CECE 2023

18th International Interdisciplinary Meeting on Bioanalysis

"… bringing people
and ideas together …"

October 23 – 25, 2023
Hotel Continental
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Organizing committee:
Frantisek Foret, Jana Lavicka, Jan Prikryl, Petr Kuban

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JRWN s. r. o., Jan Prikryl

Find the meeting history and more at www.ce-ce.org.

Foreword

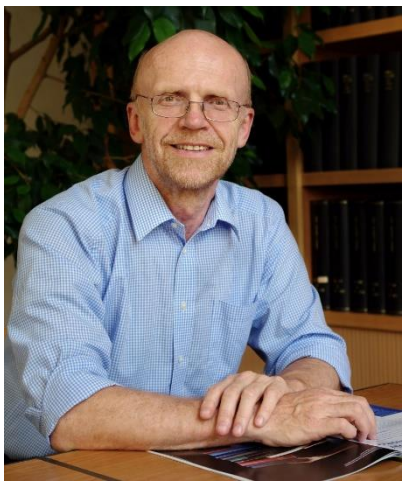
Welcome to CECE 2023, the 18th International Interdisciplinary Meeting on Bioanalysis. As in previous years, our goal is to "bring together scientists from different disciplines who may not meet at other meetings". Lectures by well-established scientists and presentations by students and postdocs will be complemented by poster sessions open during all three days. The organizers thank all speakers, sponsors, and participants for their support. Please check our web at www.ce-ce.org for more information about the history, programs, photos, and videos.

Brno, October 20, 2023

The Medal of Jaroslav Janák

The Medal of Jaroslav Janák for contributions to the development of analytical sciences was established by the Institute of Analytical Chemistry. Named after the inventor of the gas chromatograph (patented in 1952), the founder of the institute (1956), and its long-term director, the medal is awarded to scientists who have significantly contributed to the development of separation sciences.

In 2023, the Medal of Jaroslav Janák goes to **Prof. Bohuslav Gaš**. Bob studied at the Charles University in Prague. After finishing his doctorate in 1979, he worked at the Research Institute of Organic Syntheses and the Institute of Physical Chemistry and Electrochemistry of the Czechoslovak Academy of Sciences. In 1994, Bob returned to his Alma Mater to pursue the theory and instrumentation of electromigration separation methods. Since 2005, he has been a professor at the Department of Physical and Macromolecular Chemistry. He has served two terms as a dean of the Faculty of Natural Sciences. Besides serving as the deputy editor of Electrophoresis (Wiley VCH), he is also a passionate lecturer of physical chemistry highly prized by his students. In 2023, he received the Velemlok prize for the best lecture from his students. Other awards include medals for the development of Charles University and the Arnold O. Beckman medal for exceptional lifelong contributions to the theoretical understanding and development of new electro-driven separation techniques (2016). He has authored seven international patents and over 140 papers published in impacted journals, which collected over 4000 citations.



I wish to congratulate Bob on all his achievements and thank him for all his work for the analytical chemistry community and science in the Czech Republic.

Franta Foret

Program of the CECE 2023

Hotel Continental, Brno, Czech Republic, October 23 – 25, 2023

October 23, 2023 (Monday)

12:00		Opening the Registration Desk
13:00-13:15		**Opening Remarks and Award of the Medal of Jaroslav Janak** Chair: Frantisek Foret
		Session 1 Chair: Frantisek Foret
13:15-13:45	**O1**	Fate of One Equation **Bohuslav Gas**, *Charles University, Prague, Czech Republic*
13:45-14:15	**O2**	Intact Protein Analysis in Biological Matrices by Capillary Zone Electrophoresis – Mass Spectrometry **Katarina Marakova**, *Comenius University Bratislava, Bratislava, Slovakia*
14:15-14:45	**O3**	Recent Advances in Electroextraction Techniques: A Perspective from a Brazilian Research Group **Ricardo Mathias Orlando**, *Federal University of Minas Gerais, Belo Horizonte, Brazil*
14:45-15:05	**J1**	Detection of Protein Biomarkers Using Lateral Flow Immunoassays Based on Photon-Upconversion Nanoparticles **Eliska Machacova**, *Masaryk University, Brno, Czech Republic*
15:05-15:35		**Coffee Break**
		Session 2 Chair: Petr Kuban
15:35-16:05	**O4**	Integration of Metabolic Sensors in Musculoskeletal Organs-on-a-Chip in Osteoarthritis Research **Mario Rothbauer**, *Medical University of Vienna, Vienna, Austria*
16:05-16:35	**O5**	Capillary Electrophoresis with Mass Spectrometry: From Enantiomers to Nanoparticles **Jan Petr**, *Palacky University, Olomouc, Czech Republic*

16:35-16:55	**J2**	Solid Phase Extraction Methods for Purification of Positively Charged Oligosaccharides and Glycans **Denisa Smolkova**, *Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic*

October 24, 2023 (Tuesday)

	Session 3 Chair: Andras Guttman	
08:30-09:00	**O6**	Design and Development of Coating Materials with Ionic Liquids/Surfactants on Iron Oxide-based Magnetic Nanoparticles and 3D-printed Silica/Thermoplastic Polymer Composites as Extraction Sorbent Materials in Drug Analysis **Tomasz Baczek**, *Medical University of Gdansk, Gdansk, Poland*
9:00-9:30	**O7**	Noncovalent Biomolecular Interactions Studied by Affinity Capillary Electrophoresis **Vaclav Kasicka**, *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
09:30-9:50	**J3**	Tracking of the pH Gradient During cIEF-LIF Analysis Using Fluorescent Isoelectric Point Markers and Their Application in Efficient Separation of Protein Samples **Pavlina Dadajova**, *Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic*
9:50-10:10	**J4**	Body-on-a-Chip Technologies Towards Neurotoxic Monitoring and Environmental Safety/CBRN Applications **Mateo G. Vasconez Martinez**, *Vienna University of Technology, Vienna, Austria*
10:10-10:40	**Coffee Break**	
	Session 4 Chair: Tomasz Baczek	
10:40-11:10	**O8**	Non-Destructive Forensic Document Examination of Ballpoint Cationic Inks by Blotting-Capillary Electrophoresis **Doo Soo Chung**, *Seoul National University, Seoul, Korea*
11:10-11:40	**O9**	The Role of IgA *N*-Glycosylation in the Development of Oral Mucositis **Eniko Gebri**, *University of Debrecen, Debrecen, Hungary*

11:40-12:00	**J5**	Magnetic Microparticles as Potential Stationary Phase for Affinity Studies in Capillary Electrochromatography **Michal Sedlak**, *Masaryk University, Brno, Czech Republic*
12:00-12:20	**J6**	Exploring the Feasibility of Animal-Free Protein Analysis in Molecular Osteoarthritis Research **Eva Ingeborg Reihs**, *Medical University of Vienna, Vienna, Austria*
12:20-14:20	**Lunch Break – Hotel Restaurant** **Poster Session**	
	Session 5 Chair: Frantisek Foret	
14:20-14:50	**O10**	Analysis of Glycans for Disease Diagnostics **Jan Tkac**, *Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia*
14:50-15:20	**O11**	Two Columns, More Than Double Band Broadening - Dilution Issues in Comprehensive Two-Dimensional Liquid Chromatography **Petr Cesla**, *University of Pardubice, Pardubice, Czech Republic*
15:20-15:40	**J7**	A New Device for Online Nanoscale Sampling and Capillary Electrophoresis Analysis of Plant Sap Composition **Natalie Melicherova**, *Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic*
15:40-16:00	**J8**	Exploration of a Glycan Biomarker Panel for the Early Screening of Type 2 Diabetes **Rebeka Torok**, *University of Pannonia, Veszprem, Hungary*
16:00-16:30	**O12**	There and Back Again: One Person's Struggle Between Industry and Academia and How It Relates to Ionization Efficiency **Michael Volny**, *Czech Academy of Sciences, Prague, Czech Republic*
19:00	**Conference Dinner – Hotel Restaurant**	

October 25, 2023 (Wednesday)

	Session 6 Chair: Doo Soo Chung	
9:00-9:30	**O13**	Electromigration Dispersion in SDS-CGE of Proteins **Andras Guttman**, *University of Debrecen, Debrecen, Hungary*
09:30-10:00	**O14**	Automated Analyses of Dried Blood Spots by Capillary Electrophoresis **Milos Dvorak**, *Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic*
10:00-10:20	**J9**	Separation Tools and Infectious Diseases: Feasibility Study of a High Throughput Extracorporeal Virus Capture Device **Dora Szerenyi**, *University of Pannonia, Veszprem, Hungary*
10:20-10:50	**Coffee Break**	
	Session 7 Chair: Frantisek Foret	
10:50-11:20	**O15**	The Art of Liquid Biopsy: Clinical Considerations and Analytical Approaches in Noninvasive Molecular Cancer Diagnostics **Marek Minarik**, *Watrex Praha, s.r.o. and Charles University, Prague, Czech Republic*
11:20-11:50	**O16**	Separation and Analysis of Proteins Using SDS Capillary Gel Electrophoresis Online Coupled to Electrospray Ionization Mass Spectrometry **Gabor Jarvas**, *University of Pannonia, Veszprem, Hungary*
11:50-12:10	**J10**	Epitachophoresis - Stabilization of the LE/TE Border **Vanda Kocianova**, *Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic*
12:10-12:30	**J11**	GC×GC for the Determination of Hypercholesterolemic Fatty Acids in Cow Colostrum During the First Days of Lactation **Veronika Farkova**, *Masaryk University, Brno, Czech Republic*
12:30-12:40	**Closing Remarks**	
12:40	**Lunch – Hotel Restaurant** **Poster Removal**	

List of poster presentations

P1 Separation of Sodium Dodecyl Sulfate-Proteins by Capillary Electrophoresis Using Dilute and Ultra-Dilute Dextran Solutions
Felicia Auer, Andras Guttman

P2 Impact of Heavy Water on Luminescence of Quantum Dots
Jan Badin, Josef Kučera, Ivona Voráčová, Petr Táborský

P3 Optimization of Glycoproteomic Protocol for Efficient and Sensitive Analysis of *N*-Linked Glycans by CE/LIF
Janette Bobalova, Denisa Smolkova, Dana Strouhalova, Richard Cmelik, Jana Lavicka

P4 Improvement of Concentration Sensitivity in Capillary Electrophoresis-Frontal Analysis
Taťána Bržezická, Lenka Kohútová, Hana Mlčochová, Zdeněk Glatz

P5 BODIPY-based Fluorescent Tag for Efficient Oligosaccharide Labeling
Richard Cmelik, Denisa Smolkova, Michal Gregus, Hubert Vesely, Hana Pizova, Pavel Bobal, Jana Lavicka

P6 UHPLC-MS Analysis of Salivary Bile Acids in Non-Invasive Diagnostics of Barrett's Esophagus
Věra Dosedělová, Markéta Laštovičková, Jiří Dolina, Štefan Konečný, Petr Kubáň

P7 Determination of Lamotrigine in Alternative Types of Samples Utilizing Validated LC-MS Method
Viktória Ďurčová, Marta Pelcová, Jan Juřica, Zdeněk Glatz

P8 Effect of COVID-19 Disease and Vaccination on Salivary Secretory IgA *N*-Glycome
Anna Farkas, Petra Polyák, Enikő Gebri, Kinga Bágyi, András Guttman

P9 Use of Calcium Chloride for Removal of Polysaccharides During DNA Isolation from Tropical Fruit Products
Lenka Fialova, Katerina Zylkova, Ivana Marova

P10 Importance of Glycosylation Modification of Sialoglycoproteins in Dementia
Eniko Gebri, Timea Kulcsar, Andras Guttman

P11 *N*-Glycomic Identification of Novel Soft Tissue Prognostic Biomarkers for Oral Cancers
Eniko Gebri, Kinga Hogyor, Adrienne Szabo, Gabor Jarvas, Zuzana Demianova, Andras Guttman

P12 UHPLC-HDMS^E Analysis of Lipids in Glial Cells
Ivana Gerhardtova, Timotej Jankech, Petra Majerova, Dominika Olesova, Josef Jampilek, Andrej Kovac

P13 Analysis of Peptides and Proteins by Native and SDS Capillary Gel Electrophoresis Coupled to Electrospray Ionization Mass Spectrometry
Daniel Sarkozy, Andras Guttman

P14 Single Drop Microextraction for Enhanced Detection in Glycan Analysis by Capillary Electrophoresis
Eniko Gebri, Sunkyung Jeong, Anna Farkas, Doo Soo Chung, Andras Guttman

P15 Preparative Gel Electrophoresis for the Fractionation of DNA
Helena Hrušková, Roman Řemínek, František Foret

P16 UHPLC-MS/MS Strategy for Tryptophan Metabolite Analysis Based on Simple Sample Derivatization Using 2-Bromo-4'-Nitroacetophenone
Timotej Jankech, Ivana Gerhardtova, Petra Majerova, Juraj Piestansky, Josef Jampilek, Andrej Kovac

P17 Inhaled Lead Nanoparticles Cause Degenerative Alterations in Brain Tissue: Interdisciplinary Methodological Approach
Adriena Jedličková, Daniela Kristeková, Jana Dumková, Tomáš Vaculovič, Pavel Mikuška, Marcela Buchtová

P18 Synthesis of Silver-Iron Oxides Nanocomposites and Their Application for SERS
Vladimir Jonas, Jiri Volanek, Duy Hai Bui, Jan Prikryl, Thi Thanh Ngan Nguyen, Thu Vu, Anna Tycova

P19 Development of the Miniaturized Instrumentation for Caspase Activity Detection
Michael Killinger, Barbora Veselá, Eva Matalova, Karel Kleparnik

P20 Enhancing Sensitivity with Different Injection Modes for Amino Acid Structure Compounds by MEKC
Piotr Kowalski, Michał Pieckowski, Ilona Olędzka, Anna Roszkowska, Alina Plenis, Tomasz Bączek

P21 HPLC-FLD Method Development for the Determination of Alpelisib in Human Plasma
Eva Krejčířová, Marta Pelcová, Jan Juřica, Zdeněk Glatz

P22 Differentiation of Biofilm-Positive and Biofilm-Negative Candida Parapsilosis Strains by Capillary Isoelectric Focusing
Anna Kubesová, Jiří Šalplachta, Filip Růžička, Veronika Holá

P23 Characterization of the Miniaturized Platform for Cell Migration Studies
Petra Lišková, Adriena Jedličková, Michael Killinger, Marcela Buchtová, Tomáš Václavek

P24 The Ionic Liquids as Supporting Agents for Extraction and Electrophoretic Separation of Clinically Important Catecholamines
Ilona Olędzka, Natalia Kaczmarczyk, Piotr Kowalski, Anna Roszkowska, Alina Plenis, Tomasz Bączek

P25 Sample Pretreatment Methods for CZE-MS Analysis of Selected Intact Growth Factors in Various Biological Fluids
Martina Opetová, Radovan Tomašovský, Peter Mikuš, Katarína Maráková

P26 HPLC Analysis of Vancomycin and Magnetic Nanoparticles Modified with Chitosan and Vancomycin
Jakub Podhajský, Miloš Barna, Kristýna Burešová, Jan Raja, Pavel Melichercik, Eva Klapková, Jana Čepová, Julia Werle, Luděk Melich, Karel Kotaška, Richard Průša, Rene Kizek

P27 Miniaturized System for Capillary Electrophoresis Coupling with Mass Spectrometry Detection
Roman Řemínek, Tomáš Václavek, Elizaveta Vereshchagina, Andreas Vogl, František Foret

P28 Analysis of Low Numbers of Bacterial Cells by CE and MALDI-TOF MS Utilizing Roughened Capillary
Jiří Šalplachta, Anna Kubesová, Pavel Karásek, Filip Růžička, Michal Roth

P29 Capillary Electrophoresis-Mass Spectrometry Analysis of Insulin-like Growth Factor 1 in Pharmaceutical Preparations
Radovan Tomašovský, Martina Opetová, Peter Mikuš, Katarína Maráková

P30 Novel Fluorophores Applicable for Glycan Detection
Hubert Vesely, Hana Pizova, Pavel Bobal

P31 Synthesis and Modification of Magnetic Nanoparticles for Bioanalytical Applications
Jiri Volanek, Anna Tycova, Vera Dosedelova, Petr Kuban

P32 Epitachophoresis – New Insert for Horizontal Gel Electrophoresis Device
Markéta Vaňková, Ivona Voráčová, Antonín Hlaváček, František Foret

P33 New Design of APCI/APPI Ion Source for Low Flow Rates
Vladimír Vrkoslav, Kateřina Pražáková, Barbora Kloudová, Ondřej Pačes, Josef Cvačka

Acknowledgement

MEYS V4-Korea Joint Research Project 9F23002
„ATBG“

MSB 2024

May 19 – 22, 2024
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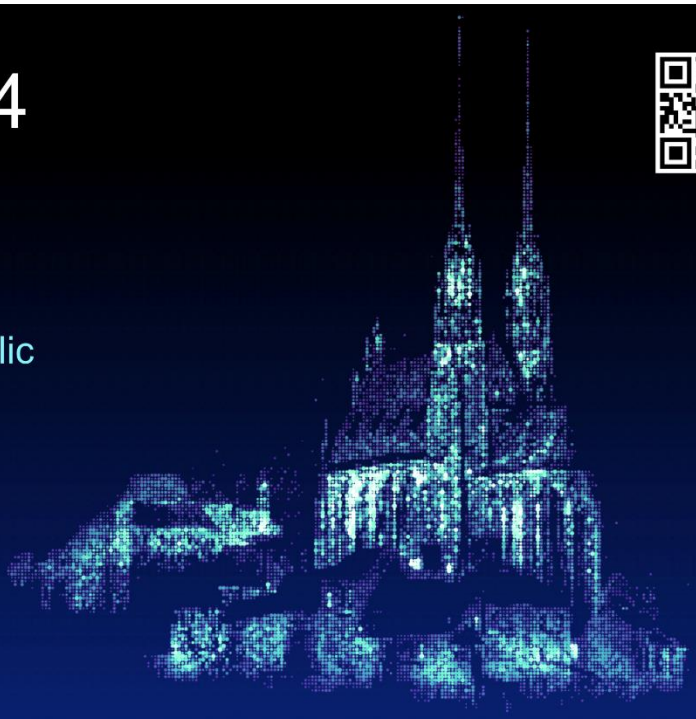

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Microscale Separations and Bioanalysis

Conference Topics

Capillary electrophoresis	Bioinformatics and AI	Pharmaceutical analysis
Liquid chromatography	Instrumentation	Biomedical analysis
Multidimensional separations	Sample preparation	Food analysis
Mass spectrometry	Miniaturization	Forensic analysis
Hyphenated techniques	Microfluidics	Environmental analysis

Abstract Submission Deadlines

Oral presentations: February 16, 2024 **Poster presentations: April 30, 2024**

Registration fees	General	Student
Early bird (until March 31, 2024)	EUR 450	EUR 300
Regular (until May 10, 2024)	EUR 550	EUR 350
On-site	EUR 650	EUR 400

Organizing Committee

Frantisek Foret (chair)
Jana Lavicka, Jan Prikryl, Petr Kuban
Institute of Analytical Chemistry of the CAS, Brno, Czech Republic

iac brno

Abstracts

O – oral presentation by senior scientists

J – oral presentation by students and junior scientists

P – poster presentation

O1

Fate of One Equation

Bohuslav Gaš
Charles University, Faculty of Science, Prague, Czech Republic, bohuslav.gas@natur.cuni.cz

Summary

Electrophoresis utilizes a difference in movement of charged species in a separation channel or space for their spatial separation. A mathematical consequence of the mass conservation law is the continuity equation which is acting whenever there is a movement of mass and which can be formulated as a partial differential equation.

The continuity equation is a beautiful law, concise in formulation and boundlessly rich in solution, which even nowadays can reveal very unexpected consequences and phenomena, such as oscillation, see Fig. 1. Attempts at the analytical solution of the continuity equation in electrophoresis go back to the Kohlrausch's days. However, in spite of effort of mathematicians, the full analytical solution has not been presented until now. Therefore, the scientists try to obtain useful results by another ways: extracting conservation functions, by means of moving boundary approximation, by linearization, and of course by numerical solution.

The presentation reviews (i) derivation of conservation functions from the conservation law as appeared chronologically, (ii) deals with theory of moving boundary equations, (iii) presents the linear theory of electromigration, (iv) shows the abilities of the last generation of numerical simulation software [1].

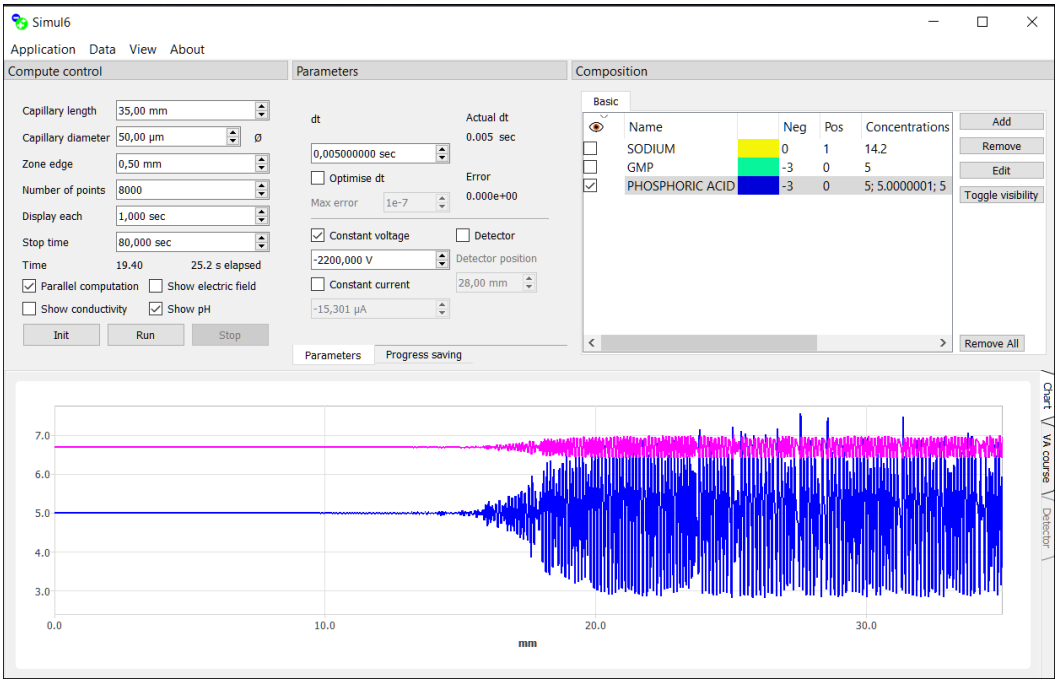

Fig. 1 Oscillation of electrolytes

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[1] B. Gaš, Theory of electrophoresis: Fate of one equation, Electrophoresis 30 (2009) S7–S15.

O2

Intact Protein Analysis in Biological Matrices by Capillary Zone Electrophoresis – Mass Spectrometry

Katarína Maráková[1,2], Martina Opetová[1,2], Radovan Tomašovský[1,2]

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Summary

Proteins are important biomolecules playing many essential roles in living organisms. Evaluation of an abnormal expression of proteins during various diseases can help with their early diagnosis and prediction of the response to pharmacological treatments. In addition, many proteins have become important biopharmaceuticals in the recent decades. However, direct quantitation of intact proteins from complex biological matrices by mass spectrometry is still a very challenging task. Liquid chromatography (LC) is the most frequently employed separation technique in top-down proteomics. Nevertheless, capillary electrophoresis (CE) has emerged as a complementary technique to LC also in proteomic analysis [1]. CE methods provide highly efficient separations, require low sample amounts and volumes per single analysis and they are greener methods compared to LC. However, there are still several challenges connected with an application of CE methods for intact protein analysis: protein adsorption on the inner wall of the fused-silica capillaries and the low concentration sensitivity due to the low injected sample volume. This can be a serious drawback if the analyte is present in complex biological matrices and at a very low concentration level. In this work, we investigate the practical possibilities, limitations, and challenges of a top-down quantitative analytical workflow for low molecular weight proteins (<30 kDa) in biological samples by CE-MS. When working with intact proteins and biological matrices, more effort has to be paid to careful optimization of instrumental methodology. Selection of the separation capillary, composition of the background electrolyte, sample preparation and preconcentration approaches were carefully optimized to obtain the best detection sensitivity of target proteins.

Acknowledgements

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O3

Recent Advances in Electroextraction Techniques: A Perspective from a Brazilian Research Group

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Summary

Over the past two decades, sample preparation techniques based on electric fields, also known as electroextraction techniques, have seen significant development. Countries such as Norway, Iran, Denmark, the Czech Republic, Spain, and China, among others, have made substantial contributions to this advancement. Electroextraction techniques encompass a wide range of sample preparation strategies that utilize electric fields as a driving force to purify and pre-concentrate analytes present in various matrices. This approach offers several advantages. Firstly, only species with an electric charge opposite to that of the electrodes are efficiently transported to the extraction phase, providing high selectivity to the process. Secondly, by using small amounts of acceptor phase, a significant level of pre-concentration can be achieved. Furthermore, most techniques based on electric fields offer high speed, advanced automation, batch extraction capacity, and low consumption of reagents and solvents. In Brazil, electroextraction techniques have a somewhat more recent history of application and development but are rapidly growing, with various modalities proposed by Brazilian researchers. This presentation will highlight the main advances in electroextraction techniques achieved in recent years by the LAMS research group at the Federal University of Minas Gerais in Brazil. Additionally, we will discuss ongoing projects in collaboration with the Department of Electromigration Methods (UIACH - Brno - Czech Republic) and outline the future prospects of these efforts.

O4

Integration of Metabolic Sensors in Musculoskeletal Organs-on-a-Chip in Osteoarthritis Research

Eva I. Reihs[1], Danny O'Hare[2], Miguel Otero[3], Torsten Mayr[4], Stefan Tögel[1], Reinhard Windhager[1], Peter Ertl[5], Mario Rothbauer[1,5]

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Summary

The current scientific agreement based on molecular studies outlines that the fundamental joint homeostasis is severely affected by osteoarthritis due to the dysregulation of metabolic pathways mediated by activation of joint tissue type-specific cells including for instance chondrocytes and synoviocytes. The entire knee joint undergoes inflammatory episodes with changes in tissue architecture, composition and function, as well as matrix degradation and remodelling [1]. Changes in cell metabolism can create synovial fluid hypoxia, reduced mitochondrial respiration, oxidative stress, and anabolic protein biosynthesis of degradative matrix proteases [2,3]. Consequently, the biological significance to investigate cell metabolism in advanced 3D in vitro cultures of human joint tissues is vital to understand the catabolic and anabolic processes that favor the onset and progression as well as late-stage osteoarthritis pathology [4,5]. Even though microsystems are discussed as the future of in vitro science, most models lack the capabilities for non-invasive inline monitoring and molecular cell analysis. Here, we present avenues how to include multi-parametric sensing strategies for advanced joint-on-a-chip systems for time-resolved metabolic profiling of anabolic and catabolic mechanisms in osteoarthritis with a focus opto- and electro-chemical biosensor integration into human patient-derived joint tissue surrogates.

References

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O5

Capillary Electrophoresis with Mass Spectrometry: From Enantiomers to Nanoparticles

Jan Petr, Daniel Baron, Marianna Nytka, Tomáš Pluháček
Palacký University Olomouc, Olomouc, Czech Republic, jan.petr@upol.cz

Summary

Capillary electrophoresis (CE) is well-known separation technique that can be combined with both "organic" and "inorganic" mass spectrometry to get more information about the sample. In this contribution, our work on electrospray ionization-based CE-MS (CE-ESI-MS) and inductively coupled plasma-based CE-MS (CE-ICP-MS) will be presented. CE-ESI-MS was used for the determination of tamsulosin enantiomers using 200 mM acetic acid titrated with NH_4OH to pH 4.0 with addition of 4.0 mg/mL sulfated-β-cyclodextrin. Stacking conditions with 40 s injection led to LOD of 1.6 nmol/L. Interestingly, this work showed the necessity of characterization of different batches of chiral selector. Commercial sulfated-β-cyclodextrin is a mixture of differently sulfated β-cyclodextrins. Due to the process of preparation, there are batch-to-batch differences including also the separation power. In our work, we characterized eight sulfated-β-cyclodextrins using CE, NMR, and cyclic ion mobility MS. We found interesting correlations between the number of $-SO_3Na$ groups, their variance and the cyclodextrin resolution power.

As known, CE is also suited for the characterization of objects as e.g., nanoparticles (NPs). In our next work, CE-ICP-MS was used for the study of interaction between carboxylated core-shell magnetic NPs and polymyxin B sulfate. Iron isotopes (^{54}Fe, ^{56}Fe, ^{57}Fe) were used for the detection of NPs, while ^{34}S was used for the electroosmosis, and ^{12}C reflected the interaction. The focus on isotopes has a positive effect on the evaluation of interactions what led us to following study. We developed a novel Taylor dispersion analysis-based CE-ICP-MS method for the investigation of behavior of NPs in the presence of other NPs, all in the aqueous high-ionic strength conditions. Carboxylated core-shell magnetic NPs, Au and Ag NPs were analyzed together by the Taylor dispersion analysis and CE-UV and CE-ICP-MS. Here, the use of ICP-MS was the key for obtaining correct diameters, comparable to common characterization techniques as e.g., DLS. To conclude, CE-MS represents a powerful tool for challenging analytical tasks.

Acknowledgement

The financial support of the research by the project IGA_PrF_2023_027 is gratefully acknowledged.

O6

Design and Development of Coating Materials with Ionic Liquids/Surfactants on Iron Oxide-Based Magnetic Nanoparticles and 3D-Printed Silica/Thermoplastic Polymer Composites as Extraction Sorbent Materials in Drug Analysis

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Summary

The work is focused on the design and development of sorbent materials selective towards extractions during drug analysis. The first extraction sorbent material is based on the structure of magnetic nanoparticles (MNPs). The second extraction sorbent material involves an application of 3D-printing technology.

MNPs can be interesting approach in the sample pretreatment due to the large surface-to-volume ratio and the possibility of their easy removal using an external magnetic field, which significantly reduces the number of steps in the extraction procedure. In the study, research utilizing different cation-anion combinations belonging to the group of ionic liquids (ILs) or surfactants in the functionalization of Fe_3O_4 MNPs is presented. The usefulness of the proposed method for therapeutic drug monitoring is proved also by the results of analysis for samples collected from the pediatric cancer patient and presented in the form of epirubicin time-concentration profile in biological fluids [1].

Using the fused deposition modeling (FDM) technology of 3D printing, sorbents can be shaped and sized to meet current laboratory requirements. A composite made of polypropylene (matrix), ABS (porogenic polymer) and C18-modified silica particles was designed and optimized. The novel PP/ABS/silica composite was designed and fabricated by FDM 3D-printing. An activated 3D printed object has a porous structure that allows access to silica particles while maintaining macroscopic size and shape. The effectiveness of the proposed composite is demonstrated on the example of glimepiride, imipramine, and carbamazepine extraction. The influence of sorbent shape is discussed, too. The 3D-printed sorbent's extraction parameters can be fine-tuned to provide satisfactory recoveries and high precision, especially for carbamazepine extraction [2].

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O7

Noncovalent Biomolecular Interactions Studied by Affinity Capillary Electrophoresis

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Summary

Two modes of affinity capillary electrophoresis (ACE), mobility shift-ACE (ms-ACE) and pressure assisted partial-filling ACE (pa-PFACE), were applied for investigation of noncovalent interactions between peptides and chiral selector and between small organic ligands and protein receptor.

Stereoselective interactions of L- and D-enantiomers of insect antimicrobial dipeptides, β-Ala-Tyr and its two derivatives, with chiral selector, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), were studied by ms-ACE using acidic background electrolyte (BGE) (32/50 mM Tris/H_3PO_4, pH 2.5) or alkaline BGE 50/49 mM $Na_2B_4O_7$/NaOH, pH 10.5 [1]. The strength of the interactions was quantified by the average apparent binding constant, K_b, of the peptide–selector complexes. The K_bs were calculated using nonlinear regression analysis of the dependences of the effective electrophoretic mobility of dipeptides on the concentration of HP-β-CD in BGE. These interactions were relatively weak; the K_bs ranged from 11.2 to 79.1 L/mol.

Interactions between an important protein hormone, human insulin (HI), and biologically relevant ligands, dopamine, serotonin, arginine, and phenol, in alkaline aqueous media were investigated by pa-PFACE. The K_bs of the HI hexamer–ligand complexes were determined from the dependence of the effective migration time changes of the above ligands on the variable zone lengths of HI hexamer dissolved in alkaline BGEs (40/40 mM Tris/tricine, pH 8.1, or 25/34 mM NaOH/tricine, pH 8.5), and hydrodynamically introduced into the bare fused silica capillary close to the UV detector [2]. The interactions of HI with the above ligands were weak to moderately strong, with the K_bs in the range 385–1 314 L/mol, and decreasing in the order HI-phenol > HI-dopamine > HI-serotonin > HI-Arg. Both ACE modes proved to be suitable methods for investigation of noncovalent interactions of biomolecules in a microscale.

Acknowledgement

The work was supported by the Czech Academy of Sciences, Research Project RVO 61388963.

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O8

Non-Destructive Forensic Document Examination of Ballpoint Cationic Inks by Blotting-Capillary Electrophoresis

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Summary

Forensic document examination plays a crucial and objective role in forensic science to establish legal evidence. The preservation of original documents is of paramount importance, leading to the adoption of spectroscopic or chemical analysis methods that minimize potential damage. Particularly with antique and historical documents, even minor damage can have significant consequences. Therefore, there is an urgent need for non-destructive chemical analysis methods to investigate document inks. One promising solution is the technique of blotting, where the ink used in document writing is transferred onto a film for subsequent chemical analysis. This approach allows for thorough document analysis without inflicting physical harm to the original item. By combining surface blotting with capillary electrophoresis (CE), a novel non-damaging chemical surface analysis method can be employed, offering valuable information on document inks while preserving their integrity. Analyzing oil-based ballpoint pen inks poses challenges for conventional CE. Nonaqueous capillary electrophoresis (NACE) is an alternative by using background electrolytes (BGEs) capable of dissolving these inks. Large volume sample stacking with an electroosmotic flow pump (LVSEP) is a powerful method for efficiently concentrating analytes without polarity switching or other complex maneuvers. Successful LVSEP requires the electroosmotic mobility (EOM) should be smaller than the analyte electrophoretic mobilities and have the opposite sign. By injecting a large volume sample of lower conductivity than the BGE and applying a voltage to create an electroosmotic flow (EOF) towards the inlet, analytes are stacked at the boundary between the sample and BGE. Subsequently, as the sample matrix of lower conductivity is removed into the inlet vial and replaced by the BGE of higher conductivity from the outlet, the average EOF decreases, automatically pushing the stacked analytes towards the detector. For LVSEP of cations, a reversed EOF needs to be generated by coating the capillary inner wall dynamically with a cationic polymer like polybrene. This scheme, LVSEP-NACE, has been successfully applied to cationic dye standard samples and real ballpoint pen ink extracts, yielding hundredfold sensitivity enhancements. Blotting-LVSEP-NACE is promising for non-destructive analysis of documents with high preservation value.

O9

The Role of IgA N-Glycosylation in the Development of Oral Mucositis

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Summary

The oral cavity, as a window, reflects most systemic changes in the whole body. Oral and enteral mucositis due to high-dose cytostatic treatment administered during autologous and allogeneic stem-cell transplantation increases mortality. Salivary secretory immunoglobulin A (sIgA) is a basic pillar of local immunity in the first line of defense. Altered salivary sialoglycoprotein glycosylation is important in oral cavity pathologies including inflammation, infection and neoplasia. We used capillary electrophoresis to assess whether changes in the salivary and serum IgA glycosylation correlated with development and severity of oral mucositis. A comparative analysis of serum and salivary IgA total N-glycans were conducted including 8 patients with autologous peripheral stem-cell transplantation (APSCT) at four different stages of transplantation (day −3/−7, 0, +7, +14) and in 10 healthy controls. Fourteen out of 31 N-glycan structures were identified in serum and 6 out of 38 in saliva, which showed significant changes upon transplantation compared with the control group. The serum core fucosylated, sialylated bisecting biantennary glycan (FA2BG2S2) showed significant differences between any two stages of transplantation (day −3/−7 and day +14; $p = 0.0279$). Our results suggested that changes in the serum IgA total N-glycan profile could serve as a disease-specific biomarker in patients undergoing APSCT, while analysis of salivary IgA N-glycan reflected the effect of APSCT on local immunity. Analysis of the N-glycosylation profile alterations in salivary, oral mucosal and gingival crevicular fluid glycoproteins would be an efficient and novel way in the diagnostics field.

O10

Analysis of Glycans for Disease Diagnostics

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Summary

The analysis of glycans (complex carbohydrates attached to protein or lipids) is increasingly important in diagnosing diseases, including various types of cancer. The main reason for this statement is the fact that genomic/proteomic profiles cannot be used to fully understand many pathological processes. Glycans are part of 70% of all intracellular proteins, with 80% of membrane proteins being glycosylated, making every cell in our body glycosylated. Thus, glycoproteins can be very effectively applied as biomarkers for various types of cancer.

Glycans are information-rich molecules involved in many physiological and pathological processes. The essential characteristics of glycans and why they can be used to diagnose cancer diseases will be presented in the presentation. We will introduce biomarkers used in clinical practice to diagnose various types of cancer, but especially prostate-specific antigen (PSA), considered the best diagnostic cancer biomarker. However, this biomarker is not very suitable for the diagnosis of prostate cancer, which is also underlined by the fact that the discoverer of this biomarker claims that this biomarker is suitable for monitoring the progression of the disease. Moreover, even the regulatory authorities are not clear on whether this biomarker should be used to diagnose prostate cancer and what the "cut-off" concentration of this biomarker in the blood should be to limit false negative or false positive results.

Analysis of glycans can not only refine the diagnosis of prostate cancer but it can be used for diagnostics of other diseases. Part of the lecture will be devoted to the way in which new biomarkers are validated and what are the hot trends in the diagnosis of various types of cancer, e.g. by exosome analysis.

Acknowledgment

The financial support received from the Slovak Research and Development Agency APVV-21-0329, APVV-20-0272 and from the Slovak Scientific Grant Agency VEGA 2/0130/20.

O11

Two Columns, More Than Double Band Broadening - Dilution Issues in Comprehensive Two-Dimensional Liquid Chromatography

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Summary

In recent decades, there has been a growing interest in the fundamental development and application of comprehensive two-dimensional liquid chromatography. The technique can separate and quantify higher number of compounds compared to unidimensional LC in a single analysis. Due to the application of two columns, preferably with different separation mechanism, the sample compounds are essentially diluted on each column and also with significant contribution of the interface connecting the two columns in the first and in the second dimension. In this work, the factors affecting dilution of the analyzed samples are addressed with special attention to the use of highly orthogonal reversed-phase and hydrophilic interaction liquid chromatographic modes in micro-scale separations. The uniform conditions, which are usually used for the fraction transfer process, can affect band broadening in the sampling loops, and/or trapping columns used within the interface. Approach of programmed conditions in the fraction transfer process has been developed and compared with fixed and active solvent modulation systems.

Acknowledgement

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O12

There and Back Again: One Person's Struggle Between Industry and Academia and How It Relates to Ionization Efficiency

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Summary

The purpose of this invited talk is to help cover the unbearable break between the rigorous scientific program and the evening reception, where alcohol is finally to be served. The author, a common mass spectrometrist and a gas-phase ion enthusiast, compares his experiences from academia and industry, with particular emphasis on trying to convince the present young audience to avoid his past mistakes and mishaps. Surprisingly enough, it is demonstrated that the life of any mass spectrometrist always depends on the elusive phenomenon known as the ionization yields. The talk should not be taken as a career advice and absolutely no guarantees, assurances nor warranties on the presented ideas will be offered. There will be no refunds on admission.

O13

Electromigration Dispersion in SDS-CGE of Proteins

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Summary

The electromigration dispersion (EMD) of the light and heavy chain subunit peaks of the therapeutic monoclonal antibody omalizumab was investigated in sodium dodecyl sulfate capillary gel electrophoresis (SDS-CGE) using borate cross-linked dextran sieving matrices. Our results suggested that the observed fronting and/or tailing peak shapes of the monoclonal antibody fragments in SDS-CGE at increasing boric acid concentrations can be considered as the result of multiple effects including changes in pH, sieving matrix pore size, viscosity and the mobility variation of the co-ionic borate adducts with the gel-buffer ingredients. While EMD mediated band broadening, in general, can be minimized via matching the effective mobility of the co-ionic species to the analyte molecules of interest, in case of borate cross-linked dextran gels, optimization of the boric acid concentration required special consideration of its gel cross-linking function. Therefore, borate gradient mediated transient mobility matching was introduced to alleviate EMD, which novel approach resulted in close to optimal peak shapes even for the distantly migrating IgG subunits within a single run, as well as unraveled the long sought possible solution to perform capillary pore-size gradient gel electrophoresis.

O14

Automated Analyses of Dried Blood Spots by Capillary Electrophoresis

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Summary

Micro-sampling of dried blood spots (DBSs) has become a viable alternative to venous blood collection and has evidenced enormous interest recently. Despite the numerous benefits of DBSs, their processing, pretreatment, and analysis are usually performed manually, are tedious, and labor- and time-intensive. DBSs are typically punched out from a sampling card, rehydrated in elution solvent(s), centrifuged, evaporated to dryness, and reconstituted, and the final extract is manually transferred to an analytical instrument. To eliminate these manual procedures, commercial systems for automated DBS processing became available in the last decade, nevertheless, they exhibit numerous drawbacks, which limit their broader use in quantitative DBS analyses. As a consequence, the development of simple and cheap solutions for unmanned DBS analyses is at the forefront of the actual research, and one such concept is discussed herein.

It uses an off-the-shelf capillary electrophoresis (CE) instrument for executing all tasks of the analytical protocol, i.e., a single CE instrument serves for DBS elution and processing, DBS eluate injection, separation, detection, and quantification. The actual concept, thus, enables fully autonomous DBS analyses of several hundred DBS samples per day for various clinical assays. Its suitability was exemplified by the determination of endogenous markers (e.g. inorganic ions, amino acids, uric acid) and exogenous species (e.g. blood anticoagulants, non-steroidal anti-inflammatory drugs) in remotely collected DBS samples. Details of a purpose-made method and sequence programming for ultra-fast DBS processing and analyses as well as the unique flexibility of CE parameters will be highlighted by the example of the determination of creatinine as a marker of renal failure.

The developed concept represents a progressive clinical tool for personalized healthcare, screening populations at risk, and monitoring the effect of medical treatment on patients. It can also be useful in critical (e.g. pandemic) situations and it might propel a shift from the actual sick-care to a prevention-based healthcare system. Moreover, due to the universal character of the elution and analytical procedures, rapid DBS processing, excellent separation efficiencies, great variability in CE separation and detection modes, and high sample throughputs, it is suitable for the determination of a wide range of analytes in various dried material spots and might play an important role in clinical, toxicological and forensic analyses in the future.

Acknowledgement

Financial support from the Czech Academy of Sciences (Institute Research Funding RVO:68081715) and the Grant Agency of the Czech Republic (Grant No. 23-05972S) is gratefully acknowledged.

O15

The Art of Liquid Biopsy: Clinical Considerations and Analytical Approaches in Noninvasive Molecular Cancer Diagnostics

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Summary

The detection and quantification of genomic aberrations in circulating tumor DNA (ctDNA) extracted from peripheral blood of cancer patients, notoriously termed as a "liquid biopsy", has gradually evolved from its early days of technological development into routine clinical use [1,2]. There are currently two fundamental approaches which correspond to separate applications. In the first case a ctDNA derived from plasma sample is subjected to tumor-agnostic testing for possible presence of a wide-range of molecular markers (typically point mutations, gene fusions or amplifications). In a second scenario a prior information of aberrations existing within the tumor tissue is utilized for targeting such aberrations. The tumor-agnostic liquid biopsy approach is broadly understood as a viable alternative to standard of tissue biopsy samples. While this has widely been utilized in performing molecular profiling of cancer that has previously been diagnosed in a patient, its application has relatively recently been loosely presented as a potential tool for early cancer detection. A variety of techniques under an umbrella of Multi-cancer early detection (MCED) are now being developed and clinically validated. In contrast the utility for methods based on a tumor-informed approached is steadily finding its way to clinical use for stratification of patients to risk groups and, most importantly, monitoring of an ongoing treatment or a detection of residual disease following surgery. The methods are specifically designed to detect individual markers with high sensitivity while maintaining reasonable cost allowing for repetitive longitudinal. Another important factor in the liquid biopsy area is the clinical utility of use. For some cancers (such as lung cancer, colorectal cancer or malignant melanoma) oncologists have a wide range of treatments available (including targeted biological therapies and immunotherapies) and its application relies on molecular diagnostics. There are, however, some types of cancers, for which the molecular testing still has limited to none clinical utility. Such is the case for pancreatic cancer or neuroblastoma. The proper combination of technology choice and a rational consideration of clinical utility in terms of patient benefit key therefore for efficient application of the various options offered by liquid biopsy technology.

Acknowledgement

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O16

Separation and Analysis of Proteins Using SDS Capillary Gel Electrophoresis Online Coupled to Electrospray Ionization Mass Spectrometry

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Summary

Capillary electrophoresis is one of the frequently used liquid phase separation techniques for the analysis of peptides and proteins, mostly utilizing UV or fluorescent detection, both in zone and gel electrophoresis modes. Hyphenation of CE with electrospray ionization mass spectrometry (ESI-MS), provides additional structural information about the separated sample components. Over the past decade, there have been many attempts to improve CE-MS coupling with highly competitive features, however, most of them still have challenging issues, such as accommodating the option to use gel filled capillaries and non-MS friendly buffer components, such as sodium dodecyl sulfate. To address these issues and further improve the versatility and robustness of CE-MS couplings, our group has developed a new approach by introducing a coaxial sheath liquid interface in which the separated sample components exiting the separation gel matrix are carried towards the ESI source by the sheath flow through a closed-circuit narrow bore flow reactor tube. This approach enabled routinely using the original factory designed ESI sources for any MS system connected to the CE unit. In addition, utilizing the flow reactor section, our design opened the possibility for using gel filled capillaries, even with non-MS friendly buffer components such as sodium dodecyl sulfate with MS detection, which was previously not possible with a direct one-dimensional online connection. The applicability of this novel approach to couple the CE and MS units was demonstrated by the analysis of peptides and proteins in both native- and SDS-capillary gel electrophoresis modes. In sodium dodecyl sulfate capillary gel electrophoresis mode, addition of a capture agent to the sheath liquid efficiently eliminated the SDS content of the sample and the background electrolyte by inclusion complexation, while maintaining good separation efficiency and ion suppression free detection.

J1

Detection of Protein Biomarkers Using Lateral Flow Immunoassays Based on Photon-Upconversion Nanoparticles

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Summary

Rapid and sensitive detection of protein biomarkers is essential for the early diagnosis and prevention of different diseases. Due to the fast and easy analysis, lateral flow immunoassays (LFIAs) are currently becoming one of the most popular forms of tests for the detection of various analytes. The high selectivity of LFIAs is given by the high specificity of antibodies. Furthermore, little to no laboratory equipment is required to perform these assays, making them suitable for point-of-care testing (PoCT). Gold nanoparticles (AuNPs) are generally used as a label for LFIA analysis, allowing for naked-eye readout. However, even though AuNP-based LFIAs are highly convenient for PoCT testing, the sensitivity is often insufficient to detect low-abundance biomarkers. Thus, different kinds of nanomaterials are being studied for use as alternative labels.

Our work focused on the application of photon-upconversion nanoparticles (UCNPs) as a label in LFIAs. UCNPs are lanthanide-doped nanocrystals exhibiting anti-Stokes luminescence; they can be excited by the near-infrared laser and detected in the visible region without optical background interferences in biological samples. UCNPs composed of $NaYF_4$ doped with Yb^{3+} and Er^{3+} were synthesized and conjugated with specific antibodies *via* copper-catalyzed click chemistry. Such conjugates enabled the development of LFIA assays for the detection of protein biomarkers human serum albumin (HSA) and prostate-specific antigen (PSA). Several optimization steps were carried out. The biggest impact was observed in the case of selection of suitable membranes for designing the LFIA strips, addition of methanol to buffer for immobilization of antibodies, and addition of sucrose to buffer for incubation of UCNP conjugate. So far, UCNP-based LFIA has been successfully used for detecting HSA and PSA from buffers, proving that UCNPs are a convenient alternative to AuNPs. Moreover, the preliminary data demonstrate great potential for real sample analysis in various body fluids (e.g., blood serum or urine). The UCNP-based LFIAs can thus enable the sensitive detection of protein biomarkers and other clinically relevant analytes, combining simplicity of the assay with high sensitivity.

J2

Solid Phase Extraction Methods for Purification of Positively Charged Oligosaccharides and Glycans

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Summary

The crucial step for the analysis of oligosaccharides and glycans by capillary electrophoresis (CE) and/or mass spectrometry (CE-MS) is their derivatization by label. The signal of the unreacted label is routinely significantly higher, indicating the need to deplete the unreacted label prior to analysis by CE. The suitability of five different solid-phase extraction sorbents (Spe-ed Amide-2, Chromabond HILIC, HyperSep Diol, Bond Elut PBA, and HyperSep Hypercarb) was tested for the clean-up and concentration of positively labeled maltooligosaccharides from the reaction mixture. Maltooligosaccharides containing four to seven glucose units were labeled by cationic tags (2-aminoethyl)trimethylammonium chloride and (carboxymethyl)trimethylammonium chloride hydrazide (Girard's reagent T). Purified samples were analyzed by CE with conductivity detection to compare the purification selectivity and efficiency using the tested SPE materials. The amide-based sorbent gave the best results and was used to process the N-linked glycans enzymatically released from the ribonuclease B sample.

Acknowledgment

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J3

Tracking of the pH Gradient During cIEF-LIF Analysis Using Fluorescent Isoelectric Point Markers and Their Application in Efficient Separation of Protein Samples

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Summary

Due to the difficulty to perform direct pH measurements during isoelectric focusing (IEF) separations, namely in the closed environment of capillary isoelectric focusing (cIEF), IEF methods rely on the use of the isoelectric points (p*I*) markers to characterize the pH gradient profile. In the past, our laboratory has already presented multiple low-molecular-mass UV and visible region absorbing compounds, that can be utilized as p*I* markers [1,2]. Currently, we present a set of fluorescein-based ampholytes for tracing the pH gradient during the high sensitivity cIEF analysis coupled with laser induced fluorescence detection (LIF), which is a continuation of a past work presenting four markers covering narrow range from p*I* 5.4 to 6.6 [3]. By attaching variety of functional groups to the molecule of fluorescein, more than 60 further candidate molecules have been synthesized since the original publication. The potential candidates were individually analyzed by cIEF-LIF and the precise p*I* values of the compounds suitable for tracing pH were determined. Various approaches how to determine the p*I*s were considered. Four of the markers, evenly covering the range 4.05 to 8.73, were selected to show usability of the markers in cIEF-LIF analysis and p*I* determination of protein samples. We choose three examples - fluorescently labeled immunoglobulin, in-house labeled erythropoietin and an extract from powdered spirulina cell lysate, which contains naturally fluorescent phycobiliproteins, namely phycocyanin in comparison to a commercial standard of phycocyanin.

Acknowledgement

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J4

Body-on-a-Chip Technologies Towards Neurotoxic Monitoring and Environmental Safety/CBRN Applications

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Summary

Organophosphates, such as Malathion, are pervasive components in pesticides and chemical weapons like Sarin. Prolonged exposure to these compounds can result in severe neurological disorders. There is an urgent need for enhanced methods to assess environmental neurotoxicity without endangering human or animal lives. This study aims to develop a portable multi-organ microfluidic platform equipped with in situ biosensors for in-field deployment and real-time acute toxicity evaluation. In this platform, we conducted characterizations of micropumps, including assessments of varying pressures, actuation frequencies, and sizes, along with an analysis of material deformation (PDMS) and liquid flow dynamics. Additionally, our study involved an investigation into the responses of tissue-specific cell lines when exposed to organ-specific toxins within both 2D and 3D environments. Specifically, we exposed liver cells (HepG2) to Paracetamol and kidney cells (Hek293) to Gentamicin, documenting their toxicity across different toxicant concentrations. We also examined the behavior of cell micromasses in an organ-on-a-chip platform when exposed to the IC50 concentration of the toxicants. Furthermore, we evaluated the toxicological response of midbrain organoids to varying concentrations of an Acetylcholinesterase inhibitor and Malathion. Overall, the current work successfully demonstrated tissue specific response for Paracetamol and liver models (IC50 2 mM), gentamicin and Kidney (IC50 4.4 g/L), as well as Acetylcholinesterase inhibitor and Malathion on midbrain organoids of 26 µM and <10 mM, respectively. To conclude, the current work being embedded in a multi-centric defense project paves the way to more predictive analytical tool on acute neurotoxicity monitoring using in vitro 3D models.

J5

Magnetic Microparticles as Potential Stationary Phase for Affinity Studies in Capillary Electrochromatography

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Summary

The instrumentation of capillary electrophoresis (CE) enables introduction of novel materials as stationary phases into the capillary. The presence of selective stationary phase allows separation of analytes based on both, their mobility in electric field and interaction with stationary phase. Due to the combination of electrophoretic and chromatographic principles, this approach is called capillary electrochromatography (CEC). Columns used in CEC are usually prepared directly inside the capillary; the stationary phase is filling its interior or is bound on its inner surface. Superparamagnetic iron oxide particles represent novel stationary phase material with useful properties, besides their applicability for immobilized enzyme reactor formation. Magnetic behavior of the particles makes it easy to separate them from the reaction mixture during stationary phase preparation on their surface and enables formation of the column inside the capillary held by permanent magnets. Moreover, the column can be easily regenerated by the magnet removal.

Convenient affinity modes of CE allow protein-ligand interactions studies in conditions close to physiological and with minimal sample consumption. Immobilization of protein on the surface of magnetic particles and preparation of affinity CEC column could expand the application potential of these methods. Protein of high interest in protein-ligand interaction studies is serum albumin. Since to its ability to bind drug molecules and its high plasma concentration, serum albumin affects significantly pharmacokinetical and pharmacodynamical profiles of drugs. According to the generally accepted “free drug” theory, only the free fraction of the drug is available for physiological processes resulting in its efficacy, and therefore it is important to understand interactions from all points of view.

Successful covalent immobilization of bovine serum albumin (BSA) on the surface of magnetic silica coated particles has been demonstrated by our group in previous studies. The immobilized protein retained its drug binding properties. In the current study, magnetic particles with immobilized serum albumin were used for the preparation of an affinity CEC column, held inside the capillary by permanent magnets. Crucial and most tricky step was formation of stable and permeable column, as well as choice of suitable analysis conditions. Several magnet arrangements as external magnetic field source were tested since the particles tend to organize themselves into structures depending on magnetic field orientation. Prepared column was evaluated for its potential application in affinity studies and its suitability was evaluated with model drugs.

Acknowledgement

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J6

Exploring the Feasibility of Animal-Free Protein Analysis in Molecular Osteoarthritis Research

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Summary

Animal-free research momentum in Europe rises due to ethics. Recent developments [1-3] stress stricter animal testing regulations for ethical transparency. EU's 1986 legislation drives alternatives like human cell-based methods to reduce animal experiments. Molecular research's challenge persists with reliance on animal-derived products for ethical models. In this innovative project, we aim to develop an animal-free methodology for investigating extracellular matrix components and signaling molecules in research on human osteoarthritis (OA). We utilize OA patient-cell-derived microfluidic biochips, including humanized 3D models of key disease-driving tissues, the synovial membrane, and articular cartilage, for this feasibility study. Our aim is to assess the 'Abby' automated capillary electrophoresis, an animal-free method, for protein analysis replacing animal-based Western blot analysis. Protein samples from intracellular lysates or cell culture supernatants of synovial and chondral biochips are analyzed. Our focus is on intracellular messenger Yes-associated-protein1 (Yap1) analysis in synovial biochips, an OA marker, and on investigating changes in cartilage-oligomeric-matrix-protein (COMP) release during a 14-day cultivation, with and without IL1β and TNFα (50 ng/mL) as disease triggers. Recombinant antibodies replace animal-derived ones, enabling animal-free detection through biotinylated primary antibodies and a Streptavidin-HRP coupled reporter. This innovative approach aims to create an ethical methodology for studying molecular signaling in human arthritis by replacing traditional animal-derived antibodies and blockers with the 'Abby' automated capillary electrophoresis method. We aim to integrate 'Abby' into animal-free analysis of disease-related signaling cascades and protein expression in established multi-tissue biochips as a near-term objective, following optimization and feasibility demonstration.

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J7

A New Device for Online Nanoscale Sampling and Capillary Electrophoresis Analysis of Plant Sap Composition

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Summary

In this work, an online sampling of plants combined with an efficient capillary electrophoresis (CE)-based method was developed and applied to study the kinetics of changes in the xylem sap composition and to assess plant fitness under stress conditions comprehensively [1,2]. A laboratory-built CE device was developed to provide online sampling and CE analysis of various ionogenic species in the plant sap during plant stress response. The short CE analysis time allows for capturing time-dependent kinetic changes in the living plant during the stress response. The developed device successfully monitored chloride, nitrate, and sulfate ions in the plant xylem during the salt stress or stress caused by nitrate deficiency.

Acknowledgement

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J8

Exploration of a Glycan Biomarker Panel for the Early Screening of Type 2 Diabetes

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Summary

Type 2 diabetes (T2D) is a complex, incurable metabolic disorder strongly influenced by genetics factors and its early detection is a significant challenge. We present this preliminary study at the glycomics level for the better understanding of the pathomechanism of T2D. This research involved the development of a novel high-throughput N-glycan analysis using capillary electrophoresis coupled with artificial intelligence capable of recognizing patterns in glycomics data in the case of T2D. This state-of-the-art methodology enables sensitive analysis of N-glycan structures in human serum samples obtained from the Prof. Andras Koranyi Hungarian Transdanubia Biobank (HTB), which is a substantial sample source from with over 9000 individuals across 1000 families. As a first step, it was necessary to pre-process HTB serum samples to get insight into the stability of the sugar molecules. The retrospective analysis of HTB serum samples focused on siblings, particularly those diverging for T2D, shedding light on potential N-glycan profile variations associated with the hereditary nature of the disease. In addition, to uncover crucial molecular pathways and signatures of complex data generation, biological network analysis and machine learning technologies were utilized. In summary, this research outlines a multidisciplinary approach to the N-glycome of human serum samples, exploring potential glycan biomarker panel to T2D. The integration of glycomics data with data mining tools, promises to advance our understanding of the molecular underpinnings of T2D and may open up avenues for novel therapeutic interventions.

J9

Separation Tools and Infectious Diseases: Feasibility Study of a High Throughput Extracorporeal Virus Capture Device

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Summary

During the epidemic, it became clear that effective extracorporeal processes can be an effective treatment of infectious diseases and intensive care. In this presentation, we demonstrate the utilization of separation science tools to fight viral infections. Based on various hemoperfusion and immune-affinity based capture systems, a blood virus depletion device has been developed that offers effective capture and removal of the targeted virions from the bloodstream, thus decreasing viral load. Recombinant single domain antibodies were immobilized on the surface of monodisperse soda-lime glass beads and utilized as stationary phase for immune-affinity mediated pathogen removal. For feasibility testing of our immune-affinity based platform, a virus particle suspension was flown through a laboratory-scaled column designed for this purpose, in a model system driven by a peristaltic pump, representing the extracorporeal circulation. The tests of the proposed approach were performed in a BSL-4 classified laboratory using the VHH-72 SARS-CoV-2 strain. The laboratory scale device specifically captured ~120,000 virions from the model circulation, which corresponded to ~15 million virus particle capture capacity of a therapeutic size cartridge. The technology was highly over-engineered with the assumption of 5 million genomic virus copies in case of a typical viremic patient. According to our results, this new therapeutic device could significantly lower virus load, prevent organotropism of virus particles and the development of more severe viremia and sepsis.

J10

Epitachophoresis - Stabilization of the LE/TE Border

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Summary

Epitachophoresis is a novel separation method based on the migration of ions in a discontinuous electrolyte system. The primary value of this method is the separation and concentration of ionic analytes from the complex matrix of the sample. So far 20 mL of solution containing DNA ladder has been concentrated into 300 µL with a recovery of up to 95% using 0.5% agarose gel [1].

Epitachophoresis works on the principle of isotachophoresis where a targeted substance is focused in a zone between leading (LE) and trailing (TE) electrolytes. Contrary to isotachophoresis, the focused zones accelerate and expand during the migration to the middle of the device. This is caused by the round design of the device. This unique circular shape allows for the injection of a large amount of the sample (up to tens of mL) and collects hundreds of µL in the middle of the device. Due to the large area of the LE/TE border, it is necessary to stabilize it.

The object of this work is the development and optimization of the epitachophoresis process for the new device design named mini ETP, especially the selection of a medium stabilizing LE/TE border. Different materials for the stabilization of this border were tested, including agarose gels, various foamed polymers, 3D printed structures, and sponges. Due to the problems with the hydrophobicity of some of these materials and analyte sorption, different surface modifications were tried out, including oxygen plasma and surfactants (MHEC, PEG, TRITON X100).

Although the mini ETP concentrates DNA from smaller amounts of the sample (units of mL), it enables a parallel run of multiple experiments. As our future focus is on the long DNA fragments, the “open” structure materials with minimum sieving effect were searched for stabilization of the buffer system during the ETP run. The most promising stabilizing medium from tested options appeared to be a 3D-printed star insert filled with 0.2% agarose gel and the PE hydrophilic disk. The DNA concentration efficiency and recovery were determined to be up to 90%.

Acknowledgement

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J11

GC×GC for the Determination of Hypercholesterolemic Fatty Acids in Cow Colostrum During the First Days of Lactation

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Summary

Bovine colostrum is an important source of a highly concentrated complex of nutrients. It contains nutritional and biologically active compounds needed for the early nutrition of a newborn calf. Due to its unique composition, bovine colostrum and colostrum-based products have recently been used as dietary, nutraceutical, and medicinal supplements for preventing and healing many human diseases. Even though the variation in the content of many nutritional and bioactive compounds in bovine colostrum has been widely studied, the information on fatty acid (FA) profile changes during the first days of lactation is lacking.

Hypercholesterolemic fatty acids include saturated FAs, where studies showed that these FAs can cause increased levels of LDL cholesterol and risk of cardiovascular diseases, hypertension, and arthritis. These saturated FAs represent around 50% of all FAs in colostrum. On the other hand, colostrum also contains hypocholesterolemic FAs represented by oleic acid and polyunsaturated FAs. The ratio between hypo- and hypercholesterolemic FAs can be used for the evaluation of the health properties of bovine milk fat, as the reduction of LDL cholesterol concentrations in the plasma of hypercholesterolemic patients has been shown to reduce the incidence of cardiovascular mortality in humans.

The goal of our work was to optimize the extraction and derivatization of FAs from colostrum and to track the changes in the levels of hyper- and hypocholesterolemic FAs in bovine colostrum and immature milk during the first four days of lactation to evaluate their possible impact on human health. All samples were analyzed by comprehensive two-dimensional gas chromatography with flame ionization detection (GC×GC-FID), which provided a better separation, identification, and semi-quantification for such a complex matrix. The colostrum was obtained from eight Czech Fleckvieh cows kept on a private dairy farm (L. Klíčová, Božice, CZ). The samples were taken from morning milking during the first four days of lactation. Detailed profiles of individual hypercholesterolemic FAs revealed that palmitic acid had the highest concentration while lauric acid had the smallest representation out of the hypercholesterolemic FAs.

The results showed that the levels of FAs were changing over time. We have demonstrated that colostrum from later phases of lactation had a more favorable composition of milk fat from a human nutrition point of view. As each fatty acid has a different metabolic function in metabolism, the day of lactation should be considered in the production of colostrum-based products with specific compositions for nutraceutical and medicinal purposes.

P1

Separation of Sodium Dodecyl Sulfate-Proteins by Capillary Electrophoresis Using Dilute and Ultra-Dilute Dextran Solutions

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Summary

Sodium dodecyl sulfate capillary gel electrophoresis is a widely used method in the biopharmaceutical industry and the biomedical field for rapid size-based separation of proteins. Albeit, numerous publications discussed this popular technique, no study reported yet about the utilization of dilute and ultra-dilute sieving matrices for SDS-protein analysis. In the present work, 1% to 0.01% dextran as well as no dextran containing background electrolytes were used in borate-based background electrolyte for the separation of protein size standards ranging up to 225 kDa, and the intact and subunit forms of a therapeutic monoclonal antibody. Significant differences were observed between the separation traces of the protein sizing standards and the components of the mAb with decreasing dextran concentration in the background electrolyte. To understand the separation mechanism, the Ferguson and reptation plots were examined. Interestingly, using high dilution level dextran solutions, linear Ferguson plots were obtained for both types of solute molecules similar to that of suggested by the Ogston theory. However, this model assumes a rigid pore structure, therefore, not appropriate to describe the separation mechanism in ultra-dilute polymer solutions where no reticulations supposedly exist. The saddle differences between the resolution of the protein size standards and the intact and subunit forms of a monoclonal antibody in ultra-dilute dextran matrices suggested the importance of shape selectivity, manifested by the adequate separation of the SDS covered intact as well as light and heavy chain subunits of the therapeutic mAb sample even at zero dextran concentration.

P2

Impact of Heavy Water on Luminescence of Quantum Dots

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Summary

The impact of normal water – deuterated water solvent exchange on the luminescence properties of fluorophores has been known for a long time. Some organic and inorganic compounds exhibit significant enhancement of luminescence signal when normal water is exchanged with deuterated one. As these solvents behave similarly, it is relatively easy to use this exchange for various applications, e.g., fluorescence microscopy, liquid chromatography, capillary electrophoresis, etc. The most observable difference between these solvents is the price. So, practical application is possible mainly in methods where only small amounts of the solvents are used.

This work is focused on the study of the luminescence properties of the quantum dots (QD), mainly the effect of deuterated water. To characterize the impact of normal and deuterated water on luminescence, emission spectra, time-resolved luminescence, and quantum yields of fluorescence were used. Several sizes of commercially available quantum dots such as graphene QD, core CdTe QD, and core/shell CdTe QD were studied.

It was found that the amplification of luminescence for graphene quantum dots is dependent on emission maxima wavelength. This leads to the interpretation, that deuterated water figures for them as a less effective dynamic quencher in comparison with normal water. The maximal observed amplification of luminescence intensity was 1.62x for the graphene quantum dot Aqua green luminescent. Core-type CdTe quantum dots also show amplification in deuterated water. The highest amplification (1.79x) was observed for CdTe 520 SIGMA QD. With increasing emission maxima, the amplification decreases. Here, we suppose a larger surface degradation in normal water than in deuterated water caused by coating-solvent interaction. For core/shell-type quantum dots, there was no distinct change in the luminescence in deuterated water. This should be explained by the stabilization of their core via the passivation layer, the shell. Based on our measurements we can conclude that luminescence enhancement in deuterated water for quantum dots is negligible.

P3

Optimization of Glycoproteomic Protocol for Efficient and Sensitive Analysis of *N*-Linked Glycans by CE/LIF

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Summary

Protein glycosylation is involved in the control of many important biological processes and structural changes in *N*-linked glycans are associated with various types of diseases. High-throughput profiling of *N*-glycans is a key step for elucidating the functions of glycans in biological processes and disease development and for discovering new diagnostic biomarkers. The low abundance of glycans in a living organism, the competitive/suppressive effect of other high abundance glycans found in the biological molecules of a living organism, and the lack of basicity of glycans lead to their extremely low sensitivity of detection in MS analysis. Therefore, efforts must be made to develop glycan derivatization reagents and to optimize glycan derivatization protocols.

In this work, we report the effect of different proteomic protocols varying in enzyme or digestion procedures followed by derivatization by Cascade Blue hydrazide (8-(2-hydrazino-2-oxoethoxy)pyrene-1,3,6-trisulfonic acid trisodium salt, CBH) on the glycan detection in CE/LIF analysis. CBH represents a more reactive analog of APTS widely used for glycan labeling before CE analysis [1]. Ribonuclease B was selected as a model glycoprotein and four digestion protocols (alkylation, in gel/in solution treatment, purification step, etc.) were applied in order to obtain highly sensitive detection of labeled glycans by CE/LIF analysis.

Acknowledgement

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P4

Improvement of Concentration Sensitivity in Capillary Electrophoresis-Frontal Analysis

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Summary

Capillary electrophoresis is a powerful technique with many advantages. However, it suffers from the lack of concentration sensitivity associated with common utilisation of UV-VIS detector. The issue can be solved by replacing the detector with a more sensitive one or incorporation a preconcentration step without changing the detection instrument. Established capillary electrophoresis method for binding studies which gives information not only about binding strength but also about number of binding sites is capillary electrophoresis-frontal analysis (CE-FA). This study is focused on interaction between plasma protein and drug as a small molecule. For improvement of concentration sensitivity in CE-FA method the contactless conductivity detector is chosen and the second strategy enhancement of the UV-VIS detection by on-line preconcentration step field amplified sample stacking which amplifies concentration sensitivity in capillary during separation is used. For each newly developed method the conventional CE-FA UV-VIS method is optimised and binding parameters are compared.

P5

BODIPY-based Fluorescent Tag for Efficient Oligosaccharide Labeling

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Summary

Among the fluorescent probes frequently used in bioanalysis, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes are very promising because of their relatively high photostability, neutral total charge, high fluorescence quantum yield, and sharp absorption and emission spectra [1]. BODIPY dyes are relatively insensitive to the polarity and pH of their solution, and small modifications in structures enable tuning of their fluorescence characteristics [2].

In this work, we present the synthesis of a novel fluorescent tag including the BODIPY core and hydrazide functional group as well as the application of the tag for oligosaccharide analysis by HPLC/FLD technique. The mentioned derivative of BODIPY was synthesized from 2,4-dimethylpyrrole, methyl succinyl chloride, and boron trifluoride diethyl etherate followed by a two-step conversion of the ester group to the hydrazide one. The synthesized fluorescent tag was fully characterized by NMR, UV/Vis, fluorescence, and mass spectrometry. The conditions of labeling reaction via hydrazone formation chemistry were optimized by labeling of maltooligosaccharide standards. In addition, the presented labeling method was used for N-linked glycan profiling of several glycoproteins (ribonuclease B, immunoglobulin G) by RP-HPLC/FLD and HILIC/FLD analysis. The proposed approach significantly improved the oligosaccharide analysis in comparison to the commonly used procedure employing 2-aminobenzamide. The analysis of labeled maltohexaose provided the limit of detection in the low tens of femtomole.

Acknowledgement

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P6

UHPLC-MS Analysis of Salivary Bile Acids in Non-Invasive Diagnostics of Barrett's Esophagus

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Summary

Barrett's esophagus (BE) is a serious complication of severe gastroesophageal reflux disease and a major risk factor for the development of esophageal cancer [1]. The diagnosis of BE currently involves invasive endoscopy with sample collection and histological examination. Bile acids, a group of steroid compounds, were previously suggested to contribute to the progression of gastroesophageal reflux disease into BE [2]. Bile acids are essential for lipid digestion in the small intestine, however due to their detergent properties they are toxic in high concentrations [3]. This work is focused on development of methodology for analysis of bile acids in saliva and its possible application for a non-invasive diagnosis of BE.

Saliva samples were collected from volunteers into a plastic container that was followed by C18 solid-phase extraction for sample purification and bile acid extraction. A sensitive reversed-phase ultra-high-performance liquid chromatography – mass spectrometry (UHPLC-MS) method was developed for the quantification of unconjugated and glycine-conjugated bile acids. It was found that levels of bile acids were significantly higher ($p < 0.05$) in saliva from patients with BE ($n = 10$) compared to healthy volunteers ($n = 10$). Moreover, UHPLC – high-resolution MS was utilized for the investigation of bile acid fragmentation patterns which led to the identification of taurine-conjugated bile acids in saliva from healthy volunteers. Salivary bile acids might serve as a possible non-invasive biomarker in diagnostics of BE, therefore a larger clinical study including analysis of taurine-conjugated bile acids is currently underway to validate our initial findings.

Acknowledgement

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P7

Determination of Lamotrigine in Alternative Types of Samples Utilizing Validated LC-MS Method

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Summary

This study presents the analytical method for antiepileptic drug analysis, focusing on the determination of lamotrigine (LTG) in saliva and dried blood spots (DBS). The significance of monitoring antiepileptic drugs, such as lamotrigine, in saliva and DBS lies in their potential applications for therapeutic drug monitoring and personalized medicine. Saliva, being a non-invasively collected and easily accessible biological fluid, offers an alternative to traditional blood sampling for determination of drug levels. DBS, as well, can offer reasonable option for the determination of drug concentrations with minimally invasive sampling from blood capillaries.

The developed LC-MS method was subjected to an essential validation process. The LC-MS separation was performed on Kinetex C 18 Polar column (3x100 mm, 2.6 mm) which was maintained at 35 °C during whole acquisition period. The mobile phase consisted of solution A – MilliQ water with 0.1 % formic acid and solution B – acetonitrile (ACN) containing 0.1 % formic acid. Linear gradient was set in the range from 5 % to 90 % B within 10 minutes. Following elution from the LC column, ESI-qTOF analysis using the maXis impact Bruker instrument enabled identification and quantification of the drug. Analytical data were processed by using Compass DataAnalysis 4.1 and Compass QuantAnalysis 2.1 softwares (both supplied by Bruker).

Liquid-liquid extraction was used for both types of biological matrix, employing ethyl acetate for LTG extraction from saliva samples and MeOH:ACN 3:1 (v/v) solution for extraction of DBS samples. The extraction efficiency of LTG and its internal standard (LTG-13C15N4) was better than 90 % for saliva and more than 53 % for DBS. Limit of the quantification was 60 ng/ml in saliva and 30 ng/ml in DBS (based on signal-to-noise ratio 10:1). The detector response was linear over the concentration range 0.5-20 mg/ml for saliva and 1-18 mg/ml for DBS with satisfactory results, i.e. coefficient of determination was better than 0.996 in both matrices. Matrix effects and carry-over were measured and no effect on acquired signals was found. Analyte stability in processed and unprocessed samples was undertaken for different time spans, from overnight up to 1 month, and results fulfilled validation criteria according to European Medicines Agency.

The validated LC-MS method was successfully applied for LTG determination in patient saliva and DBS samples. The most of samples were obtained from patients on polypharmacotherapy (the study was approved by local Ethical committees, No. 32/2022 and 03V/2023). The presented method is suitable for the quantification of LTG in alternative matrices, DBS and saliva.

Acknowledgement

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P8

Effect of COVID-19 Disease and Vaccination on Salivary Secretory IgA *N*-Glycome

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Summary

Although the vaccines developed during the pandemic caused by the SARS-CoV-2 virus reduced the number and severity of diseases, the course of the disease showed a high degree of variability between individuals. Due to the frequent mutation of the virus, there are many variants of it even today, which differ in their infectivity. Oral immunity plays a fundamental role in the susceptibility to infection and the severity of the disease. The aim of our research was to examine the changes in the *N*-glycosylation profile of salivary secretory immunoglobulin A (sIgA) as a result of the vaccinations used and the infection experienced. Six people from 3 age groups (25-34 years; 35-59 years; ≥60 years) without acute or chronic diseases were included in the study. Four patients had confirmed SARS-CoV-2 infection within 1-6 months before sample collection, 2 were asymptomatic contacts; 5 patients were vaccinated and 1 was unvaccinated. The *N*-glycosylation pattern of sIgA purified by affinity chromatography from resting mixed saliva samples obtained from patients before (2018) and after (2022) the pandemic was analyzed using capillary electrophoresis with a laser-induced fluorescence detector (CE-LIF). Student's t test was used for statistical analysis. All experiments were approved by the Regional Institutional Research Ethics Committee, Clinical Centre, University of Debrecen (Ethical license: DE RKEB/IKEB 4948-2018, 6051-2022; 5570-1/2018/EKU). The sIgA *N*-glycosylation profiles of vaccinated patients differed significantly ($p \leq 0.05$) from those of unvaccinated patients at both time points. Changes in some *N*-glycan structures (up or down) were observed between unvaccinated and vaccinated patients after infection. The sIgA *N*-glycosylation profile of the vaccinated contacts without clinical symptoms showed the most significant changes. It can be concluded that both the SARS-CoV-2 infection and the applied vaccine resulted in a permanent, detectable change in the N-glycosylation pattern of salivary sIgA. Changes in the resting mixed saliva sIgA *N*-glycan profile may reflect the severity of the course of the disease, susceptibility to infection, and effectiveness of vaccination. Further investigation on larger patients cohort is planned.

P9

Use of Calcium Chloride for Removal of Polysaccharides During DNA Isolation from Tropical Fruit Products

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Summary

Food products made out of certain fruits (such as mango) may be subject to adulteration. The adulteration may be detected by various methods, including PCR-based ones. However, PCR-based methods are susceptible to inhibition if the DNA sample is contaminated with certain polysaccharides. Furthermore, polysaccharide contamination is notoriously difficult to remove. In this work, we demonstrate the use of calcium chloride for successful removal of potentially inhibitory polysaccharides from samples which are known to contain them (mango and banana puree). We also address the potential inhibitory effect of calcium chloride itself and we show a way in which polysaccharide precipitation by calcium chloride may be added to a DNA isolation protocol without hampering the amplifiability of the isolated DNA.

Acknowledgement

This work was supported by project no. FCH-S-23-8330 „Current problems and approaches to research in modern food sciences“ at the Faculty of Chemistry, Brno University of Technology.

P10

Importance of Glycosylation Modification of Sialoglycoproteins in Dementia

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Summary

More than 50 million people are affected by dementia worldwide. This number is expected to reach 150 million in 2050, which is not only a global health problem, but also an economic burden. More than 70% out of all dementia cases is Alzheimer's disease (AD). Not only effective cure but early non-invasive biomarker is also unknown regarding AD. AD has multifactorial ethiopathogenic origin. Extracellular Aβ plaques are the major feature of AD, followed by the intracellular neurofibrillary plaques (NFTs) formation caused by hyperphosphorylation of Tau protein. Hyperactivation of immune cells (e.g. microglia) can also be detected in patients with Alzheimer's disease. Considering the fact that most proteins involved in the etiopathogenesis of Alzheimer's are either glycosylated or participate in the regulation of glycosylation, thus glycomics provides new insight into mapping the complexity of AD. Alteration in cerebrospinal fluid (CSF) glycosylation may occur prior to AD clinical onset. Glycans showed significantly different levels in hippocampus compared to cortex in both control and AD brain. Protein sialylation has also been reported to be altered in AD, and a decrease in sialyltransferase (ST) has been identified in AD patients. Mild increases in free sialic acid levels are associated with AD pathology in vulnerable brain regions such as the temporal lobe. Key differences have been observed between cerebellar and temporal lobe sialylation in AD. Accurate diagnosis of AD can be set up only post mortem, earlier examinations are more invasive and not informative and specific enough. The wider mapping of early, non-invasive diagnostic methods and effective biomarker research are especially important nowadays. Saliva and immunoglobulins are an active area for this field of research. Immunoglobulins (Igs) are large molecular size glycoproteins. Immunoglobulin G (IgG) is the most abundant immunoglobulin with several immunological functions. Immunoglobulin A (IgA) represents 15–20% of the immunoglobulins in sera, although it is basically secreted on the surface of various mucous membranes, playing a key role in the first line of mucosal defense. Both IgA and IgG have a pivotal role in neuroinflammation. Increased abundance of non-fucosylated IgG1 and IgG2 was observed in patients with AD compared to controls. Furthermore, all immunoglobulin glycopeptides were increased in AD patients. Previous studies described significant difference in the amount of salivary IgA between patients with dementia and controls. Qualitative glycoanalysis of IgA has not been done in this patient population yet. The aim of this poster presentation is to summarize the role of glycomics in the ethiopathogenesis of dementia and identify novel insigths into biomarker research.

P11

N-Glycomic Identification of Novel Soft Tissue Prognostic Biomarkers for Oral Cancers

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Summary

The mortality rates of oral cancers have increased six-fold in the last 50 years. As the number of malignant diseases proliferates, more than 377,000 oral squamous cell carcinoma (OSCC) new cases and consequently 170,000 deaths are diagnosed worldwide annually. In addition, OSCC is an aggressive disease with a glycoproteomically unmapped disease progression and disheartingly low five-year survival rate. Besides the most commonly known risk factors such as alcohol consumption, tobacco, poor oral hygiene, HPV infection, long-term immunosuppressant therapies may also increase the risk and change the therapeutic response of secondary malignancies. Alterations of protein N-glycosylation have a pivotal role in tumorigenesis and metastasis formation. Thus, the aim of our study was to identify novel glycobiomarkers to predict more precise prognosis suggesting more efficient therapeutic alternatives for oral cancers. In our experiment oral mucosal soft tissue samples were obtained by using incisional biopsy from five patients with OSCC, both from the malignant and the opposite healthy gingival sides, as well as from seven age-sex matched healthy controls with the appropriate Ethical Permissions and Informed Patient Consents (DE RKEB/IKEB: 6152-2022). The collected tissues were properly homogenized (BeatBox, PreOmics, Munich, Germany), followed by N-glycan profiling of endoglycosidase released and fluorophore-labeled carbohydrates using capillary electrophoresis coupled with ultra-sensitive laser-induced fluorescent detection (CE-LIF, Beckman Coulter, Brea, CA). Ten out of the 39 identified N-glycan structures showed significant ($p<0.05$) differences between the malignant tissue samples of OSCC patients and the healthy controls. Comparing the healthy and the positive control oral mucosal samples two significantly different N-glycan structures have been revealed, while there were no differences between the N-glycan profiles of the malignant tumor and the positive control samples. We can conclude that the high-resolution CE-LIF-based glyocoanalytical method reported in this presentation proved to be an efficient and sensitive workflow for glycobiomarker-based molecular diagnostics of oral malignant lesions.

P12

UHPLC-HDMS^E Analysis of Lipids in Glial Cells

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Summary

Lipids are an important class of biomolecules involved in many vital cellular processes, they play three major roles: energy metabolism, structural and signaling. They have an irreplaceable function in human health as well as brain function. The brain contains a high number of lipids, and their imbalance is related to many neurological disorders and neurodegenerative diseases.

Lipidomics is a part of metabolomics focusing on the profiling and quantification of lipids. In the last decade, lipidomics has allowed better understanding of the metabolic processes associated with rare disorders and it can be used as a powerful tool for their clinical investigation. Most lipidomic identifications and quantifications are performed using liquid chromatography coupled with mass spectrometry.

In this work, we identified lipids from different classes (ceramides, hexosylceramides, dihexosylceramides, ceramides phosphate, sphingomyelins, phosphatidylcholines, phosphatidylethanolamines, phosphatidic acids, lyso-phosphatidylcholines, lyso-phosphatidylethanolamines, cholesterylesters, di- and triacylglycerols) in rats glial cells samples and compared peak areas of individual lipids in 2 groups of samples – control samples and samples treated with lipopolysaccharide (LPS). Sample analysis was carried out using ultra-high performance liquid chromatography (UHPLC) coupled with high-definition mass spectrometry (HDMS^E) working in positive ionization mode. Results were evaluated using Skyline 22.2 and Metaboanalyst 5.0. Responses of individual lipids were different in LPS-treated samples from control samples, we proved that neuroinflammation can cause changes in the lipid profiles.

Acknowledgement

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P13

Analysis of Peptides and Proteins by Native and SDS Capillary Gel Electrophoresis Coupled to Electrospray Ionization Mass Spectrometry

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Summary

Capillary electrophoresis (CE) is one of the frequently used techniques for the analysis of peptides and proteins, mostly utilizing UV or fluorescent detection both in zone (CZE) and gel electrophoresis (CGE) modes. Hyphenation of CE with electrospray ionization mass spectrometry (ESI-MS), however, provides additional structural information about the separated sample components. In the past decades, various CE-MS interfaces have been developed including sheath flow, sheathless and liquid junction based approaches, but none of them supported CGE-ESI-MS for SDS-proteins. Coupling capillary gel electrophoresis to a mass spectrometer via the Coaxial Sheath Flow Reactor Interface only required a cut-to-size rugged bare fused silica separation capillary filled with the corresponding gel-buffer compositions for native- and SDS-CGE modes. The setup could be readily connected in just a few minutes to any commercial ESI-MS via the closed-circuit flow reactor tube [1]. The excellent potential of this novel setup was shown by comparing UV and MS detections under identical separation conditions for peptide and protein analysis. This arrangement also offered the option of the application of post column reactions in the flow reactor section, e.g., to remove non-MS friendly background electrolyte components. In SDS-CGE mode, inclusion complexation was utilized in the flow reactor section having γ-cyclodextrin in the sheath liquid to remove the SDS content from the sample and the background electrolyte before entering the MS unit. While maintaining good separation efficiency, the decreased ion suppression made possible the long sought MS detection after separating SDS-proteins by CGE. A simple and widely applicable Coaxial Sheath Flow Reactor Interface was designed and implemented for easy and robust connection of liquid-phase microseparation methods to mass spectrometric detection, especially for capillary gel electrophoresis analysis of proteins and peptides, including SDS-CGE-ESI-MS.

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P14

Single Drop Microextraction for Enhanced Detection in Glycan Analysis by Capillary Electrophoresis

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Summary

Capillary electrophoresis (CE) handles complex samples with excellent resolution, but it has poor sensitivity due to the small detection volume and suffers from destacking of high-conductivity samples. Therefore, sample preparation is often required to clean up the matrix and enrich the analyte(s). Despite advancements in high performance analytical instruments, sample preparation remains indispensable for extracting the analyte(s) of interest in a concentrated form to facilitate high sensitivity analysis. In this paper, single drop microextraction (SDME) is evaluated for sample preparation, aiming to enhance sensitivity in CE analysis of glycans. In our study a single drop hanging at the inlet of the separation capillary was used as the acceptor phase in SDME of carbohydrate samples ranging from monomers to oligomers of up to a dozen units. The enriched sample in the single drop was injected into the separation capillary for subsequent high resolution analysis. This in-line coupling of SDME and CE demonstrated the advantages of reducing sample loss and providing convenience. SDME was first evaluated for preconcentrating the neutral fluorophore carbohydrate tag, 2-aminoacridone (AMAC). Following the successful enrichment of the neutral fluorophore, standard carbohydrates such as maltose and maltooligosaccharides up to a degree of polymerization of 12 with AMAC label were used to optimize the SDME method for glycans. We can conclude that this paper presents an operationally-simple sample preparation approach that can be easily adopted without modifying the commercial CE instrument with complex interfaces. SDME has proven to be a powerful tool for analyzing glycan samples, especially for trace analysis in complex biological matrices.

P15

Preparative Gel Electrophoresis for the Fractionation of DNA

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Summary

Liquid biopsy is a relatively new non-invasive tool used for diagnosis and screening. It is based on the collection of the body liquids and detection of the certain biomarkers. In the case of tumor diseases, blood can be used for these purposes and the detection of the disease is based on the evaluation of the presence of circulating tumor DNA (ctDNA) [1,2].

Detection of ctDNA fragments in the blood samples can be achieved by PCR. Nevertheless, the matrix of the blood sample can hinder the possibility of the ctDNA analysis. Especially large non-tumor fragments occurring in high quantities can be problematic and decrease PCR sensitivity. For that reason, we decided to fractionate blood DNA and selectively extract ctDNA (short DNA fragments) from the sample.

In our study, we developed a new 3D-printed device for DNA fractionation. Using this device, we optimized and partially validated a gel electrophoretic method for the preparative DNA separation. The recovery was ranging between 69-80% and repeatability between 20-31% (RSD, n=48). We also tested the method on the DNA spiked samples of blood serum and blood plasma. In this case, we achieved recovery of 48% and 60% (n=11).

Acknowledgement

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P16

UHPLC-MS/MS Strategy for Tryptophan Metabolite Analysis Based on Simple Sample Derivatization Using 2-Bromo-4'-Nitroacetophenone

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Summary

L-Tryptophan (TRYP) is an essential amino acid and precursor of many biologically active substances. TRYP catabolism occurs mainly through kynurenine, the serotonin pathway, and bacterial degradation. Concentration changes of metabolites from these pathways are associated with several neurodegenerative diseases (i.e., Alzheimer's or Parkinson's disease). The most effective tool for reliable and accurate determination of metabolite levels is liquid chromatography (LC) coupled with mass spectrometric (MS) detection. Despite technological progress, a chemical modification called derivatization is often used to increase sensitivity or improve the chromatographic properties of analytes. In this work, we developed and optimized the ultra-high performance liquid chromatography–tandem mass spectrometry (RP-UHPLC-MS/MS) method for determination of TRYP and its 12 metabolites (picolinic acid, quinolinic acid, nicotinic acid, kynurenic acid, xanthurenic acid, anthranilic acid, 3-hydroxyanthranillic acid, kynurenine, 3-hydroxykynurenine, indole-3-lactic acid, indole-3-acetic acid and 5-hydroxyindoleacetic acid) using the pre-column derivatization with 2-bromo-4’-nitroacetophenone (BNAP). The conditions of the derivatization reaction, such as time, temperature, concentration, and volume of BNAP, were modified. Finally, the optimal derivatization of all analytes was performed under mild conditions (50°C, 60 min). Separation of derivatives was achieved within 6 minutes on the C18 column. The method was successfully applied to the analysis of biological samples (urine, plasma) and creates a promising premise for the future for its optimization and subsequent validation for the analysis of these matrices.

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P17

Inhaled Lead Nanoparticles Cause Degenerative Alterations in Brain Tissue: Interdisciplinary Methodological Approach

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Summary

Elevated concentrations of lead nanoparticles (PbNPs) pose a significant risk to living organisms, with their impact on brain tissue remaining poorly understood. In this study, mice were exposed to insoluble lead oxide and soluble lead II nitrate nanoparticles for 6 and 11 weeks in whole-body chambers. We conducted a multi-disciplinary analysis combining analytical chemistry, histology, electron microscopy, and protein/mRNA expression studies across various brain regions. ET-AAS analysis uncovered a notable increase in Pb level in brain tissue for both inhaled PbNP types. ICP-MS revealed distinct distribution patterns of Pb and alterations in essential element distribution within the brain. Transmission electron microscopy identified inhaled PbNPs in different brain regions, alongside cytoskeletal ultrastructural changes confirmed histologically. Additionally, we determined the alterations in the expression of selected molecules of PI3K/Akt/mTOR signaling pathway on protein and mRNA levels across different brain regions. Moreover, we noticed deregulation of Tau phosphorylation. To confirm the direct impact of PbNPs on neural tissue, we established an in vitro model, primary cell cultures of trigeminal ganglia, for the detection of intracellular calcium levels. The values were elevated after 24 hours of PbNPs exposure, which was probably associated with cytoskeleton disruption due to Pb's ion substitution ability. In conclusion, our study, encompassing a diverse range of methodological approaches, provides valuable insights into the intricate molecular mechanisms governing the actions of PbNPs within brain tissue. These findings highlight the importance of comprehending the health risks associated with PbNP exposure.

P18

Synthesis of Silver-Iron Oxides Nanocomposites and Their Application for SERS

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Summary

Surface-enhanced Raman spectrometry (SERS) belongs to optical methods, which signal encodes information on molecular vibrations of an analyte. It operates with the enhancement of inelastically scattered light (Raman scattering) on nanostructured plasmonic metal, which might push sensitivity of the measurements down to a single molecule level. Bimetallic nanocomposites are an interesting alternative to traditional metallic substrates, as they combine abilities of two type of metals. This allows tuning of the experimental conditions for superior SERS analyses.

We focus on the combination of silver (enhancing the Raman scattering) and iron oxides, which selectively attract phosphorylated substances. Phosphorylation is the most common post-translational modification, usually responsible for activation/deactivation of enzyme, hence, the metabolism regulation. In this work, we compare two Ag-Fe_xO_y nanocomposites prepared with different protocols. As both procedures are based on alkalic coprecipitation of iron oxides and subsequent reduction of silver by hydroxylamine hydrochloride, the main difference lies in the washing procedures and in the implementation of silanization. We compare both nanocomposites in terms of their overall morphology and their capacity for the surface enhancement of Raman signal.

Acknowledgement

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P19

Development of the Miniaturized Instrumentation for Caspase Activity Detection

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Summary

Cell cultures provide important information about the mechanisms of hard tissue development, regeneration, and disease by using bulk analysis of average protein levels in cell populations. However, the cell heterogeneity at the single-cell level has been recognized to be vital for an understanding of various life processes during animal development and disease progress. In this study, we are focused on caspases, the cysteine proteases, which are traditionally associated with cell death via apoptosis but have recently been discovered as important factors in hard tissue differentiation.

A miniaturized device based on bioluminescence detection was created to measure their activity even in individual cells. The enzymatic activity of selective caspases (caspases – 3/7, 6, 8, 9) was measured inside different cell types including osteoblasts, osteosarcoma cells and periodontal ligament stem cells. The different activity of these enzymes occurred in the various cell lines. For instance, the activity of caspase-3/7 was elevated during osteoblast differentiation.

The results can potentially confirm the role of caspases as targets for the simulation of cell differentiation in supportive therapies for osteoporosis or simulated differentiation of stem cells into requested cell lineages. Moreover, these findings support one of the putative key mechanisms of non-apoptotic functions of pro-apoptotic caspases based on fine-tuning of their activation levels. Due to these aspects, caspases present a potential target for future clinical applications in hard tissue regeneration.

Acknowledgement

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P20

Enhancing Sensitivity with Different Injection Modes for Amino Acid Structure Compounds by MEKC

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Summary

Due to the short optical path and limited volume of the sample injected into the capillary, electromigration techniques are not characterized by similar sensitivity for the determination of compounds using UV detection compared to chromatographic techniques. To overcome these difficulties, various injection methods have been tried, manipulating the composition of both the sample and the separation buffer and using different types of injection [1]. The analytes were molecules with an amino acid structure; compounds of the kynurenine pathway (four kynurenines, phenylalanine and tyrosine) and selected thyroid hormones. The novelty of the work is the combination of the pressure-assisted electrokinetic injection (PAEKI) technique with the on-line capillary preconcentration technique, which involves the use of mixed micelles to lower the detection limit (obtaining clear, symmetrical and sharp peaks at the nanogram level). The determination of analytes was performed by micellar electrokinetic capillary electrophoresis (MEKC) using a separation buffer consisting of: 20 mM borax, 2 mM Brij-35, 20 mM SDS (pH = 9.2) and 20 % (v/v) MeOH. DAD spectrophotometric detection at an analytical wavelength of 200 nm was used to determine selected analytes. An attempt was made to isolate the tested compounds from urine samples using several extraction techniques, but the most satisfactory results were obtained by the method based on SPE using Strong Cation Exchanger (SCX) columns. A method of isolation from artificial urine samples was developed due to the presence of a number of endogenous compounds in natural urine samples. The limits of quantification for most analytes were set in the range 5-10 ng/mL and the limit of detection were in the range at 1.5-3 ng/mL. The research for a method ensuring the lowest possible level of detection was important due to the fact that analytes occur physiologically in very low concentrations, of the order of ng or pg per milliliter. The developed method can be successfully employed for further optimization procedures that will allow for their quantification in human biological fluid samples

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P21

HPLC-FLD Method Development for the Determination of Alpelisib in Human Plasma

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Summary

Alpelisib (ALP) is a selective phosphatidylinositol 3-kinase (PI3K) inhibitor approved for breast cancer treatment. Dysregulation of the PI3K signal pathway is involved in progression of cancer and is therefore a target for antineoplastic therapy [1]. Another indication approved only in the United States is the treatment of severe manifestations of PIK3CA-Related Overgrowth Spectrum, including venous malformations. ALP exposure shows considerable inter-individual variability and adverse effects and therefore therapeutic drug monitoring (TDM) is beneficial [2]. Fluorescence detection is a more affordable alternative to LC-MS/MS methods and provides sufficient sensitivity for TDM and pharmacokinetic profiling [1].

ALP bioanalysis was performed on an Agilent 1200 Series liquid chromatograph combined with a fluorescence detector. The excitation and emission wavelengths were set to 311 nm and 384 nm, respectively. A Kinetex Core-Shell C18 column (100 × 3 mm, 2.6 µm) heated to 40 °C was used for separation. The mobile phase consisted of ammonium acetate (10 mM, pH 6.6) and acetonitrile at 65:35 (v/v) in isocratic mode. The flow rate was 0.5 mL/min, the total time of single run was 8 min. Analytical data were processed using Agilent OpenLAB CDS software. After optimizing the separation conditions, a suitable liquid-liquid extraction procedure was tested. The highest extraction yield was obtained with a mixture of dichloromethane-isopropanol, 9:1 (v/v). The method was validated in terms of linearity, precision, accuracy, and stability according to the European Medicines Agency guideline and these parameters were within the acceptable limits. The limit of detection for ALP was determined to be 1.1 ng/mL, and the limit of quantification was 3.6 ng/mL (the limits were based on the signal-to-noise ratio of 3 and 10, respectively). The developed HPLC-FLD method was applied to determine ALP in patient plasma samples. Quantification was based on the peak area ratio of the analyte to the internal standard (glibenclamide) and the obtained concentrations were within the chosen calibration range (10–1000 ng/mL). This method is sensitive enough for the TDM of ALP in human plasma even for non-standard dosing schedules (e.g. off label use, children).

Acknowledgement

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P22

Differentiation of Biofilm-Positive and Biofilm-Negative Candida Parapsilosis Strains by Capillary Isoelectric Focusing

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Summary

One of the opportunistic pathogens that can cause life-threatening infections is *Candida parapsilosis*. It may colonize both natural and artificial surfaces in the host's body because of the production of a robust, adhesive biofilm layer. Patients with candidemia have considerably greater rates of mortality when exposed to biofilm-positive strains of *Candida parapsilosis* than when exposed to biofilm-negative strains. Techniques used to determine the ability to form biofilms are usually based on growth on suitable culture surfaces or microtiter plates. These procedures take a long time and a change in the cultural environment can easily affect the results. As a different approach, the physico-chemical characteristics of the cell surface, particularly in a cell surface charge, can be used to differentiate between biofilm-positive and biofilm-negative *Candida parapsilosis* strains. Determination of isoelectric points of *Candida parapsilosis* strains by capillary isoelectric focusing seems to be a useful technique to distinguish whether or not *Candida parapsilosis* strains form biofilm. This determination process is very fast taking a few minutes. The calculation and determination of isoelectric points was achieved by comparing the migration times of the tested yeasts and pI markers.

Acknowledgement

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P23

Characterization of the Miniaturized Platform for Cell Migration Studies

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Summary

Microfluidic devices enabling real-time visualisation of cell dynamics are improving our understanding of the migration of tumour cells to specific tissues or processes such as chemotaxis. Cell migration includes a variety of biophysical and biochemical activities, which are intensively studied in many laboratories. The migration of tumour cells from pathological to healthy tissues determines tumour invasiveness and plays an important role in cancer cell metastasis.

This work aims to design and fabricate a polydimethylsiloxane (PDMS) microfluidic cell-culture platform for testing natural cell mobility. The main feature is a circular arrangement of micropillars in a structure that forms an artificial barrier. The dimensions of micropillars and gaps can be easily adjusted in our design, and typically multiple devices are produced in one casting step, as the resulting PDMS cast is cut into individual cell-migration devices. Multiple cell-migration devices are then bonded to a single Petri dish so it serves as a powerful model for characterising malignant cells by label-free mechano-sorting. This platform will be used to test the ability of different tumour cells to change their shape and squeeze across the gaps between the pillars. In addition, the platform will be used for co-culture experiments with different populations of cells separated by micropillars or extracellular gels.

Acknowledgement

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P24

The Ionic Liquids as Supporting Agents for Extraction and Electrophoretic Separation of Clinically Important Catecholamines

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Summary

Nowadays, ionic liquids (ILs) are perceive as very promising solutions in various fields of analytical chemistry. It is worth to emphasize the contribution of ILs to the improvement of purification of the samples by extraction and improvement of reproducibility of migration times during separation. The extraction efficiency depends on the optimal parameters affecting the extraction efficiency [1]. In turn, one of the helpful solutions to improve reproducibility of migration times, could be the application of ILs as background electrolyte additive [2,3]. The first goal was to develop and validate MEKC method for determination of catecholamines (CAs) extracted from urine samples by SPME procedure using methanol with the tetrafluoroboran 1-ethyl-3-methylimidazolium as a desorbent. Next, this method was used to quantify adrenaline (A), noradrenaline (NA), dopamine (DA), L-tryptophan (L-Tryp) and L-tyrosine (L-Tyr) in real urine samples from pediatric patients suffering from neuroendocrine tumors [1]. The second goal was the comprehensive study evaluating the effectiveness of two alkylimidazolium ILs as additives to BGE for determination of another important catecholamines by the MEKC method. IL-cation can be adsorbed on the capillary wall and modify the electroosmotic flow and prevent the sorption of analytes at the same time. This project allows to assess if selected ILs such as ([HMIM+Cl-] and [HMIM+BF4-]) added to BGE at a concentration of 1 mM would affect the migration times and separation efficiency for homovanilic acid (HVA), vanillylmandelic acid (VMA), normetanephrine (NM) and metanephrine (M). The proposed MEKC method was used to analyze real urine samples from eight pediatric patients diagnosed with neuroblastoma (NBL) [2,3].

Acknowledgement

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P25

Sample Pretreatment Methods for CZE-MS Analysis of Selected Intact Growth Factors in Various Biological Fluids

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Summary

Growth factors are a heterogeneous group of proteins that are secreted by various tissues in the human body. Their presence in biological matrices may indicate the development of various diseases [1], thus there is a growing need to develop new high-performance analytical methods and procedures for their reliable identification and quantification. However, these substances are present in biological fluids at very low concentration levels compared to other high-abundant proteins that can mask the presence of growth factors and make their analysis impossible [2]. The gold standard for the pretreatment of proteomic samples is the use of immunoaffinity methods, which, however, are relatively expensive, their effectiveness also depends on the quality of the antibodies used, and their high selectivity can be a disadvantage in cases where we want to determine several proteins in one analysis [3,4]. Research efforts in pretreatment workflows for protein analysis are currently focused on developing more versatile pretreatment procedures that could be used for a broader range of proteins.

This work aims to develop a sample pretreatment method for the purification of complex biological samples before their analysis by on-line coupling of capillary zone electrophoresis and mass spectrometry (CZE-MS). Optimization and comparison of different conditions for solid phase extraction and protein precipitation to reach the compatibility with the CZE-MS method and in-capillary sample preconcentration via transient isotachophoresis (tITP) was performed on biological fluids (urine, plasma and serum) fortified with a mixture of three selected intact growth factors, namely IGF-1 (insulin-like growth factor-1), EGF (epidermal growth factor), and TGF-α (transforming growth factor- α).

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P26

HPLC Analysis of Vancomycin and Magnetic Nanonoparticles Modified with Chitosan and Vancomycin

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Summary

One of the most severe post-surgery complications in orthopaedic surgeries are bacterial infections. Vancomycin (VANCO) is used as an antibiotic of choice, and monitoring its concentrations is necessary to ensure its proper dosing. Analytical methods including liquid chromatography with UV/Vis or MS detections are eligible, because of their great selectivity and sensitivity. Modifications enhancing efficiency of antibiotic treatment, such as nanotransporters, are in severe need [1]. The aim of this study was to synthetize a nanotransporter (Superparamagnetic iron oxide nanoparticles (SPIONs)/Chitosan (chito)/VANCO) to improve antibacterial efficiency and monitor the release of drugs by the nanoparticles by the HPLC method. Chemical synthesis of nanoparticles, Chito (12.5 mg/L) and VANCO (250 a 500 μg/mL) were modified for 24 hours. VANCO analysis was determined on a HPLC system. Growth curves of *Staphylococcus aureus* (MSSA and MRSA) were measured (at 450 nm, 1 min, 25 ℃, 300 rpm, USA). In the experiment, VANCO was quantified by HPLC using gradient elution in Zorbax 5 μm, 4,6 x 250 mm. Symmetric and reproducible signal VANCO was detected at 250 nm. By the set of conditions VANCO signal was in RT: 5.2±0.1 min. Dependence on the concentration (0 – 1200 μg/mL) was linear (y = -6.1 + 0.097x; r = 0.9998; QC = 2.71). We calculated VANCO LOD 78 μg/mL and LOQ 234 μg /mL with RSD 2.8 %. We determined, that the size of SPION/Chito/VANCO was around 150 nm and zeta potential was around -30 mV. Release of VANCO from SPION/Chito/VANCO was tested in model conditions simulating the environment of the stomach, blood and the large intestine. From the curves of cumulative release of VANCO we observed the fast increase of VANCO under 100 min. The described transporter ensures longer release of VANCO. In this biological experiment we determined that SPION without modification will not cause any bacterial inhibition. Low molecular chitosan showed bacterial inhibition in concentrations around 10 mg/L. SPION/Chito (12.5 mg/L) in tested strains of MSSA and MRSA are causing an inhibition of growth by 60 to 80 %. Application of SPION/Chito/VANCO resulted in a significant growth inhibition of about 80 – 90%. We also observed the effect of added amount of nanoparticles. When we used SPION/Chito/VANCO with concentrations above 10 μg/mL, the inhibition of growth occurred in 90 % of MSSA and MRSA.

Acknowledgement

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P27

Miniaturized System for Capillary Electrophoresis Coupling with Mass Spectrometry Detection

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Summary

Mass spectrometry (MS) is a universal analytical technique continuously expanding towards the biopharmaceutical, life sciences, and other industries. MS analysis is often used as a golden standard for benchmarking of novel analytical devices and sensors. For its coupling with separation techniques, such as capillary electrophoresis (CE) or liquid chromatography, it is necessary to transfer the analytes from the liquid to the gas phase. Among a variety of available approaches, electrospray ionization (ESI) is the most frequently used. Whereas the standard sheath liquid ESI interfaces are commercially obtainable, the potential of nanoESI systems has not been fully explored. Due to ultra-low flow rates, typically below 100 nL/min, the nanoESI technology holds a promise of superior ionization efficiency and reduction of ion suppression.

For these reasons, a miniaturized system for coupling CE with MS detection via nanoESI was developed as one of the main goals of the uBIOSEP project (ubiosep.com). The system consists of two fundamental parts: *i*) a nanosprayer manufactured using Si technology and *ii*) a module for positioning the interface in front of an MS system and enabling the connection of CE and nanosprayer by the principle of the liquid junction. The microfabricated nanosprayers are based on Si wafers (150 mm in diameter, 400 µm thick). The fabrication process relies on the combination of thin film deposition, deep reactive ion etching (DRIE), and lithographic techniques. On its surface, 2.5 µm thick thermal oxide is grown. The nanosprayers are approximately 10×10 mm^2 after dicing, and each of them comprises of a pointed emitter, a transport channel, and a self-aligning liquid junction structure. The mechanical adjustment of the separation capillary before the analysis is therefore not needed. In the liquid junction, the separation capillary and transport channel are connected by a small gap filled with a spraying buffer. The system, thus, enables the use of the separation buffer optimized for a desired resolution of analytes and the spraying buffer selected for optimal ESI performance. The nanosprayer is positioned in a module with securing ports for connection of the CE and ESI current source, as well as for automated flushing of both the liquid junction and the separation capillary. The functionality and performance of the system were tested, and high-impact applications shall follow to demonstrate its potential as a versatile CE-nanoESI/MS platform for various relevant applications in the life and pharmaceutical sciences.

Acknowledgement

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P28

Analysis of Low Numbers of Bacterial Cells by CE and MALDI-TOF MS Utilizing Roughened Capillary

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Summary

Detection and identification of low amount of pathogens in the biological samples is of increased interest in recent years. This study presents rapid and sensitive method for identification of pathogenic bacteria in biological samples based on a combination of capillary electrophoresis (CE) with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). A part of single-piece fused silica capillary was etched with supercritical water with the aim of using it for adhesion (static or dynamic) of bacterial cells from large sample volumes. Adhered bacterial cells were desorbed and concentrated from the roughened capillary surface by transient isotachophoretic stacking. Charged cells were separated in micellar electrokinetic chromatography. Concentrated and separated bacteria were collected from the capillary and off-line analyzed by MALDI-TOF MS. Dynamic adhesion allowed concentration of bacteria from large sample volumes with the limits of detection in the range from 1.8×10^2 to 1.7×10^3 cells mL^{-1}, depending on the sample origin. Nevertheless, the limits of detection were the same for all the examined bacteria. The recovery of the method was about 83 % and it was independent on the sample matrix. A combination of CE with MALDI-TOF MS required at least 4×10^3 cells mL^{-1} to obtain reliable results.

Acknowledgement

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P29

Capillary Electrophoresis-Mass Spectrometry Analysis of Insulin-like Growth Factor 1 in Pharmaceutical Preparations

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Summary

Insulin-like growth factor-1 (IGF-1) is a 70-amino acid single-chain polypeptide. IGF-1 has found application in diagnostics as a biomarker of growth hormone disorders and a therapy for growth failure. Due to its strong anabolic effects, athletes often abuse it for doping purposes in various pharmaceutical forms, including tablets, capsules, and injectable solutions.

We developed an online method for the determination of IGF-1 in pharmaceutical matrices by coupling capillary zone electrophoresis-mass spectrometry with electrospray ionization (CZE-ESI-MS). Efficient separation and well-shaped peaks with favorable migration times (<15 min) were achieved by optimizing the background electrolyte and separation voltage. Highly efficient, accurate, repeatable, sensitive (sub μg/mL levels) and selective analysis of IGF-1 was achieved with further optimization of a sheath liquid composition, sheath liquid flow rate, and mass spectrometry conditions (nebulizing gas pressure, drying gas temperature, its flow rate, and capillary voltage). Optimized and validated CZE-ESI-MS method was successfully applied for the determination of IGF-1 in injectable solutions (Increlex®) and its presence was also confirmed in nutritional preparations (tablets and liquid colostrum).

This is the first work that uses the validated CZE-ESI-MS method for the determination of IGF-1 in pharmaceutical matrices and reveals the potential of capillary electrophoresis for its use in drug quality control laboratories with benefits such as high separation efficiency, high-speed analysis, low sample consumption, as well as environmental and cost aspects compared to the more established liquid chromatography methods.

Acknowledgement

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P30

Novel Fluorophores Applicable for Glycan Detection

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Summary

Changes in the composition of glycans correlate with the progression of many diseases; therefore, they are investigated as disease markers. Unfortunately, their analysis is complicated because they do not contain a chromophore or charges that would enable their electrophoretic separation. Our idea was to develop a new fluorescent marker covalently bound to glycans providing fast labeling kinetics, high quantum yield, increased detection sensitivity using MS, and at the same time, carrying a charge in the structure, which will enable the studied glycans to be separated electrophoretically.

The synthesis of the proposed structure of new fluorophores was based on the connection of two building blocks, synthon A and synthon B (Fig.1). The plan is to prepare variants of the fluorophore carrying electron-donating or electron-accepting groups in synthon A. These substitutions allow for variability in the absorption maximum wavelength and, thus, fluorescence wavelength. The molecule also holds a dimethylamino functional group responsible for electrophoretic mobility after protonation and a propanoate chain, which is necessary to attach the fluorophore to the glycan.

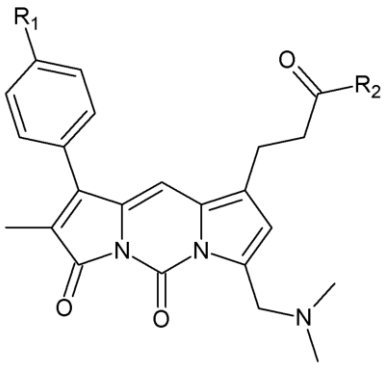

Fig. 1. Structural formula of the proposed structure of fluorophore.

The prepared substances will also be characterized in cooperation with the Institute of Analytical Chemistry of the Academy of Sciences of the Czech Republic. The usability of the newly developed fluorophores will be demonstrated by profiling glycoproteins associated with breast cancer.

Acknowledgment

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P31

Synthesis and Modification of Magnetic Nanoparticles for Bioanalytical Applications

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Summary

Magnetic nanoparticles are attractive in bioanalysis, because their physical separation by a magnet from the sample is simple and they can be used for analyte preconcentration, provided the analyte interacts with their structure. Firstly, the magnetic nanoparticles were prepared via coprecipitation of iron salts by addition of ammonia, followed by the addition of a stabilizing agent – sodium citrate [1]. The product was typically a brownish colloid, which has been sonicated to achieve uniform particle size - approximately 100 nm. The effect of supernatant removal and magnetic separation time was studied. The magnetic material was further modified, to obtain magnetic nanocomposites. To prepare a gold-iron oxide nanocomposite, modified Turkevich gold nanoparticle synthesis method [2], as well as a method developed by Lo et al. [3] were used. The nanocomposites were of an orange-reddish and reddish-pink color, respectively. The first type of synthesized nanocomposites did not have a gold plasmon peak on UV-Vis spectra, however, the latter did, at 556 nm. The behavior of the bond between the iron oxide and gold nanoparticles was further studied. The future application of this product is selective and fast magnetic separation and preconcentration of biological thiols from biological fluid samples. Iron oxide nanoparticles were modified by silver as well, to use this material in surface-enhanced Raman spectrometry (SERS). The reduction of silver onto the magnetic core was performed by adding sodium borohydride [4]. The size of the prepared silver-iron oxide nanocomposite was approximately 170 nm. The application of the prepared material to SERS seems to be possible since 100nM malachite green as well as 50µM riboflavin were detected.

Acknowledgment

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P32

Epitachophoresis – New Insert for Horizontal Gel Electrophoresis Device

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Summary

Electromigration separation techniques include a large group of separation methods used in many areas of human activity. One of them is DNA analysis, where these methods can be applied for both analytical and preparative purposes. The development of new purification and isolation techniques is potentially beneficial in a wide range of applications. It is assumed that the purification and isolation of selected DNA fragments from biological samples could increase the efficiency of their determination.

This work focuses on the use of epitachophoresis (ETP) for the separation, purification, concentration, and isolation of DNA fragments. A new type of ETP device was developed and tested. The uniqueness of the new device is in its design and structure since it will be used as a removable extension for horizontal gel electrophoresis devices. Generally, the presented device can be used for DNA extraction, either in isotachophoretic or electrophoretic mode. Depending on the initial conditions, DNA concentration and/or separation is possible. Firstly, the functionality of the new ETP device was tested. The following steps were selecting a gel stabilization medium, optimizing DNA sampling, and determining DNA yield. The DNA fragments were visualized using SYBR Gold fluorescent stain.

The presented device allows injection of up to 2.5 mL of sample and collection of 750 µL or 250 µL depending on the collection space size. Both separation modes were tested. Isotachophoretic mode allows the concentration of all DNA fragments into one or two fractions with a recovery of 65%. Using electrophoretic mode, DNA fragments were separated as in planar gel electrophoresis. Moreover, the DNA fragments were concentrated and collected in several fractions. The composition of collected fractions was controlled by Bioanalyzer.

P33

New Design of APCI/APPI Ion Source for Low Flow Rates

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Summary

Commercial atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) ion sources are designed for high flow rates, making them unsuitable for low-flow applications, e.g., capillary and nano HPLC. In the past two decades, work has been done on developing ion sources for low flow rates. Downscaled ion sources offer advantages such as low consumption of solvents, low sample amounts, and higher sensitivity. However, the ion sources mostly worked in ambient (open-air) conditions. Atmospheric oxygen and moisture can significantly affect ionization processes in such ion sources. In this work, we have developed an enclosed APCI/APPI source with the ionization region protected from ambient air. The ion source comprises the following parts: a nebulizer, a vaporizing chamber for drying the aerosol, an ionization chamber, and an element focusing the ions into the inlet capillary of the spectrometer. Nebulization of the sample is achieved using a recently introduced gas dynamic virtual nozzle [1]. The ionization chamber accommodates a UV lamp (APPI mode) or a corona discharge needle (APCI mode). In APPI mode, photons from the deuterium UV lamp (Hamamatsu) pass the MgF_2 polished window and ionize gas-phase molecules. In APCI mode, nitrogen is ionized by a corona discharge, and the nitrogen ions start the reaction cascade, leading to analyte ions. The device can be heated up to 260 °C. The ion source was mounted on LTQ Orbitrap XL (Thermo Scientific). The device was tested by direct infusion of samples ionizable by APCI and APPI, such as verapamil, caffeine, squalene, cholesterol, cholesteryl esters, and wax esters. The abundance of the molecular peaks increased with the flow rate, indicating a mass-sensitive detector. The ion source temperature significantly affected the efficiency of ionization and the formation of radical cations. APPI provided more complex spectra than APCI. From calibration curves of verapamil and caffeine measured in the concentration range of 0.5 to 500 µM and the noise level, basic analytical parameters were estimated.

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References

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