting a mixture of honey and water in a certain ratio. Honey fermentation is triggered by the addition of water, which causes the natural yeasts present in the honey to begin the fermentation process, so the production of mead appeared relatively early in human history [1]. The honey used affects the taste and colour of the final product. Additional spices, fruits, and fruit juices may be added at this stage to further enhance flavours and aromas of meads [2,3].

Most of the tests developed to measure 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 frequently used methods for antioxidant activity determination. The DPPH radical is neutralized by accepting an electron and a hydrogen atom, a reaction with a colour change that is measured spectrophotometrically at λ = 517 nm [4,5]. The Folin-Ciocalteu test is frequently used for the determination of polyphenol content of food samples. This method is based on electron transfer, where a reagent containing phosphomolybdic or phosphotungstic acid reacts, resulting in a blue chromophore, which is measured spectrophotometrically at λ = 765 nm [4].

The aim of this work was to investigate the antioxidant activity and polyphenol content of selected meads available in Slovakia. We tested the antioxidant activity and polyphenol content of five selected meads: two craft and three commercial meads. We found that craft meads with higher alcohol content have higher antioxidant activity and total polyphenol content. The highest values were measured for craft barrique mead.

Key words: polyphenol content, antioxidant activity, spectral methods, mead

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Application of Microchip Electrophoresis Techniques Coupled with Ion Mobility Spectrometry to the Analysis of Liquid Biological, Environmental, Food and Pharmaceutical Samples

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Microchip electrophoresis (MCE) and ion mobility spectrometry (IMS) are analytical separation techniques based on the different mobility of ions in the electric field. For MCE analysis liquid samples are required, while IMS analysis takes place in gaseous phase. In this study, the development of hyphenated MCE-IMS methods and their application to the analysis of complex liquid samples is demonstrated. Online two-dimensional MCE-IMS methods can be used to overcome the limitations of the MCE and IMS as separate techniques for the analysis of complex samples.

Two different MCE techniques, zone electrophoresis and isotachophoresis, were used in combination with the IMS. A thermal spray-based interface was used to vaporize the sample components separated by the MCE and introduce them into the IMS analyzer. Diluted electrolyte solutions used for electrophoretic separations were used as an auxiliary liquid to transfer separated sample components from the microchip to the thermal spray-based unit. Various analytical parameters such as sensitivity, linearity and precision were evaluated for the developed MCE-IMS methods. MCE-IMS methods were applied to the analysis of carboxylic acids in various environmental, food, biological and pharmaceutical samples.

Key words: Microchip electrophoresis, Ion mobility spectrometry, Complex liquid samples

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NMR structure elucidation of synthetic cannabinoids sold on the internet

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Besides classic illegal drugs, numerous designer drugs, also called New Psychoactive Substances (NPS) are available on the global drug market. One of the bigger and fast-growing substance classes comprises the synthetic cannabinoids. According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 245 out of 930 monitored substances, belong to this group of NPS, with 24 new cannabinoids registered for the first time in 2022.[1]

For purchase it is not necessary to use the dark web. Due to the structural differences compared to the illegal Δ^9-THC (delta-9-tetrahydrocannabinol) the legal status changes and the synthetic cannabinoids are available via clear web pages. They are available as powders or dissolved and sprayed onto non-psychoactive layers such as cannabidiol hemp, industrial hemp or also on other plant material like herbal tea mixtures or dried flowers. These adulterated plant parts can be smoked as a substitute to the psychoactive and in many areas illegal Δ^9-THC-containing hemp.

The main task of this project was to check the identity of online available synthetic cannabinoids and to generate experimental data using a combination of gas and liquid chromatography with mass selective detection and NMR (nuclear magnetic resonance) structure elucidation. More than 50 % of the acquired substances were falsely declared. In total, 23 synthetic cannabinoids in solid state were bought from various online shops. They turned out to be 21 different substances after analysis.

Key words: NPS, designer drugs, cannabinomimetics

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Mechanochemical synthesis of MIPs: the case study of atenolol

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Molecular imprinting enables the fast, versatile, robust and cost-effective synthesis of biomimetic polymeric receptors with tailored selectivity for a wide variety of target molecules. Solvents are a critical component in the synthesis of molecularly imprinted polymers (MIPs), both as a porogen and a reaction media, however their use comes with additional challenges. On a larger scale, especially the nonpolar ones, may imply environmental concerns. Some solvents can interfere with the binding of the target molecule to the polymer matrix, leading to reduced binding efficiency or selectivity. And last, but not least some polymerization methods, such as precipitation polymerization, require the use of specific solvents or solvent mixtures. To address some of the above-mentioned issues, but also to explore potential opportunities or further constraints, we report the first solvent-free mechanochemical synthesis of MIPs via liquid-assisted griding [1].

The successful synthesis of the imprinted polymer has been functionally demonstrated measuring its template rebinding capacity, as well as the selectivity of the molecular recognition process in comparison with the ones obtained by the conventional, non-covalent molecular imprinting process in liquid media. The proof-of-concept study demonstrated similar binding capacities towards the template molecule and superior chemoselectivity compared to the conventional MIP synthesis method.

The adoption of green chemistry principles with all its inherent advantages in the synthesis of MIPs, not only alleviates potential environmental and health concerns associated with their analytical (e.g. selective adsorbents) and drug delivery (e.g. drug carriers or reservoirs) applications, but might also offer a conceptual change in the molecular imprinting technology.

Key words: Molecularly imprinted polymers, Mechanochemistry, Liquid-assisted grinding

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Optimization of sample preparation for bacterial identification with MALDI-TOF MS

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Currently, many bacterial identification methods are available for both diagnosis and research. These include culture-based, biochemical, and serological methods as well as polymerase chain reaction (PCR), sequencing, and matrix-assisted laser desorption (MALDI-TOF MS) [1]. In clinical microbiology, there are many advantages to being able to identify bacterial strains as quickly as possible for subsequent therapeutic selection [2]. The biggest breakthrough in this field in recent decades has been MALDI-TOF MS, which has significantly reduced identification costs [3]. Despite its many advantages, its shortcomings include the identification of closely related bacteria at the species level [4].

In this study, we sought to determine whether there is a sample preparation method for bacteria isolated from the environment that can be used prior to MALDI-TOF MS, which, although more time-consuming, significantly increases identification efficiency. In our study, we compared three sample preparation methods: direct digestion, formic acid pretreatment, and a relatively longer pretreatment procedure using formic acid and acetonitrile. The aim of this study, based on data from our experiments, is to present a method that can be used more effectively for bacterial identification in the future. From our experiments, we have concluded that of the three methods compared, pretreatment with formic acid and acetonitrile has proven to be the most effective.

Key words: bacterial identification, MALDI-TOF MS, sample preparation

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Exploring cortisol detection in biological fluids: a customized electrochemical aptasensor

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Cortisol (COR) is an active endogenous hormone involved in several physiological and pathological processes, with levels fluctuating in biological fluids due to numeorus factors, including some disorders with an inflammatory component. Hence, the accurate and selective detection of COR is crucial for assessing both the diagnosis of the disorder and the effectiveness of treatment. Electrochemical methods represent an advantageous approach for analyzing biomarkers, such COR, in biological samples due to their low-cost, high sensitivity and specificity, suitability for miniaturization, and possibility for *in situ* analysis [1].

The main objective of the study was to design a customized platform for COR specific electrochemical detection in biological samples with prospects for wearable biomedical applications. Flexible, customized carbon electrodes were in-lab printed and the surface of the working electrodes was modified using Au and Pt to increase the sensitivity, and an aptamer to increase the specificity for COR detection. Lastly, the aptasensor was incubated with COR. All modification steps were confirmed using cyclic voltammetry and electrochemical impedance spectroscopy and the detection of COR was performed by cyclic voltammetry. The elaborated platforms were analyzed regarding the analytical performances (detection limit, limit of quantification, and sensitivity for COR) and used for real sample analysis. The elaborated sensor allows COR specific detection and it can be further developed for wearable medical applications.

Key words: aptasensor, cortisol, biological samples

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HPLC- ESI-Q TRAP-MS/MS analysis of flavonoids and nonflavonoids in red wine

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In this study, a high performance liquid chromatography (HPLC) coupled to tandem mass spectroscopy (MS/MS) operated in electrospray ionization (ESI) and quadrupole linear ion trap (Q TRAP) negative mode was applied for simultanous determination of nonflavonoids and flavonoids in Kratošija red wine. A total of 27 phenolic compounds, including 13 nonflavonoids (10 phenolic acids, 2 stilbenes and 1 stilbenoid) and 14 flavonoids (6 flavan-3-ols, 4 flavonols, 2 flavones, 1 flavanone and 1 flavanonol) were identified within 55 minutes by using a Phenomenex Luna C18(2) column (3 μm, 100 Å, 100 x 2 mm), with flow rate at 0.2 mL/min, column temeperature at 30°C and a gradient elution system of 8 mM formc acid in water (eluent A) at pH 2.8 and acetonitrile (eluent B). The relative amounts of phenolic compounds were calculated as areas of the ion extracted peaks of the individual compounds in wine. Gallic acid was the dominant phenolic acid, followed by p-coumaric and syringic acids. Resveratrol-3-glucoside (piceid) was present in highest amount compared to resveratrol and viniferin. From the group of flavonols, myricetin was the dominant component and from flavan-3-ols, procyanidins B1 and B3 dominated in wine. Flavones chrysin and luteolin, flavanone naringenin and flavanonol taxifolin were reported for the first time in Macedonian red wine.

Key words: flavonoids, nonflavonoids, HPLC- ESI-MS/MS, Kratošija red wine

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Microbial secondary metabolite detection from faecal samples with GC-MS

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The gut microbiome is a dynamic ecosystem of microorganisms that live in the intestines of the host organisms. The composition of the gut microbiota depends on many factors, including host genome, dietary intake, and disease conditions. The metabolic pathways of the host are influenced by microbes through microbial secondary metabolites, such as short-chain fatty acids (SCFAs) [1]. Many diseases have been shown to be correlated with an imbalanced microbiota, including rheumatoid arthritis (RA) and type 2 diabetes mellitus (T2DM). Studies targeting microbial small metabolites, so-called metabolomic studies, have resulted in promising data on host-gut microbiota interactions [2].

The aim of the present study was to determine microbial secondary metabolites from faecal samples of diseased patients such as RA and T2DM.

Faecal samples were preserved with sodium hydroxide, extracted with the addition of MeOH, and derivatized with 1-trimetylsilyl-imidazole. The derivatized samples were analysed with GC-MS. GC-MS analysis was performed using a 7890b gas chromatograph/5977a mass-selective detector (Agilent Technologies, Santa Clara, CA, USA) with an HP-5 ms capillary (Agilent Technologies). The flow rate of helium carrier gas was maintained at 20 mL/min. One microliter of the derivatized sample was injected with a 5 min solvent delay time. The initial column temperature was 60 °C, held for 1 min, then finally increased to 320 °C at a rate of 10 °C/min and maintained at this temperature for 10 min. Ionization was performed in electron impact (EI) mode at 70 eV. The MS data were acquired in full-scan mode from m/z 50–600 [3]. The detection of SCFA from faecal samples using the above-mentioned protocol was not successful, and other secondary metabolites, such as lactic acid (C3), pentanoic acid (C5), and hexanoic acid (C6) were detected.

Key words: GC-MC, gut microbiota, secondary metabolites.

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Preparation, characterization and stability of NAB:β-CD systems in solid state

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In our previous work we have investigated the influence of β-cyclodextrin (β-CD), hydroxypropyl-β-CD (HP-β-CD), randomly methylated-β-CD (RM-β-CD), and sulfobutylether sodium salt β-CD (SBE-β-CD) on solubility of poorly soluble drug Nabumetone (NAB) in various aqueous media. Inclusion complexes NAB:β-CD in solution were characterized in detail by several techniques.[1]

In this study, we aimed to prepare NAB:β-CD systems in the solid state by mechanochemical activation *i.e*. by grinding in high-energy ZrO_2 and steel vibrational mills. The grinding process was monitored by calculating the relative degree of NAB crystallinity based on the results of differential scanning calorimetry analyses. The final products obtained by milling were additionally characterized by powder X-ray diffraction and FT-IR ATR spectroscopy. Saturation solubility and *in-vitro* dissolution experiments were conducted in simulated gastric media at pH 1,2. Selected NAB:β-CD binary systems were subjected to long-term stability and photostability testing according to ICH Q1A(R2) and Q1B guidelines respectively.

Key words: Nabumetone, cyclodextrins, solid state, mechanochemical activation

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Polyethylene oxide/dextran all-aqueous emulsions stabilization using silica/PDADMAC aggregates

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Aqueous Two-Phase Systems, so called ATPS systems are formed when two incompatible and water-soluble compounds, for example two polymers or a polymer and salt are mixed in water. Polyethylene oxide (PEO) along with dextran (Dex) are both water soluble and incompatible polymers, which makes them perfect candidates in forming ATPS systems [1]. They have huge potentials in many fields, which caused a rise in the number of studies in recent years [2]. The nontoxic compounds of these systems make them beneficial for example in biomedical or pharmaceutical applications [3].

ATPS systems are known for their thick interface and the extremly low interfacial tension between the two aqueous phases. These qualities make their stabilization challenging. For a successfull stabilization of the ATPS system it's necessary to have large enough particles with appropriate interfacial properities and interparticle interactions to enable their adsorption at the water-water interface in time [4].

In this study, the agglomerates of silica nanoparticles with adsorbed polycations (called Poly diallyldimethylammonium chloride (PDADMAC) were used for stabilization [4] of the ATPS emulsions formed in the aqueous mixtures of PEO and Dex. The dextran we used had 450-650 kDa average molar mass and we used PEO with three with different average molar masses of 4, 20 and 100 kDa. Our aim was to compare these ATPS emulsions and analyse their stability in the function of different parameters including the concentrations of the stabilizing components and the details of the mixing protocols. It was shown that the initial separation of the PDADMAC and that of the silica particles in the starting media of PEO and Dex solutions and that of the molar mass of polymers had a crucial impact on the stability and structure of the formed emulsions.

Key words: Aqueous Two-Phase System, ATPS emulsions, silica nanoparticles, polycations, Poly diallyldimethylammonium chloride (PDADMAC)

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Challenges faced optimizing the electrochemical nano structuring of gold screen-printed electrodes into SERS substrates

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Environmental health represents a serious issue nowadays, pollution being one of the main causes for the deterioration of the living standard. Considering the variety of compounds and the number of chemical structures, the need for a versatile detection technique arises.

Raman spectroscopy offers fingerprint-like information for a variety of molecules, and so it represents a powerful tool in both qualitative and quantitative analysis. For low analyte concentrations, substrates that can enhance Raman signals are essential. However, commercially available substrates are often expensive and have limited specificity towards certain substances and laser wavelengths due to the size and arrangement of nanoparticles on their surfaces.

The study objective was obtaining such substrates by electrochemical means using screen printed gold electrodes, which are cheaper and can produce similar amplification of the Raman signal to the commercially available SERS substrates. Usually, substrates are being evaluated in terms of performance using molecules with an intense Raman signal and good interactions with the metallic surface, like colorants or compounds with thiolic groups.

In the optimization process for this study, 4-aminothiophenol (4-ATP) was used for evaluation, but when testing the obtained substrate for other substances well-known for their strong Raman signal like propranolol (PRNL) and thiabendazole (TBZ), the results were suboptimal.

This confirmed that although using a Raman reporter substance like 4-ATP, provides an intense SERS signal, the substrate might not exercise the same performance when using other analytes. The electrochemical process should be optimized for individual analytes in order to obtain maximum performance.

Key words: SERS, electrochemical, optimization

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Comparison of extraction methods for natural yellow dyes from historical fabrics

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Acid extraction is a common method used to recover dye from the fabric to the solution previously attached during dyeing. Recent developed methods focus on obtaining information emphasising complex forms of flavonoids. Their qualitative composition is an information about the source of the dyeing. The most used acids for acidic extraction are two acids with different properties: formic acid and hydrochloric acid.

The aim of this project was to compare acid extraction methods for yellow dyes of natural origin. Solutions of hydrochloric and formic acids were used. High-performance liquid chromatography-mass spectrometry (LC-MS/MS) was used to identify the compounds. The analysis was carried out an experiment of solutions of Reseda luteola and Genista tinctoria as well as wool dyed in these solutions. Both of the proposed extraction methods were used to identify the dyes in a 19th century historical fabric.

In extracts of *Reseda luteola* and fabric dyed in it were identified: luteolin-7-O-glucoside, luteolin, apigenin-8-C-glucoside (vitexin), apigenin-O-glucoside and apigenin. *Genista tinctoria* extracts and fabric dyed in confirmed the presence of luteolin-7-O-glucoside, luteolin, apigenin-8-C-glucoside (vitexin), apigenin, genistein-7-O-glucoside (genistein), genistein. These are characteristic sets of compounds for these dyeing sources.

In the antique fabric from the 19th century, identification of compounds was easier to receive after the hydrochloric acid was used for extraction. Significantly higher signal intensities confirmed the presence of luteolin, luteolin-7-O-glucoside, vitexin and apigenin, which may indicate that the *Reseda luteola* was used for dyeing this fabric.

Key words: dyeing, flavonoids, yellow dyes, acid extraction, HPLC, mass spectrometry, antique fabric

Strengths and limitations of MALDI-TOF MS in microbial identification

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Rapid identification, which is accurate and cost-effective for microorganisms isolated from the environment, is indispensable for applied microbiology. Several tools have been developed in the past few years for rapid microbial identification, including spectrophotometry (Biolog system), DNA sequencing, gas chromatography (GC-FAME), and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS has revolutionized diagnostics in culture-based microbiology [1]. Several studies have focused on the effect of pretreatment on the identification of microorganisms using MALDI-TOF MS. Owing to the sensitivity of MALDI-TOF MS, different preparation methods have led to changes in microbial protein fingerprints [2].

In this study, we evaluated the performance of a MALDI-TOF MS system (Bruker MALDI Biotyper) in a head-to-head comparison with 16S rRNA gene sequences as a reference for the identification of environmental bacteria. The isolates were identified by MALDI-TOF MS as *Bacillus sp., Pseudomonas sp., Lysinibacillus sp., Micrococcus sp., Variovorax sp., Serratia sp., Artrobacter sp*., *Variovoax sp*., and *Microbacterium sp..* The same genera were identified through sequence analysis and differences were observed mainly at the species level. Several sample preparation methods have also been used for Bruker MALDI Biotyper identification to improve its effectiveness. From the direct colony transfer method, on-target extraction method (pretreatment with formic acid), and in-tube extraction method (formic acid and acetonitrile), direct colony transfer was the least effective, regardless of whether the in-tube extraction method was the most effective in the case of the soil environment-derived bacteria.

Key words: bacterial identification, MALDI-TOF MS, 16S rDNA

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Synthesis of a novel cationic covalent organic framework with potential biomedical applications

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Covalent organic frameworks (COFs) are a class of crystalline porous organic polymers known for their permanent porosity and highly ordered structures.[1] Unlike other polymers, COFs are notable for being structurally predesignable, synthetically controllable, and functionally manageable.[1] In COFs, crystallinity, stability, and functionality compete with each other.[2] Dynamic imine chemistry provides a good balance between crystallinity and stability under mild thermodynamic and catalytic conditions. COFs have been recognized as promising candidates for biomedical applications, including their potential use as antimicrobial agents.[3]

The research aimed to obtain a cationic organic covalent framework with imine connections by the reaction between 2-((5-(trimethylammonium)pentyl)oxy)benzene-1,4-diammonium hydrochloride and benzene-1,3,5-tricaboxaldehyde. Cationic COFs can interact with negatively charged areas of bacteria's membrane, inhibiting cell-to-cell communication and growth. Starting from 1,4-Phenylenediamine and benzene-1,3,5-tricaboxaldehyde, characterization of intermediates by Thin-Layer Chromatography, Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR), High-resolution Mass Spectrometry and Single-crystal X-Ray diffraction was performed. Cationic COF was obtained under solvothermal conditions, further being characterized by Fourier-Transform Infrared (FT-IR) Spectroscopy, X-Ray diffraction spectroscopy (XRD), Differential scanning calorimetry and Scanning Electron Microscopy.

Key words: cationic covalent organic framework, imine-linked covalent organic framework, antibacterial agents.

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Recent advances in microchip electrophoresis for bioanalysis

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Microchip electrophoresis (MCE) is one of the simplest miniaturized separation techniques, which offers high separation efficiency, high throughput, easy automat ion, low consumption of reagents, reduction of waste production and low running costs [1]. MCE performed on the microchip with coupled channels is a multifunctional tool, which facilitates online integration of sample pretreatment with two-dimensional separation and utilization of various detection techniques. Practical examples of separation and determination of analytes present in complex samples of biological, food and pharmaceutical origin are demonstrated.

For example, a microanalytical method based on an online combination of isotachophoresis (ITP) and zone electrophoresis (ZE) with conductivity detection was developed for the determination of nitrite and nitrate in cerebrospinal fluid for the purpose of indication of various neurological diseases [2]. A photometric detector coupled to the microchip using optical fibers was used for the ZE determination of carminic acid (natural red food dye) in various food and pharmaceutical samples [3]. The ITP method performed on the microchip and combined with surface enhanced Raman spectrometry for the separation and detection of synthetic dyes present in various pharmaceuticals was developed [4]. The ITP separation followed by ion mobility spectrometry detection was used for the determination of carboxylic acids in various food, pharmaceutical and biological samples which delivered good repeatability and accuracy [5].

Presented results show a great analytical potential of the MCE for the analysis of complex biological, food and pharmaceutical samples.

Key words: Microchip electrophoresis, Bioanalysis, Detection techniques

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The stability study for the pharmaceutical form used in hypokalmia treatment

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Introduction. The stability of a drug is the degree to which a product retains the same properties and characteristics over a long period of time, which is established. The stability testing represents a routine procedure carried out on the active pharmaceutical ingredient (API) and finished pharmaceutical form (medicine). According to the FDA and ICH guidance state the requirement of stability testing data, the stress testing is forced degradation study, induced within a short period of time by different factors, to show the degradation pathway, that could have the potential to carry out during manufacture, long-term storage, distribution, and use [1,2]. So, it is a systemic test performed on APIs and medicines to demonstrate the quality, efficiency and intrinsic stability of APIs in formulation to various environmental factors. The stability test is an important step in the development of stable medicine in ensuring safety, stable, effective medicine by determination of the shelf life, selection of packaging and storage conditions.

Aim of the study. To determine the stability of potassium orotate (OK) in combined powders to the influence of stress factors by spectrophotometry.

Materials and methods. The present study was performed using a single beam Ultraviolet-Visible spectrophotometer (Agilent 8453, SUA) with 10.0 mm matched quartz cells. All absorbance measurements were carried out at 20 ± 1ºC at λ_{max} = 286±2nm, using 0.1M NaOH as a reference solution. All weights were taken on an electronic balance (Model Radwag), sonicated by UltraSonic Bath (Sapfir). APIs (OK, potassium aspartate, magnesium aspartate) were obtained from Sigma-Aldrich, spironolactone - Acros Organic and OK standard -European Pharmacopoeia Reference Standard. Analytical grade reagents and solvents (Chem-Lab): 1M HCl, 1M NaOH, 0.1%-, 1%- and 3% H_2O_2 solutions and daylight and 240 nm UV lamp chamber were used to carry out the study. Each solution was prepared in triplicate.

Results and discussions. The present paper describes application of the UV spectrophotometric method to determine the quantitative content of OK in elaborated powder samples, which were exposed to acidic (1M HCl) and basic (1M NaOH), thermal (60^{0}C temperature), photolytic (daylight and UV), oxidative (0.1%-, 1%- and 3% H_2O_2 solutions) and hydrolytic stress. The solutions were analyzed at time

intervals: 0 minutes, 3 hours and 24 hours after the action of the stress factors. The results show degradation processes with increasing concentration due to the formation of decomposition and absorption products in the same spectral range, with 33% in acidic medium (0 min - 123.60%, 3 h - 126.94%, 24 h - 133.60%) and 20.22% in basic medium (0 min - 100.20%, 3 h - 122.12%, 24 h - 120.42%). The same processes occurred at sunlight action with 17.7% (0 min - 101.04%, 3h - 107.53%, 24h - 118.74%), and UV irradiation with 15.59% (0 min - 101.04%, 3h - 102.42%, 24h - 116.63%). At oxidant action, the degradation process evolved rapidly, with OK decomposition until the impossibility of detecting absorbance at the specific wavelength.

Conclusions. The research has shown that OK is vulnerable to the action of stress factors and is subject to degradation, which justifies the need to protect the pharmaceutical form by selecting appropriate packaging.

Key words: Potassium orotate, stability, degradation, stress factors

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Chiral separation of Mirabegron by capillary electrophoresis

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Introduction:

Mirabegron is a selective beta-3 receptor agonist used for treating frequent urination and overactive bladder, as it reduces the number of daily urinations. The compound has a single asymmetric carbon atom, and the S-enantiomer is marketed. Chiral separation methods are necessary for the detection and quantification of the distomer, as a potential impurity.

Objectives:

The aim of our work is to develop a simple and quick capillary electrophoretic method that allows the chiral separation of mirabegron enantiomers.

Material and methods:

An initial cyclodextrin (CD) screening was applied for separation of the enantiomers in a phosphate buffer at neutral pH, using the following chiral selectors: hydroxypropyl-β-CD, hydroxypropyl-γ-CD, sulfated-β-CD, carboxymethyl-β-CD, succinyl-β-CD, carboxyethyl-β-CD, heptakis(2,3-dimethyl-6-sulfo)-β-CD, octakis(2,3-dimethyl-6-sulfo)-γ-CD, heptakis(2-methyl-3,6-disulfo)-β-CD. Dynamic capillary coating using polybrene, polydiallyldimethylammonium chloride (PDADMAC), polyethylene oxide (PEO) and covalently coated capillaries with linear polyacrylamide (LPA) and polyvinyl alcohol (PVA) were employed for the migration order reversal of the enantiomers

Results and discussion:

The quick separation of the two enantiomers was achieved using sulfated-β-CD as the chiral selector and LPA-coated capillary. It was found that between pH 2.5-6.5, there is no separation, and chiral separation can be achieved over pH 7.0, with separation improving as pH increases. However, above pH 8.5, the LPA capillary coating begins to detach.

Conclusion:

The chiral separation of mirabegron can be achieved by capillary electrophoresis using sulfated-β-cyclodextrin and an LPA-coated capillary at basic pH, in under 4 minutes of runtime.

The role of lipopolysaccharide biosynthesis in antibiotic basic research

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Introduction: Gram-negative bacterial surfaces are covered by lipopolysaccharide molecules. While lipopolysaccharides provide a structural barrier around the bacteria, they also have role in thermosensitivity and biofilm forming ability. This project demonstrates the significance of this molecule group, its biosynthesis and the connection to the so called bacterial two-component systems, suggesting new possible targets on Gram-negative bacteria.

Materials and methods: Whole genome of two Shigella sonnei species were sequenced by IonTorrent PGM. De novo assembly of genomes were performed by SPAdes v3.1 [1] and scaffolds of the draft genome were reordered by Mauve [2] software and MeDuSa web server [3]. Closest relatives were found by phylogenetic analysis of 16S rRNA, adk, fumC, gyrB, mdh and purA sequences by BLASTn [4]. Lipopolysaccharide biosynthesis genes were identified using KEGG database [5]. Bacterial two-component system contributors were identified using KEGG and literature data.

Results: Hotspots in lipopolysaccharide biosynthesis were identified. A network between bacterial two-component system contributors and lipopolysaccharide biosynthesis enzymes were described, tailored to the observed strains. A possible target of gmhD by Closantel was tested by negative result.

Conclusion: Bacterial two-component systems can play a role in the regulation of lipopolysaccharide biosynthesis by controlling the expression of genes involved in the biosynthesis, while the testes Closantel had no effect on the expression of gmhD.

Key words: *Shigella sonnei*, lipopolysaccharide, bacterial two-component system

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Differentiation of 24 Chickpea Cultivars by Targeting Microsatellites and Single Nucleotide Polymorphisms using High Resolution Melting Analysis

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Cultivation of chickpeas is becoming more interesting with the progressing climate change, as chickpeas grow better in a warmer environment and are less sensitive to water limitations [1,2]. Additionally, chickpeas provide a good source of protein, soluble and insoluble fibers, show a low content of fat and sodium and contain no cholesterol [2]. With the rising trend of vegan products and meat alternatives, food containing chickpeas becomes more popular in European supermarkets.

This study aimed to differentiate 24 chickpea cultivars belonging to the three types desi, kabuli and gulabi, most of them originating from Europe. Apart from the visual differences between desi, kabuli and gulabi, no morphological distinction is possible among cultivars belonging to one of these three types. We developed DNA-based assays including polymerase chain reaction (PCR) to amplify chickpea DNA containing microsatellites or single nucleotide polymorphisms (SNPs), followed by high resolution melting (HRM) analysis.

Multiple primer pairs targeting specific microsatellites in the chickpea genome were chosen from literature, sorted out with multiple in-silico tests, and 20 primer pairs were tested in the lab [3,4]. With one singular primer pair most of the 24 cultivars can be distinguished. However, most of the remaining cultivars can be differentiated with other primer pairs.

For SNPs, primer pairs were newly designed. With the aim to obtain more complex melt curves, we tried to target multiple SNPs with one and the same primer pair. The locations of SNPs being in close vicinity and their surrounding bases were identified with the help of literature [5,6]. Nine primer pairs passed the in-silico tests and were tested in the lab. To gain more insight into the melting curves, the amplicons containing SNPs were sequenced. Similar to the assays targeting microsatellites, one primer pair distinguished the majority of the 24 cultivars and most of the remaining samples are complemented by other tested primer pairs.

By combining the results of both types of molecular markers all cultivars except four pairs can be differentiated.

Key words: Chickpeas, Cultivar differentiation, PCR, HRM, Pyrosequencing

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Nature-Inspired Nano-system for Synergetic Anticancer Therapy, Sensing and Modulation of Tumor Microenvironment

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Fibrotic changes in solid tumors, constitute a major obstacle to cancer treatment and diagnosis. Here, we emphasized the challenges posed by transforming growth factor (TGF-β), the chief driver for several transformational changes within the tumor microenvironment (TME), such as the infiltration of cancer-associated fibroblasts (CAFs), the remodeling of extracellular matrix (ECM) and fibrosis. TGF-β also regulates Smad2/3 pathway, PI3K, MEK/ERK, JNK/MAPK, RhoA/Rock and Wnt/β-catenin pro-EMT signaling, linked with cancer resistance, aggression and metastasis. Based on these facts, we propose the urgent need for cancer innovative therapy – such that can tackle the intrinsic malignant cell-autonomous signaling and the extrinsic TME-associated facilitators. Evidences show that natural saponins, e.g., panax ginsenoside Rg1 can significantly inhibit the growth and proliferation of cancer cells, blocking the TGF-β1/Smad signaling network, interstitial fibrosis and pro-EMT mechanisms (1,2).

As contribution, we developed MnO2/Saponin-based nano-system (NS) that can synergize activity with conventional anticancer – Sorafenib (SOR)), for improved tumor-targeting, delivery and modulation of the TME. Our results showed an improved Zeta potential after coating the particles with natural saponin – values, jumped from -32.1mV to 17.1mV, and particle size 161nm to 364nm (ave.), respectively. In addition, a higher release of SOR was recorded *in vitro* at pH 5.5 than pH7.4; likewise, the capacity of NS to generating molecular oxygen. Therefore, we conclude that NS could be an ideal system for targeting hypoxic TME via MnO2 catalase-like-action on H_2O_2; also, for modifying the dense, stiffened ECM, for an improved drug penetretability and cancer theragnostic (3).

Key words: MnO2-Saponin nano-system, cancer therapeutics, anti-fibrotic, TGF-β1 signaling

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Polyphenols and flavonoids from *Galium verum* L. Species

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Introduction. *Galium verum* L. (popularly known as lady's bedstraw or yellow bedstraw) is a perennial plant in the Rubiaceae family. Lady's bedstraw has a long history of use in folk medicine for the treatment of many diseases and illnesses, and it has been used as a diuretic, choleretic, antidiarrheal, spasmolytic, in the treatment of gout and epilepsy, as a wound healing remedy [1,3], and a non-specific use of the species was as a sedative [2]. To the present day, several classes of bioactive compounds, such as iridoid glycosides, phenolic compounds, anthraquinones and triterpenes, as well as small amounts of tannins, saponins and essential oils, have been isolated from *G. verum* [3].

Aim of the study. To determine the content of polyphenols and flavonoids of *Galium verum* species from the spontaneous flora of the Republic of Moldova.

Materials and methods. Various plant organs (stems, leaves, flowers and herbs) collected during the flowering period from *G. verum* sp. from the wild flora of the Republic of Moldova were used as biological material for our study. The collected material was dried and conditioned according to the technical normative requirements. Polyphenols and flavonoids were extracted with 60% ethanol solution for 30 min, respecting the ratio of 1:10, on water bath with condenser. The both chemical group of substances were determined spectrophotometrically, total phenolic contents was determined by the Folin-Ciocalteu method with gallic acid as reference substance and flavonoids content was determined with $AlCl_3$, by six consecutive measurements at 430, 425 and 340 nm, expressed as rutoside, quercetine, apigenine equivalents, measured in mg/100 g vegetal product (VP).

Results and discussions. The received data show, that the highest values of polyphenols are found in the leaves of *G. verum* species (28.91 mg/GAE), followed by herbs (27.56 mg/GAE), and flowers (27.10 mg/GAE), with a lower content in the stems (15.86 mg/GAE in 100 g VP). The measurements determined a higher concentration of flavonoids in flowers of *G. verum*, with the highest concentration expressed in apigenin (21.76 mg/g VP), quercetin (11.75 mg/g VP) and rutoside (2.01 mg/g VP), followed by herb, leaves and stems.

Conclusions. The obtained results give the possibility to explore the species *G. verum* from the spontaneous flora of the Republic of Moldova as a source of polyphenols and flavonoids for the local pharmaceutical industry. Project: Development of new pharmaceutical products from local raw material (Nr. 080301).

Key words: *Galium verum*, polyphenols, flavonoids

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The Long Journey of Peptides as a Drugs

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The pioneering medical application of peptides as therapeutic agents began about a century ago (insulin, oxytocin, etc.), yet they remain clinically relevant candidates garnering more attention on the drug development agenda. This is also reflected in the continuous approval of peptide-based agents by the Food and Drug Administration.

Peptides represent an intriguing area of therapy that lies between the two extremes of molecular weight, small molecules and biologics, combining the best features of both. They have been widely studied across the therapeutic spectrum, exhibiting various biological properties, including antitumor, antioxidant, antimicrobial, anti-inflammatory, antihypertensive, as well as currently widely studied antiviral activity.

In recent decades, the application of various peptide synthesis technologies has successfully overcome the limitations of peptide drugs and highlighted their attractive pharmacological profile, including their remarkable efficacy, selectivity, and low toxicity.

In this presentation, we will endeavour to show the century-long journey of peptide-based drug discovery, integrating the current state of the art, various developments and emerging perspectives in peptide research.

Key words: peptides, peptide drugs

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Polysuccinimide-salt electrospun composite scaffold as a potential wound dressing material

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In our research, we have used electrospinning to create a two-component biodegradable polymeric scaffold containing polysuccinimide (PSI) and antibacterial salts. Antibacterial agents applied for therapeutical purposes mostly contain silver ions. Still, their use is associated with high environmental impact and, in some cases, may cause undesired immune reactions, so it is essential to develop other systems with similar effects.

In our work, nanofibrous systems contain antibacterial and tissue-regenerating salts of zinc acetate or strontium nitrate in different concentrations, whose structures may be suitable for developing biomedical wound dressing systems in the future. Antibiotic-resistant bacteria are a huge problem worldwide, so instead of antibiotics, we are using inorganic salts.

Several experiments have been conducted to optimize the physicochemical, mechanical, and biological properties of the scaffolds developed for application as wound dressings. The scaffold systems obtained by synthesizing PSI, then adding salts, and performing fiber formation were first investigated using scanning electron microscopy (SEM) images. In almost all cases, different salts caused a decrease in the fiber diameter of the PSI polymer-based systems (<500 nm). Fourier transform infrared (FTIR) spectroscopy was used to investigate whether salts were present in the scaffolds and to determine the interaction between the salt and the polymer. When creating a wound dressing, it is important to investigate the mechanical properties, as the wound dressing is subjected to several impacts on the skin surface. Our result showed that the mechanical properties of the polymer scaffold changed under the influence of the salts, both in terms of its specific load capacity and relative elongation values. In addition, dissolution experiments were carried out, as salts can exert their antibacterial properties when leaching from the scaffold. The whole amount of strontium-nitrate could dissolve from the scaffold after 8 hours, but in the case of zinc acetate, approximately 50 % of the salt content could dissolve. In addition, antibacterial activity tests were performed on four different bacterial strains relevant to skin surface injuries. These results showed that, in most cases, inhibition zones appeared around the scaffold discs. We also investigated the potential cytotoxicity of the scaffolds to find out the effects on human

tumor and healthy cells. Except for the zinc acetate salt-containing ones, the scaffolds are not at all cytotoxic either to tumor or healthy cells.

Key words: polysuccinimide, electrospinning, antibacterial activity, wound dressing

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Immobilized enzymes in continuous flow microfluidic systems

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This research is focused on the use of a microfluidic device created by different technics such as CNC and laser engraving, respectively, which is suitable for testing the activity and selectivity of prepared biocatalysts. Within the microreactor, catalysts such as enzymes, whether in their native or immobilized forms, are housed in its inner channels. This setup allows starting materials to flow through, facilitating reactions and enabling precise control over parameter optimization [1].

In this investigation, lipase B from *Candida Antarctica* (CaL-B) yeast was immobilized onto several different carriers such as Immobead T2-150, magnetite and sol-gel. The immobilization rate and the synthetic activity of the produced biocatalysts were determined using the UV-Vis spectroscopy. As miniature devices, microreactors function as a small vessel designed to contain and facilitate chemical reactions in a confined space [2]. The efficacy of these biocatalysts was evaluated within a continuous flow microfluidic system using a self-designed and manufactured aluminium and polycarbonate-based microchip in enzymatic acylation reaction. To evaluate the selectivity and activity of the immobilized enzyme, the acylation reaction of *rac*-1-phenylethanol with vinyl acetate in hexane was used.

Due to optimization, it was found that parameters such as substrate concentration, flow rate and temperature significantly influence the efficiency of the microfluidic system.

Key words: enzyme immobilization, gas chromatography, continuous flow microfluidic system

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Establishing the origin of Volatile Organic Compounds in urban Air: Regional Cross-Border Study between North Macedonia and Kosovo

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Volatile Organic Compounds (VOCs) are a group of organic chemicals that are emitted into the air from various sources, including natural ones like vegetation, wildfires, and volcanic activities, as well as anthropogenic sources such as vehicle emissions, industrial processes, solvents, paints, and household products like cleaners and air fresheners.

Based on their chemical properties the VOCs can be classified, such as aliphatic, aromatic, halogenated, oxygenated, and nitrogenated compounds, all of which can pose short-term and long-term health risks to humans. Additionally, they contribute to the formation of ground-level ozone and smog, which adversely affect air quality and can worsen respiratory conditions, especially in urban areas.

The aim of the study was to establish the origin of VOCs in urban air at nine cross-border sites between North Macedonia and Kosovo, using Radiello® passive and diffusive samplers, and gas chromatography-mass spectrometry (GC-MS).

The GC-MS analysis revealed that VOCs originating from gasoline fuel components (BTEX: benzene, toluene, ethylbenzene, and xylenes) and diesel fuel components (*n*-undecane, *n*-dodecane, *n*-tridecane, *n*-tetradecane, and other linear alkanes) accounted for approximately 60% of total VOCs present in the outdoor air at the sampling sites. Fossil fuels, particularly automotive fuels, were identified as the primary source of VOCs across all sampling locations and seasons, with only a minor fraction attributed to biogenic emissions, predominantly monoterpenes. Additionally, the ratios of benzene to toluene (B/T) and xylenes to ethylbenzene (X/E) were utilized to assess the origins of VOC emissions in the outdoor urban air at the monitored sites.

Key words: VOCs, origin, urban air, cross-border study, GC-MS

Application of Different Sorbents for preconcentration of Selected Phthalates

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Phthalic acid esters, called phthalates (PAEs), are a group of chemical compounds that are commonly used as plasticizers. These compounds are added to plastics (PVC, PET) to improve their flexibility and strength. Phthalates are not chemically bound to the matrix and can be released from plastic materials, thus contaminating the air, soil, water and food. Some phthalate compounds may have harmful effects on human health. They have been found to have carcinogenic, teratogenic, endocrine-disrupting properties and cause diseases of the circulatory, nervous and respiratory systems.

The aim of the research was to develop a method for phthalates determination in drinking water and beverage samples using high-performance liquid chromatography with spectrophotometric detection. Due to the low concentrations of phthalates in the selected samples, a preconcentration step is necessary. Two preconcentration methods were used in the study: classic solid-phase extraction (SPE) and solid-phase extraction using nanoparticles of materials exhibiting magnetic properties (MSPE).

The concentration process was optimized using the solid phase extraction method, comparing the recovery values obtained on various sorbents: HLB Oasis, C18 Sep-Pak Vac, Extract-Clean C8, SPE Phenyl, Supelclean Envi-carb. The influence of various parameters (e.g. type of solvent, sample volume) on the concentration process was examined. For MSPE, nanoparticles with a magnetic core, consisting of two crystalline phases: Fe and Fe3C, covered with graphite were applied. This material was first used to concentrate phthalates. Both, the adsorption and desorption of phthalates from this deposit were studied.

Key words: Phthalic acid esters, SPE, HPLC

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Analysis of loratadine-cyclodextrin complexes by high resolution mass spectrometry

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Loratadine (LOR) is a second-generation antihistaminic agent characterized by its low solubility and high permeability [1]. Cyclodextrins (CD) are cyclic oligosaccharides known to form inclusion complexes with various drugs, improving their physico-chemical properties, such as solubility and stability [2]. High resolution mass spectrometry can be used to confirm formation of inclusion complexes in a gas phase, which can be correlated with the behaviour in the solution [2]. To our knowledge, only inclusion complexation of LOR and dimethyl β-CD was studied by mass spectrometry [1].

In this work we present a detailed study of complexation of LOR with β-CD and its derivatives hydroxypropyl-β-CD (HP-β-CD), methyl-β-CD (RM-β-CD) and sulfobutylether-β-CD sodium salt (SBE-β-CD) by high resolution mass spectrometry.

Key words: Loratadine, cyclodextrins, high resolution mass spectrometry

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Association of *MGMT* enhancer methylation with *MGMT* promoter methylation, MGMT protein expression and clinical parameters in glioblastoma

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In glioblastoma, high activity of the protein O^6-methylguanine-DNA methyltransferase (MGMT) interferes with the anti-cancer effect of the alkylating chemotherapeutic agent temozolomide (TMZ). Since *MGMT p*romoter methylation is commonly found to be negatively correlated with MGMT protein expression it has been established as a predictive biomarker for the responsiveness of glioblastoma (GBM) patients to TMZ. However, this negative correlation is not given for all GBM patients [1, 2]. In our first study we reported associations of *MGMT* enhancer methylation with MGMT protein expression, *MGMT* promoter methylation and/or overall survival of GBM patients [3]. In the present study, we expanded the number of analyzed enhancers and CpG sites to gain an improved understanding of the subject.

Primers targeting CpG sites in five intergenic and four intragenic enhancers of *MGMT* were designed. Genomic DNA of primary cell line samples, derived from tumor tissue of GBM patients, was bisulfite converted and amplified by polymerase chain reaction. Methylation levels were determined by pyrosequencing.

For all four intragenic enhancers we found negative correlation of methylation with *MGMT* promoter methylation as well as significantly higher methylation levels in MGMT expressing than MGMT non-expressing samples. Two intragenic enhancers turned out to be suitable for prediction of overall survival of GBM patients. Our results indicate that *MGMT* enhancer methylation contributes to *MGMT* regulation and might serve as a potential prognostic biomarker for GBM patients.

Key words: DNA methylation, glioblastoma, MGMT

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Applications of electrochemical techniques in pharmaceutical formulation development

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Chemotherapy, while effective, has certain limitations like systemic side effects. Thus, novel methods for targeted drug delivery are essential for overcoming these drawbacks. This can be achieved through passive transport, relying on tumor tissue properties, or active transport, using drug delivery systems (DDS) functionalized with antibodies, enzymes, or aptamers. Characterization via various analytical methods is crucial for DDS development. As DDS evolve, analytical methods for chemotherapeutic quantification must progress in tandem.

The present work proposes four different nano-DDS for the targeted delivery of several chemotherapeutics: nanosomes loaded with (i) doxorubicin [1] and (ii) carboplatin [2] for passive delivery and magnetic (iii) nanoparticles or (iv) nanoclusters for the active delivery of sorafenib.

Electrochemical methods for the detection of the three chemotherapeutics were developed in parallel to determine the amount of drugs loaded and released from the DDS. The results obtained using the electrochemical methods were compared to those obtained by UV-Vis spectrophotometry and good correlations were observed. This demonstrates the potential of electrochemical methods to be used as alternative quality control strategies in pharamaceutical formulation.

Key words: nanosomes, magnetic nanoparticles, electrochemical sensors

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Practical and theoretical study of weak interactions between molecules

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In this thesis, we reviewed a specific type of weak intermolecular interactions, the so-called charge-transfer (CT) or electron donor-acceptor (EDA) complexes. In a theoretical overview of CT complexes, we have defined the donor and acceptor molecules that form such complexes. Based on the literature on EDA complexes, we selected tetracyanoethylene TCNE as an excellent acceptor molecule and aniline and his derivatives as a donor molecules.

Using Gaussian 16 [1] and GaussView software, [2] we also modelled these molecules.

The UV-VIS spectrophotometric analysis of the CT complexes TCNE-aniline and TCNE-2-chloroaniline and TCNE-o-toluidine is presented. Considering the solvent effect, [3] dichloromethane solvent was used for the measurements. In our work we also recorded the spectra of TCNE, aniline, 2-chloroaniline and o-toluidine in the visible range at T_1=295.15 K to see how the absorbance of CT complexes formed from donor and acceptor molecules changes in comparison. Based on the measurements, the value of the TCNE-2-chloroaniline CT complex λ_{max} was 578 nanometres, the maximum absorbance value was obtained at this wavelength, while the maximum absorbance of the TCNE-aniline CT complex was obtained at 588 nanometres.

The CT complexes of TCNE-aniline, TCNE-2-chloroaniline and TCNE-o-toluidine were also tested at 4 different temperatures: T_1=293.15K; T_2=295.15K; T_3=301.15K; T_4=303;15K for 5 different concentrations. The concentration of the TCNE acceptor molecule was 0.01 mol/dm^3 in all samples, and the concentrations of the aniline, 2-chloroaniline and o-toluidine donor molecules were 0.01mol/dm^3, 0.02 mol/dm^3, 0.03 mol/dm^3, 0.04 mol/dm^3 and 0.05 mol/dm^3 in the 5 different samples at all 4 different temperatures.

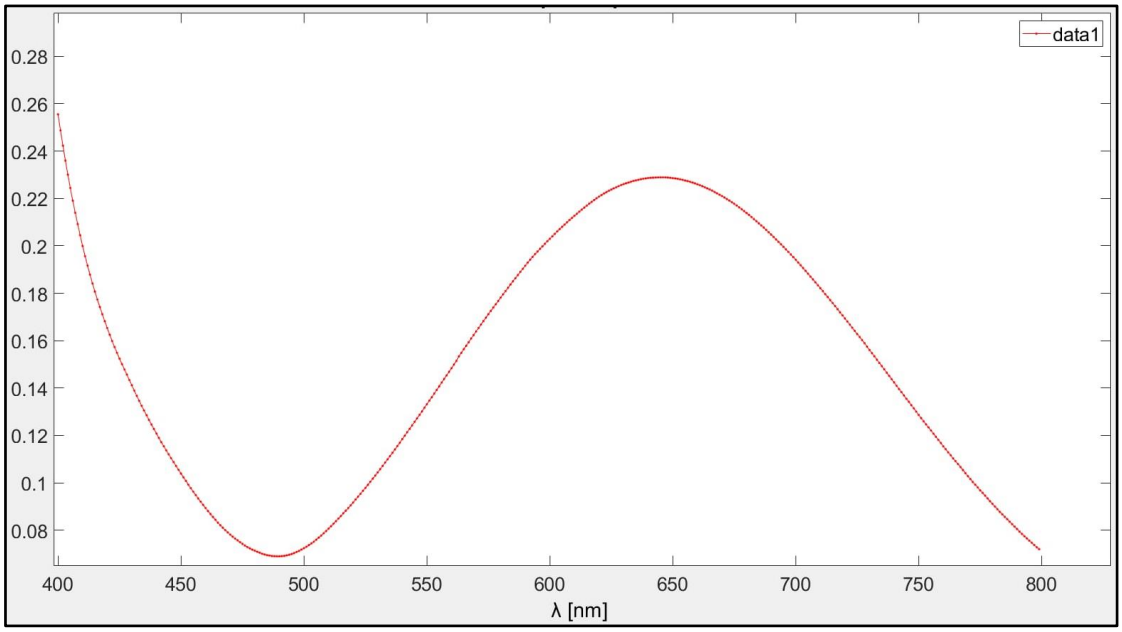

Figure 1 *Absorption spectra of the TCNE and o-toluidine CT complex at concentration of o-toluidin c_D=0.01M and concentration of TCNE c_A=0.01M at T_1 =295.15 K*

Source: own editing in MATLAB

Our aim in increasing the temperature was to investigate the effect on the stability of the complex and, based on this, to determine the thermodynamic state determinants of the complexation process. Using the linearized Benesi-Hildebrand or Scott equation, [4-5] we calculated the equilibrium constants K and the molar extinction coefficient ε and lnK values of the CT complexes TCNE-aniline, TCNE-2-chloroaniline and TCNE-o-toluidine at each temperature.

For the EDA aniline-TCNE complex, we estimated the following values: ΔH_x^0= -66.11 kJ/mol and process entropy ΔS_x^0 = -233 J/(K.mol). For the 2-chloroaniline-TCNE EDA complex, that provides the following values of thermodynamic state parameters: ΔH_x^0= -88.74 kJ/mol and process entropy ΔS_x^0 = -282 J/ (K. mol). Thus, we see that in the case of the 2-chloroaniline-TCNE complex, this process leads to a more significant decrease in entropy, i.e. we obtain a more disordered system. The enthalpy values indicate that the process is exothermic, with a decrease in enthalpy, so that the values of these two quantities suggest that the formation of CT complexes is a spontaneous process in which the rate of decrease in enthalpy is compensated by an increase in the entropy of the process.

The results have been compared with those available in the literature [6-7] and new results obtained in our study have been attempted to be justified in the light of these results.

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From Inception to Implementation: biNivo Biosimilar Product Development

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The development of biosimilars involves the creation of biological medicinal products that are highly similar to licensed reference products. This process focuses on reproducing the original active substance while allowing for minor differences that do not significantly impact clinical outcomes. Unlike generic drugs, biosimilars are complex proteins that may differ in structure and properties from the reference product due to the intricate manufacturing process involving the transfection of target cells and purification steps. The development process for biosimilars necessitates extensive analytical testing, including molecular structure evaluation, impurity profiling, and biological activity assessments *in vitro* and in human subjects. Bridging studies play a crucial role in expanding the reach of approved biosimilars to new regions. To this end, innovative statistical methodologies are required to design and analyze these studies. In conclusion, adherence to scientific and regulatory standards is essential for successfully developing and implementing biosimilar products with equivalent clinical profiles to innovative biotherapeutics. This poster presents the journey of a successfully implemented biosimilar monoclonal antibody from design to implementation.

Key words: Biosimilar, Product, Development

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Selective Biotransformations Mediated by hCAII

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Chiral alcohols are important building blocks widely used in the synthesis of pharmaceuticals, agrochemicals and fine chemicals and have received considerable attention in the field of catalysis. Human carbonic anhydrase II (hCAII) was reported that exhibited excellent catalytic activity and enantioselectivity in the reduction of prochiral ketones using silanes as hydride donor [1]. Carbonic anhydrases (CAs; EC 4.2.1.1) are mostly zinc-containing metalloenzymes, catalyzing the reversible hydration of carbon dioxide through a ping-pong mechanism. The CAs have been extensively studied due to their broad physiological importance in all kingdoms of life and clinical relevance as drug targets [2].

It was demonstrated that hCAII is a versatile biocatalyst for the abiotic reduction of variously substituted aryl-ketones. Various substrates were tested to monitor enzyme activity, selectivity and substrate tolerance. While the nature of the substrates had a slight impact upon the activity of the enzyme, the stereoselectivity of the reaction was strongly influenced by the size and the polarity of substituents. Normal-phase HPLC methods were developed for the chiral separation of the enantiomers of the produced alcohols to determine the enantiomeric excess, using different columns.

Key words: human carbonic anhydrase II, biocatalysis, chiral alcohols

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Chiral separation of synthetic cathinones by HPLC-UV using a Lux® i-Amylose-3 column

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In 2024, 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 Monitoring Centre of Drugs and Drug Addiction (EMCDDA) in recent years [1]. Among them, synthetic cathinones, which are often misleadingly traded as "bath salts," play an important role. They usually possess a chiral center, leading to the existence of two enantiomers with presumably different pharmacological properties. For newer compounds, little is known about the distinct effect of the enantiomers. The aim of this study was to test a commercially available Lux® i-Amylose-3 column by HPLC-UV for enantiorecognition of cathinone derivatives. Overall, 80 compounds were tested in normal phase mode, where 75 substances were separated under initial conditions. After method optimization, at least partial separation was achieved for the remaining compounds. The same set of substances was measured in polar-organic mode, where 63 analytes were resolved into their enantiomers under initial conditions with very short retention times. All measurements were carried out under isocratic conditions, and intraday and interday repeatability tests were performed. Both modes showed complementary results for the individual compounds [2]. Furthermore, the method in normal phase mode was tested on a real-life sample, the synthetic cathinone N-cyclohexyl methylone, which was identified in the course of a local drug checking program in 2023.

Key words: enantioseparation, amylose tris(3-chloro-5-methylphenylcarbamate), high-performance liquiid chromatography (HPLC), NPS

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Evaluation of the effectiveness of selenomethionine supplementation in patients with autoimmune thyroiditis

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Selenium in the form of the amino acid selenocysteine is a component of the active site of the enzymes glutathione peroxidase, thioredoxin reductase and iodothyronine deiodinase, which have an antioxidant effect, influence the immune system and play an important role in the metabolism of thyroid hormones. In areas with selenium deficiency, the occurrence and development of autoimmune diseases of the thyroid gland is more frequent due to the reduced activity of these enzymes. This study investigated the efficacy of selenomethionine supplementation in patients with Hashimoto's thyroiditis in relation to plasma selenium concentrations.

The efficacy of selenomethionine supplementation in patients with Hashimoto's thyroiditis was investigated in a study group of 43 subjects who were divided into three groups according to thyroid peroxidase antibody levels (TPOAb). A decrease in TPOAb levels was observed in 35% of the subjects after three months of supplementation, with the average baseline level decreasing by 15.5 %. The best effect was observed in subjects with a TPOAb concentration > 1000 IU/ml, with the TPOAb concentration decreasing in 53% of subjects. During this period, the average plasma Se concentration increased by 24%. A greater decrease in TPOAb concentration was observed after 6 months of supplementation, with TPOAb concentration decreasing by 29.5% of baseline concentration in 51% of subjects and by 17.8% in the last 3 months. The increase in selenium concentration in the second trimester was less significant compared to the first trimester. During supplementation, the concentration of thyroid hormones remained almost unchanged. In the placebo group, the plasma concentrations of Se and the antibody levels remained unchanged after three and six months of supplementation. Three months after the end of supplementation, the TPOAb concentration increased by 25.3%, while the selenium concentration almost returned to the initial value.

Key words: Hashimoto's thyroiditis, selenomethionine, thyroid antibodies

Optimization of DNA extraction from fruit juices for the differentiation of cultivars using high resolution melting analysis

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Fruit juices are one of the most consumed beverages worldwide. In the EU alone, 9.2 million litres of fruit juice and nectars were consumed in the year 2017. However, fruit juices are a common target for economically motivated adulteration, also known as food fraud. Therefore, it is of great interest to develop methods, which detect food adulteration. But previous studies show, that DNA extraction from juices is problematic due to their low concentration of intact DNA. Therefore, it is of great importance to optimize DNA extraction in order to obtain a sufficient amount of intact DNA for the analysis.

In this study, peel, pulp and four kinds of pear juices (with or without peel; high or low fibre-content) from three different pear cultivars (Anjou, Cheeky and Williams) were used. The pear juices were produced in-house. For DNA extraction, two commercial kits (NucleoSpin Plant II (Macherey-Nagel) and DNeasy mericon Food Kit (Qiagen)) were tested. The extracts were analysed using polymerase chain reaction (PCR) coupled with high resolution melting analysis (HRM). Two primer pairs targeting microsatellites, one common apple primer pair and one pear primer pair, were used.

Our results indicate that with DNeasy mericon Food Kit a higher concentration of intact DNA was obtained, leading to reproducible melting curves for peel, pulp and the four juices. The melting curves for peel, pulp and the four juices of the same variety had the same shape but differed from those obtained for the other two cultivars. Thus, our DNA extraction method combined with HRM analysis is applicable to differentiate pear cultivars in fruit juices.

Key words: food fraud, authenticity, fruit juices, DNA extraction, high resolution melting (HRM)

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How to develop a HPLC separation method to compare drug content of original Viagra samples with counterfeits

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This Workshop is intended to give a general overview how to develop a suitable HPLC method for quality control of active pharmaceutical ingredients. First, general considerations have to be made with respect of choosing the suitable equipment along with the appropriate detection system. For this purpose, the properties of the API to be analyzed have to be studied.

After best choice of the HPLC instrumentation a method has to be created or adapted from existing prescriptions. For this, the rule is to develop a method, which is as short as possible, but long enough to separate possible other ingredients. Several parameters can be altered to obtain optimal results. Also, the correct way to obtain reference standards has to be taken into account. The entire procedure will be presented by means of sildenafil citrate as an example. For a reliable separation method, method validation has to be performed.

In the second part of the workshop, the method to check for sildenafil content in tablets will be tested for its suitability by means of different formulations compared to the original formulation. Short clips of a working HPLC apparatus will be shown along with a suitable mobile phase. Parameters will be discussed and set, and sample preparation will be presented. A calibration curve will be created by means of different concentration points following the correct range. Then, the extracted and filtered samples will be analyzed and their areas under curve as well as retention times will be compared to those obtained with pure standards. Finally, values will be typed into an Excel sheet to display results.

In case, troubleshooting will be presented and explained.

Volatile and Semi-volatile Organic Compounds in the urban atmosphere of Skopje

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Emission of volatile (VOCs) and semi-volatile organic compounds (SVOCs) leads to a significant decrease in air quality and has a negative impact on climate change, human health and the biosphere. The main focus of the work was the monitoring of volatile organic compounds (VOCs) and semi-volatile organic compounds (polycyclic aromatic hydrocarbons, organochlorine pesticides and polychlorinated biphenyls) in the urban air of Skopje, Macedonia during different seasons in 2021 and 2022 using passive diffusion on Radiello® and polyurethane foam adsorbents, respectively.

In total, 82 volatile and 37 semi-volatile organic compounds were identified and semi-quantified. Most of the VOCs can be attributed to emissions from transport fuels and their derivatives, such as BTEX (benzene, toluene, ethylbenzene and xylenes) and alkanes (*n*-undecane, *n*-dodecane, *n*-tridecane and *n*-tetradecane). Terpenes (α-pinene, β-pinene and limonene) were detected in many samples, which may originate from natural sources, waste or cleaning agents. The identified semi-volatile organic compounds included 12 polycyclic aromatic hydrocarbons (PAHs), 18 organochlorine pesticides (OCPs) and 7 polychlorinated biphenyls (PCBs). OCPs and PCBs are hardly degradable chemicals emitted from the landfill of the former OHIS factory in Skopje and the detected PAHs can be attributed to various anthropogenic activities in the city (such as incomplete combustion of fossil fuels, motor vehicle emissions, domestic heating, waste incineration, industry etc.).

The estimated concentrations of the detected VOCs were below the reference concentration levels that cause critical effects established by the EPA. However, for the semi-volatile compounds, the highest concentration levels were observed for hexachlorocyclohexane (HCH) isomers (α-HCH, β-HCH, and γ-HCH), exceeding the maximum acceptable toxicant concentration (MATC) in the period from April to August 2022 near the former factory. Among the 12 detected PAHs, naphthalene, phenanthrene, fluoranthene and pyrene were most abundant in the air during the sampling period and their presence and levels should be regularly monitored.

Key words: Volatile Organic Compounds, Semi-volatile Organic Compounds, Air Pollution

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The support for this study received from the Macedonian Ecological Society within the project "Development and application of methods for monitoring of volatile and semi-volatile organic compounds in the air of Skopje" financed under the grant program for young ecologists is gratefully acknowledged.

Taylor-Aris Dispersion Assisted ESI-MS applied in a simple flow injection system

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Our research group have recently demonstrated the utility of the Taylor–Aris (TA) dispersion in avoiding serious interference issues commonly occurring in the electrospray ionization-mass spectrometric (ESI-MS) determination of protein samples with high, MS incompatible matrix content without pre-separation or sample purification. It was pointed out that the conditions needed for TA dispersion and the utilization of this technique can be easily accomplished using commercial CE-MS instrumentation. The proposed Taylor–Aris dispersion-assisted mass spectrometric (TADA-MS) analysis used a 65 cm long 50 μm i.d. capillary, 0.5 μL sample was injected and pumped toward the MS with 1 bar pressure. This is an efficient procedure for the direct injection analysis of components having low diffusion coefficients such as proteins that are present in complex matrices (small organic or inorganic compounds) [1].

In this work, we demonstrate the usability of a simple, low-pressure liquid apparatus replacing the CE device to simplify the system. This was achieved by setting a syringe pump to the appropriate speed to creating a constant and low flow rate in a capillary. One end of the capillary was connected to a sampling valve and the other end to the mass spectrometer. The sample was inserted into the sampling valve with a syringe. The characteristic Taylor–Aris dispersion was observed in this liquid system. We use the developed system for the direct analysis of intact proteins under denaturing and native conditions.

Key words: Mass spectrometry, Taylor-Aris dispersion, Proteins

Acknowledgements

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Study of Flow Conditions for Taylor–Aris Dispersion Assisted Mass Spectrometry

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Electrospray ionization mass spectrometry (ESI-MS) serves as an essential tool for analysing protein samples. However, ESI-MS incompatible matrix components typically hinder direct analysis without prior separation or sample purification. We have recently demonstrated the utility of Taylor-Aris Dispersion in the direct injection analysis of large molecules in MS incompatible matrices, such as mAbs in their original formulation or native protein complexes in PBS solution, facilitating simple, rapid and cost-effective analysis [1].

Finding the ideal conditions for these analyses can be challenging since Taylor-Aris Dispersion is affected by numerous parameters. In Taylor Dispersion Analysis the diffusion coefficient of the analyte is determined by the observed peak variance based on a formula describing the theoretical concentration distribution [2]. We found that with slight adjustments, this formula can be used to describe the expected taylograms under different conditions. The signal suppression caused by matrix components during ionization were described according to Donelly et al. [3]. Utilization of these formulas enabled the in-silico investigation on the effects of conditions for Taylor-Aris Dispersion Assisted Mass Spectrometry.

Key words: Mass spectrometry, Taylor-Aris dispersion, Proteins

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Aptasensing approaches for the electrochemical detection of *Staphylococcus aureus*

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Introduction and objectives: Worldwide, a *silent pandemic* threatens the healthcare systems, as antimicrobial resistance (AMR) cases are reported to grow exponentially. Among the bacterial species that are highly resistant to the available antibiotherapy, *Staphylococcus aureus* (*S.aureus*) is responsible for almost 70% of the reported cases of resistant hospital aquiered infections, along with *Escherichia coli* and *Klebsiella pneumoniae* [1]. As conventional detection methods can take up to 72 hours to establish a diagnostic, new analytical strategies are necessary to fight AMR. Electrochemical techniques, combined with nanomaterials and biorecognition elements (such as aptamers), may represent a solution in terms of rapidity and selectivity, by incorporating them into portable sensors. This study focused on the development and optimisation of an electrochemical aptasensor for the fast detection of protein A (PrA) from the surface of the bacterial wall of *S.aureus* [2].

Material and methods: The sensor was developed on gold screen printed electrodes (AuSPE), on which the specific aptamer for PrA was immobilised via multipulse amperometry (MPA) and the unbound sites were covered using a blocking agent. The optimised version of the sensor implied using complementary DNA sequences (cDNA) to enhance the selectivity and titanium carbide (Ti_3C_2 MXene) structures to improve the analytical response [3] . The electrode surface was analysed using pulse voltammetry (DPV), cyclic voltammetry (CV) and electrochemical impedance spectroscopy.

Results and discussions: A wide range of concentrations of the target (PrA) were incubated on the surface of the sensor, being tested in both spiked TRIS buffer solution and real samples (bacterial cultures and artificial human serum).

Conclusions: Electrochemical biosensors using an aptamer sequence and cDNA/MXene were optimised and developed for the fast detection of *S.aureus* and its specific marker (Pr A) from both enriched samples and real bacterial culture and human serum samples.

Key words: protein A, *Staphylococcus aureus*, aptamers, electrochemical methods, real samples

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CFTR Drug Interactions and Their Impact on Ion Conduction

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CFTR Cystic Fibrosis Transmembrane Regulator is a cyclic AMP-regulated ion channel that transports small ions like Cl^- and HCO_3^- across epithelial cells. Loss-of-function mutations of the transporter give rise to Cystic Fibrosis (CF), a multiorgan disease that declines patient health and quality of life. Our work is focused on mutations, which are related to dysfunctionality produced by improper folding. The most broadly studied mutation and the most abundant alteration in CF patients is F508del, which reduces the thermal stability of NBD1 and uncouples the NBD1 and TMD2 interface, interfering with folding. Other mutations like G551D directly affect the function of the transporter, producing diminished ion conduction.

Current drugs like Ivacaftor (VX-770) potentiate chloride transport. This compound with correctors Elaxacaftor (VX-445) and Tezacaftor (VX-661) is commercialized as TRIKAFTA to improve efficacy in rescuing F508del. These targeted drugs are promising therapeutics to increase the life span of CF patients. However, they produce intense side effects, and their complete mechanism of action at an atomic level remains unknown.

We aim to better understand the drug effects in ion conduction likely produced by allostery since the drug-binding sites are neither close to the channel pore nor the mutation located in NBD1. Current CF-drugs affect channel conformations, increasing ion conduction. One of the factors driving the transport of chloride ions is the permeability of the pathway, which decreases as a function of anion size; molecules with a diameter larger than 5.3 Å cannot pass the narrowest part of the pore. The pore is usually sampled in its closed conformation, so exploring open conformations is crucial to elucidate more atomic-level details about the transport phenomena.

To sample open conformations, we retrieved CFTR protein structures from the RCSB experimental database or modelled them using AlphaFold2 DeepMind. All structures were subjected to equilibrium MD simulations. We used Python and Bash scripts to calculate the contact maps of the amino acids 4.5 Å around Ivacaftor (VX-770), Elaxacaftor (VX-445), and Tezacaftor (VX-661). We evaluated amino acid conformational changes around the binding sites of CF-drugs. Our preliminary results provide insights into the intermolecular interactions that lead to channel opening. This work is crucial for designing targeted treatments that address broader mutational defects with fewer side effects, potentially leading to more effective therapies for CF patients.

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Artificial metabolic systems for synthetic or analytical purposes using microfluidic enzymatic devices

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Nanotechnology and flow chemistry are among the fastest developing fields of science. Magnetic nanocatalysts containing porphyrin Fe(II)-complexes was successfully applied for biomimetic oxidation of antihypertensive drug amlodipine in batch and continuous-flow reactors (Fig. 1).

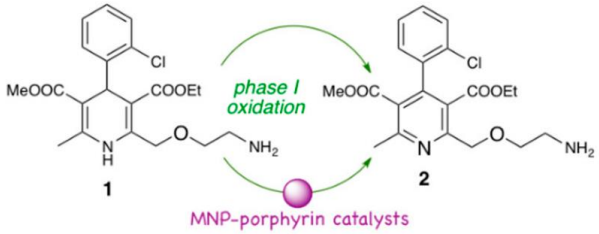

Figure 1.

The immobilized catalysts integrated in a continuous-flow magnetic chip reactor was also efficiently used for the synthesis of new pharmaceutically active derivatives and liver related phase I oxidative major metabolite of another widely used drug, the antiarrhythmic amiodarone (Fig. 2).

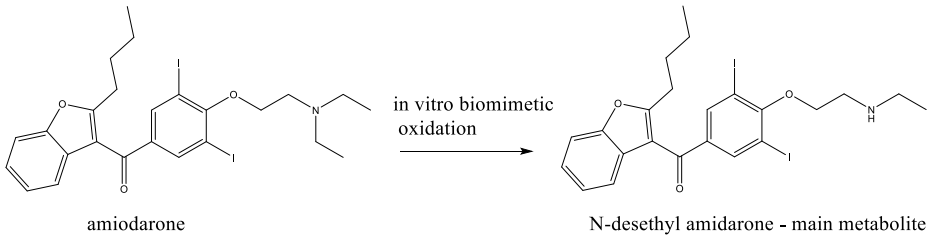

Figure 2.

With the integration of metalloporphyrines into microfluidic magnetic chip reactors drug metabolites can be produced effciently in extremely small reactor volumes, resulting an excellent volumetric productivity. In addition, not only the in vivo major metabolite can be easily synthetized by the microfluidic magnetic "Liver-on-a-chip" system, but also new derivatives can be produced opening up unique novel opportunities for modern drug discovery.

Key words: magnetic nanoparticles, drug metabolism, chip reactor

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Influence of volatile oils on the bioavailability and efficacy of topical fixed-dose combinations

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Introduction and objectives: Volatile oils are essential oils known for their aromatic properties and therapeutic benefits. They have been used in traditional medicine for centuries and are now being incorporated into modern pharmaceutical formulations. The integration of volatile oils in topical fixed-dose combinations (FDCs) has garnered significant interest due to their potential to enhance drug bioavailability and therapeutic efficacy. Volatile oils, such as eucalyptus, basil, lavender, or tea tree oil, possess unique physicochemical properties that facilitate transdermal drug delivery [1]. By carrying out a review of the most recent publications with reference to the properties of volatile oils, it was proposed to elucidate some specific features of the mechanisms and the possibilities of their involvement on the bioavailability and effectiveness of topical preparations at the stage of development and research.

Results and discussion: The mechanisms through which volatile oils influence the bioavailability and efficacy of active pharmaceutical ingredients (APIs) in topical FDCs are different. Enhancement of skin permeability is one of the main mechanisms, thus, volatile oils can disrupt the stratum corneum, the outermost layer of the skin, enhancing the permeability and facilitating deeper penetration of APIs. Also, they can act as solvents for lipophilic drugs, improving their solubility and, consequently, their absorption through the skin. Some volatile oils exhibit anti-inflammatory and antimicrobial properties, which can synergize with APIs to enhance therapeutic outcomes. Studies have shown that volatile oils can significantly increase the bioavailability of drugs in topical FDCs. For instance, eucalyptus oil has been demonstrated to improve the penetration of anti-inflammatory agents, leading to higher local concentrations at the site of action. The efficacy of topical FDCs containing volatile oils is often superior to formulations without them. Clinical trials and in vivo studies indicate that the inclusion of volatile oils can lead to faster and more pronounced therapeutic effects, particularly in the treatment of skin conditions like eczema, psoriasis, and localized infections [2].

Topical therapy of inflammatory ear infections (otitis, otomycosis) can also be improved by including volatile oils in fixed-dose combinations of antibacterial and antifungal drugs. Many volatile oils possess intrinsic anti-inflammatory and analgesic properties. These properties can reduce local inflammation and pain in the ear. By decreasing inflammation, volatile oils can reduce swelling and

congestion in the ear canal, improving the diffusion and bioavailability of the ear drop's active ingredients. Reduced pain and inflammation can lead to increased patient compliance with the treatment regimen, indirectly enhancing the overall efficacy of the treatment. Volatile oils often have strong antimicrobial properties. When combined with antibiotics or antifungal agents, volatile oils can enhance the antimicrobial effect by attacking the pathogens through multiple mechanisms, most of the time volatile oils can prevent the formation of biofilms, which are protective layers created by bacteria. This makes the bacteria more susceptible to the ear drops. Synergistic effects can result in increased potency (allowing for lower doses of the primary drug while achieving the same or better therapeutic outcomes), or broad-spectrum activity (the multi-faceted approach of volatile oils can target a wider range of pathogens and conditions, making the ear drops more effective overall). Some volatile oils can act as natural preservatives, enhancing the stability and shelf-life of ear drop formulations by preventing oxidation and inhibiting microbial growth.

Basil volatile oil, derived from Ocimum basilicum, is renowned for its aromatic and therapeutic properties. Recent studies carried out within the Scientific Center of Medicines of the Nicolae Testemiţanu State University of Medicine and Pharmacy from the Republic of Moldova demonstrated its potential to enhance the efficacy of antibacterial and antifungal agents, possess a synergistic effect that can improve treatment outcomes.

Basil volatile oil is rich in bioactive compounds such as eugenol, linalool, and methyl chavicol, which exhibit significant antimicrobial properties. The synergistic effects of these compounds when combined with conventional antimicrobial agents can offer a potent alternative in managing bacterial and fungal infections of ear.

Studies have demonstrated that basil volatile oil can significantly enhance the antibacterial activity of ciprofloxacine against strains such as *Staphylococcus aureus* and *Escherichia coli*. Basil volatile oil has also shown synergistic effects with antifungal agents such as fluconazole, econazole and ketoconazole. This combination is particularly effective against fungal strains like *Candida albicans* and *Aspergillus niger*, where the oil enhances the antifungal agent's penetration and disrupts fungal cell wall integrity.

Conclusion. The synergistic effects of basil volatile oil with conventional antibacterial and antifungal agents highlight its potential as an adjunct therapy in the treatment of microbial infections.

Keywords: volatile oils, bioavailability, topical fixed-dose combinations

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Intact Protein Analysis of Snake Venoms with CZE-MS

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Venoms consist of several biologically active components, primarily peptides and proteins. these toxin components have the potential to cause lethal effects [1]. Top-down mass spectrometric technique when combined with capillary zone electrophoresis, becomes highly effective in studying the structural and dynamic characteristics of intact proteins. This methodology can be utilized for the analysis of complex protein molecules such as snake venom [2]. In this research work we demonstrated the potential analytical performance of capillary zone electrophoresis coupled with mass spectrometry (CZE-MS) for the intact protein analysis of similar venom samples. Using 1 M formic acid (pH=1.9) as BGE, minimal adsorption and narrow peaks shapes - thus good separation efficiencies - were obtained for the protein components of the venom samples. The precision of migration times and peak areas were 1.9-2.8 RSD% and 0.8-7.2 RSD%, respectively and the theoretical plate numbers were 32000-238000 for peaks having signal-to-noise ratio (S/N) larger than 50.

More than 250 different toxin components (7-10 kDa) were detected in the venoms obtained from snakes of 9 different subspecies (belonging either to Naja or Dendroaspis species). The protein contents of the venoms of the same subspecies collected from different geographical regions are similar and differ only in a few (less than 10%) components. However, the venoms collected from different organism (within the same species) exhibit very different protein patterns.

Notably, our findings revealed discrete protein patterns among venoms from different subspecies, highlighting the unique fingerprinting potential of venom in various snake populations.

Key words: snake venom, intact protein analysis, capillary electrophoresis-mass spectrometry

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Structural and Nanomechanical Analysis of Membrane Disruption Induced by Photosensitization

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This study investigates the impact of reactive oxygen species (ROS) on supported lipid bilayers (SLBs) to enhance understanding of ROS effects on membranes in disease and therapy. We used a porphyrin derivative dye, irradiated at disease sites, to generate ROS, which are confined to areas where the photosensitizer accumulates. Since photosensitizers typically localize in lipid membranes, we focused on photoinduced damage to these membranes. SLBs were created by depositing DPPC or DPPC/DOPC (70/30 molar %) liposomes on mica. Topological changes were mapped using non-contact mode AFM imaging in buffer solution, while nanomechanical alterations were assessed through force spectroscopy. Direct ROS addition via hydrogen peroxide was also tested. AFM images showed that ROS formation disrupted porphyrin-bearing DPPC-DOPC membrane integrity, creating nanoscopic pores post-irradiation. Force spectroscopy indicated membrane stiffening, with rupture forces increasing after irradiation, especially in the presence of DOPC, highlighting unsaturated lipids' role in ROS-mediated disruption. FTIR spectra were recorded to analyze chemical changes.

Key words: ROS, membrane damage, AFM

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