30TH INTERNATIONAL SYMPOSIUM

PBA

ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019
DAN PANORAMA HOTEL
TEL-AVIV, ISRAEL

ABSTRACTS BOOK

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NMR SPECTROSCOPY IN QUALITY ANALYSIS OF DRUGS AND EXCIPIENTS – A HOLISTIC APPROACH?

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Jonas Urlaub, *Institute of Pharmacy, University of Wuerzburg, Wuerzburg, Germany*
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Bernd Diehl, *Spectralservice, Cologne, Germany*

In former time, NMR spectroscopy was mainly used for structure elucidation and confirmation of newly synthesized compounds as well as natural products. More recently, quantitative NMR spectroscopy was increasing reported as a valuable tool for pharmaceutical quality analysis. On the one hand, drugs and excipients can be identified by 1D and 2D NMR techniques and structurally similar compounds, being e.g. synthesis or degradation impurities, can be recognized. Furthermore, counterfeit drugs can easily be identified. On the other hand, NMR spectroscopy can quantitatively be used to determine the content of a drug, its related compounds, residual solvents and in some cases counterions. Since this can often be done in one run, NMR spectroscopy is able to outclass separation techniques such as HPLC and capillary electrophoresis with regard to time necessary for a measurement and structure information, which can simultaneously be retrieved. In the talk the requirement for qNMR as well as different techniques to be applied will explained and the applicability demonstrated by a number of pharmaceutical examples.

Prof. Dr. Ulrike Holzgrabe

received her diploma degree in Chemistry at the University of Marburg in 1979, her approbation in Pharmacy in 1982, and her PhD in Pharmaceutical Chemistry in 1983 both at the University of Kiel. After habilitation in Pharmaceutical Chemistry in Kiel 1989 she became an associate professor at the University of Bonn (1990-1999). In 1988 she was a post-doc fellow at the University of Bath, UK, with Alan Casy, in 1994 a visiting professor at the University of Erlangen with J. Gasteiger, and 1995 at the University of Illinois at Chicago with A.F. Hopfinger. Since 1999 she holds a full professorship at the University of Würzburg (declining offers from the universities of Tübingen, Münster and Berlin, and the CEO position of the BfArM). She served as vice-rector of the University of Bonn from 1997-1999, as a president of the German Pharmaceutical Society from 2004 till 2007 and as a vicepresident of the University Wuerzburg since 2018. She is a member of the German and European Committee of the Pharmacopoeia (BfArM, EDQM), and a member of the scientific boards of the BfArM, BPS of FIP, and the HIPS as well as a collegiate of the DFG. The research interests are the development of antiinfectives and GPCR ligands as well as quality analysis of drugs.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

THE ENDOCANNABINOID SYSTEM - A LOOK BACK AND AHEAD

Raphael Mechoulam, *Center for Drug Research, Hebrew University, Jerusalem, Israel (mechou@cc.huji.ac.il)*

Over the last few decades research on the cannabinoids has gone through several distinct phases:
A. Research on plant cannabinoids [mostly on tetrahydrocannabinol (THC) and cannabidiol (CBD)].
B. Research on endogenous cannabinoids [mostly on anandamide and 2-arachidonoyl glycerol (2-AG)]
C. Research on endogenous anandamide-like endogenous fatty acid amides with amino acids and ethanol amines.

A. Plant cannabinoids. While many dozens of plant cannabinoids are known today, most research is still on THC and CBD. THC has been approved as a drug for enhancement of appetite, and is also used to prevent vomiting due to cancer chemotherapy. However of particular interest is CBD, which does not cause the typical cannabis psychoactivity, but is a potent anti- epileptic drug and is used in many countries in pediatric epilepsy. It is being evaluated in other therapeutic areas (graft versus host disease, schizophrenia, auto-immune diseases).
B. Endogenous cannabinoids. The endogenous cannabinoids anandamide and 2-arachidonoyl glycerol (2-AG) were discovered in the 1990's. Both compounds bind to the cannabinoid receptors CB1 and CB2. They are involved in a very large number of diseases, mostly as neuroprotective entities. However neither anandamide nor 2-AG have been administered to humans and although they are highly promising as potential drugs clinical trials have to be undertaken.
C. Anandamide-like endogenous fatty acid amides with amino acids and ethanol amines. A large number of compounds of these types have been discovered in the brain and other tissues, and some of them have been shown to be of major importance in a large spectrum of biological functions and diseases. Thus, oleoyl serine is an anti-osteoporotic molecule, arachidonoyl serine is a vasodilator and lowers brain damage and oleoyl glycine has anti addiction properties.

Professor Raphael Mechoulam

- 1930 Born Sofia, Bulgaria
  M.Sc. in Biochemistry, Hebrew University, Jerusalem
- 1953-1955 Army Service
- 1955-1958 Ph.D. at Weizmann Institute, Rehovot. Research on steroid synthesis. Advisor: Professor F. Sondheimer
- 1959-1960. Postdoctoral research at Rockefeller Institute, New York. Research on structure of triterpenes.
- 1960-1966. Junior and later Senior Scientist, Weizmann Institute. Research on the chemistry of natural products, including cannabinoids, terpenes, alkaloids.
- 1966- Hebrew University, Jerusalem; 1968 – Associate Professor; 1972 –Professor
- 1975. Endowed chair: Lionel Jacobson Professor of Medicinal Chemistry
- 1979-1982. Rector (Academic Head) of Hebrew University
- 1993-94Visiting Professor, Departments of Pharmacology and Medicinal Chemistry, Virginia Commonwealth University, Medical College of Virginia, Richmond, USA
- 1999-2000 President of the International Cannabinoid Research Society
- 2007-2013 Head, Natural Sciences Section, Israel Academy of Sciences.

Research interests: chemistry and biological activity of natural products and synthetic drugs. Published ca. 450 scientific articles

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

WONDERFUL OPPORTUNITIES FOR DRUG DEVELOPMENT BASED ON PRODRUGS AND CODRUGS

Abraham Nudelman, *Chemistry - Medicinal Chemistry Diviison, Bar Ilan Unniversity, Ramat Gan, Israel (nudelman@biu.ac.il)*

To overcome many of the problems encountered in the course of drug development, such as; poor aqueous solubility, toxicity, lack of specificity, instability, unpleasant taste or odor, etc., two major approaches have been developed, involving the use of prodrugs and codrugs as suitable clinical candidates. Prodrugs are defined as inactive substances, which upon metabolic activation release the active drug. Codrugs are substances wherein two or more active agents are covalently linked, that when metabolized release the synergistically active components of the administered substance. In recent years, prodrugs and codrugs comprise a major group of substances approved for clinical use. Our investigations led to the discovery of biologically active compounds in a variety of fields including: water soluble prodrugs; antischizophrenic codrugs; prodrugs and codrugs of various types of anticancer agents; codrugs of analgesics; codrug inducers of hemoglobin biosynthesis; lipophilic prodrugs of depigmenting agents. These areas of research will be discussed in the presentation.

Abstract Reference & Short Personal Biography of Presenting Author

Prof. Abraham Nudelman received his Ph.D. under the direction of Nobel Laureate Prof. Donald Cram at UCLA. Established the Division of Medinal Chemistry at the Chemistry Department of Bar Ilan Universitty. Published about 150 papers most of them in Medicinal Chemistry and Drug Development. He has under his name some 80 patents.

In collaboration with Dr. Hugo Gotlieb he published the artcle: NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. H. E. Gottlieb, V. Kotlyar and A. Nudelman. J. Org. Chem.1997, 62, 7512-7515", which as become the "Most Read" paper in the history of the Journal of Organic Chemistry, and has been cited by close to 4000 times.

A large volume of his investugations have dealt with the dvelopment of prodrugs and codrgus, three ofwhich have reached human clinical trials.

Among his discoveruies is a Novel, Patented Process for the Synthesis of Etoposide - The process was licensed to Teva Pharmaceuticals, which synthesized and marketed this compound for many years.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

METABOLOMICS WORKFLOW: RECENT DEVELOPMENTS IN THE ANALYTICAL PROCESS

Coral Barbas, *CEMBIO, Universidad San Pablo CEU, Madrid, España (cbarbas@ceu.es)*

In an analytical environment it is curious to see how many researchers consider that either "omics technologies" are "magic technologies" where you introduce data and get information by using software tools or just the opposite, "fairy tale technologies" where everything is unreliable. Indeed metabolomics is the omics with a closer relationship to Analytical Chemistry. Even more, I would say that Metabolomics is producing an evolution in analytical terms and concepts. Metabolomics workflow mimics the classical steps in the "Analytical Process" and our group has been developing tools to increase analytical quality and confidence at every step of the workflow, while at the same time applying our developments in real world studies because the path is made by walking.

Examples of different studies to avoid black boxes in untargeted metabolomics will be presented.

1.-**Sampling and sample collection**: Brain tissue obtained from autopsy is practically the only source of normal brain in humans. The post-mortem time (PT)-induced changes that may occur at both the metabolomics and anatomical levels in the brains will affect the conclusions obtained(1). In another study a popular method of cell isolation (fluorescence-activated cell sorting (FACS)) was considered. The impact of FACS on the cell metabolome was deeply investigated (2).

2.-**Identification**. CEU Mass Mediator (CMM) is an on-line open source tool developed at CEMBIO (3). It aims aiding researchers when performing metabolite annotation. In addition, CMM scores the annotations which matched the query parameters using 122 rules based on expert knowledge (4). The information from external databases has been refreshed, and an in-house library with oxidized lipids not present in other sources has been added. This has increased the number of experimental and predicted metabolites. Furthermore, new taxonomy and ontology metadata have been included. CMM has expanded its functionalities with a service for the annotation of oxidized glycerophosphocholines, a service for spectral comparison from MS2 data, and a spectral quality-assessment service.

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Abstract Reference & Short Personal Biography of Presenting Author

PROF.CORAL BARBAS is currently Full Professor of Analytical Chemistry at Pharmacy Faculty, Universidad CEU San Pablo, Madrid (Spain) and Director for the "Centre for Metabolomics and Bioanalysis" (CEMBIO) at this Faculty. She is also Director for CEU International School of Doctorate (CEINDO); Visiting Professor at Imperial College London, Department of Surgery and Cancer. As previous appointments she was Vice-

Chancellor for Research at Universidad CEU San Pablo and Marie Curie Fellow at Kings College London and visiting Professor at Bialystok Medical University.

She is the author of more than 250 papers, with current research interests in all the steps in metabolomics workflow: experimental design, sample pretreatment, analytical methods for targeted and untargeted metabolomics, method validation, data treatment, compound identification and interpretation. Her researcher is focused on multiplatform analysis with GC-MS, LC-MS and CE-MS of all kind of biological samples searching for disease biomarkers, prognostic biomarkers, mechanisms of action of a drug, diet, etc.

Her awards include Angel Herrera Research Award and Teaching Award, the medal of Bialystok Medical University and she was named to the 2016 Power List, the 50 Most Influential Women in Analytical Chemistry in the World, *The Analytical Scientist* and recently she has received the award of the Belgian Society of Pharmaceutical Sciences (BSPS 2018) and a *Honoris causa* doctorate in Bialystok Medical University Member of different boards of international Committees and Editor for Journal of Pharmaceutical and Biomedical Analysis.

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RECENT DEVELOPMENTS IN ENANTIOSEPARATION OF CHIRAL DRUGS

Bezhan Chankvetadze, *Chemistry, Tbilisi State University, Tbilisi, Georgia (jpba_bezhan@yahoo.com)*

This presentation summarizes the recent developments in analytical and preparative scale enantioseparation of chiral drugs. The techniques discussed include high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), nano-liquid chromatography (nano-LC), capillary electrochromatography (CEC) and capillary electrophoresis (CE). Chiral drugs and drug-like compounds discussed include arylpropionic acid derivatives, chiral sulfoxides, amino acids, etc. Various fundamental and applied aspects will be discussed in each techniques.

In HPLC part novel chiral selectors, mobile phases, mobile phase additives and inert carriers, as well as some unusual effects and approaches for a better understanding of the chiral recognition mechanisms will be summarized [1, 2]. This part of the presentation will highlight the efforts of our group with regard to separation of enantiomers in the liquid phase with the highest possible coverage of analytes, separation selectivity, plate numbers and the shortest analysis time [3]. In order to achieve these goals, the systematic optimization of the composition of polysaccharide-based chiral selectors (CS), the structure of studied analytes, composition of the mobile phase, mobile phase additives and separation temperature have been performed. In a parrallel project, the particle size pof the silica, its morphology (porosity and the pore size), the nature of the CS and its content in chiral stationary phase (CSP) were optimized in order to achieve the highest possible column performance. In SFC part our recent results on separation of enantiomers of native amino acids with polysaccharide-based CSPs will be shortly discussed [4]. The CE part will focus on chiral recognition mechanisms with cyclodextrin-Type CSs [5].

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Abstract Reference & Short Personal Biography of Presenting Author

Bezhan Chankvetadze is Full Professor for Physical Chemistry and the director of the Institute of Physical and Analytical Chemistry at Tbilisi State University, Tbilisi, Georgia.

B. Chankivetadze has published over 230 research papers in peer reviewed journals, many review papers, book chapters and holds over 10 patents of Japan, Germany, and USA. He has authored and co-authored several books and serves sínce 2005 as one of the editors of the Journal of Pharmaceutical and Biomedical Analysis. B. Chankvetadze has given over 250 presentations on international conferences. His current citation number is around 8500 and H-index 52.

B. Chankvetadze is the recipient of "Journal of Chromatography Top Cited Article Awards" in 2005, 2006 and 2010, the recipient of "2006 Belgian Society of Pharmaceutical Science Award of Recognition", "The Scientist of the Year 2016" award of Georgia, and the joint Csaba Horvath Memorial Award of the Hungarian Separation Science Society and Connecticut Separation Science Council in 2017.

Prof. B. Chankvetadze is Full Member of the Georgian National Academy of Sciences.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

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BIOANALYTICAL APPLICATIONS OF MATRIX COMPATIBLE SOLID PHASE MICROEXTRACTION

Janusz Pawliszyn, *Chemistry, University of Waterloo, Waterloo, Canada (Janusz@uwaterloo.ca)*

Development of matrix compatible coatings for solid-phase microextraction (BioSPME) has enabled direct immersion microextraction of small molecules from complex matrices, including body fluids and even live tissues. Such coating consists of appropriate sorbent particles, such as HLB phases dispersed in a biocompatible binder, such PAN forming well defined wettable porous structure. The direct immersion mode of BioSPME when utilized in conjunction with such morphology extraction phases facilitates clean extraction of a wide range of analytes from complex matrices without the occurrence of fouling or coating saturation as well as elimination of matrix effects when combined with LC/MS. Such "chemical biopsy" process isolates only small molecules from the matrix followed by introduction to the instrument clean mixtures containing target analytes without any sample matrix.

SPME workflows for automated high throughput diverse bioanalytical applications of body fluids based on 96 well plate using coated-blade format combined with LC/MS will be described. It has been demonstrated that the approach meets the required LODs. The clean extraction property of this approach led to diverse BioSPME-MS strategiesresulting in fast and simple determination of target compounds in diverse matrices. These technologies include Coated Blade Spray (CBS) and SPME- open-port probe (SPME-OPP). Unlike direct-sample-to-MS approaches, BioSPME-MS provides a cleaner extract which allows for long-term operation of the instrument with minimal maintenance and reliable quantification. Sampling/sample-preparation is performed either by spotting the sample onto the SPME-device, or by immersing the SPME-device on a vial containing the sample. Despite short extraction times, limits of quantitation ranging between low-pg/mL to sub-ng/mL were obtained, while good accuracy, and linearity were attained.

Important bioanalytical applications of BioSPME include in vivo investigations. In particular, tissue analysis remains the bottleneck of such studies due to the invasiveness of presently used sample collection based on biopsy, as well as the laborious and time-consuming sample preparation protocols. In the proposed BioSPME technology, the flexible metal acupuncture size needle containing recess is coated with the coating having morphology described above. In this approach, only molecules of interests are extracted onto biocompatible sorbent from the investigated system, however no tissue is not removed. Therefore, the direct coupling of the "chemical biopsy" probe to mass spectrometry is feasible as well. Such hyphenation facilitates rapid quantification of the compounds of interests resulting in possibility of close to real time monitoring. This approach has potential in biological and clinical on-site investigations including bedside medical diagnosis.

Anal. Chem. 2018, 90, 302−360

Abstract Reference & Short Personal Biography of Presenting Author

The primary focus of Professor Pawliszyn's research program is the design of highly automated and integrated instrumentation for the isolation of analytes from complex matrices and the subsequent separation, identification and determination of these species. The primary separation tools used by his group are Gas Chromatography, Liquid Chromatography and Capillary Electrophoresis coupled to variety of

detections systems, including range of mass spectrometry techniques. Currently his research is focusing on elimination of organic solvents from the sample preparation step to facilitate on-site monitoring and in-vivo analysis. Several alternative techniques to solvent extraction are investigated including use of coated fibers, packed needles, membranes and supercritical fluids. Dr. Pawliszyn is exploring application of the computational and modeling techniques to enhance performance of sample preparation, chromatographic separations and detection. The major area of his interest involves the development and application of imaging detection techniques for microcolumn chromatography, capillary electrophoresis and micro chip separation devices.

Professor Pawliszyn Hirsch Index (H-index) is 91. He is a Fellow of Royal Society of Canada and editor of Analytica Chimica Acta, Trends in Analytical Chemistry. He initiated a conference, "ExTech", focusing on new advances in sample preparation and disseminates new scientific developments in the area, which meets every year in different part of the world. He received the 1995 McBryde Medal, the 1996 Tswett Medal, the 1996 Hyphenated Techniques in Chromatography Award, the 1996 Caledon Award, the Jubilee Medal 1998 from the Chromatographic Society, U.K., the 2000 Maxxam Award from Canadian Society for Chemistry, the 2000 Varian Lecture Award from Carleton University, the Alumni Achievement Award for 2000 from Southern Illinois University, the Humboldt Research Award for 2001, 2002 COLACRO Medal, in 2008 he received A.A. Benedetti-Pichler Award from Eastern Analytical Symposium, 2008 Andrzej Waksmundzki Medal from Polish Academy of Sciences, 2008 Manning Principal Award, 2010 Torbern Bergman Medal from the Swedish Chemical Society, 2010 Ontario Premier's Innovation Award,2010 Marcel Golay Award, 2010 ACS Award in Separation Science and Technology, 2011 PittCon Dal Nogare Award, 2012 E.W.R. Steacie Award, 2013 CIC Environmental Research and Development Award, 2013 CICLeSueurMemorial Award, 2015 Maria Skłodowska-Curie Medal from Polish Chemical Society, 2015 Halász Medal Award from the Hungarian Society for Separation Sciences, 2017 Pittsburgh Conference Analytical Chemistry Award, the 2017 Eastern Analytical Symposium Award for Outstanding Achievements in the Fields of Analytical Chemistry, 2018 ACS Award in Chromatography, 2018 North American Chemical Residue Workshop Excellence Award and 2019 Talanta medal.

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KEYNOTE LECTURERS

NOVEL BIOSENSORS FOR PHARMACEUTICAL ANALYSIS

Zeynep Altintas, *Institute of Chemistry, Technical University of Berlin, Germany, Berlin, Germany (zeynep.altintas@tu-berlin.de)*

The high consumption of drugs unavoidably leads to their release into the environment and this has been a subject of interest for many years. A vast number of reports in the last decade have highlighted the occurrence of drugs and their metabolites in aquatic systems, waste waters and water treatment plants. Their presence in aquatic environment and drinking waters is a result of unsuccessful elimination during sewage treatments. The use of milk products contaminated with antibiotics is another drawback, leading to allergic reactions and sensitivity in human. Also, long-term consumption of milk products containing low levels of the last resort antibiotics may cause the relevant bacteria to build up resistance. Sensitive, rapid and effective quantification and monitoring systems play a key role for pharmaceutical determination in water and milk products. Such quantification systems are also needed to determine the new drugs in tropical plant extracts and for use in pharmacokinetic studies. Hence, we have addressed these needs by developing novel sensors for pharmaceutical analysis in various samples, including plant extracts, human serum, water, and milk.

To achieve this goal, we design and synthesize biomimetic molecular receptors towards pharmaceuticals in nano- and electro-MIP formats, which are used for sensor development. The two different polymerization techniques, including solid phase synthesis and electropolymerization, are performed to obtain the drug specific receptors. The affinity between the receptors and the target drugs (dissociation constant < 1.8 nM) is mostly superior to natural and other synthetic receptors. We have applied these receptors for different transducing systems, such as electrochemical, surface plasmone resonance and piezoelectric sensors. In addition to utilizing these sensors in pharmaceutical monitoring from water and milk samples, they find application for the determination of certain drugs (*e.g.* artemisinin) in plant extracts and for pharmacokinetic studies. The novel sensor systems offer rapid, sensitive and cost-effective analyses for pharmaceuticals, which are extremely simple, robust, and do not require pre-sample treatment. The developed sensors may have immense impact in pharmaceutical and health sectors.

Keynote Speaker & Short Personal Biography of Zeynep Altintas

Dr. Zeynep Altintas is the head of the Biosensors and Receptor Development Group at the Technical University of Berlin, Germany. Her background is biomedical engineering and biotechnology. She leads an interdisciplinary research group in the domain of optical, piezoelectric and electrochemical biosensors, bioreceptor development, and molecular modelling. Her specialization also includes but not limited to implementation of biosensors for the diagnosis of disease biomarkers, pharmaceuticals, and food and environmental pathogens. She has more than 100 publications in these fields, including a book, journal articles, book chapters, patent applications, and conference papers. She has delivered numerous invited talks at international conferences and world-renowned institutes. She has built a reputation in her areas of expertise that is recognized by several international awards to her research. She serves as an expert reviewer for EU and Wisconsin Groundwater Coordinating Council (USA) funded projects, in addition to acting as an editorial board member and a reviewer for several important journals in her areas of expertise. She is also a member of the Royal Society of Chemistry.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

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INVESTIGATING AMYLOID AGGREGATION AND ITS INHIBITION: IMPLICATION OF REVERSIBLE AND NON-REVERSIBLE ADDUCTS FORMATION

Vincenza Andrisano, *for Life Quality Studies, University of Bologna Italy, Rimini, Italy (vincenza.andrisano@unibo.it)*
Angela De Simone, *Department for Life Quality Studies, Rimini Campus, Alma Mater Studiorum Università di Bologna, Italy*
Marina Naldi, *Department of Pharmacy and Biotechnology, Alma Mater Studiorum Università di Bologna, Italy*
Center for Applied Biomedical Research (C.R.B.A.), S. Orsola-Malpighi Hospital, Bologna, Italy
Daniele Tedesco, Department of Pharmacy and Biotechnology, Alma Mater Studiorum Università di Bologna, Italy
Manuela Bartolini, *Department of Pharmacy and Biotechnology, Alma Mater Studiorum Università di Bologna, Italy*
Vincenza Andrisano, *Department for Life Quality Studies, Rimini Campus, Alma Mater Studiorum Università di Bologna, Italy*

Inhibition of aggregation of protein and peptides is a focus of therapeutic development for several diseases [1]. In particular, amyloid peptide 1-42 (Ab_{1-42}) self-assembly is a central event in the pathogenesis of Alzheimer's disease, leading to the formation of amyloid plaques, involving several toxic intermediates. In this talk, a multi-methodological approach will be introduced, which allowed the elucidation of the diverse assembly species formed during the Ab_{1-42} aggregation process [2,3]. The combination of mass spectrometry (MS), circular dichroism (CD) and fluorescence spectroscopy and atomic force microscopy (AFM), as a fundamental part of this multi-methodological approach, enabled the setup of a reliable experimental protocol, whichwas applied to the chemical and morphological characterization of the self-assembly species during the Ab aggregation process and inhibition [4-6].

This approach resulted to be a successful tool to elucidate the mechanism of action of known and new inhibitors, because could highlight the formation of reversible and irreversible adducts at the basis of the inhibition process. A discussion of several examples of inhibitors will follow [7].

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Abstract Reference & Short Personal Biography of Presenting Author

Vincenza Andrisano received her 5 years Degree in Chemistry and Pharmaceutical Technology from the University of Bologna (Italy) and a 2 years post-lauream Diploma from Scuola di specializzazione in Scienze e Tecnologie Cosmetiche Università di Ferrara (Italy). As part of her postdoctoral training, she spent two years as a research assistant at the Sydney University Australia (Department of Biochemistry), researching on the enzymatic properties of new mechanism based substrates and inhibitors for dihydrofolate reductase in the search of anticancer drugs (1987-88). She has been visiting professor at McGill University, Division de Pharmacocinétique, Department D'Oncologie, Montréal (Canada) (1995) (stereoselective interaction between drug-target protein by biochromatography) and at the Department of Pharmacology Georgetown University Medical Center Washington DC (USA) (2000)(enzyme immobilization for the development of glyceraldheyde 3-phosphate dehydrogenase inhibitors in the search of anti-tripanosomial drugs).

Since 2012 she is full professor in medicinal chemistry at the Department for the Life Quality Studies, Rimini Campus, University of Bologna.

VA's present research covers four main lines: (i) structural characterization of protein targets (i.e. amyloid peptides) and candidate leads by circular dicroism in solution and HPLC-MS, for the development of new drugs for the treatment of degenerative diseases (Alzheimer's disease and cancer) : enzyme kinetics: determination of mechanism of action of new potential acetylcholinesterase, beta-secretase, Glicogeno Synthase Kinase 3 beta inhibitors with dual function, inhibition studies of b-amyloid fibril formation by circular dichroism, fluorescence spectroscopy, mass spectrometry (ii) development of analytical methodologies in the design, synthesis and biological evaluation of new bioactive compounds (iii) characterization of the ligand/target peptide/protein/enzyme interactions (classical and multiwells spectroscopic kinetic methods, biochromatography, analysis through optical biosensor, HPLC-MS) (iv) Immobilisation of target enzymes on solid matrices which are then inserted in fluidic and chromatographic systems for binding studies.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

THE CONTRIBUTION OF DIFFERENTIAL SCANNING CALORIMETRY TO PHARMACEUTICAL DEVELOPMENT

Judith Aronhime, *J.A.Consulting, Rehovot, Israel (juditharonhime@gmail.com)*

Successful formulation development requires fundamental knowledge of the physicochemical properties of the active pharmaceutical ingredients.

Differential scanning calorimeter (DSC) has been traditionally a valuable element in the physicochemical analysis of active pharmaceutical ingredients. It is being used for some decades as a complementary tool to X-ray powder diffraction and spectroscopic methods for understanding and characterizing polymorphic systems, in particular their thermodynamic stability. Other uses include quick screening of drug-excipient interactions, characterization of racemic mixtures, purity determinations and quantitative analyses.

The use of the DSC technique is very common due to the cost accessibility and its wide range of uses. Also, it requires only very small amounts of material for analysis and practically no sample treatment prior to analysis.

This presentation will review some of the important applications of DSC to identify the most critical solid-state parameters of active pharmaceutical ingredients, including those applications aimed at characterizing polymorphic systems.

Abstract Reference & Short Personal Biography of Presenting Author

Dr Judith Aronhime is a consultant in the field of pharmaceutical polymorphism and pharmaceutical solid state science. She has accumulated 27 years experience in her former position in Teva TAPI global R&D management as responsible for all the solid state aspects of the active pharmaceutical ingredients (APIs). In this key role she gained expertise in scientific analysis, characterization, development and research of polymorphism and all solid state attributes of API's, along with the scientific support provided to patent litigations, prosecutions and clearances.

She was a key contributor to TEVA worldwide leadership position in the intellectual property domain by searching new crystal forms of APIs. Part of those became API ingredients in the marketed TEVA products.

Author and contributor to more than 100 patents and current applications.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

STRUCTURAL AND FUNCTIONAL PROFILE OF HUMAN SERUM ALBUMIN: CLINICAL IMPLICATIONS AND PROGNOSTIC RELEVANCE

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Department of Hepatology and Gastroenterology, Aarhus University Hospital, Denmark

Notwithstanding human serum albumin (HSA) is one of the most studied plasma proteins, it continues to attract the research interest. Along with its oncotic properties and thanks to its peculiar structure, HSA is endowed with many “non oncotic properties” which include the fine regulation of the plasma redox state, the modulation of the inflammatory and/or immunological responses and the capacity of binding several endogenous or exogenous molecules [1]. Moreover, HSA plasma concentration has shown to be a valuable biomarker in many diseases and pharmaceutical grade formulation of this protein is widely used for the treatment of shock, hypoproteinemia and burns. Unfortunately, in pathological but also in physiological conditions HSA structure may undergoes several post-translational modifications (PTMs) such as reversible and irreversible oxidation, truncation and glycation; recent studies have shown that extensive PTMs associated with liver cirrhosis affect HSA structure and functionality [2].

In the light of the key physiological role of HSA, the assessment of disease-related PTMs is of pivotal interest. To this aim, we have developed and validated an analytical methodology based on reverse phase liquid chromatography (RPLC) coupled to electrospray ionization mass spectrometry (ESI-MS) for the identification of HSA structural alterations as well as HSA dimerization occurring in a large cohort of cirrhotic patients (n=144). Interestingly this study showed a fine correlation between altered HSA isoforms and disease severity, clinical complications and patients’ survival. Moreover, the analysis of altered HSA structure in a population of patients affected by alcoholic hepatitis (AH) (n=20) showed that the severe oxidative stress experienced by AH patients caused profound changes in circulating HSA micro-heterogeneity

Abstract Reference & Short Personal Biography of Presenting Author

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Full Professor of Medicinal Chemistry University of Bologna, Italy. Visiting Professor at Oregon State University, Corvallis, Oregon, USA: 1980, 1982, 1984, 1985; at The University of São Paulo, School of Pharmacy at Ribeirão Preto, Brazil, 1989, 2006, 2008, 2010, 2011, 2012; at McGill University, Montreal, Canada, 1995; at the University of Bordeaux, Francia, 1997; at the Georgetown University, Washington DC, USA, 2000.

The scientific activity of Prof. Carlo Bertucci mainly deals with the structural characterization of drugs and proteins, the study of the biorecognition mechanisms in the drug/target protein and protein/protein binding. In particular: enantioselective HPLC and circular dichroism based detection system; absolute configuration assignment to chiral drugs; structural characterization of peptides and proteins by spectroscopic and spectrometric techniques; drug binding to target proteins by biocromatography, SPR biosensor and chiroptical spectroscopy; monitoring and modulation of conformational transition of peptides and proteins having physiological or pathological relevance.

He is member of the Editorial Advisory Board of the Journal Chirality and of the Journal of Pharmaceutical and Biomedical Analysis.

He is the author, or co-author, of some 200 papers or reviews in international journals, 1 patent, 8 chapters in books, 1 entry in an encyclopaedia.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

QSRR STUDY OF AMINO ACIDS AND LIPIDS ON THE POLAR-EMBEDDED STATIONARY PHASES ACCORDING TO THE STRUCTURAL SIMILARITIES

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Magdalena Skoczylas, *Chair Of Environmental Chemistry And Bioanalytics, Faculty Of Chemistry, Nicolaus Copernicus University In Torun & Interdisciplinary Center For Modern Technologies, Toruń, Poland*

In the case of separation techniques the evaluation of separation medium comprises an essential part of analytical methods improvement. Significant efforts have been made over the last few years to achieve stationary phases imitated natural matter (e.g. biological membrane) as well as endogenous compounds (e.g. amino acids). Specificity of chemically bonded ligands in the case of new materials enables receiving a so-called dedicated stationary phases. Moreover, structural similarity of the immobilized ligands with the desired group of analytes may determine the high selectivity of the prepared stationary phases. Therefore, the investigations in accordance with the "3S" assumption – *similarity, selectivity, and specificity* – allow the development of a new generation of separation materials.

The QSRR study comprises one of the approach that can give some insights into the separation mechanism occurring at the molecular level. The elementary objective of QSRR investigations is to determine a mathematical model that relates the analyte retention to physicochemical and structural parameters. Aside of molecular separation mechanism, the QSRR study allow to evaluating stationary phase properties, predicting retention for a new solutes, accelerating the phase selection of chromatographic method development etc.

The aim of this study was to develop prediction models using multiple linear regression that could accurately describe the retention behavior of a group of structurally related compounds – amino acids – on peptide-silica stationary phases operated in HILIC and – lipid compounds (phospholipids and sphingomyelin) – on the polar-embedded stationary phases operated in HILIC and RP HPLC. The QSRR methodology has been applied according to the „3S" assumption - structural similarity of analytes and chemically bonded ligands. The research also aimed at using the prediction models to provide insights on the retention mechanism in the studied system.

The derived mathematical models showed that the number of hydrogen bond acceptors is a main retention predictor for the peptide-silica columns, signifying that hydrogen bonding is crucial mechanism. On the other hand, the QSRR studies showed that the dominant analyte descriptor influencing retention on the alkyl-amine stationary phase was the logarithm of the octanol-water partition coefficient. For the phospho-diol and alkyl-amide stationary phases, the dominant analyte descriptor influencing retention was the molar volume and solvent accessible area of the analyte, respectively.

Acknowledgement

This work was financially supported by the National Science Center (Poland) as a part of the PRELUDIUM 12 project No. 2016/23/N/ST4/00369.

Abstract Reference & Short Personal Biography of Presenting Author

Professor Bogusław Buszewski is the head of the Chair of Environmental Chemistry & Bioanalytics at the Faculty of Chemistry, Nicolaus Copernicus University. He is also the president of the *Central European Group for Separation Sciences* and the chairmen of the *Committee of Analytical Chemistry of Polish Academy of Sciences. Former, He was the* president of the *Polish Chemical Society* and *European Society for Separation Science.* He was awarded by numerous national and international organizations. His main scientific interests are concerned with separation science including theoretical and practical aspects in chromatography and related techniques, adsorption, sample preparation, bianalytics and bioanalysis (metabolomics, proteomics, biomarkers), nanotechnology and chemometrics. He is authored or co-authored 15 books, patents and more than 567 scientific papers (over 10.000 citations, h= 46) and member of the editorial boards of 26 national and international journals in field of analytical chemistry and separation sciences.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

DEVELOPMENT OF CHROMATOGRAPHIC PLATFORMS FOR IMERS CHARACTERIZATION AS PROTOTYPES FOR FLOW-CHEMISTRY APPLICATIONS

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***Marco Corti**, Drug Sciences, University of Pavia, Pavia, Italy*
Gabriella Massolini, *Drug Sciences, University of Pavia, Pavia, Italy*
Paola Conti, *Germanyddepartment of Pharmaceutical Sciences, University Of Milan, Milan, Italy*
Marco Terreni, *Drug Sciences, University of Pavia, Pavia, Italy*

Enzymes are increasingly used as biocatalysts for the production of fine chemicals and pharmaceutical products. In this context biocatalyzed reactions performed in a flow system can benefit from improved mass transfer, temperature control and, importantly, continuous substrate feed and product removal, thus limiting the possible substrate/product inhibition of the enzyme activity.

Flow reactors can be used for different scopes: analytical scale synthesis (i.e. for screening purposes and rapid optimization of the reaction conditions), lab scale synthesis for pilot studies or large scale synthesis (e.g., industrial production of APIs). In the recent years low-volume immobilized enzyme reactors (IMERs) and analytical IMER-based platforms have found several applications in the drug discovery field with a special emphasis on enzyme inhibitors screening and on-line proteolysis of proteins prior to analytical characterization. However, the potentiality of IMERs for reaction optimization studies on analytical scale as prototypes for biosynthetic purposes (very low sample consumption, short analysis time and the possibility of connecting them to different separation and detection systems) has not been fully exploited.

In this presentation two families of enzymes are selected: nucleoside phosphorylases and ω-transaminases. Nucleoside phosphorylases have shown their powerfulness in the synthesis of modified nucleosides with clinical relevance as antiviral and antitumor agents. The synthesis of these products can be of great interest for small or medium pharmaceutical industries because the low dosage of most of the antiviral and antitumor nucleosides (and nucleotides) necessitates small production sizes of high value products. On the other hand, ω-transaminases are powerful enzymes for the preparation of enantiomerically pure chiral amines, which represent useful synthetic intermediates for the preparation of several different classes of APIs. Enzymes belonging to the two different families were immobilized on different analytical supports (monolithic silica and poly-HIPEs supports) and used in chromatographic platform for IMERs characterization. The flow-synthesis of nucleosides catalyzed by immobilized Purine Nucleoside Phosphorylases was carried out on analytical scale in an integrated systems for reaction control and product purification.

A chromatographic platform based on liquid chromatography has been also developed to facilitate studies on the biocatalytic properties of an ω-transaminase useful for chiral amines synthesis (ATA117). ATA117 was covalently immobilized on different supports. The developed enzymatic reactor was coupled through a switching valve to an analytical column for separation and quantitation of the hydrolysis products. The enantiomeric excess was determined to follow the kinetically controlled enantiomer-differentiating reaction.

Abstract Reference & Short Personal Biography of Presenting Author

Curriculum Vitae

Prof. Enrica Calleri got her master degree in Chemistry and Pharmaceutical Technology in 1996 at the University of Pavia and in 2000 she defended her PhD thesis titled “development of new protein-based chiral stationary phases”. She spent part of her PhD course at Georgetown University, Washington DC (USA), under the supervision of Prof. I.W. Wainer. In 2006 she worked at the Gerontology Research Centre NIA/NIH, Baltimore, carrying out a research project aimed at the development and characterization of new stationary phases based on purinergic receptors for binding studies.

Since 2014 she is Associated Professor of Pharmaceutical Analysis at Pavia University. From 2000 to 2016 she was member of the scientific committee of the Summer School on Pharmaceutical Analysis supported by the Division of Medicinal Chemistry of the Italian Chemical Society and by the European Federation for Medicinal Chemistry. She has been a member of the organizing committees of the 10th and 14th Editions of the International Meeting RDPA (International Meeting on Recent Developments in Pharmaceutical Analysis). From 2011 to 2013 she was Member of the teaching board of the PhD course in “Chimica e Tecnologia Farmaceutiche” and from 2013 to 2016 Member of the teaching board of the PhD course in “Scienze Chimiche e Farmaceutiche” at the University of Pavia.

Prof. Calleri is author of more than 65 publications in peer-reviewed journals and she has a strong analytical background, mainly on the use of separation techniques for molecular recognition and drug discovery. Her scientific interests have been focused on:

1. Development and validation of novel HPLC and CE methods in pharmaceutical analysis;
2. High Performance Affinity Chromatography with immobilized receptors (membrane and nuclear receptors) for binding studies and affinity selection of new potential drug candidates;
3. Development of biochromatographic systems based on immobilized enzymes for proteomic applications, quality control of biopharmaceuticals and chiral resolution of drugs;
4. Development of bioreactor for the flow-synthesis of Active Pharmaceutical Ingredients.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

RAPID LIGAND FISHING ASSAY FOR IDENTIFICATION OF ENZYME LIGANDS IN NATURAL PRODUCT LIBRARY

Quezia B. Cass, *Chemistry, Federal University of São Carlos, Sao Carlos, Brasil (qbcass@gmail.com)*

Natural product combinatorial libraries afford a diversity of molecular framework with different levels of stereochemical complexity and biological activity and are considered a promisor source of leads for the development of new drugs.[1]They possess, however, high level of difficulty for disclosing active compounds which usually involves time-consuming, high complex, and ineffective procedures either to identify or to isolate bioactive compounds. In this respect, affinity-based screening assays emerged as a useful tool for monitoring binding events aiming identification, characterization and/or isolation of active small molecules.[2] The use of magnetic beads coated with biological targets such as enzymes and receptors, for instance, are used to identify weak but selective binders from natural products extracts, enabling identification of ligands directly from fractions or crude extracts. There are several advantages associated with this approach, as reutilization of the immobilized proteins (which are generally expensive), rapidly chemical characterization of ligands and investigation of the specificity of the interactions.[3]As an alternative to classical bioassay guide fractionation of natural product crude extracts, we have been exploring ligand fishing assays by the use of magnetic beads coated with a series of important targets enzymes such as: acetylcholinesterase from *E. electricus* (used as a model), phosphoenolpyruvate carboxykinase (PEPCK) from *T. cruzi* and arginase from *L. amazonenses*. Classical inhibition methods for these enzymes are based in the use of either colorimetric and/or conjugated enzyme assays, which are susceptible to false negative and positive results and interferences. Thus, for all of them, we have developed direct analysis by LC-MS/MS, in which the enzyme activity is directly monitored through the quantification of the formed product. These activity-based assays allow evaluation of the immobilized enzymes kinetics and interactions of identified ligands with the enzyme catalytic site. For the fishing assay, in order to chemically characterize the identified ligands, we have developed natural product extracts fingerprint by liquid chromatography hyphenated with high-resolution mass spectrometry (LC-HRMS). For this presentation, a critical overview of these assays will be sightseen.

Abstract Reference & Short Personal Biography of Presenting Author

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Quezia B. Cass is a full professor at the Chemistry Department of Federal University of São Carlos, SP, Brazil and coordinator of Separare – Chromatography Research Nucleus. Separation of drugs plays an important role in her research projects and a number of methods were developed for studies of achiral and chiral drugs of medical and pharmaceutical interest by multidimensional mode. Zonal bioaffinity chromatography and ligand-fishing assays has been, lately, one of her main research interests. She has been working for prospecting ligands from natural or synthetic libraries associated with drug discovery programs and green chemistry procedures. In this respect, she has been recognized for her contribution to the development of cutting-edge analytical techniques. She has authored more than 160 articles that have been published in professional scientific journals; supervised more than 52 Masters and PhD candidates, 10 research fellows and 24 undergraduate research students. She has presented more than 50 seminars in national and/or internationally recognized conferences and has worked as editor of 3 books and authored 4 book chapters. She is a member of the international scientific committee of the Pharmaceutical and Biomedical Analysis Symposium series.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

GOOD SCIENCE PRACTICES AND QUALITY BY DESIGN APPLIED TO ACADEMIC AND NON-GLP/GMP R&D ORGANIZATIONS

Dionigio Franchi, *Pharmaceutical Professional, Milano, ITALY (dionigio.franchi@gmail.com)*

In the Pharmaceutical Industry GLPs/GMPs/GCPs provide guidance for documentation, materials, equipment, etc. through a matrix of principles and strategies to ensure the Safety, Strength, Purity and Quality of the Product.

In Academic and non-GLP/GMP R&D Organizations, non-GxP activities are governed by Good Science Practices (GSP) applyied to Experiments and Data, Methods and Processes, Technology Transfer, Publications, Thesis and Grants etc.

Quality by Design (QbD), is a new paradigm for the development of pharmaceutical products and it provides "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". Quality is 'designed into' the product and manufacturing process as opposed to 'tested into'.

The lecture will discuss how to establish and build a Quality Culture into non-GLP/GMP R&D and Academic Organizations by implementing procedures and strategies for the critical systems involved.

Practical incorporation of GLP/GMP Quality concepts and Quality by Design guidance into Academic and R&D Organizations will be presented.

This will bring into non-GLP/GMP R&D and Academic Organizations consistency and control, remove variables and minimizes errors, add confidence to interpretations and conclusions, strengthen the overall quality & reliability of the data, data integrity and good documentation practices, technology transfer process efficient and effective with the enhancement of the overall value.

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Abstract Reference & Short Personal Biography of Presenting Author

Dr. Dionigio Franchi, *Pharmaceutical Professional, Italy*

- Consultant to multinational and startup Healthcare and Research organizations in the field of GMP, Quality System and Quality by Design, dosage form development and filing, project reviews and definition of product development strategies.
- Consultant at Fondazione Telethon, a major biomedical charity in Italy, whose mission is to advance biomedical research towards the cure of rare genetic diseases.

- Consultant at Research Center Pharmaceutical Engineering GmbH; Graz, Austria.
- Member of the Teaching Council and Faculty at the second level Master course for "Pharmaceutical Analytical Expert", Department of Drug Sciences, University of Pavia, Italy

Professional Summary

- Up to December 2010, Director Pharmaceutical Development Dept. R&D GlaxoSmithKline - Verona, Italy
- With more than 25 years experience in GlaxoSmithKline Research and Development, working in highly productive and dynamic R&D Verona, Pre-Clinical worldwide organization with a solid track record of delivery projects from Candidate Selection to filing and launch.
- Experience in a wide range of therapeutic areas including Anti-infectives, Cardiovalscular and CNS areas profiling and developing New Chemical Entities up to regulatory file submission.
- Trainer and lecturer on various pharmaceutical related topics such: biopharmaceutics and drug delivery system; Pharmaceutical Quality System and Stability.
- Registered Qualified Person (more than 10 year in the role for both Active Product Ingredient and Finished Product).

Education and Professional Qualification

Doctor in Chemistry, University of Padova, Italy (1976); Post-Doc, University of Padova, Italy (1978); Research Associate, Dept of Biochemistry, Medical School, University of Maryland, USA (1978 -1979); Researcher University of Padova, Italy (1979 – 1980).

Academic and external involvement

- Faculty at Istituto Universitario di Studi Superiori (IUSS), Pavia Italy - Course on Creating Medicines to Medical Students (2010, 2011, 2012, 2013)
- Faculty at University of Padova - Italy, School of Pharmacy - Course on Drug Development, University Degree: Informazione Scientifica del Farmaco (2004 – 2007)
- Active member of the EUFEPS Committee for Industrial Relationships (CIRR) (2000 – 2010)

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

QUALITY BY DESIGN STRATEGY AS A RISK-BASED FRAMEWORK FOR THE OPTIMIZATION OF FAST AND SELECTIVE CHROMATOGRAPHIC METHODS FOR THE ANALYSIS OF BIOACTIVE MIXTURES: POTENTIALITY AND CRITICAL ISSUES

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B. Pasquini, *Department of Chemistry "Ugo Schiff", University of Florence, Italy*
C. Caprini, *Department of Chemistry "Ugo Schiff", University of Florence, Italy*
M. Del Bubba, *Department of Chemistry "Ugo Schiff", University of Florence, Italy*
G. Pieraccini, *Mass Spectrometry Center (CISM), Department of Health Sciences, University of Florence, Italy*
S. Pinzauti, *Department of Chemistry "Ugo Schiff", University of Florence, Italy*

Quality assurance (QA) is a process that ensures the customer that the final product meets predefined requirements. It has a key role in the pharmaceutical field to ensure efficacy and safety of drugs. Quality control, within the QA process, requires a series of investigations aimed at identifying any non-conformances in the deliverables. However, it is increasingly important, in the face of a rising culture of quality, to develop analytical methods and processes according to the principles of Quality by Design (QbD), that envisages the definition of all the factors potentially critical for the process and the definition of an experimental space where the quality of the process is assured at a predefined level of probability.

In the last years, US Food and Drug Administration documents have underlined the importance to adopt risk management strategies to ensure the quality of pharmaceutical processes. QbD framework has been outlined in International Council for Harmonization guidelines, ICH Q8-Q11, focusing on pharmaceutical product and process control. Even if the adoption of analytical QbD concept has been significantly rising over the years, it still needs to be effectively encouraged within both the academic and the industrial research. In this context, the development of new ICH Q14 guideline on Analytical Procedure Development will represent an opportunity to further spread this risk-based strategy, which also enables to lay scientific basis for flexible regulatory approaches to post-approval Analytical Procedure changes. In this presentation, examples of QbD applied to the development of chromatographic methods will be presented. Attention will also be paid, in a critical way, to how the research in this field can be useful in sectors where controllable variables are difficult to be established.

Abstract Reference & Short Personal Biography of Presenting Author

Sandra Furlanetto is an Editor of the Journalof Pharmaceutical and Biomedical Analysis (Elsevier).
She is Associate Professor of Analytical Chemistry at the Department of Chemistry of the University of Florence, Italy.
She is Rector's Delegate for Students Orientation at the above University.
The current research of her group is focused on the development and validation of separative analytical methods and on the application of chemometrics for their optimization.

CHIRALITY AT THE DRIED BLOOD SPOT: ENANTIOSELECTIVE ANALYSIS OF PHARMACEUTICALS ON SUB TWO MICRON COLUMNS

Ashraf Ghanem, *University of Canberra, Australia*

The pathology blood testing in the majority of commercial labs involves a whole venous blood sample in a tube from which the serum or plasma portions are separated, and run on an immunochemistry or biochemistry analyser. Until today, these testings are very efficient, however, the assays are generally expensive and not sensitive. Furthremore, the current technology for testing very much restricts a direct-to-consumer approach for providing test information because it requires the professional taking of blood by a skilled nurse. Dried blood spot has been introduced especialy in the doping in sports arena. This method is designed to detect and deter the use of substances and methods on the World Anti-Doping Agency (WADA) prohibited list. The WADA list prohibits all stimulants in competition and names about 60 examples. The most notorious is the chiral amphetamine, which stimulates the central nervous system by releasing neurotransmitters, such as dopamine, into the synapse between neurons and consequently combat fatigue. Many other substances including chiral β2 agonists and chiral β-blockers. In this presentation, we investigate the use of two recently commercialized sub-2 µm columns Chiralpak IG-U® and Chiralpak ID-U® for the fast HPLC enantioseparation of a set of drugs extracted form a dried blood spot.

Abstract Reference & Short Personal Biography of Presenting Author

Ashraf Ghanem (University of Canberra) was honored with a Citation of the RACI for his contributions to the chemistry profession. Ghanem
studied at Helwan University, Cairo and the University of Stuttgart, and worked with Volker Schurig at the University of Tbingen for his PhD
(completed in 2002). He subsequently carried out postdoctoral research with Paul Mller at the University of Geneva (2002–2004), and was Head of the Biomedicinal Chemistry Unit at King Faisil Specialist Hospital and Research Centre, Riyadh (2004–2008). From 2008–2010, he was a research fellow and lecturer with Paul

Haddad at the University of Tasmania, and after a visiting professorship with Nobuo Tanaka at the Kyoto Institute of Technology, he joined the faculty at the University of Canberra, where he was made professor in 2012. Ghanem————————————s research interests lie in the area of asymmetric and enantioselective catalysis and analysis. He has published a Microreview in the European Journal of Organic Chemistry on rhodium- catalyzed enantioselective CH functionalization reactions.

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

QUANTITATIVE COMPARISON OF THE N-GLYCOSYLATION OF PROTEIN THERAPEUTICS USING THE GLYCOSIMILARITY INDEX (IGLYCOS)

Andras Guttman, *AOK-MMKK, Horvath Csaba Memorial Institute of Bioanalytical Research, Debrecen, Hungary (a.guttman@neu.edu)*

Glycosylation is one of the most important critical quality attributes (CQA) of glycoprotein biotherapeutics. Biosimilars are biologic medical products, which are similar but not identical copies of already authorized biotherapeutics. The carbohydrate moieties of most glycoprotein based biological drugs play essential roles in serum clearance and such major mechanisms of actions as antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and anti-inflammatory functions. In addition, alteration in glycosylation may influence the safety and efficacy of the product. Glycosylation, therefore, is considered as one of the critical product quality attributes (PQA) of glycoprotein biotherapeutics, and consequently for their biosimilar counterparts. In this presentation a rapid and efficient sample preparation workflow is introduced in conjunction with a high resolution capillary gel electrophoresis based separation process to compare and quantitatively assess the glycosylation aspect of biosimilarity (referred to as glycosimilarity) between the innovator and a biosimilar versions of several high profile biotherapeutics, based on their N-linked carbohydrate signatures. The term of glycosimilarity index is introduced, based on the averaged biosimilarity criterion.

Abstract Reference & Short Personal Biography of Presenting Author

András Guttman leads the biopharma related separation-application efforts at SCIEX, also heading the Horváth Csaba Memorial Laboratory of Bioseparation Sciences in University of Debrecen, Hungary. His work is focused on capillary electrophoresis and CESI-MS based glycomics and glycoproteomics analysis of biopharmaceutical and biomedical interests, with special attention to biosimilars and new modalities in the industry. Dr Guttman has over 300 scientific publications, wrote 35 book chapters, edited 4 textbooks and holds 23 patents. He is an external member of the Hungarian Academy of Sciences, on the board of several international scientific organizations editor-in-chief of Current Molecular Medicine and serves as editorial board member for a dozen scientific journals. He has been recognized by numerous awards including the Analytical Chemistry Award of the Hungarian Chemical Society (2000), named as Fulbright Scholar (2012), received the CASSS CE Pharm Award (2013), the Arany Janos medal of the Hungarian Academy of Sciences, the Pro Scientia award of the University of Pannonia and the Dennis Gabor Award of the Novofer Foundation in 2014. Dr Guttman also received the Dal Nogare Award of the Delaware Valley Chromatography Forum (2017), the Grand prize of the Swedish Chamber of Commerce (2017) and the Molnar Award (2019).

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

OPTIMIZING THE DRUG DEVELOPMENT PROCESS USING PHARMACOMETRIC TOOLS: A CASE STUDY ON TYPE 2 DIABETES PATIENTS

Serge Guzy, *Ph.D., Professor of Phamacometrics, VP Pharmacometrics, Bioforum*

Pharmacometrics can be defined as the analysis and interpretation of data produced in pre-clinical and clinical trials. It includes Population Pharmacokinetic/Pharmacodynamic modeling, Disease progression modeling and Clinical Trial Simulation.

Population pharmacokinetic and Pharmacodynamic modeling involves the analysis of data from a group (population) of individuals, with all their data analyzed simultaneously to provide information about the variability of the model's parameters.

Disease progression models are mathematical models to describe, explain, investigate and predict the changes in disease status as a function of time. It incorporates the functions of natural disease progression as well as Drug action which reflects the effect of a drug on disease status.

Clinical Trial simulation provides a data set that will resemble the results of an actual trial that has not been conducted yet. Multiple replications of a clinical trial simulation can be used to make statistical inferences like estimate the power of the trial (Predicting p-value) and/or estimate the expected % of the population that should fall within a predefined therapeutic range.

A sponsor has a proprietary antibody that was engineered as a broad anti-inflammatory agent, with the potential as a treatment for many diseases, including diabetes (Type 2 Diabetes, T2D), rheumatoid arthritis, acute gout, systemic juvenile idiopathic arthritis (sJIA), and perhaps for the treatment of cardiovascular disease. This study concentrates on T2D patients where a Pharmacometric analysis was performed to Select the right Pharmacokinetic (PK) model based on Preclinical PK data, Characterize the PK correlation with a Pharmacodynamic (PD) biomarker, based on Phase 1 data, Propose the optimal trial conditions for the upcoming Phase 2 trial.

Based on Preclinical Pharmacokinetic study on cynomolgus monkeys, first time in man trial (Phase 1) was proposed using combined Pharmacokinetic/allometric scaling modeling.

Phase 1 data included both IV and SC route as well as single and multiple dose regimens. The PD marker was a surrogate of the disease progression with the drug inhibiting the PD marker production. A Population PK/PD modeling was performed and revealed that maximum inhibition effect is itself reduced by the drug (feedback inhibition).

Being aware of that feedback inhibition phenomena, the design of the Phase 2 trial was assessed using simulations. The goal of the simulation procedure was to quantitatively assess the dose response relationship for the monthly SC dosing and decide the different arm conditions for Phase 2.

The results of this complex PK/PD analysis lead to an optimal study design of the upcoming Phase 2 trial. The trial would include Five arms (Four treatments groups and 1 Placebo Group). The simulations predict enough separation between the different Dosing arms and the ability to show there is indeed a Dose Response relationship, key objective in order to optimize the probability of success in having the drug approved in Phase 3.

Abstract Reference & Short Personal Biography of Presenting Author

Serge Guzy acquired his Chemical Engineering Degree in 1982 from the University of Brussels and received his Master's Degree in Chemistry and Biophysics in 1985 from the Weizmann Institute. Serge obtained his Doctorate in Biomedical Engineering in 1990 from Technion and, one year later, got his Post-doctorate Degree from UC Berkeley in Chemical Engineering.Serge held a faculty position at UCSF School of Pharmacy between 1991-1996.

Serge expertise includes services includes mathematical modelling, statistical modelling and simulation of clinical trials, Population Pharmacokinetics, Pharmacodynamics, compartment analysis, design of experiments and optimization algorithms development. Serge founded POP-PHARM in 2004, with the goal of providing consulting and software development in support of drug development. With more than 30 years of experience with modeling and simulation, Serge Guzy established new methods for statistical population approaches in drug development, based on Monte Carlo simulation algorithms. The resulting MC-PEM methodology and population software development made him internationally recognized. These new tools have already been well utilized in drug development.

Serge brings vast professional experience. Serge is currenlty associated with Bioforum as VP of Pharmacometrics, Serge serves also as President and CEO of Pop Pharm. He is also Affiliate Professor at the University of Maryland, Adjunct Professor at the University of Minnesota, Courtesy Professor at the College of Pharmacy, Florida and Adjunct Professor at the University of Denver.

PREPARATION OF MOLECULARLY IMPRINTED POLYMERS FOR PROMAZINES AND DETERMINATION OF CHLORPROMAZINE AND ITS METABOLITES IN RAT PLASMA BY COLUMN-SWITCHING LC

Jun Haginaka, *Mukogawa Womens University, Nishinomiya, Japan (haginaka@mukogawa-u.ac.jp)*

Molecularly imprinted polymers (MIPs) could be utilized for selective extraction of a target compound and its structurally related compound(s) from complex matrices. One of their disadvantages is a leakage problem, where the leaked template molecule prevents the accurate and precise assay for a target compound. In order to overcome this problem, a structurally related analogue or a deuterated molecule (a dummy-template molecule) has been used to prepare MIPs. In this study, MIPs for chlorpromazine (CPZ) and bromopromazine (BPZ), MIP_{CPZ} and MIP_{BPZ}, were prepared using methacrylic acid as a functional monomer and ethylene glycol dimethacrylate as a crosslinker by multi-step swelling and polymerization. The retention and molecular-recognition ability of MIP_{CPZ} and MIP_{BPZ} was evaluated in reversed-phase LC. Each MIP recognized the template molecule the most, while CPZ metabolites, desmethyl CPZ (DM-CPZ), CPZ sulfoxide (CPZ-SO) and 7-hydroxy CPZ (7-OH-CPZ), were moderately recognized on MIP_{CPZ} and MIP_{BPZ}. Furthermore, both MIPs gave similar retention and molecular-recognition ability for CPZ and its metabolites. For avoiding the template-leakage problem, MIP_{BPZ} was used for the determination of CPZ and its metabolites in rat plasma samples as the pretreatment column in column-switching LC with UV detection. In addition to DM-CPZ and CPZ-SO, didesmethyl CPZ (DDM-CPZ) and CPZ *N*-oxide (CPZ-NO) were speculated as the metabolites in rat plasma after administration of CPZ using LC-ESI-TOF-MS, while 7-OH-CPZ was not detected as the metabolite. Finally, we validated the column-switching LC method and applied it to the determination of CPZ and its metabolites, DM-CPZ, DDM-CPZ, CPZ-SO and CPZ-NO, in rat plasma samples after intravenous or oral administration of CPZ.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Jun Haginaka is a pharmacist from Kyoto University, Kyoto, Japan with a PhD in pharmaceutical Sciences in 1982 from the same University. He promoted to Full Professor in 1994 at Mukogawa Womens University through Lecturer and Associate Professor. His research interests include analysis of drugs and their metabolites in biological fluids by chromatographic and electrophoretic methods; preparation and application of protein-based stationary phases; and preparation and application of molecularly imprinted polymers. He has published about 250 original research papers, 30 review articles and 30 book chapters, and has held 25 patents. As from 1 January 2002, he became an editor of Journal of Pharmaceutical and Biomedical Analysis. His awards include the Japan Society for Analytical Chemistry Award for Young Scientists, the Pharmaceutical Society of Japan Award for Young Scientists, the Society for Chromatographic Sciences Award, the Japan Society for Analytical Chemistry Award and the Molecular Chirality Award.

BIOMIMETIC PEPTIDE FUNCTIONALIZED AFFINITY MATERIALS FOR MABS CAPTURE AND ANALYSIS

Zhengjin JIANG, *Institute of Pharmaceutical Analysis, College of Pharmacy, JINAN UNIVERSITY, Guangzhou, china (jzjjackson@hotmail.com)*
Qiqin WANG, *Institute of Pharmaceutical Analysis, College Of Pharmacy, Jinan University, Guangzhou, China*
Xiao LIU, *Institute of Pharmaceutical Analysis, College Of Pharmacy, Jinan University, Guangzhou, China*
Hao HUANG, *Institute of Pharmaceutical Analysis, College Of Pharmacy, Jinan University, Guangzhou, China*

Therapeutic monoclonal antibodies (mAbs) are the fastest-growing category of new therapeutic drugs. In recent decades, antibody purification and capture were mainly performed using affinity chromatography. Protein A or G from bacterial sources are commonly used affinity ligands. However, there are still some drawbacks, such as high cost, leakage of ligands and not selective for specific mAbs[1], to be overcome.

In comparison with protein-based ligands, biomimetic peptides are particularly attractive because of their better chemical stability, lower cost and immunogenicity, rich functionality, wide structural diversity etc.. These peptides can be designed for specifically binding to Fc or Fab region of mAbs. In our recent studies, a series of biomimetic peptide ligands were successfully developed based on the complex of mAbs-antigen or mAbs-protein A. Biomimetic peptide ligands functionalized affinity materials were then fabricated through radical initiated co-polymerization. All affinity materials showed high affinity to their corresponding mAbs. For example, DAAG peptide functionalized monolith was successfully applied to specifically enrich and purify trastuzumab from CHO cell culture supernatant and IgG from human serum [2]. HH24 peptide binding to Fab region of trastuzumab could precisely distinguish between IgG and trastuzumab. The peptide (HH24) functionalized biomaterial could successfully capture trastuzumab in 3-folds diluted spiked human serum with high purity and recovery. Moreover, a simple detection method based on this novel biomaterial was also developed for the quantification of trastuzumab in the spiked human serum samples. In summary, biomimetic peptide functionalized affinity materials hold great promises for the purification and bioanalysis of target mAbs in complex samples.

Abstract Reference & Short Personal Biography of Presenting Author

1 Darrouzain F, Bian S, Desvignes C, et al. Therapeutic drug monitoring, 2017, 39(4): 316-321.
2 Wang X, Xia D, Han H, et al. Analytica chimica acta, 2018, 1017: 57-65.

Dr. Zhengjin Jiang received his PhD degree in the Institute of Elemento-Organic Chemistry, Nankai University in 2001. After graduation, he worked for one year in Unimicro (Tianjin) Technologies, Inc, followed as postdoctoral researcher or research fellow in The University of Tuebingen in Germany, King's College London, Pfizer (Sandwich) Research Centre, Novartis Horsham Research Centre, respectively. In 2011, he returned to China and became a full professor of pharmacy at Jinan University, China. His research has been focused on separation science and their applications in pharmaceutical analysis. So far, he has published over 100 scientific publications in international peer-reviewed journals and given more than 30 oral presentations at international/domestic conferences (including 20 keynotes). He is also the holder of 7 patens. He also served as chair of the 27th International Symposium on Pharmaceutical and Biomedical Analysis in 2016, permeant board member of steering committee of PBA and international scientific committee member of ISC2018, PBA2018 and PBA2019, APA2017 etc.. Current, he serves as editorial board member of Journal of Pharmaceutical and Biomedical Analysis, Chinese Journal of Pharmaceutical Analysis, Journal of Pharmaceutical Analysis etc.

ANALYTICAL TOOLS TO MEASURE PROTEIN-PROTEIN INTERACTIONS AND DEVELOPMENT OF INHIBITORS ACTIVE IN VIVO

Joachim Jose, *PharmaCampus, Insitute of Pharmaceutical and Medicinal Chemistry, Westfälische Wilhelms-Universität, 48149 Münster, Germany (joachim.jose@uni-muenster.de)*

Protein-Protein-Interactions (PPI) are involved in basically all biological processes within the human body. Recent studies estimate the human interactome to consist of around 130.000 – 650.000 different PPI. Addressing selected protein-protein interactions (PPIs) by small molecules that bind and interfere appears to be a promising and emerging target for drug discovery. In contrast to the active site of an enzyme or the ligand binding site of a receptor, however, the PPI interface in general does not represent a cavity, but rather a surface exposed domain or area of the protein. Hydrophobic patches are important in such interfaces, but the number and role of hydrogen bonds and water molecules can be rather different from classical cavities. Furthermore, the influence of protein flexibility and induced fitting is more difficult to predict, than it is enzymes or a receptors.

Applying bacterial surface display, click chemistry and flow cytometry we developed screening assays to identify small molecule PPI inhibitors for human cancer target proteins. The assays developed could be used to identify inhibitors of heterologous PPIs, as in the case of human protein kinase CK2 [1] and transcription factor Myb [2,3], as well as inhibitors of homologous PPI as in the case of human HSP90 [4,5]. K_D values of the new compounds identified with the corresponding PPI targets were determined by microscale thermophoresis (MST). This strategy led to new potent small molecule PPI inhibitors in the nanomolar range that turned out to be active in a mouse model as well as in primary human leukemia cells.

In conclusion, the strategy as applied here, by combining bacterial surface display, click chemistry and flow cytometry for the rapid screening for PPIs and IC_{50} determination, followed by MST for K_D measurement of the best candidates appears to be a practical and valuable way to identify novel small molecule inhibitors of PPIs.

Acknowledgments: I am grateful for the valuable contributions of my colleagues Karsten Niefind, Karl-Heinz Klempnauer, Holger Gohlke, Julia Hauer and their groups, as well as to my co-workers involved in this work.

References:

[1] ACS Chem. Biol. **8** (2012) 901-907; [2] Mol. Cancer. Ther. **14** (2015)1276-1285; [3] Blood **127** (2016)1173-1182; [4] BBA Gen. Subj. **1860** (2016) 1043-1055; [5] Blood, **132** (2019 307-320.

Abstract Reference & Short Personal Biography of Presenting Author

Joachim Jose studied biology at the University Saarbrücken, and graduated in 1994 with a thesis on the reaction mechanism of bacterial ureases. During his Post-Doc at the MPI for Biology in Tübingen and the MPI for Infection Biology, Berlin (1994-97), he was mainly involved in the discovery of a new family of secreted proteins: the Autotransporters. From 1998 until 2004, he was an Assistant Professor (C1) at Saarland University, Saarbrücken and obtained his habilitation with a thesis on the evolutive drug and biocatalyst design by bacterial surface display. 2004 he accepted a call for an Associate Professor in Bioanalytics at the Institute of Pharm. Med. Chemistry at Heinrich-Heine-University Düsseldorf, where he was appointed Head

of the Institute in 2008. Since 2011 he is Full Professor and Chair in Pharm. Med. Chemistry at the PharmaCampus of the Westfälische Wilhelms-University Münster, and is executive Director of the Institute.

His publication record includes > 177 papers, 18 patent and patent applications, 94 invited lectures and 298 conference contributions. In addition he was mentor of 34 completed PhD theses.

His awards include the GDCh/DPhG Innovation Award in Medicinal Chemistry (1998) and the SaarLB Science Award (2004). In 2009 he was elected corresponding member of the French National Academy of Pharmacy and in 2013 he obtained the Medal of the Faculty for Biology and Pharmacy of the University Claude Bernard, Lyon, France.

Joachim Jose is co-founder of two start-up companies in the field of 'drug screening and selection' (Pharmacelsus, founded in 2000) and 'biocatalytic synthesis and evolutive drug design' (Autodisplay Biotech, 2008).

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

MEMORIAL PROF. ROMAN KALISZAN SESSION

23 December 1945 – 9 May 2019

The Memorial session for Prof. Roman Kaliszan will be organized
in the morning, on Tuesday, **17 September**, immediately after **Plenary Session 4.**
The Chair of **Memorial session: Prof. Michal Markuszewski**, Medical University of Gdansk, Poland
If you would like to contribute a **short oral talk (~10 min)** for this session,
please contact him, by 15 June, to e-mail: markusz@gumed.edu.pl,
keeping in the loop the Chair and the Secretariat of the Symposium:
Vladimir Ioffe. ioffe@teva.co.il and **Reut Lazar** reutl@bioforum.co.il.

Professor Kaliszan was born on December 23, 1945 in Przybysław (Jarocin District). In the years 1963-1968 he studied at the Faculty of Pharmacy of the Medical Academy in Gdańsk (currently the Medical University of Gdańsk), and in 1973 he graduated from the Faculty of Mathematics, Physics and Chemistry of the University of Gdańsk.

He received his doctorate in pharmaceutical sciences in 1975, habilitation in 1982 and professorship in 1990. In 1999 he became the vice-rector for science at GUMed, and from 2005 to 2008 he was the rector of the university.

Professor Kaliszan was also the winner of many awards, including Foundation for Polish Science, the Minister of Health and the Prime Minister of the Republic of Poland. He also sat in many scientific forums, including Polish Academy of Sciences and Polish Academy of Learning.

In 2012 he received the title of doctor honoris causa of the Medical University. Karol Marcinkowski in Poznan. He was also awarded the Commander's Cross of the Order of Polonia Restituta (2011) and the Officer's Cross of the Order of Polonia Restituta (2002).

The text above is taken from the web site Wyborcza.pl, a local news portal of Gdansk / Trojmiasto

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CHEMOMETRICALLY PROCESSED CHROMATOGRAPHIC DATA IN MEDICINAL CHEMISTRY, MOLECULAR PHARMACOLOGY AND LABORATORY DIAGNOSTICS

Roman Kaliszan, *Medical University of Gdańsk, Gdańsk, Poland (roman.kaliszan@gumed.edu.pl)*

Physicochemical interactions, which do not cause formation of new or breaking of the existing covalent bonds in the interacting molecules of a drug and respective components of biological environment are at the basis of drug action. Analogous fundamental intermolecular interactions between analytes and components of both stationary and mobile phases determine chromatographic retention. Hence, data on chromatographic behavior of drug analytes can be used for modeling their activity in biological systems. However, elicitation of systematic information from sets of diverse retention data requires proper chemometrics processing. The widely applied approach is analysis of Quantitative Structure-Retention Relationships (QSRR), proposed by our group in 1977. Example QSRR will be presented allowing prediction of properties of compounds, which determine their "druglikeness", *i.e.* their pharmacokinetics (ADMETox) and pharmacodynamics, including biological barriers' permeation and binding to pharmacological receptors. Emphasis will be put on combination of QSRR with mass spectrometric data in proteomics and metabolomics. QSRR models will be presented for the prediction of retention of peptides and verification of their identification, based on semiempirical structural descriptors demanding determination of retention of only 7 out of 20 existing natural amino acids. Profiles of chromatographic retention data of urine sample components will be shown to discriminate healthy subjects from cancer patients. Another QSRR model will be presented to support procedure of identification of bioanalytes of relevance for doping control, based on retention parameters in combination with the molecular descriptors, derived by calculation chemistry solely. QSRR analysis will be demonstrated to provide biorelevant information on drugs and other xenobiotics.

Short Personal Biography of Presenting Author & Abstract Reference

Roman Kaliszan, Full Member of the Polish Academy of Sciences (PAN) and the Polish Academy of Science and Arts (PAU), Foreign Member of the Academy of Sciences and Arts of Vojvodina (VANU), Foreign Member of the National Scientific Qualification Committee (ANVUR) of Italy, honorary doctor of the Medical University of Poznań. He obtained both Ph.D. and D.Sc. in Medicinal Chemistry from the Medical University of Gdańsk, where he is a Full Professor since 1990 and where he was Vice-Rector (1999-2005) and Rector (2005-2008). He received scientific prizes of Prime Minister of Poland (twice), Hevelius Prize of Gdańsk, the Prize of the Foundation for Polish Science and the Interdisciplinary Prize of the Polish Ministry of Science. He is recipient of 2018 Tswett-Nernst Award of the European Society for Separation Sciences. He authored about 400 papers, which get *the h* index of 51 and about 9000 citations.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

TRANSIENT INCOMPLETE SEPARATION FACILITATES FINDING ACCURATE EQUILIBRIUM DISSOCIATION CONSTANT, KD, OF PROTEIN–SMALL MOLECULE COMPLEX

Sergey Krylov, *Department of Chemistry, York University, Toronto, Canada (skrylov@yorku.ca)*
Nicolas Sisavath, *Department Of Chemistry, York University, Toronto, Canada*
Jean Luc, *Department Of Chemistry, York University, Toronto, Canada*
J. C., Sciex, *Concord, Canada*
Victor Galievsky, *Department Of Chemistry, York University, Toronto, Canada*
Jiayin Bao, *Department Of Chemistry, York University, Toronto, Canada*
Sven Kochmann, *Department Of Chemistry, York University, Toronto, Canada*
Alexander Stasheuski, *Department Of Chemistry, York University, Toronto, Canada*

Current practical methods for finding the equilibrium dissociation constant, K_d, of protein–small molecule complexes are mainly biosensoric and calorimetric. They have inherent sources of inaccuracy; immobilization of molecules on sensors and heat of side reactions often lead to errors in K_d as large as 2–3 orders of magnitude. We introduce "Accurate Constant *via* Transient Incomplete Separation" (ACTIS), a non-biosensoric and non-calorimetric approach for finding K_d, which appears to be free of inherent sources of inaccuracy. Conceptually, in ACTIS, a short plug of the pre-equilibrated protein–small molecule mixture is pressure-propagated in a capillary, causing fast transient incomplete separation of the complex from the unbound small molecule. A superposition of signals from these two components is measured near the capillary exit and used to calculate a fraction of unbound small molecule, which, in turn, is used to calculate K_d. In this work, we prove ACTIS validity theoretically, verify its accuracy by computer simulation, and demonstrate its practical use. Owing its suggested accuracy, ACTIS has a potential to become a reference-standard method for finding K_d of protein–small molecule complexes.

Abstract Reference & Short Personal Biography of Presenting Author

Prof. Sergey N. Krylov obtained his M.Sc. degree in Physics in 1987 and Ph.D. degree in Biophysical Chemistry in 1990, both from Lomonosov Moscow State University. His postdoctoral training was with Prof. Norman J. Dovichi at the University of Alberta. In year 2000, he accepted a position of Associate Professor at York University in Toronto, where he was promoted to Professor in 2006. In 2003-2013, he held Canada Research Chair, Tier II, in Bioanalytical Chemistry. In 2015 he was appointed York Research Chair in Bioanalytical Chemistry, Tier I. He is a founder and Inaugural Director of the Centre for Research on Biomolecular Interactions at York University. He is recognized internationally for his pioneering work in the fields of kinetic analysis of affinity interactions, and methods for selection and applications of oligonucleotide aptamers. He is the author of over 170 peer-reviewed papers. His research contributions have been recognized with a number of awards including Petro Canada Young Investigator Award, Premier's Research Excellence Award, President's Research Excellence Award (York University) and two recognitions from the Chemical Society of Canada: McBryde Medal (2007) and Maxxam Award (2014). For more information see http://www.yorku.ca/skrylov/index.html

ANALYSIS OF COMPLEX DISEASES USING MASS SPECTROMETRY BASED PROTEOMICS

Yishai Levin, *The Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Rehovot, Israel (yishai.levin@weizmann.ac.il)*

Proteins are the key building blocks of every organism. They also play a central role in most biological and pathological processes. Therefore, proteins are ideal molecules for disease biomarkers as they are influenced both by genetic factors and environmental factors.

Mass spectrometry based proteomics is now a mature technology used in many areas of life science and biomedical research. With significant improvement in sample preparation, instrumentation and software, it is now possible to measure entire repertoires of proteins across dozens of complex samples, including tumor tissue and biofluids, which is particularly useful for prognostic and diagnostic biomarker discovery.

Several case studies will be presented highlighting the state of the art in proteomic analysis, including some of the latest improvements in sample preparation and instrumentation.

Abstract Reference & Short Personal Biography of Presenting Author

Yishai holds a B.Sc. in physics and completed a Ph.D. in Biotechnology at the University of Cambridge UK. There he co-developed the first blood based diagnostic test for schizophrenia.

In 2010 he joined the Weizmann Institute and now heads the Protein Profiling platform of the Nancy and Stephen Grand Israel National Center for Personalized Medicine. His main interest is applying mass spectrometry technologies for solving clinical questions, what is known as clincal proteomics.

METABOLOMICS FOR ANALYSIS OF CLINICAL SAMPLES

Michał Markuszewski, Department of Biopharmacy and Pharmacodynamics, Medical University of Gdansk, Hallera 107, 80-416 Gdansk, Poland, markusz@gumed.edu.pl

Nowadays, due to widespread access to high-throughput, ultrasensitive bioanalytical methods, metabolomics is the most dynamically developing branch of biomedical research. Of particular interest is medical metabolomics, which consists of the determination of qualitative and quantitative metabolite profiles of clinical materials. Among potential applications of metabolomics, special attention has been devoted to the search of pathomechanisms of the diseases and medical diagnostics, allowing detection of diseases at the stage of preliminary laboratory analysis. Taking in mind the vast complexity of the pathophysiological and physiological processes it is not surprising that sets of different metabolites might represent more comprehensively the changes in an organism related to the disease than a single selected compound would.

The leading causes of death globally are cardiovascular diseases (CVD), including hypertension and cancer diseases. There is a need for the development of specific diagnostic methods, more effective therapeutic procedures, as well as drugs, which can decrease the risk of deaths in the course of diseases. For this reason, better understanding and explanation of molecular pathomechanisms of them are essential. In the shown studies, the current status of our finding related to those two major health problems would be presented and discussed.

Abstract Reference & Short Personal Biography of Presenting Author

Prof. Dr. Michał Markuszewski is the head of the Department of Biopharmacy and Pharmacodynamics of the Medical University of Gdańsk since 2016. He has graduated from the Faculty of Pharmacy, the Medical University of Gdańsk in 1995 and obtained a Ph.D. degree under the supervision of Prof. Dr. Roman Kaliszan in 2009. He has got a habilitation degree in pharmaceutical analysis in 2007, and he received the title of professor in 2017. In years 2000-2002 he joined a postdoc position in the group of Prof. Shigeru Terabe at Himeji Institute of Technology (currently University of Hyogo) in Japan. He has got scientific training also in the University of Bremen in Germany, the Eindhoven University of Technology in the Netherlands and Vrije Universiteit Brussel in Belgium. His scientific interest comprises the metabolomics, cancer biomarkers, pharmacokinetics, application of separation techniques for pharmaceutical analysis (LC-MS, GC-MS, CE-MS) and bioinformatics. Author of ca. 120 published peer-reviewed publications. He was invited lecturer during international symposia and conferences (more than 20). He has coordinated/participated in several research projects funded by Polish and international institutions over the past ten years in the areas of metabolomics, pharmaceutical analysis, and analytical chemistry. Prof. Dr. Michał Markuszewski is the author and co-author of more than 120 peer-reviewed, original research papers (May 2019: the overall IF > 300, citations according to Web of Science > 2000 and excluding self-citations > 1900, h-index = 26). In the years 2012-2016 he was the vice-dean of the Faculty of Pharmacy of the Medical University of Gdańsk, and since 2016 prof. Markuszewski is a dean of the Faculty of Pharmacy of the Medical University of Gdańsk.

APPRAISING THE ENTOURAGE EFFECT

David (Dedi) Meiri, *Biology Faculty, Technion Israel Institute of Technology, Haife, Israel (dedimeiri1@gmail.com)*

In the course of the past 20 years, the interest into the therapeutic benefits of medical *Cannabis* has been rapidly growing all around the world. Many countries now allow or are considering allowing, the medical use of *Cannabis* or cannabinoids in some form.

Cannabis is a complex plant composed of several hundred compounds of various chemical classes and wide concentrations. These are biosynthesized as secondary metabolites mainly in glandular trichomes which are abundant on the surface of the female inflorescence. An increasing body of evidence over the last decade has shed light on the therapeutic potential of many of these metabolites, mainly phytocannabinoids, terpenoids and flavonoids. More recent studies suggest also of synergistic effects between the different compounds which boost therapeutic efficacy in the treatment of many physiological and pathological conditions, a phenomenon known as "*The Entourage Effect*". As a result, the interest into the therapeutic benefits of medical *Cannabis* has been rapidly growing all around the world, and many countries now allow or are considering allowing the medical use of *Cannabis* or cannabinoids in some form. Nonetheless, there is still a large gap in the current knowledge as to the mechanisms of action of these secondary metabolites that needs to be filled, before distinct *Cannabis* chemovars could be safely and beneficially applied for the treatment of specific conditions.

Therefore, in the Cancer Biology and Cannabinoid Research Laboratory in the Technion, we aim to determine the therapeutic effects of *Cannabis* specific compounds, and to elucidate their mechanisms of action in cancer, neurodegenerative diseases, the immune system, pain, sleep and more. In order to characterize the *Cannabis* extracts used in our research, we have established an array of analytical chemistry methods for comprehensive identification, extraction, isolation and quantification of whole metabolites in *Cannabis*. One of the major questions that we are trying to answer using all the developed tools, is do we actually need all the different compounds in one specific extract, or whether a small number of specific compounds exists which exhibit the same therapeutic effects as the whole plant.

Abstract Reference & Short Personal Biography of Presenting Author

David (Dedi) Meiri, PhD, is an Assistant Professor at the Faculty of Biology at the Technion Israel Institute of Technology and a member of the Technion Integrated Cancer Center (TICC). Dr. Meiri's scientific background is highly diverse. He holds a M.Sc. in biochemistry and a Ph.D. in plant biotechnology from Tel Aviv University. Dr. Meiri conducted his post-doctoral fellowship at the Ontario Cancer Institute where he focused on the role of the GEF-H1 protein in tumor invasion and metastasis. During his post-doctoral studies, he expanded his knowledge in human biology and cancer pathogenesis and succeeded within a very short time frame to receive worldwide recognition as an expert in the fields of G-protein coupled receptors (GPCRs) and small GTPases. Upon completion of his post-doctoral fellowship, Dr. Meiri took a position at the Technion Israel Institute of Technology, where he heads the "Laboratory of Cancer Biology and Cannabinoid Research".

Presently, his lab investigates the therapeutic potential of phytocannabinoids, the unique active compounds of the *Cannabis sativa* plant. On top of other research being conducted in the lab, the main focus of his

research is to determine the antitumor effects of cannabinoids, including the anti-metastatic and pro-apoptotic effects of phytocannabinoids.

In addition to the laboratory cannabis research performed on-site, Dr. Meiri collaborates with cannabis growers, clinicians, major manufacturers and distributors of medical cannabis for the purpose of revolutionizing cannabis treatment. He is operating the "Cannabis Database Project" and his lab is currently involved in eight clinical trials covering diverse aspects of cannabis treatment such as colon disease, pain prevention, cancer treatment and epilepsy.

Dr. Meiri is also highly involved in governmental regulations and is a residing member in several Israeli Ministry of Health committees which seek to advance the fundamental understanding of optimal cannabis usage and minimization of adverse side effects.

SELENIUM CHEMISTRY AS A TOOL TO STUDY PROTEIN SCIENCE

Norman Metanis, *Institute of Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel (metanis@mail.huji.ac.il)*

Selenium occurs rarely in natural proteins, but is becoming a commonly used element in unnatural contexts to aid in the study of protein structure and function. In its natural context, selenium's role remains uncovered in half of the 25 human selenoproteins. With the aid of chemical protein synthesis, a full characterization of many of these proteins looms close on the horizon. In unnatural contexts, selenium serves as a traceless handle in native chemical ligations and as a “chaperone” for oxidative protein folding. New amino acids containing selenium allow previously unfavorable protein syntheses to occur in good yields. We will discuss selenium's contributions to protein chemistry thus far, as well as its potential in future applications.

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Abstract Reference & Short Personal Biography of Presenting Author

Norman Metanis was born in Maghar 1978, and earned his B.A. degree in Chemistry in 2000 (*Cum Laude*) from the Technion - Israel Institute of Technology, Haifa, Israel. After one year as a visiting student at The Scripps Research Institute (TSRI), La Jolla, he returned to the Technion and completed his M.A. degree in 2004 (*Cum Laude*). Then he moved back to TSRI where he worked with Prof. Ehud Keinan and Prof. Philip Dawson on a joint program between the Technion and TSRI for his Ph.D. studies, which he completed in 2008. Dr. Metanis joined the group of Prof. Donald Hilvert at ETH Zurich as a postdoctoral research associate. In 2013, Dr. Metanis joined the Institute of Chemistry at the Hebrew University of Jerusalem as an Assistant Professor., and was promoted to Associate Professor in 2018. Dr. Metanis is the winner of the Miklós Bondanszky Award (2018), given by the European Peptide Society (EPS), he was also elected as the National Representative at the European Peptide Society (EPS) (2018), selected as outstanding teacher for "Organic Chemistry for Medical Students" (2017), received the “Thieme Chemistry Journal Awardee” (2017) and Ma’of Fellowship for Outstanding Arab Lecturer (2013).

His major research interests (http://chem.ch.huji.ac.il/metanis/research.html) are bioorganic chemistry, protein science, chemical synthesis of proteins, the development of chemoselective reactions applied to peptide and protein chemistry, therapeutic peptides and proteins, protein posttranslational modifications as well as protein functions.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

NATURAL PRODUCT SCREENING USING LIGAND FISHING AND BIOAFFINITY CHROMATOGRAPHY

Ruin Moaddel, *Baltimore, Baltimore, USA (rmoaddel@hotmail.com)*

Natural product extracts can be considered an invaluable source of biologically active secondary metabolites. It has been demonstrated that the screening of natural products is a viable approach for the identification of bioactive compounds and can be a successful approach for the discovery of new drug leads. Ligand fishing and bioaffinity chromatography offers new opportunities for the rapid identification of active metabolites from complex matrixes. Examples of this approach will be discussed

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

IMPURITIES IN DRUG SUBSTANCES AND DRUG PRODUCTS

Raphael Nudelman, *Chemical & Computational Toxicology, Teva Pharmaceuticals, Netanya, Israel (raphael.nudelman@teva.co.il)*

Impurities are inevitably present in drug substances and drug products. They can result from residual carryovers of the synthetic process or form as degradation products during formulation of the drug product or during storage of the drug substance or drug product. The toxicity of such impurities is of a concern to drug manufacturers as well as for regulators who have provided various guidelines to assist in the analysis and control of impurities. The presentation will describe these guidelines and provide practical examples for coping with challenges that the industry is faced with.

Abstract Reference & Short Personal Biography of Presenting Author

Raphael completed his Ph.D. in organic chemistry from the Weizmann Institute of Science in Israel, followed by postdoctoral positions in the US Air Force Research Lab in Aberdeen Proving Ground, Maryland USA and in Duke University Medical Center, North Carolina USA. He joined Teva Pharmaceutical Industries' Medicinal Chemistry department in 2003 and in 2010 he established the Chemical & Computational Toxicology group which he currently heads.

A GENERAL PLATFORM FOR ANTIBODY PURIFICATION UTILIZING ENGINEERED-MICELLES

Guy Patchornik, *Chemical Sciences, Ariel University, Ariel, Israel (guyp@ariel.ac.il)*

We introduce a new concept and a potentially general platform for antibody (Ab) purification that does not rely on chromatography nor specific ligands; rather, it makes use of detergent aggregates capable of efficiently capturing Ab's while rejecting hydrophilic impurities. Captured Ab's are then extracted from the aggregates in pure form (>95%) without co-extraction of hydrophobic impurities or aggregate dissolution. The aggregates studied consist of conjugated "***Engineered-micelles***" built from the nonionic detergent, Tween-20; the hydrophobic metal chelator, bathophenanthroline (batho) and Fe^{2+} ions. When tested in serum-free media with/without BSA (or HSA) as additives, human or mouse IgG's were recovered at high overall yields (85-90%). Extraction of IgG's with 7 different buffers at pH 3.8 sheds light on possible interactions between captured Ab's and their surrounding detergent matrix. Extracted Ab's preserve their secondary structure, specificity and monomeric character as determined by CD, ELISA and DLS, respectively. Possible integration of the approach within industrial-scale downstream processing of therapeutic grade mAb's, will be discussed.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Patchornik received his B.Sc. and M.A degrees in Chemistry from Bar-Ilan University. He earned his Ph.D. in Biochemistry from the Weizmann Institute of Science and continued to a Postdoctoral position at UCLA. Upon his return to Israel, he co-founded a biotech company (Affisink Biotechnology, Israel) and served as its CTO for five years. Since 2009, Dr. Patchornik is a faculty member in the Department of Chemical Sciences at Ariel University where his research focusses on the development of alternative purification and crystallization approaches for integral membrane proteins.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

NEW INSIGHTS FOR THE COMPREHENSIVE ANALYSIS OF BIOACTIVE COMPOUNDS IN CANNABIS SATIVA L.

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Cannabis sativa L., a dioecious plant belonging to *Cannabaceae* family, is known to contain many bioactive compounds. The most representative ones are cannabinoids, flavonoids and terpenes. Among cannabinoids, Δ^9-tetrahydrocannabinol (Δ^9-THC) is responsible for *Cannabis* psychoactive effects. Other main cannabinoids include tetrahydrocannabinolic acid (THCA), cannabinol (CBN), cannabidiol (CBD) and cannabidiolic acid (CBDA). For what concerns other phenolic compounds, several flavonoids have been identified in *C. sativa*, belonging mainly to flavones and dihydrostilbenoids. In particular, cannflavin A and B represent hemp-specific methylated isoprenoid flavones. Terpenes represent the largest group of *Cannabis* components and they are responsible for its aromatic properties.

Some biological activities of cannabinoids are known to be enhanced by the presence of other secondary metabolites in *C. sativa* extracts, such as in the case of sleeping disorders and anxiety. This effect has been attributed to a strict interaction between cannabinoids and terpenes, resulting in a synergistic action. Also flavonoids may modulate the pharmacokinetics of some cannabinoids, by means of the inhibition of hepatic cytochrome P450 enzymes.

In this view, the development and application of advanced analytical methods is highly recommended to guarantee a rational use of *Cannabis* for therapeutic purposes. In the light of all the above, the present study was aimed at the multi-component analysis of the bioactive compounds present in *C. sativa* female inflorescences by means of innovative chromatographic methods. In particular, the profiling of cannabinoids in ethanolic extracts obtained by dynamic maceration was carried out by means of a HPLC-UV/DAD, ESI-MS and MS^2 method, together with a selective extraction protocol, by taking advantage of the innovative fused-core technology of the stationary phase. A new RP-HPLC-UV/DAD, ESI-MS and MS^2 method, together with an optimized extraction procedure, was developed as well and applied for the determination of phenolics (including cannflavin A, cannflavin B and canniprene). The study on *Cannabis* volatile compounds was performed by developing a new method based on HS-SPME coupled with GC-MS and GC-FID.

The methods described above were applied to recreational-type and drug-type samples, together with fibre-type *Cannabis* samples; the first ones, being rich in psychoactive Δ^9-THC, are used for recreational or medicinal purposes, while the second ones, rich in CBD or related compounds and almost devoid of Δ^9-THC, are used for textile or food purposes. These procedures were found to be suitable for the comprehensive chemical analysis *C. sativa* inflorescences in order to ensure their quality, efficacy and safety.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Federica Pellati graduated cum laude in 2000 in Pharmaceutical Chemistry and Technology at the Faculty of Pharmacy of the University of Modena and Reggio Emilia. In 2004 she got a PhD degree in Pharmaceutical Sciences. Then she had a post-doctoral fellowship position in Medicinal Chemistry and in 2006 she got a position of Assistant Professor in Medicinal Chemistry at the University of Modena and Reggio Emilia. In 2014, she got the Italian Professorship Qualification (ASN 2012) as an Associate Professor in Medicinal Chemistry.

The research activity of Dr. Pellati is focused on the development of innovative techniques for the extraction and analysis of bioactive natural products, and on the isolation of new bioactive compounds of natural origin. She has a number of national and international research collaborations and she participates to peer-reviewed research projects.

Dr. Pellati is the author of more than 70 papers in ISI indexed international journals, n. 3 book chapters, n. 4 proceedings in international journals, n. 2 patents and more than 90 congress communications (oral and poster).

She is an associated editor, an editorial board member and a reviewer for international Journals in the area of Pharmaceutical, Plant and Food Analysis.

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CURRENT CHALLENGES IN METABOLOMICS: FROM ANALYTICAL COVERAGE TO STRATEGIES AND TOOLS FOR DATA INTEGRATION IN CHRONIC KIDNEY DISEASE

Serge Rudaz, *School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland (serge.rudaz@unige.ch)*

Metabolomics, one of the omics technologies that aims to comprehensively analyse the metabolic complexity of biological systems, constitutes a representative example of fast moving research fields taking advantage of recent technological advances to provide extensive sample monitoring. Today, untargeted metabolomics allows a quite large coverage of metabolites (mass < 1000 Da) for phenotype modifications assessment caused by pathologies. As no single technique offers a holistic monitoring of all metabolites in a biofluid, the use of multiple analytical platforms is still needed. Reversed-phase chromatography (RPLC) and hydrophilic interaction chromatography (HILIC) coupled to high resolution mass spectrometry (HRMS) are among the complementary techniques commonly used for their coverage of apolar and polar metabolites, respectively. This strategy based on the integration of data obtained from various analytical approaches was applied to plasma samples collected from a clinical study designed to evaluate the metabolic impact of chronic kidney disease (CKD). CKD is a renal disorder characterized by progressive loss of kidney function leading to end-stage renal disease and high risk of cardiovascular morbidity and mortality, and is one of the major public health issue. Due to the irreversibility of the disease progression, hemodialysis and transplantation are mandatory at end-stage. The first aim was to evaluate metabolic alterations related to CKD severity and highlight new biomarkers which could improve diagnosis. More than 250 annotated compounds were investigated thanks to the fusion of datasets generated from multiple platforms using an in-house database. Then, a study of the metabolome was performed just after dialysis session to assess the impact of hemodialysis on end-stage renal disease (ESRD) patients. Finally, kidney transplantation, which tends to restore healthy near-to-normal kidney filtration, was investigated trough the study of transplanted patients, but also volunteers before and after kidney donation. As presented in this lecture through the CKD example, accounting properly for the structure and inherent properties of metabolomic datasets is mandatory for harnessing their complexity and provide relevant information. In that perspective, advances in analytics as well as chemometrics have a central role to play in the choice of an appropriate methodology. Multivariate datasets originating from multiblock data collections and longitudinal studies demonstrated the interest of methodological improvement for metabolomics in patient monitoring.

Abstract Reference & Short Personal Biography of Presenting Author

Serge Rudaz studied pharmacy in Switzerland, where he obtained his PhD in 1997 with Prof. Jean-Luc Veuthey. Later, he joined the National Research Center in Roma (Italy) for a post-doctoral position concerning the application of capillary electrophoresis (CE) hyphenated to mass spectrometry (MS) for chiral separation in biological fluids. From 1998 to 2011, he was a master-assistant in Phytochemistry at the University of Lausanne and then Maître d'Enseignement et de Recherche (MER), where he started to promote new strategies for untargeted MS analyses. He was promoted to Associate Professor in 2012 at the School of Pharmaceutical Sciences, University of Geneva, where he leads the biomedical and metabolomics analysis (BMA) group. Serge Rudaz contributed to the field of analytical sciences with diverse activities, including invited lectures and invited professorships at various Universities. He is a member of several scientific societies and scientific boards. In addition to acting as a research group leader and member of the management board of the Swiss Centre for Applied Human Toxicology (SCAHT) Foundation, he is also President of the Swiss Metabolomics Society (SMS) and vice-president of the Competence Center in

Chemical and Toxicological Analysis (ccCTA). Currently, he is interested in metabolomics, (UHP)LC and CE coupled to MS, advances in sample preparation, analysis of pharmaceuticals and falsified medicines, biological matrices, and clinical and preclinical studies, which include metabolism and toxicological analysis. Serge Rudaz is an expert in a variety of chemometric approaches, including experimental design (DOE) validation and regulation (ISO17025), as well as multivariate data analysis (MVA) for metabolomics. He is a (co)author of over 10 book chapters (>10) and 285 peer-reviewed papers, with an H-index (Scopus) of 51. He was chair/co-chair of several national or international congress, such as Chimiométrie 2015, SEP 2017 and MSB 2020.

The group of Serge Rudaz is developing new strategies for targeted and untargeted metabolomics analyses and specializes in the analysis of low molecular weight compounds in biological matrices. Since 2010, the group has also focused on developing chemometric approaches dedicated to the analysis of data produced by MS couplings, including CE. Aspects of dimensionality reduction and multi-table analysis are addressed through collaborative projects in the fields of toxicology, biology, biochemistry, and pharmacology.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CAPILLARY ELECTROPHORESIS IN DRUG STEREOISOMER ANALYSIS

Gerhard Scriba, *Pharmaceutical/Medicinal Chemistry, Friedrich Schiller University, Jena, Germany (gerhard.scriba@uni-jena.de)*

The importance of the stereochemistry of pharmaceutical drugs is well recognized because stereoisomers often differ in their pharmacological, toxicological and/or pharmacokinetic profiles. As a consequence, the majority of small molecule drugs of the top 10 products according to their worldwide sales in 2016 are single enantiomer drugs [1]. Consequently, for drug development and quality control, the determination of the stereoisomeric composition and purity of a compound requires sensitive and accurate analytical methods in order to determine the stereoisomer ratios in synthetic products, pharmaceutical formulations and biological samples. Besides HPLC, capillary electrophoresis (CE) has become an attractive alternative for this purpose.

In CE, the chiral selector is added to the background electrolyte acting as a pseudostationary phase, which is also mobile in contrast to chromatographic methods. Consequently, two stereoselective principles contribute to stereoisomer separations: (1) the formation of transient diastereomeric complexes between analyte enantiomers and the chiral selector (also referred to as the thermodynamic or chromatographic enantioselective mechanism) and (2) the mobility of the complexes (electrophoretic enantioselective mechanism). Both principles can cooperate or counteract each other.

The current presentation will discuss recent applications of chiral CE methods for the determination of the enantiomeric purity of pharmaceutical drugs including the effects of analyte complexation and mobility of the selector-analyte complexes on the enantioseparation. Precise and accurate methods were developed using a quality by design (QbD) approach and design of experiment (DoE) methodologies and eventually applied to the analysis of bulk drug or pharmaceutical formulations.

Abstract Reference & Short Personal Biography of Presenting Author

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Gerhard K. E. Scriba is a full professor at the Department of Medicinal/Pharmaceutical Chemistry at the Friedrich Schiller University Jena, Germany. He studied Pharmacy at the University of Bonn and received his Ph.D. in 1984 from the University of Münster. Between 1986 and 1988 he worked as a post-doc at the Department of Pharmaceutical Chemistry of the University of Kansas, Lawrence, Kansas, USA before returning to the University of Münster where he finished his habilitation in 1995. Since 1999 he holds his current position at the University of Jena where he served as head of the School of Pharmacy from 2005 to 2013 and as Dean of Study Affairs of the Faculty of Biological Sciences from 2010 to 2013. He received the Rottendorf-Prize for Pharmaceutical Sciences in 1995 and the Johann-Wolfgang-Döbereiner-Prize of the German Pharmaceutical Association in 1997.

Prof. Scriba has published over 175 research and review papers and more than 20 book chapters. He is editor of the book *Chiral Separations* (2nd and 3rd edition) and co-editor of the journal *Chromatographia*. Furthermore, he is and a member of the editorial boards of the journals *Electrophoresis*, *Journal of Separation Science* and *Journal of Pharmaceutical and Biomedical Analysis* and regularly serves as guest

editor of the paper symposia "Pharmaceutical Analysis" of *Electrophoresis*. Prof. Scriba is a member of the working group Pharmaceutical Chemistry of the German Pharmacopoeia, the scientific commission of the German Drug Codex (DAC) and an advisor for the World Health Organization (WHO) on quality control and pharmacopoeial specifications for medicines. Between 2007 and 2016 he served as a member of the scientific advisory board of the German Federal Institute for Drugs and Medical Devices (BfArM).

The research focuses on the analysis of drugs and peptides including stereoisomer analysis by capillary electrophoresis and HPLC as well as mechanistic studies on the interaction between selectors and solutes. A further research topic covers capillary electrophoresis-based enzyme assays.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

HILIC-MS FOR BIOPHARMACEUTICAL ANALYSIS AND (GLYCO)PROTEOMICS

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Thomas Senard, *Center For Proteomics And Metabolomics, Leiden University Medical Center, Leiden, The Netherlands*
Guusje van Schaick, *Division Of Bioanalytical Chemistry, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*
Gestur Vidarsson, *Department Of Experimental Immunohematology, Sanquin Research And Landsteiner Laboratory, Amsterdam, The Netherlands*
Rob Haselberg, *Division Of Bioanalytical Chemistry, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*
Manfred Wuhrer, *Center For Proteomics And Metabolomics, Leiden University Medical Center, Leiden, The Netherlands*
David Falck, *Center For Proteomics And Metabolomics, Leiden University Medical Center, Leiden, The Netherlands*
Elena Dominguez-Vega, *Division Of Bioanalytical Chemistry, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*
Andrea Gargano, *Division Of Bioanalytical Chemistry, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*

Liquid chromatographic (LC) methods that provide both efficient separation of intact proteins and compatibility with mass spectrometry (MS) are of essential importance in biopharmaceutical analysis and top-down proteomics. Recently, hydrophilic interaction liquid chromatography (HILIC) using amide stationary phases has shown highly suitable for analysis of intact proteins. This presentation outlines HILIC-MS methodologies for protein analysis highlighting the following main aspects:

- HILIC is suitable for the separation of a wide range of proteins, exhibiting a selectivity orthogonal to RPLC.

- Using appropriate eluent gradients, HILIC-MS shows a unique selectivity towards protein glycosylation, providing high resolution of intact glycoforms.

- Computer-aided method development facilitates and strongly accelerates optimization of glycoform separations by HILIC.

- Capillary HILIC-MS employing trap-columns for pre-concentration and dopant-gas for enhanced ionization substantially boosts sensitivity, allowing detection of low-abundant proteins from biological samples.

The attainable HILIC-MS performance will be illustrated by characterization of pharmaceutical proteins such as interferon-beta, erythropoietin, antigen glycoconjugates and monoclonal antibodies, but also by analysis of a complex cell lysate and detailed profiling of IgGs in human plasma.

Abstract Reference & Short Personal Biography of Presenting Author

Govert Somsen is full professor of Biomolecular Analysis/Analytical Chemistry at the Vrije Universiteit Amsterdam, The Netherlands. He obtained his doctorate in Amsterdam, and subsequently was assistant and associate professor at the University of Groningen and at Utrecht University in The Netherlands. His current research interests include the compositional and conformational characterization of intact biomacromolecules, and the bioactivity screening of compounds in complex samples. His group built an internationally recognized expertise in the hyphenation of separation techniques with mass spectrometry and optical spectroscopy for the analysis of intact proteins. Somsen is (co)author of over 185 peer-reviewed papers and has an H-index of 39. He is editor of Journal of Chromatography B.

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

IN SEARCH OF BIOMARKERS FOR THE EARLY DETECTION OF RENAL CYSTIC DISEASES

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Asmin Andries, *Pharmaceutical Analysis, Ku Leuven, Leuven, Belgium*
Charlotte Keulers, *Pharmaceutical Analysis, Ku Leuven, Leuven, Belgium*
Djalila Mekahli, *Pediatric Nephrology, University Hospitals Leuven, Leuven, Belgium*

This study is situated in the field of renal cystic diseases that may occur under the form of autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive polycystic kidney disease (ARPKD) or tuberous sclerosis complex (TSC). More specifically, ADPKD will be investigated. It is the most common hereditary renal disease (incidence between 1:400 and 1:1000 live births) and is mainly related to mutations in the *PKD1* or *PKD2* gene. The cyst development starts early in childhood and a decline in kidney function occurs from the third decade, eventually leading to end-stage renal disease.

The overall objective of this study is to find early markers, specific of this disease. These are needed as a tool for early diagnosis and monitoring of disease progression. Various analytical techniques were used for urine samples, as urine is a convenient and easily accessible patient matrix.

The first aim was to measure oxidative stress in children with ADPKD and compare with healthy controls (by HPLC-UV). A bioanalytical method for the determination of allantoin and adenosine in urine was optimized and fully validated. Because of the rather long run time, efforts were done to shorten the method by applying core shell particles. A significant number of control and patient samples were analyzed, and the obtained data were statistically evaluated. A critical discussion of the results will show whether these compounds can act as early predictors of the ADPKD disease.

Future studies will tackle the next objective, namely to focus on the mTOR pathway by measuring the excreted urinary amino acids (by LC-MS/MS).
In a further step, it will also be important to find out the serum levels of the investigated compounds in order to support biological hypothesis testing.
If the investigated substances indeed appear to be of relevance, they might turn out to be suitable early predictors of ADPKD.

Abstract Reference & Short Personal Biography of Presenting Author

Ann Van Schepdael obtained a degree of Pharmacist in 1986, and a PhD in 1990 (KU Leuven, University of Leuven, Belgium, Medicinal chemistry). Following a post-doc at KU Leuven and the Barnett Institute (Northeastern University, Boston, 1993) she was appointed lecturer in Leuven in 1997. Since 2007 she is a full professor at the Pharmaceutical Analysis division, and heads the latter lab since 2010. Since 1990 her research focus is on analytical techniques, mainly capillary electrophoresis. Besides interest in electrophoretically mediated microanalysis (EMMA) and in immobilized enzymes, also liquid chromatography for bioanalysis is within her research focus. She teaches courses on instrumental analysis and analytical tools for drug development, as well as practical pharmaceutical analytical chemistry exercises in the bachelor and master programs at the school of pharmacy KU Leuven. She has published over 300 publications in international peer-reviewed journals, has been the (co)-promoter of 32 PhD theses and is currently promoter of 3 PhD students and co-promoter of 4 PhD students.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

HPLC SEPARATION STRATEGIES FOR COMPOUNDS WITH MULTIPLE CHIRAL CENTERS AND THE PRACTICAL PHARMACEUTICAL CONSIDERATIONS

Kelly Zhang, *Genentech, South San Francisco, United States (zhang.kelly@gene.com)*

With the increasing complexity of pharmaceutical molecules, the availability of pure chiral starting materials and the advances of asymmetric synthesis, there are more and more new drug candidates come with multiple chiral centers. Each of the chiral entity has to been strictly quantified and monitored in order to ensure drug safety and quality. Frequently, multiple methods have to be used in order to characterize the chiral purity of a sample and sometimes even the combination of multiple methods is still not necessarily able to provide the required information.

In this lecture, we will present our fast HPLC method development and sample analysis strategies based on the results of our recent screening studies of compounds with multiple chiral centers. We will present our strategies of fast method development. The impact of different stationary phases including phase chemistry, particle size, noncovalently vs covalently bonded chiral phases will be presented, together with the impact of other factors such as mobile phases, gradient vs isocratic etc. to the separation efficiency. Strategic application of multi-dimensional separation for compounds with multiple chiral centers will be demonstrated. We will also discuss the practical considerations of chiral separation method, sample preparation and analysis for pharmaceutical samples.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Kelly Zhang is a Principal Scientist at Genentech in South San Francisco, California. She leads analytical chemistry R&D teams for drug substance and drug formulation, moving novel therapeutic molecules forward from research to clinical trials through collaboration with cross-disciplinary teams. Her current research interests are chiral separation, multi-dimensional separation, universal detection and high throughput automation. She was named one of the top 50 most influential women in analytical science by The Analytical Scientist. She is a member of the Permanent Scientific Committee of the international HPLC conference series.

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30TH INTERNATIONAL SYMPOSIUM
PBA
ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

ORAL PRESENTATIONS

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CHEMICAL SPECIFIC PARTICLE SIZING OF THE ACTIVE PHARMACEUTICAL INGREDIENT IN COMPLEX FORMULATIONS BY HYPHENATED SPECTRO - MICROSCOPIES

Yousif Ayoub, *Analytical Technologies Unit, Solid State Characterization, Global R&d, Teva Pharmaceutical Industries, Ltd, kfar Saba, Isarel (yousif.ayoub@teva.co.il)*

The Particle Size Distributions of Active Pharmaceutical Ingredient (API) in final drug products is one of the most important and critical parameters for pharmaceutical end products function and bioavailability.

The delivery of low soluble API and nasal drugs is highly controlled by the particle size and its monitoring is even more crucial.

The regulatory agencies are requesting today more thorough characterization of the final products in general and more specifically of the size distributions of the API in drug product.

We will focus on chemical-specific particle size identification and distribution of multiple components in complex pharmaceutical matrixes of drug products, specifically on imaging Raman spectroscopy coupled with optical imaging and scanning electron microscopy (SEM). Additional approaches such as micro computational tomography will also be discussed.

Abstract Reference & Short Personal Biography of Presenting Author

Yousif Ayoub PhD. is Associate Director & R&D Team Manager at Global R&D, Teva Pharmaceuticals. Where he is the director of the team specialized in Solid State Characterization, which include polymorphism, particle size distribution (PSD), advanced microscopy and spectroscopy technologies, thermal properties of the material as well as his responsibility for the reverse engineering team.

Yousif holds a PhD in Physical Chemistry from the Hebrew University of Jerusalem since 2009.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CURCUMIN-BASED ANTICANCER DRUG DELIVERY

Andrii Bazylevich, *Chemical Sciences, Ariel University, Ariel, Israel (andriib@ariel.ac.il)*

Since ancient times curcumin (CMN), the yellowish-orange pigment from turmeric, has been used as a natural substance with diverse curable merits. Its biological and chemical properties have been extensively investigated by scientists yielding many publications on treatments for a variety of diseases, including cancer, inflammation and Alzheimer. Here we present a development of novel anticancer curcuminoids and biolable curcumin (CMN) drug loaded platforms for delivery applications to treat cancer. When linked to the drug by one side and to the given carrier (peptide) by another, these platforms are likely to provide combined cytotoxic effect (drug + curcuminoid). The leading curcuminoid presented antioxidant activity similar to curcumin but with increased cytotoxicity *in vitro* in agreement with the augmented blockage of the NF-kB cell survival pathway. In addition, the ON-OFF switchable fluorescein-CMN platform for visualization of curcumin release will be presented.

Abstract Reference & Short Personal Biography of Presenting Author

PhD student; lab of Prof. Gary Gellerman, dep. of chemical sciences, Ariel University.

Abstract ref:

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CANNABINOIDOMICS – AN ANALYTICAL TOOL TO UNDERSTAND THE EFFECT OF MEDICAL CANNABIS TREATMENT IN CLINICAL AND PRE-CLINICAL STUDIES

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Cannabis is a complex plant composed of several hundred compounds of various chemical classes and wide concentrations. Among these, phytocannabinoids, the natural cannabinoids found in Cannabis, are unique to this plant. Their biological mechanisms of action are attributable mainly to their interactions with the endocannabinoid system (ECS), either by activating/inhibiting cannabinoid and non-cannabinoid receptors, metabolic (biosynthesizing or degrading) endocannabinoid enzymes and/or fatty acid binding proteins. In order to study the pharmacological effects of whole Cannabis extracts on the endocannabinoid metabolome we have developed an accurate high-resolution liquid chromatography-mass spectrometry (HR-LC-MS/MS) tool for identification and quantification of endocannabinoids and other endogenous cannabimimetic lipids, phytocannabinoids, and their metabolites in various biological matrices.

In this study, identification of target compounds by HR-LC-MS/MS was performed according to the retention times and MS/MS fragmentation patterns of available analytical standards as a reference for the identification of additional compounds from all subclasses. Overall, we identified (a) over 90 different phytocannabinoids from all 10 different phytocannabinoid subclasses by screening various natural and decarboxylated Cannabis flowers; (b) over 90 endocannabinoids and cannabimimetic lipids from 20 different lipid families by screening mice serum and tissues (brain, liver and spleen); and (c) 20 (-)-Δ9-trans-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) metabolites in mice serum and liver samples following treatment with Cannabis extracts. Extraction methods were then developed and validated for quantifying the identified compounds in various biological fluids, tissues, and cells. This cannabinoidomic tool can be used to accurately determine changes in the levels of endocannabinoid metabolites as a result of Cannabis treatment for diverse physiological and pathological conditions.

Abstract Reference & Short Personal Biography of Presenting Author

Paula has a BSc in Biotechnology Engineering, and MSc and PhD in Environmental Engineering, all from Ben Gurion University of the Negev in Israel. For the last three years Paula has been working as a postdoctoral fellow at the Laboratory of Cancer Biology and Cannabinoid Research in the Technion - Israel Institute of Technology (advisor: Prof. David Meiri). Paula's research involves the development of LC/MS/MS methods to study the effects of *Cannabis* on the endocannabinoid metabolome by means of “cannabinoidomics” (phytocannabinoid and endocannabinoid “omics”).

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

INSULIN AGGREGATION ASSESSMENT USING SDS-FREE CAPILLARY GEL ELECTROPHORESIS: COMPARISON WITH SIZE-EXCLUSION CHROMATOGRAPHY

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Size-exclusion chromatography (SEC) is a method of choice for the analysis of protein aggregates. The United States and European Pharmacopoeias currently use a SEC method with an acidic mobile phase to assess the content of aggregates in insulin formulations.

In this study, aggregated human insulin samples were analyzed and under neutral conditions, both SEC (nSEC) and capillary gel electrophoresis (CGE) were found to lead to similar results for aggregate content, unlike SEC under acidic conditions (aSEC) [1]. Polymeric complexes were detected using aSEC while they were not observed with nSEC and CGE. During method development, the effects of arginine and acetonitrile addition on SEC profiles were studied. In CGE, the impact of SDS on disruption of non-covalent insulin aggregates was confirmed and the benefit of sodium deoxycholate addition into the sieving gel was examined. The three methods were applied to the analysis of an insulin formulation and similar results to those obtained for human insulin in pure form were observed. Finally, the CGE method was used to study the stability of human insulin under different storage conditions.

In view of the results obtained, which emphasize the importance of the mobile phase composition and pH in SEC, one may question the relevance of the current pharmacopoeial method for insulin aggregation assessment. The new CGE method is an easy approach for studying non-covalent aggregates of insulin, which could be applied to other proteins.

Abstract Reference:
A. Demelenne, A. Napp, F. Bouillenne, J. Crommen, A.C. Servais, M. Fillet, Talanta, 199 (2019) 457-463.
Short Personal Biography:
Jacques Crommen is currently Professor Emeritus at the University of Liege, Belgium and Guest Professor at Jinan University, Guangzhou, China. He was Full Professor and Head of the Laboratory of Analytical Pharmaceutical Chemistry at the University of Liege from 1991 to 2010. He was also Guest Professor at the Catholic University of Louvain (UCL), Belgium, from 1997 to 2003. He was Editor of the Journal of Pharmaceutical and Biomedical Analysis from 1999 to 2003. He is Vice President of the Belgian Royal Academy of Medicine, and Honorary Member of the Hungarian Pharmaceutical Society and the Belgian Society of Pharmaceutical Sciences. He was awarded the degree of Doctor honoris causa from Iuliu Hatieganu University, Cluj-Napoca, Romania. His current research interests include chiral separations, analysis of counterfeit drugs by HPLC and CE, and quantification of biomolecules by miniaturized separation techniques coupled to mass spectrometry.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

RAMAN IMAGING AS A NEW ANALYTICAL METHOD TO MONITOR OSTEOGENIC DIFFERENTIATION IN FORMING BONE TISSUE

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Pierre-Yves Sacre, *Pharmacy, University Of Liege, Liege, Belgium*
Philippe Hubert, *Pharmacy, University Of Liege, Liege, Belgium*
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Eric Ziemons, *Pharmacy, University Of Liege, Liege, Belgium*

Tissue engineering and stem cells technology are emerging fields in modern medicine, aiming to produce living tissues in vitro to replace, regenerate or repair in vivo damaged tissues or organs. In this study, adipose-derived stem cells (ASCs) are used to produce 3D bone tissue grafts [1,2]. The safety and the feasibility of use of these grafts has been demonstrated by several clinical applications as it is largely described by Vériter *et al.* [2]. Here, Raman imaging is investigated as a non-destructive and non-invasive method to monitor the synthesis of extracellular matrix by the cells and its progressive mineralization during formation of an osteogenic tissue.

Raman imaging is a vibrational technique allowing to acquire spectral fingerprints of molecules while visualizing their spatial distribution within the sample. The fast data acquisition time of this technique further allows time-resolved analyses.

Here, Raman imaging is used for the first time to monitor extracellular matrix formation and mineralization by human cells in a live 3D structure. Our attention was focused on Raman bands related to this matrix, namely phosphate, phenylalanine and hydroxyproline, which are very distinctive and intense [3]. Several batches of ASCs were cultured in a bone tissue differentiation medium then sampled and analyzed using Raman imaging at different time points. From the Raman spectra, mineral to organic matrix ratios (MTMR) were calculated from phosphate and hydroxyproline signal intensities to evaluate the formation of mineral deposits accompanying extra-cellular matrix synthesis which is indicative of an ongoing osteogenic differentiation process [3]. Is was observed that these ratios peaked between day 35 and 49 but also the spatial distribution of individual signal intensities vary in the forming 3D structures whilst maintaining a same MTMR ratios at the end of the culture process. A study was conducted to evaluate the influence of the position of the analyzed samples in the forming tissue in vitro to define a protocol to acquire the Raman data for future analyses. Finally, the repeatability and the specificity of this Raman imaging method were evaluated.

To conclude, Raman imaging allows a time-resolved and non-invasive monitoring in vitro of the mineralization of extracellular matrix during osteogenic differentiation.

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Abstract Reference & Short Personal Biography of Presenting Author

Charlotte De Bleye was graduated in Pharmaceutical Sciences at the University of Liege in 2011. Thereafter, she had a grant holder form the F.R.S.-FNRS and started a Ph.D. thesis dealing with the investigation of quantitative performances of surface-enhanced Raman scattering (SERS) in the laboratory of Pharmaceutical Analytical Chemistry (LCAP) of Professor Philippe Hubert. She received her Ph.D. in Biomedical and Pharmaceutical Sciences in 2016. Nowadays, she is still working in the LCAP and her research is focused on SERS and Raman imaging on pharmaceutical and biological materials.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

A NEW APPROACH TO "ABNORMAL DISSOLUTION PROFILE" PROBLEMS FOR EXTENDED RELEASE DRUG PRODUCTS

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Behavior of certain drug products in dissolution profile testing, in several cases, when the profile is especially long, may exhibit abnormal profile: sometimes, in a specific sampling point, drug release may be unexpectedly lower than in a previous. This may affect release rate of API from a dosage form. Such a behavior is often characteristic for API's which have limited stability in aqueous solutions due to functional groups susceptible for hydrolysis.

Moreover, this may be followed by emerging of unknown impurities / degradation products. Reliable quantitation in such cases is usually problematic, especially when relative response factors (RRF) for emerging impurities has not been yet determined, or it is impossible for several reasons.

Our attitude in using diverse analytical tools (compatibility of materials, chromatographic, spectral, protection from environment influence, etc.) will be presented, to monitor and control such degradation processes, if occur.

One of the applied tools is the "Isosbestic point evaluation" approach. Wavelength of HPLC method is chosen to represent the same (or most similar) extinction coefficient for API and its degradation product(s). As a result, degradation, or increase of impurities during stability studies of extended release products, can be reliably assessed.

Abstract Reference & Short Personal Biography of Presenting Author

Bar Edelshtein is Analytical Project Coordinator at Global R&D, Teva Pharmaceuticals, Ltd. He is responsible for developing analytical methods for small molecules API and drug products in generic development and NTE at Kfar-Saba R&D site. Bar has joined Teva in 2008 and he involved in development of wide range of generic products and complex projects utilizing various analytical methods and technologies.

Bar holds a B.Sc. degree in Biotechnology engineering from Ort Braude College of Engineering, Carmiel, Israel.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

ANTICANCER POTENTIAL OF DIETARY FLAVONOID APIGENIN IN BREAST CANCER CELL

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Josip Madunić, *Faculty of Science, University of Zagreb, Zagreb, Croatia*
Maja Antunović, *Faculty of Science, University of Zagreb, Zagreb, Croatia*
Mladen Paradžik, *Laboratory for Cell Biology and Signalling, Ruđer Bošković Institute, Zagreb, Croatia*
Davorka Breljak, *Molecular Toxicology Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia*
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Vera Garaj-Vrhovac, *Mutagenesis Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia*

Cancer represents the disease of the millennium and is regarded as one of the major public health problems. The proliferation of tumour cells, angiogenesis, and the relationship between the cancer cells and the components of the extracellular matrix are important in the events of carcinogenesis, and these pathways are being used as targets for new anticancer treatments. For over 50 years, natural products have served us well in combating cancer with one of the main sources of these successful compounds coming from plants. The search for novel drugs is still a priority goal for cancer therapy, due to the rapid development of resistance to chemotherapeutics. Moreover, the high toxicity usually associated with some chemotherapy drugs and their undesirable side-effects increase the demand for novel anticancer drugs active against untreatable tumours, with less side-effects and higher therapeutic efficiency. Apigenin is a natural flavonoid found in several dietary plant foods such as vegetables and fruits. A large number of studies conducted over the past years have shown that this particular natural compound has several beneficial properties, generating a great deal of interest as a possible therapeutic modality. Therefore, to investigate potential anticancer properties of apigenin on human breast cancer, ER-positive MCF-7 and triple-negative MDA MB-231 cells were used. Moreover, toxicological safety of apigenin towards normal cells was evaluated in human lymphocytes. Cytotoxicity of apigenin towards cancer cells was evaluated by MTT assay whereas further genotoxic and oxidative stress responses were measured by the comet and lipid peroxidation assays in addition to the type of cell death induced by apigenin. Toxicological safety towards normal cells was evaluated by the cell viability and comet assays. After the treatment with apigenin, we observed changes in cell morphology in a dose- (10 to 100 µM) and time-dependent manner. Moreover, apigenin caused cell death in both cell lines leading to significant toxicity and dominantly to apoptosis. Furthermore, apigenin proved to be genotoxic towards the selected cancer cells with a potential to induce oxidative damage to lipids. Of great importance is that no significant cytogenotoxic effects were detected in normal non-target cells. The observed cytogenotoxic and pro-cell death activities of apigenin coupled with its low toxicity towards normal cells indicate that this natural product could be used as a future anticancer modality. Therefore, further analyses to determine the exact mechanism of action and *in vivo* studies on animal models are required to enable its translation from bench to bedside.

Goran Gajski, PhD is a Research Associate at the Institute for Medical Research and Occupational Health in Zagreb, Croatia with the scientific background in biochemistry and molecular biology. In the frame of his work he use various methodological approaches, both *in vitro* and *in vivo*, on different cell and animal models with a goal of investigating the effects of different physical and chemical agents on organisms, tissues, cells and cell structures with special emphasis on DNA molecule. Moreover, the scope of his work also comprises human biomonitoring studies which aim at revealing the impact of various physical and chemical agents on human population both in environmental and occupational settings. Up till now, Goran Gajski has published a large number of scientific publications in peer-reviewed books and journals, and has received several national and international awards for his work.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

OLD YET NEW – SFC AS AN INTERESTING TECHNIQUE FOR NATURAL PRODUCTS ANALYSIS

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The supercritical stage is reached above the critical temperature and pressure of a substance, the resulting fluid shows liquid like density and the viscosity of a gas. It has high solvating power but creates less backpressure, rendering it to an ideal mobile phase for chromatography. Since first reports in the 1960´s Supercritical Fluid Chromatography (SFC) has evolved dramatically, and today rugged instruments are available, together with a variety of HPLC-type stationary phases and detectors [1].

The potential of SFC for natural products analysis is highlighted in this presentation. Based on successfully conducted studies in our laboratory it will be shown that the separation of diverse compound classes is possible with high speed, separation efficiency and ease. After a short introduction to the technique itself, SFC applications describing the analysis of coumarins in *Angelica dahurica* [2], lactones in kava-kava [3], and alkaloids in *Cinchona* bark [4] will indicate the benefits of this increasingly popular technique. Exemplarily, the latter was possible using an Acquity UPC^2 Torus DEA 1.7 µm column, with a mobile phase comprising CO_2, acetonitrile, methanol and diethylamine. Method validation confirmed that the procedure is selective, accurate (recovery rates from 97.2% to 103.7%), precise (intra-day ≤ 2.2%, inter-day ≤ 3.0%) and linear (R^2 ≥ 0.999); at 275 nm the observed detection limits were always below 2.5 µg/ml. In terms of technical performance there are no differences to state of the art techniques like UPLC; however, the use of an environmental friendly, “green” mobile phase renders SFC unique and preferable actually.

Abstract Reference:

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Personal Biography:

Dr. Ganzera studied pharmacy at the University of Innsbruck, Austria, where he also obtained his PhD in 1997. Thereafter he was more than 3 years post-doc and research scientist at the National Center for Natural Products Research at the University of Mississippi in Oxford, USA, interrupted by a 6 month stay at the Hoechst Rousell Vet. company, Vienna, Austria. In 2002 he returned to the University of Innsbruck, where he finished his habilitation in Pharmacognosy 2006 and was appointed to Associate Professor in 2012. He spent research internships at the Food and Drug Administration (College Park, USA), The Lawrence Berkeley Lab (Berkeley, USA) and at the University of Buffalo (Buffalo, USA).

His main research interest is the analysis of natural products by conventional (HPLC, LC-MS, GC) as well as less common (CE, CE-MS, CEC, SFC) techniques, including method development, validation and application to (medicinal) plants and products thereof. He has authored more than 125 scientific papers in international journals, and since 2019 he is editor of Journal of Pharmaceutical and Biomedical Analysis.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

APPLICATION OF ISAYAMA–MUKAIYAMA COBALT CATALYZED HYDROPEROXYSILYLATION FOR THE PREPARATION OF RITONAVIR HYDROPEROXIDE

Sharon Gazal, *Teva Pharmaceutical Industries. KS, Israel*
Priya Gupta, *AMRI (Albany Molecular Research Inc.)*
Siva Ramakrishna Gunturu, *AMRI (Albany Molecular Research Inc.)*
Matthew Isherwood, *AMRI (Albany Molecular Research Inc.)*
Matthew E. Voss, *AMRI (Albany Molecular Research Inc.)*

We report the preparation of thiazol-5-ylmethyl ((2S,3S,5S)-5-((S)-2-(3-((2-(2-hydroperoxypropan-2-yl) thiazol-4-yl)methyl)-3-methylureido)-3-methylbutanamido)-3-hydroxy-1,6-diphenylhexan-2-yl)carbamate, a hydroperoxide impurity of ritonavir also known as ritonavir USP impurity F. In many cases, though listed in USP, impurity standards are not available either from USP or from other commercial sources. This also applied to ritonavir USP F. Due to the complexity of ritonavir's structure and abundance of oxidation susceptible functional groups, forced degradation was found to be a non-selective and inadequate tactic. Therefore, a multistep synthesis was applied. The overall strategy involved initial introduction of a propenyl moiety to the terminal thiazole which enabled selective oxidation using Co(thd)2 (0.1 equiv)/O2 (Isayama–Mukaiyama cobalt catalyzed hydroperoxysilylation) following structural assembly.

Abstract Reference & Short Personal Biography of Presenting Author

S. Gazal et al. / Tetrahedron Letters 57 (2016) 5099–5102
Sharon Gazal Ph.D
Dr. Sharon Gazal received his BSc in Chemistry at 1995 from the Hebrew university of Jerusalem (HUJI). His master degree and Ph.D (1998 and 2003 respectively) were also awarded from HUJI at the Department of Organic Chemistry. Between 2003-2005 was a post-doc fellow at the University of Minnesota. His industrial experience includes, among all, Medicinal Chemistry research in drug discovery companies which comprised design and synthesis of peptide and small molecules drug candidates. In addition to drug discovery, he also took part in Nanotechnology field which included deign and preparation of molecular imprinted polymers applications. Currently, Dr. Gazal holds a Principal Scientist position at Teva Pharmaceutical industries (generic R&D).

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

VISUALIZATION OF ANTI-CANCER TARGETED DRUG DELIVERY USING NIR FLUORESCENT SWITCHABLE REPORTERS

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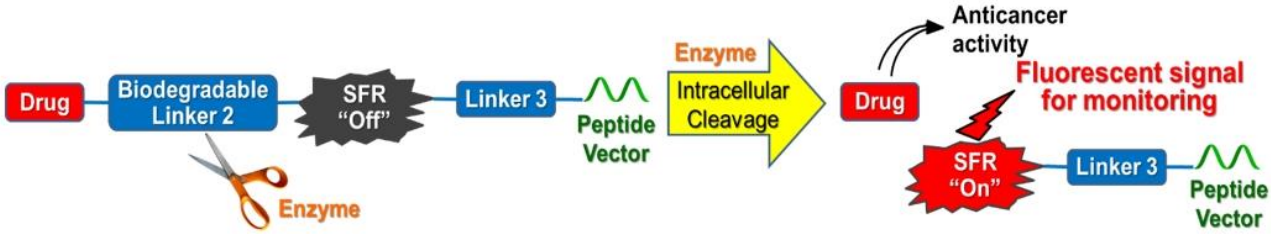
Leonid Patsenker, Chemical Sciences, Ariel, Ariel, Israel

Visualization of a drug delivery is an essential topic in effective monitoring of drug release. It is especially important requirement in the targeted drug delivery (TDD) for securing the release of the drug at the target and for further optimization of drug conjugation method. This research aims to provide a new approach for designing novel theranostic tools — peptide drug conjugates for targeted therapy of cancer. These conjugates, having a dimension of about 5 nm, comprise an anticancer drug linked biodegradably to a novel near-infrared dye — switchable reporter (SFR is in the non-fluorescent "Off" form), conjugated with a cancer-specific peptide. Peptide carrier is specific to the overexpressed receptors in solid tumors, providing receptor specific mediated drug delivery. Cleavage of the biodegradable linker in targeted cancer cells results in the release of drug and the increase of the SFR fluorescence intensity ("On" form).

Such an approach will provide a NIR light controlled, targeted therapy of cancer cells, reduced side-effects, and fluorescence monitoring.



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Abstract Reference & Short Personal Biography of Presenting Author

Prof. Gary Gellerman received his Ph.D degre from Tel Aviv University in Organic Chemistry in 1994. After completing his doctorate, Prof. Gellerman engaged in research in the field of drug development in pharmaceutical companies. In 2005 he received a position in Ariel University as a senior Lecturer in department of Biological Chemistry. Since 2015, Professor Gellerman holds a deanship of the Faculty of Natural Sciences. His main research interests are: drug design and targeted drug delivery.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

ENANTIOSELECTIVE AND MULTI-DIMENSIONAL HPLC ANALYSIS OF AMINO ACIDS AS THEIR FREE/PROTEIN BOUND FORMS FOR THE SCREENING OF NOVEL BIOMARKERS

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Enantioselective analysis of amino acids is gathering high attention recently for the screening of new physiologically active substances and/or biomarkers in mammals. However, the amounts of these chiral amino acids, especially the minor D-forms are extremely low, and the sensitive and selective analytical methods are essential. In the present study, therefore, multi-dimensional chiral HPLC systems combining a reversed-phase column, an anion-exchange/mixed-mode column and an enantioselective column have been designed and applied to the analyses of biological samples including human clinical samples.

Prior to the HPLC analysis, amino acids were derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) and detected by a fluorescence detector and also by a tandem mass spectrometer. Following the derivatization, NBD-amino acids were injected into the three-dimensional (3D) HPLC system and separated by a reversed-phase column (KSAARP) as a first dimension. The target fractions were collected to a multi-loop device, and introduced into the second dimension (KSAAAX or KSAAMX columns), and the target amino acids were separated again as their scalemic mixtures. The target fractions were transferred into the third dimension where D and L amino acids were separated by the enantioselective columns (KSAACSP). The second dimension could be omitted when the selectivity is sufficient or the tandem mass spectrometer is used as a detector.

By using the present multi-dimensional HPLC concept, chiral amino acids in a variety of biological samples including human serum/plasma and urine were analyzed. In the human serum/plasma, small amounts of D-Ala, D-Asn, D-Pro and D-Ser were observed, and the amounts of these D-amino acids, especially D-Asn and D-Ser have clear correlation to the kidney function. In the urine, the presence of non-negligible amounts of various D-amino acids (D-Ala, D-Arg, D-Asn, D-Asp, D-Glu, D-*allo*-Ile, D-Ser and D-*allo*-Thr) were shown. Concerning the protein bound type D-amino acid residues, proteins were hydrolyzed in the presence of deuterium oxide plus deuterium chloride. The resultant amino acids were derivatized with NBD-F, and analyzed by a 2D HPLC-MS/MS system. In a variety of proteins, several D-amino acid residues were observed. In ovalbumin, small but clear peak of D-Ser was detected, and D-Asn/Asp residue was found in mouse lysozyme. The present multi-dimensional HPLC concept is useful for the determination of trace levels of D-amino acids in biological samples, and further applications are in progress.

Kenji HAMASE graduated from The University of Tokyo in 1991, and received a Master of Pharmacy in 1993. He then obtained his Ph.D. degree in Pharmaceutical Sciences from The University of Tokyo in 1996. Throughout the doctoral course, he obtained research fellowships from the Japan Society for the Promotion of Science for Young Scientists (1993-1996). Subsequently, he began his academic carrier as an Assistant Professor at Kyushu University, and was promoted to an Associate Professor in 2001, and to the full Professor in 2016. He is a Program Officer of Research Center for Science Systems, Japan Society for the Promotion of Science (2018-2020). He received The Japan Society for Analytical Chemistry Award for Young Scientists in 2003, and The Pharmaceutical Society of Japan Award for Young Scientists in 2006. His current research interests focus on the development of analytical methods for chiral amino acids and the study on their physiological functions, diagnostic values and the design of functional foods.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

THE ANALYTICAL PROCEDURE LIFECYCLE: A MODERN APPROACH TO MANAGING YOUR METHODS

Stephanie Harden, *Waters Corporation, Baden-Dättwil, Switzerland (stephanie_harden@waters.com)*

The pharmaceutical industry relies on data generated by analytical methods for many critical decisions, however, the traditional approach to methods consisting of distinct exercises - method development, validation, transfer and verification - provides limited understanding of the effect of procedural variability on method performance.

The Method Lifecycle Management (MLCM) approach, as proposed in USP <1220> is a holistic approach encompassing all activities from method development to validation, routine use, change control and retirement of the method. This enhanced approach facilitates improved method understanding and performance, facilitates method transfer, can lead to fewer out-of-specifications results (OOS), and has the potential to lessen the regulatory burden.

In this seminar, we will explore some of the key concepts of method lifecycle management and discuss the benefits of adopting a more modern approach to methods.

Abstract Reference & Short Personal Biography of Presenting Author

Dr Stephanie N. Harden

Pharmaceutical Business Unit Manager & Strategic Partnerships Advisor, EMEIA

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Stephanie is responsible for the development of Waters' small molecule pharmaceutical business, the largest revenue-generating market segment within Waters' European organisation. In this role, she is responsible for planning and coordinating growth strategies to ensure Waters' continued success, and works closely with her internal and external customers, establishing and maintaining partnerships to ensure mutual benefit.

Stephanie is a highly cited researcher and University of Bristol graduate with a PhD in chemistry.
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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CHARACTERIZATION OF PLANTS' EXTRACTS USED FOR HERBAL PRESCRIPTION BY CHROMATOGRAPHIC TECHNIQUES COUPLED WITH MASS SPECTROMETRY

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Due to the presence of various secondary metabolites in plants' cells, extracts obtained from herbal plants possess various biological properties as antioxidant, anti-inflammatory, and anti-diabetic activities. Mentioned components are widely spread in other herbs, fruits as well as honey. Their isolation, separation and analysis becomes a great interest in several fields, such as pharmaceutical and food industries, biochemistry, medical cell biology, and biotechnology research or nutrition. The most used approaches involved in the last ten years for selective isolation, pre-concentration, and separation of biologically active compounds especially polyphenols and cyclitols in a range of complex matrices are widely discussed. Various conventional and non-conventional sample preparation methods have been used for extraction of mentioned compounds. Otherwise chromatographic techniques, which are characterized by high sensitivity, selectivity and versatility are widely applied for the determination of mentioned biologically active compounds. Through most used of them are gas chromatography and liquid chromatography coupled with mass spectrometry (GC-MS, LC/MS).

The main aim of presented study was to prepare methodologies for the separation and determination of biologically active compounds from various part of plants (roots, stems, leaves and flowers). Some plants of importance in medicine, pharmacology, cosmetics as well as animals feeding like *Medicago sativa* L., *Solidago canadensis* L. *Solidago gigantea* Aition, *Phacelia tanacetifolia* Benth., *Trigonella foenum-graecum* L., *Fagopyrum* Mill. have been taken into account. Various methods like solid phase microextraction (SPME), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) and classical solvent extraction allow to obtain extracts rich in volatile and non volatile compounds and characterized by a high antioxidant activity. The method of rapid assessment of the qualitative analysis of extracts by means of chromatographic techniques GC/MS, LC-MS^n and TLC-MS was proposed.

Acknowledgements

This work was financed in the framework of grant entitled: ”Cultivated plants and natural products as a source of biologically active substances assign to the production of cosmetic and pharmaceutical products as

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Abstract Reference & Short Personal Biography of Presenting Author

Magdalena Ligor has completed her DSc from Nicolaus Copernicus University and postdoctoral studies at Innsbruck Medical University. She has published more than 62 papers in the journals and 4 chapters to books. She is serving as an reviewer member of repute. Her research interests focusing chromatographic techniques (GC/MS, HPLC/MS^n, TLC-MS), sample preparation methods (SPME, SPE, LLE), analysis of plant materials, food and water, determination of VOCs, biomedical chemistry, trace analysis. Others duties: study of effects and mechanisms of biologically active compounds actions, which are occurring in plants and natural products; evaluation of the relationship between the concentration of polyphenols, flavonoids, carotenoids and their antioxidant activity; the development of specific validated methodologies for the extraction of biologically active compounds from plant materials and natural products and the determination of analytes by use of chromatographic techniques.

NEW LUMINESCENT BIOMARKERS BASED ON CARBON DOTS

Raz Jelinek, *Ben Gurion University, Israel*

Carbon dots (C-dots) are small (on the order of 10 nm or less), quasi-spherical graphitic nanoparticles and have attracted considerable and growing interest due to their unique structural, electronic, and optical properties, as well as their biocompatibility. In particular, C-dots exhibit multicolor, tunable fluorescence emissions, dependent upon both the nanoparticles' surface units and their molecular environments. In this presentation I will describe recent applications we introduced for using C-dots as a powerful vehicle for biosensing and imaging. Among the systems we developed are C-dots and C-dot-conjugates for bacterial labeling, cell staining, ROS detection and others.

Raz Jelinek
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- Education
  - B.Sc. (summa cum laude) 1985-1988, Hebrew University of Jerusalem, Jerusalem, Israel, Department of Chemistry.
  - Ph.D. 1988-1993, University of California, Berkeley, California, USA. Department of Chemistry.
- Employment History
  - 1993-1996
    Cancer Research Institute Post-doc Fellow, University of Pennsylvania
  - 1996 – present
    Ben-Gurion University, Department of Chemistry
    Current rank: Full Professor
    Current position: Vice President and Dean for R&D
  - 2015, 2018
    Visiting Professor
    NYU Shanghai, China
- Research students
  - Currently: 6 post-doc fellows; 7 PhD students; 6 MSc students
- Recent Awards
  - 2008 – 2010 - Ruth L. Kirschstein National Research Service, Senior Fellowship Award, the National Institutes of Health (NIH), USA
  - 2009 Toronto Prize for Research Excellence, Ben-Gurion University
  - 2011 Distinguished Lecturer Award, Ben Gurion University
- Scientific Publications
  - Author of four textbooks
  - Editor of two scientific books
  - Published 180 papers in peer-reviewed scientific journals
- Patents and Patent Applications
  - 12 patents awarded / submitted
- Research Grants
  - Over $8M grant support in the past 10 years from varied agencies (Israel Science Foundation, US-Israel Binational Science Foundation,
  - US-Israel Binational Agricultural Foundation, DFG, Human Frontiers Science Program, others)

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

A NOVEL ELECTROCHEMICAL MICROFLUIDIC CHIP BIOSENSOR FOR MULTICOMPONENT ANALYSIS IN RENAL FUNCTION EXAMINATION

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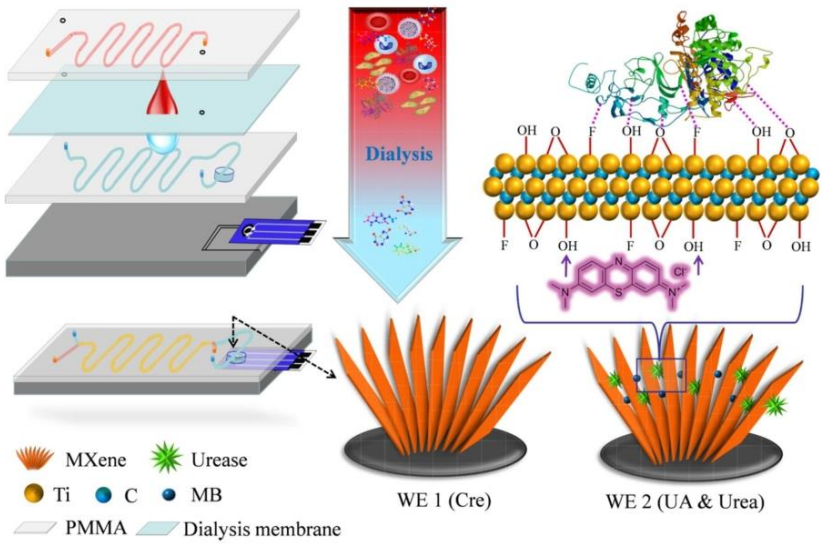
Real-time detection of various chemical and biological substances is crucial for implementing precision medical diagnosis and treatment. In spite of tremendous efforts, several challenges still accompany current techniques and instruments. In this contribution, a novel MXene-based electrochemical microfluidic chip combining initial dialysis of whole blood with subsequent detection was firstly fabricated and applied in on-line renal function test. Thanks to the intriguing behavior of MXene nanosheets and the elaborately designed testing chip platform and strategies, simultaneous quantification of three biomarkers was realized in one device and the multi-component detection proves to be accurate, reliable and interference-free, which can perfectly meet the clinical and civil demands. In the novel bio-sensing system, MXene is demonstrated as a suitable material for constructing electrochemical sensor due to its excellent electrocatalytic property, admirable enzyme-loading capacity, good biocompatibility, and abundant surface terminating groups. Additionally, a simple and versatile ratiometric sensing approach is established via the strong adsorption of MXene nanosheets for probe molecules. Continuous on-line monitoring of uric acid, urea and creatinine in whole blood was carried out by using the newly developed sensor platform, which fills the vacancy in body condition evaluation during hemodialysis treatment. The microfluidic chip also displays great potential as a promising assay device for point-of-care test (POCT) in terms of cost, stability, adaptability in different/adverse detection environments, miniaturization, automation of tests, etc. Our work expands the biomedical applications of 2D materials and millions of patients will benefit from the findings.

Figure 1. Schematic illustration of fabrication of MXene-enabled microfluidic chip.



Keywords: MXene; microfluidic chip; electrochemical sensing; renal function examination; whole blood test; therapeutic drug monitoring

Abstract Reference & Short Personal Biography of Presenting Author

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[2] Jiang Liu, Yingchun Li*, Han Zhang*, et al. MXene-enabled electrochemical microfluidic bio-sensor: applications towards multi-component continuous monitoring in whole blood. Adv. Funct. Mater., 2018(1807326): 1-9

Yingchun Li received her B.Sc. from Shihezi University in 2003, and M.Sc. from Xi'an Jiaotong University in 2006. She continued her Ph.D. study in Halle University in Germany and received her doctor degree in 2011. Then she joined Shihezi University as one scientist of the "Recruitment Program of Global Experts" (1000 Talent Plan). Now, Prof. Li continued her research in Harbin Institute of Technology (Shenzhen), and her research field is in development of advanced functional materials and sensors for serving pharmaceutical and biomedical analysis. As an independent PI, Prof. Li has published 50 research articles in well-known journals and got 4 national patents of invention. She is also the winner of Young Scientist Competition in the 27th International Symposium on PBA in the year of 2016

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CHARACTERIZATION OF PLANTS' EXTRACTS USED FOR HERBAL PRESCRIPTION BY CHROMATOGRAPHIC TECHNIQUES COUPLED WITH MASS SPECTROMETRY

Magdalena Ligor, *Nicolaus Copernicus University, Toruń, Poland (mligor@umk.pl)*
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Due to the presence of various secondary metabolites in plants' cells, extracts obtained from herbal plants possess various biological properties as antioxidant, anti-inflammatory, and anti-diabetic activities. Mentioned components are widely spread in other herbs, fruits as well as honey. Their isolation, separation and analysis becomes a great interest in several fields, such as pharmaceutical and food industries, biochemistry, medical cell biology, and biotechnology research or nutrition. The most used approaches involved in the last ten years for selective isolation, pre-concentration, and separation of biologically active compounds especially polyphenols and cyclitols in a range of complex matrices are widely discussed. Various conventional and non-conventional sample preparation methods have been used for extraction of mentioned compounds. Otherwise chromatographic techniques, which are characterized by high sensitivity, selectivity and versatility are widely applied for the determination of mentioned biologically active compounds. Through most used of them are gas chromatography and liquid chromatography coupled with mass spectrometry (GC-MS, LC/MS).

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

ECHO MS: HIGH THROUGHPUT SCREENING WITH A POTENTIAL OF 3 SAMPLES PER SECOND

Volker Kruft, *Sciex, Darmstadt, Germany*

Pramlintide acetate (SYMLINTM) is a synthetic analogue of the human hormone amylin developed as an adjunctive therapy for patients with type 1 and 2 diabetes. With nearing patent expiry dates, and recent research indicating a role for amylin in Alzheimer's Disease models, interest in amylin and amylin agonists is rising.

Hydrophobic peptides such as pramlintide often suffer from non-specific binding (adsorption) to any labware samples come into contact with (plates, pipette tips, etc…). This can make method development difficult as it can lead to poor recovery, loss of analyte, and poor limits of detection. This work describes optimization and development of a selective sample preparation strategy and LC-MS/MS analysis to achieve LLOQs of 25 pg/mL from 100 µL of serum.

Abstract Reference & Short Personal Biography of Presenting Author

Volker Kruft received his PhD from the Max-Planck-Institute of Molecular Genetics under the guidance of Prof. H.-G. Wittmann, working on structure-function relationships of the prokaryotic ribosome. He joined Sciex in 1993 as a product specialist for protein analysis. Since then, he has held various positions at Sciex in support and technical marketing. Currently he holds the position of Senior Business Development Manager for the high growth regions in Africa, Middle East and Eastern Europe/Russia.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

IDEAL-FILTER CAPILLARY ELECTROPHORESIS (IFCE) AS A UNIVERSAL METHOD FOR SELECTION OF DNA APTAMERS FROM OLIGONUCLEOTIDE LIBRARIES

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Sergey N., *Chemistrty, York University, Toronto, Canada*

Aptamers revolutionized many areas of analytical and medical chemistry ranging from diagnostics of diseases to drug development. One of the popular methods of selection of DNA aptamers from random-sequence DNA libraries is non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM), which offers a simple and rapid separation of protein–DNA complexes from the unbound DNA molecules in an electric field. However, sometimes we find that while the majority of DNA molecules migrate with predictable velocities, there is a fraction of DNA molecules that migrates with a wide range of irregular velocities. This irregular fraction of DNA can be quantitated by qPCR detection. We proved that the interaction between DNA and its condensed counter ions caused this non-uniform migration of DNA in electric field. Since protein targets often require relatively high salt concentrations, selection of DNA aptamers in low salt conditions is not desirable. The study of DNA velocity profiles in solutions containing physiological levels of salt was critical for selection of aptamers. To resolve a problem of the presence of irregular fraction of DNA we suggested moving protein–DNA complexes and the unbound DNA in the opposite directions in electric field in an uncoated capillary. This idea led us to the development of Ideal-Filter Capillary Electrophoresis (IFCE) as a uniquely efficient method for selection of affinity ligands from oligonucleotide libraries, such as random-sequence RNA and DNA libraries as well as DNA-encoded libraries (DELs)*****. In this approach, the efficiency of partitioning was optimized by changing the running buffer's ionic strength to reach a value of 10^9. This value is 10^4 times higher than the highest previously reported value for NECEEM and 10^7 times higher that the best values achievable with practical solid-phase partitioning methods (e.g. with a target protein immobilized on magnetic beads) used in aptamer selection.

* A. T. H. Le, S. M. Krylova, M. Kanoatov, S. Desai, S. N. Krylov. *Angew. Chem. Int. Ed.* **2019**, *58*, 2739 –2743.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Krylova obtained her PhD from the Russian Academy of Sciences. She has over ten years of research leadership experience in the area of medical diagnostics and drug development in biotechnology and pharmaceutical companies in Canada. She has been a contract faculty member at York University in Toronto since 2008. Dr. Krylova is also leading research projects in the area of Bioanalytical Chemistry as a Senior Research Associate in the Centre for Research on Biomolecular Interactions at York University.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

URINARY METABOLIC PROFILING IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD)

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Background: The disease course of ADPKD is highly variable and the advent of renoprotective treatment requires early risk stratification. The markers to select the patients at high-risk of rapid progression in the early stages of their disease are highly needed. Here, we applied targeted, quantitative metabolic profiling to evaluate whether changes in the urinary metabolome are associated with estimated GFR (eGFR) and with disease progression (eGFR decline) in ADPKD.

Methods: Targeted, quantitative metabolic profiling (^{1}H NMR-spectroscopy) was performed on spot urine samples using the KIMBLE workflow[1]. A discovery ADPKD cohort (n=338) was used for model building and tuning and an independent cohort (n=163) was used for validation. Multivariate modelling and linear regression were used to dissect and validate the associations between metabolic composition of urine and the annual change in eGFR.

Results: Twenty-nine known urinary metabolites were quantified from the spectra using a semi-automatic quantification routine (KIMBL). A correlation analysis of the quantified metabolites revealed a strong association with eGFR in the discovery cohort. We applied a model optimization routine resulting in selection of four metabolites. In combination, they served as a good predictor for actual eGFR. The annual change in eGFR was best described a single metabolic ratio. The model built on this predictor outperformed the models built only on the clinical risk markers (eGFR and total kidney volume) and remained significant after adjustment for potential confounders. The associations between urinary metabolites and eGFR, and with eGFR decline over time were validated in an independent cohort.

Conclusion: Quantitative NMR profiling enabled identification of two non overlapping sets of the urinary metabolic markers: one that describes a baseline eGFR and another describing annual change in eGFR or disease progression (eGFR decline) in ADPKD. The finding showed strong additional value beyond that of clinical risk markers for the management of ADPKD.

[1] Verhoeven et al., KIMBLE: A versatile visual NMR metabolomics workbench in KNIME, Analytica Chimica Acta, 2018

Abstract Reference & Short Personal Biography of Presenting Author

PhD, associate professor, graduated from the University of St. Petersburg (St. Petersburg, Russian Federation) and obtained his PhD from the Institute of Evolutional Physiology and Biochemistry of the Russian Academy of Science (St. Petersburg, Russian Federation) in 1992. After completing his post-doctoral trainings at the Technische Universität Carolo-Wilhelmina of Braunschweig and the University of Magdeburg he moved to The Netherlands where he joined the Department of Parasitology, currently the Center of Proteomics and Metabolomics, of the Leiden University Medical Center. His current interests are the application of metabolomics in epidemiological studies as well as data analysis.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

BEYOND THE CHIP: DEVELOPMENT AND APPLICATION OF A MICRO-PHYSIOLOGICAL FLUX ANALYZER

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Organ-on-chip technology aims to replace animal toxicity testing, but thus far demonstrated few advantages over traditional methods. Current methods to evaluate toxicity rely on end-point assays measuring tissue damage and cell death, resulting in limited kinetic and mechanistic information. We present the Tissue Dynamics platform capable of maintaining vascularized 3D liver, cardiac, and neural tissues for over a month in vitro. Tissues acquire physiological structure, physiological activity and show complex metabolic zonation. Tissue-embedded metabolic sensors for oxygen, glucose, lactate and glutamine permit the real-time quantification of intracellular fluxes and tissue level function. Change in metabolic function is the first indication of physiological stress, preceding any detectable damage. Using the Tissue Dynamics platform, we show a new CYP450-idependent mechanism of acetaminophen toxicity that may be responsible for clinically observed nephrotoxicity. We also show that troglitazone, a drug withdrawn from the market due to idiosyncratic toxicity, induces harmful metabolic changes at below the observed threshold for toxic damage. These metabolic changes may underlie troglitazone's observed idiosyncratic toxicity. More recently, we studied the dynamics of human liver response to the epilepsy drug valproate. Our platform demonstated a rapid disruption of metabolic homeostasis below the threshold of cellular damage, and an increase in lipogenesis rather than distruption of beta-oxidation. Our work marks the importance of tracing function in real-time, demonstrating specific advantages in predicative toxicology.

Abstract Reference & Short Personal Biography of Presenting Author

Prof. Yaakov Nahmias
Founder & CSO, Tissue Dynamics
Founder & CSO, Future Meat Technologies
Professor of Bioengineering, The Hebrew University of Jerusalem (HUJI)

Prof. Yaakov "Koby" Nahmias is a bioengineer and innovator, whose breakthroughs ranged from the first 3D printing of cells to the first commercial human-on-chip technology. He is a Magna Cum Laude graduate of the Technion, Israel Institute of Technology, and is the founding director of the Grass Center for Bioengineering of the Hebrew University of Jerusalem. Nahmias is a recipient of a NIH career award, two European Research Council (ERC) grants, the Kaye Innovation Award and the prestigious Rappaport Prize in Biomedical Research. He is the first scientist outside Britain to win the Rosetrees Trust Prize. Nahmias is the co-founding director of BioDesign-Israel. An entrepreneurship program that educated over 120 fellows, raising $3M in investment and 10 startup companies including Guide In Medical, CardioVia and VenoVision. Nahmias is the founder and CSO of two biotechnology startups, including Tissue Dynamics that is developing a groundbreaking human-on-chip instruments for drug development, and the Tyson Foods-funded Future Meat Technologies focusing on the cost-effective production of cultured meat.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

NUCLEIC ACID APTAMERS COMBINED WITH A SIGNAL AMPLIFICATION PROBE, DRAMATICALLY INCREASES SENSITIVITY IN ELISA LIKE AND OTHER IMMUNO-DIAGNOSTIC ASSAYS

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AptaTeck bio proprietary platform technology is an amplification probe for developing aptamers and antibodies based highly sensitive immuno-diagnostic assays.

Aptamers potentially can be used for monitoring targets by attaching to them a signaling element such as biotin, fluorescent dyes, electric reagent and others. However, an aptamer can carry only one such element and therefore signals obtained from such immune recognition are low and result in low sensitivity or the needs for sophisticate detection methods and readers, and therefore are usually not suitable for conventional immune assays, such as the gold standard ELISA like assays.

A proprietary platform technology developed at AptaTeck is based on a unique universal "Signal Enhancement" probe, which can easily be attached to either nucleic acid aptamers or antibodies.

This "Signal Enhancement" element is a randomized synthetic dsDNA fragment carrying a large number of detection components (biotin, fluorophore, etc.). The probe can be adjusted in length and signaling element capacity. It can easily be attached to aptamers or antibodies that are used as recognition elements. Employing Aptamers bound amplification Probe for sandwich ELISA, results in an amplified signal, leading to reliable highly specific, reproducible and sensitive assays with low limit of detection.

The aptamers-based system was tested in ELISA assays, modes employing antibodies or aptamers as for capturing and detection. In all modes, low background and highly sensitive detection of target antigens were obtained, maintaining the assay specificity and low limit of detection.

For Thrombin, the limit of detection was found to be 0.4ng/ml (20pg), while the limit of detection of a Thrombin detection ELISA kit was 4.7ng/ml (470pg). For PDGF, the limit of detection was found to be 0.32ng/ml (16pg), while the limit of detection of a PDGF detected in ELISA kit which was 0.5ng/ml (50pg). The use of aptamers together with our efficient signal amplification probe makes aptamers feasible as antibody replacement in immune diagnostic tests such as ELISA. In addition, the system can be applied and integrated in other immuno-diagnostic platforms including Flow Cytometry, Lateral Flow, Microfluidity, Immuno arrays and Immunohistochemistry.

Abstract Reference & Short Personal Biography of Presenting Author
Ofer Nussbaum PhD.

- PhD at the institute of life science, the Hebrew university of Jerusalem. Subject: The mechanism of virus penetration and infection.
- Post doctoral position at NIH-NHLBI: Molecular Biology and Gene-Therapy.
- Investigator at NIH-NIAID: HIV penetration – mechanism and specifications.
- Head of Molecular Biology, XTLbio, Rehovot Israel.

For the past 6 years, specialized in RTF techniques: assay development and validated diagnostic procedures.

- Senior Scientist & Director of Clinical affairs at MND DIAGNOSTICS Ltd.
- Co-Founder and CSO at AptaTeck bio LTD, developing nucleic acid aptamers-based diagnostics.

Today:

- Co-Founder and CEO at AptuCure bio LTD, developing nucleic acid aptamers-based drugs.
- Scientific advisor

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

LC-MS BASED-METABOLOMICS OF SERUM SAMPLES FROM NEWBORNS EXPOSED TO ZIKA VIRUS: A PILOT STUDY IN BRAZIL

Regina V. Oliveira, *Departamento de Química, Universidade Federal de São Carlos (UFSCar), São Carlos, Brazil (oliveirarv@ufscar.br)*
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Zika virus (ZIKV) is a flavivirus transmitted by *Aedes* genus mosquitoes that was identified almost 70 years earlier in Africa, the Zika virus was thought to cause only mild disease. Throughout 2015, increasingly worrisome reports trickled out of Brazil about obscure virus called Zika, which became associated with birth defects, specifically microcephaly, a brain anomalie, and progressive form of paralysis known as Guillain-Barré syndrome, transforming the Zika threat into a worldwide public health emergency. In Brazil, more than 2,100 babies have been born with microencephaly and other birth defects linked to Zika. ZIKV is primary transmitted by the bite of an infected mosquito from *Aedes* genus, however, it can also be transmitted through sexual intercourse and from mother to fetus during pregnancy (congenital) or around the time of birth (perinatal). Signs and symptoms of ZIKV infection are often mistaken with other common viral infections ranging from no symptoms to severe illness. In light of these, better understanding the pathophysiological mechanism of ZIKV infection and its relation to microencephaly is critical for delivering improved patient care. This work employed metabolomic tools for understanding alterations caused by ZIKV infection in serum from newborns with/without microcephaly. Forty-six serum samples from newborns were divided into groups: 1-Newborns from ZIKV-infected mothers but without microcephaly (n=17) and 2-Newborns from ZIKV-infected mothers and with microcephaly (n=29). Metabolites were analyzed in reversed phase and HILIC separation modes with an UHPLC system (Nexera®, Shimadzu) coupled to an Impact HD QTOF™ mass spectrometer (Bruker Daltonics) equipped with ESI operating in negative or positive ion mode. The MS and MS/MS data were processed through DataAnalysis and MZmine. The chemometric models were obtained through MetaboAnalyst and the identification of features were performed through the Mass Mediator (http://ceumass.eps.uspceu.es/). Statistical analyses were performed by unsupervised (PCA) and supervised (PLS-DA) chemometric tools based on mass spectral data. Results showed a large number of metabolites identified in the two studied groups, suggesting alterations in important biochemical pathways. Using VIP scores >1.5, 430 features were identified in positiv

e ion mode and 260 features in negative ion mode. The biomarkers detected were classified as aminoacids, lipids, biogenic amines, sugars and other serum metabolites. Correlation of the identified markers with biochemical pathways indicated alterations in serotonin, noradrenaline, adrenaline, and histidine degradation pathways. These data provided information to the biochemical events in newborns exposure to ZIKV, from which it may direct further research during gestational period.

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Abstract Reference & Short Personal Biography of Presenting Author

Dr. Oliveira is a Senior Research Associate Professor at the Chemistry Department of the Federal University of São Carlos, Brazil where she supervises Senior Researchers, Graduate students, and leads a Bioanalytical Research Laboratory. She works and collaborates on projects including qualitative and quantitative analysis of small and large molecules, natural products, biologics molecules, and biomarkers.
Her main research interest includes drug metabolism, metabolomics, bioanalytical methods for small and large molecules, mass spectrometry, biomarkers assays and cutting-edge bioanalysis methods by LC-MS/MS at the intersection of Analytical Chemistry with Biology, Biochemistry and Chemistry.

Dr. Oliveira joined the National Institute of Health, National Institute on Aging (Baltimore, MD), as a Postdoctoral Research Fellow to investigate new approaches for drug discovery using on-line screening of target molecules and bioaffinity chromatography. She also worked as a Visiting Research Scientist at Quintiles Inc (Ithaca, NY) and acted as a Senior Research Investigator at Bristol-Myers Squibb (Princeton, NJ) in the Pharmaceutical Candidate Optimization Department, where she worked with MS-based assay development for exploratory biomarkers in PK/PD and toxicology studies, collaborating to Discovery and Clinical Research activities.
She has authored or co-authored over 100 journal articles, book chapters and oral/poster presentations and has served as editorial board for the Journal of Pharmaceutical and Biomedical Analysis.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

DEVELOPMENT OF NEW SPONGY MONOLITH FOR SELECTIVE SEPARATION OF EXTRACELLULAR VESICLES

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Seiya Kato, *Department Of Material Chemistry, Kyoto University, Kyoto, Japan*
Toyohiro Naito, *Department Of Material Chemistry, Kyoto University, Kyoto, Japan*
Takuya Kubo, *Department Of Material Chemistry, Kyoto University, Kyoto, Japan*
Kazunari Akiyoshi, *Department Of Polymer Chemistry, Kyoto University, Kyoto, Japan*

An exosome is one of the extracellular vesicles containing specific proteins, nucleic acids, and sugars. The components of exosomes are not fully identified because the effective isolation method for exosomes based on their chemical characteristics has not been established. To facilitate the exosome studies, we focus on the specific separation of exosomes by the recognition of sugar chains on the surface of exosomes. For typical separations based on sugar chains, lectin affinity chromatography (LAC) is usually employed, which is currently used to purify glycoproteins, but the pore size of the LAC separation media is too small to separate exosomes.

To achieve effective exosome separations, a spongy monolith (SPM), which consists of poly(ethylene-co-glycidyl methacrylate) (PEGM), as a separation medium for LAC. The SPM contains large through pores of >10 µm in diameter, thus exosomes are assumed to be passed through easily. In addition, we previously achieved the affinity separation of biomolecules under an ultra-high flow rate using the SPM.

In this study, to separate exosomes by the recognition of the surface glycans, we prepared two kinds of LAC columns made of the SPM with immobilized *sambucus sieboldiana* agglutinin (SSA) or concanavalin A (ConA). Additionally, insulin was employed to block the hydrophobic surface of the SPMs. After preparing the columns, chromatographic analyses were carried out with bovine serum albumin (BSA) to evaluate the hydrophilicity and permeability of the SPMs. Since a sufficient amount of BSA was passed, it suggested that the SPMs were substantially hydrophilized. The interaction between the lectins immobilized on the SPMs and sugar chains was then evaluated. As results, glycoproteins (transferrin and glucose oxidase) were effectively interacted with their respective lectins. Furthermore, the desorption procedures were optimized by changing the elution conditions. And then, we evaluated the interaction with liposomes, which consist of lipid bilayer and have the sugars on the surface, as well as exosomes. Along with glycoproteins, mannose-integrated liposomes were also interacted and rapidly desorbed by using the ConA-immobilized SPM. These results suggest that the lectin-immobilized SPMs will be useful for the separation of exosomes based on recognition of the surface sugar chains.

[1] Kubota, K.; Kubo, T.; Tanigawa, T.; Naito, T.; Otsuka, K. *Sci. Rep.* **2017**, *7*: 178.

Abstract Reference & Short Personal Biography of Presenting Author

Koji Otsuka is a professor of Analytical Chemistry of Materials, Department of Material Chemistry, Graduate School of Engineering, Kyoto University, since 2002. He received his Ph. D. from Kyoto University in 1986 under the supervision of Prof. Teiichi Ando and Dr. Shigeru Terabe. After receiving the JSPS Fellowship for Young Scientists (1986–1988), he joined Osaka Prefectural College of Technology as a senior lecturer (1988–1990) and an associate professor (1990–1995), then moved to Himeji Institute of Technology as an associate professor of the Prof. Terabe's Lab (1995–2002), followed by moving to Kyoto University.

His research interests include the development of micro/nano scale high performance separation techniques using electrophoretic and chromatographic methods. He has published approximately 180 papers and book chapters.

He received the Award of the Society for Chromatographic Sciences (SCS), Japan (2006), the Award for Creative Work of the Chemical Society of Japan (2009), the Award of the Japan Society for Analytical Chemistry (JSAC) (2014), and the Terabe Shigeru Prize (2014). He is a member of the Permanent Scientific Committees of the HPLC and ITP Symposium Series and an editor of the Journal of Separation Science (Wiley-VCH). Currently he is serving as the president of the SCS as well as the chair of HPLC2019 Kyoto.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

FLUORESCENT REPORTERS FOR BIOMEDICAL APPLICATIONS

Leonid Patsenker, *Ariel University, Ariel, Israel (leonidpa@ariel.ac.il)*

Fluorescent reporters are the dye molecules or molecular systems that provide fluorescence signal for detection. These fluorescent compounds and materials are widely used in biomedical and related applications such as clinical diagnostics, biological imaging, photodynamic therapy (PDT), pharmaceutical research, high-throughput screening, food control, and environmental pollution monitoring. These materials are represented by the non-covalent probes, covalent labels, classification (coding) dyes, photosensitizers, and fluorescent standards. The current presentation focuses on the resent achievements of our research team and challenging tasks in the field of the development of fluorescent reporters for biomedical applications. The emphasis is on the near-IR spectral rang, brightness, sensitivity, stability, long and environment sensitive fluorescence lifetimes.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Leonid Patsenker is an Associate Professor at the Department of Chemistry at the Ariel University, Israel. He studied Chemistry at the Kharkov State University, USSR and received his Ph.D. in 1989 from this University. Between 1986 and 2016 he worked at the State Scientific Institute for Single Crystals of the National Academy of Sciences of Ukraine, where for 10 years held the position of the Head of the Department of Organic Luminophores and Dyes. His research interests include the development of fluorescent dyes for biomedical applications, sensitizers for photodynamic therapy (PDT), organic scintillators, materials for LEDs, laser dyes, and sunlight converters. More then 100 developed fluorescent products have been commercialized. He has been the PI of about 30 national and international projects supported by the USA, EU, Canadian and Ukrainian Governments and companies. He has published over 100 research and review papers, 2 book chapters, and has held 24 patents. Upon his immigration to Israel in 2016, he started to work in the Department of Chemical Sciences at the Ariel University. The current research focuses on the development of fluorescence based systems for targeted drug delivery monitoring. This work is supported by the Israeli Scientific Foundation.

FLUORESCENT REPORTERS FOR BIOMEDICAL APPLICATIONS

Leonid Patsenker, *Ariel University, Ariel, Israel (leonidpa@ariel.ac.il)*

Fluorescent reporters are the dye molecules or molecular systems that provide fluorescence signal for detection. These fluorescent compounds and materials are widely used in biomedical and related applications such as clinical diagnostics, biological imaging, photodynamic therapy (PDT), pharmaceutical research, high-throughput screening, food control, and environmental pollution monitoring. These materials are represented by the non-covalent probes, covalent labels, classification (coding) dyes, photosensitizers, and fluorescent standards. The current presentation focuses on the resent achievements of our research team and challenging tasks in the field of the development of fluorescent reporters for biomedical applications. The emphasis is on the near-IR spectral rang, brightness, sensitivity, stability, long and environment sensitive fluorescence lifetimes.

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

NUCLEIC ACID APTAMERS AS AN ALTERNATIVE TO ANTIBODIES IN DIAGNOSTIC AND THERAPEUTICS

Dr. Gregory Penner, *NeoVentures Biotechnology Inc., London, Canada, gpenner@neoventures.ca*

The concept that single stranded oligonucleotides (aptamers) had the capacity to mimic antibodies by binding to target molecules was discovered 30 years ago. Since then the science of aptamer identification has continued to grow but commercial applications have been disappointingly scarce. There are many reasons for this lack of commercial success to date. Some of these reasons are intrinsic to aptamers, there are things that antibodies implicitly do better than aptamers are capable of. A lot of the reasons for the lack of commercial success however are due to poor aptamer identification strategies, and a lack of imagination in terms of the chemistry required to maintain activity when immobilized, or to consider approaches for detection.

In this lecture, Dr. Penner will draw from his experience in working with aptamers at NeoVentures for the past 17 years, including the first successful commercialization of diagnostic aptamers. He will provide an update on their use of advanced mathematical models to guide and optimize aptamer selection, discuss how best to use next generation sequence analysis to identify candidate aptamers, and elaborate on strategies for the selection of aptamers that neutralize cellular receptors. In addition, he will provide an overview of effective chemical approaches to aptamer immobilization while retaining activity, use of gold nanoparticles functionalized with aptamers, and the effective use of specific antisense oligos in lateral flow detection. He will also provide insights into the novel use of aptamers themselves as biomarkers, with the aptamarker platform that his company in Paris, (NeoNeuro) has pioneered.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Gregory Penner is the President & CEO of NeoVentures Biotechnology Inc. (London, Canada) a global leader in the development of aptamers on a fee-for-service basis, and President & Founder of NeoNeuro SAS (Paris, France), where he has led the invention of the aptamarker platform for prediction of Aβ brain lesion deposition from blood analysis, and more recently cancer treatment outcomes from biopsy tissue. Dr. Penner was trained in crop genetics, and led the wheat genomics effort globally for Monsanto before turning his attention to aptamers and health care. Dr. Penner was a pioneer in development of molecular breeding, enabling decisions made in crop selection based on genotypic markers and statistical models without the need to know the biological basis for such selections. He is now bringing this agnostic approach to diagnosis to health care. Let's diagnose disease with greater accuracy, so that we can provide more effective treatments.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

MOLECULAR EPITOPE DETERMINATION OF APTAMER COMPLEXES OF THE MULTIDOMAIN PROTEIN CMET BY AFFINITY-MASS SPECTROMETRY

Michael Przybylski, *Steinbeis Centre for Biopolymer Analysis and Biomedical Mass Spectrometry, Rüsselsheim am Main, Germany (michael.przybylski@stw.de)*
Loredana Lupu, *Steinbeis Centre For Biopolymer Analysis And Biomedical Mass Spectrometry, Rüsselsheim Am Main, Germany*
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Friedemann Völklein, *Dept. Engineering, Rhein Main University, Rüsselsheim Am Main, Germany*
Günes Barka, Sunchrom Gmbh, Friedrichsdorf, Germany
Alexander Lazarev, *Pressure Biosciences Inc., Boston, Usa*
Maxim Berezovski, *Dept. Of Biochemistry, University Of Ottawa, Ottawa, Canada*

We present here a new approach for identification of biomarkers of pathophysiological target proteins, and alternative to pathophysiological antibodies, by molecular epitope identification of DNA-aptamers. C-Met protein has been recognized as a biomarker in cancer diagnosis by transmitting intracellular signals due to a unique multi-substrate docking site, and has been found to be aberrantly activated leading to tumorigenesis. C-Met aptamers have been recently considered a valuable tool for detection of cancer biomarkers. Aptamers are single-stranded DNA or RNA oligonucleotides that are readily produced and show stabilities and affinities comparable to monoclonal antibodies. A molecular interaction study of human C-Met protein expressed in kidney cells was performed with two DNA aptamers of 60 and 64 bases (CLN003 and CLN004), prepared and isolated using the SELEX procedure. Specific epitope peptides of the C-Met aptamers were identified by proteolytic affinity- mass spectrometry in a new combination with an SPR biosensor (PROTEX-SPR-MS), using high pressure proteolysis as an efficient tool for proteolytic digestion-epitope extraction of native and denatured C-Met. High affinities with binding constants K_D of 80 - 510 nM were determined for aptamer- C-Met complexes, with a two-step binding pathway suggested by kinetic analyses. A single linear epitope peptide, C-Met(381-393) (NSSGCEARRDEYR) was identified for CLN-0004, while the CLN-0003 aptamer revealed an assembled epitope comprised of two specific peptide sequences[1]. Epitope specificities and affinities were ascertained by characterization of synthetic epitope peptides. The high specificities and affinities of C-Met aptamers in this study suggest high potential for molecular diagnostics. Moreover, on the basis of the epitope identifications, aptamers with high affinity can be developed for therapeutic intervention such as neutralization of pathophysiological antibodies[2] against therapeutic proteins.

References

[1] Lupu, L. et al. **2018,** Proc**.** Int. Mass Spec. Conf., 241.

[2] Kukacka Z., et al. **2018**, ChemMedChem. 13: 909-915.

Abstract Reference & Short Personal Biography of Presenting Author

Michael Przybylski studied chemistry and completed his PhD dissertation at the University of Mainz. He spent two years as a Visiting Scientist at the National Cancer Institute, NIH/USA, and was appointed in 1989 to the Chair of Analytical Chemistry at the University of Konstanz, where he was the Director of the Laboratory of Analytical Chemistry and Biopolymer Structure Analysis. Michael Przybylski is currently Director of the Steinbeis Centre for Biopolymer Analysis and Biomedical Mass Spectrometry, located for 8 years at the University of Konstanz, and located in Rüsselsheim am Main since 2016. Since 2 years the Laboratory is located in Rüsselsheim am Main; it is associated with the Rhein Main University Rüsselsheim, Dept. of Engineering Sciences. The laboratory's current research is focused on the development of biosensor and mass spectrometry technologies for elucidation of antibody epitopes; applications of mass spectrometry to structure elucidation and and pathophysiological modifications of lysosomal proteins; structure analysis of therapeutic antibodies; development of aptamers as surrogate antibodies; affinity-mass spectrometry of biopolymer interaction epitopes. His laboratory has made numerous new developments and applications of biopolymer mass spectrometry methods, and has invented proteolytic- affinity mass spectrometry for the elucidation of protein-ligand interaction structures, and peptide/protein epitopes. He has been member of Scientific Committees of several International Conferences, Editor and Editorial Board member of several International Journals, and has been President of the German Society for Mass Spectrometry. He has published over 400 scientific publications in peer reviewed International Journals; 6 monographs/reviews; ca. 25 patents; and given ca. 150 invited lectures.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

HARACTERIZING THE SMALL-MOLECULE CORONA OF NANOPARTICLES BY CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY: A NEW APPROACH IN NANOSCIENCE

Rawi Ramautar, *Biomedical Microscale Analytics, Leiden University, Leiden, The Netherlands (r.ramautar@lacdr.leidenuniv.nl)*

The use of nanomaterials in biomedical and biotechnological applications is growing, and such materials are also released into the environment as consumer products. Due to their high surface free energy, nanomaterials adsorb biomolecules on contact with biological samples. In particular, proteins bind to the surface of nanoparticles to form a coating around the nanoparticle, known as protein corona. The small-molecule or metabolite corona is a much overlooked aspect of the nanoparticle corona. The corona affects the biological identity of the nanoparticle and may thus affect biomedical applications or modulate nanotoxicological effects. Therefore, in the development of nanomaterials for any kind of biological or biomedical applications, it is essential to understand the formation and evolution of the corona.

In this work, we show that capillary electrophoresis-electrospray ionization mass spectrometry (CE-MS) using a low-flow interfacing technique offers an exciting platform to characterise the polar and charged compounds found within the metabolite corona. This work is the first to quantitativly assess the metabolite corona of various nanoparticles incubated in academic test solutions and human plasma. Initial findings suggest that each studied nanoparticle has a unique small-molecule fingerprint dependent upon their physico-chemical properties and that the presence of proteins has a significant impact upon the formation of the metabolite corona.

Overall, these results represent the first application of CE-MS to nanoparticle corona characterisation yielding highly sensitive and reproducible data for highly polar and charged metabolites. Thus, opening a new area of coronal analysis while simultaneously introducing CE-MS-based metabolomics to the nanosciecne community.

Abstract Reference & Short Personal Biography of Presenting Author

Rawi Ramautar obtained his PhD on the development of capillary electrophoresis-mass spectrometry methods for metabolomics from Utrecht University, the Netherlands, in 2010. Intrigued by metabolomics for disease prediction and diagnosis, Rawi switched to the Leiden University Medical Center to broaden his horizon on this topic. In 2013 and 2017, he received the prestigious Veni and Vidi research grants from the Netherlands Organization for Scientific Research for the development of CE-MS approaches for volume-restricted metabolomics. Currently, he is a principal investigator at the Leiden University where his group is developing microscale analytical workflows for sample-restricted biomedical problems. Rawi Ramautar was recently selected for the Top 40 under 40 Power List of the Analytical Scientist.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CANNABIS-FROM HERBAL INTO A DOSAGE FORM ANALYTICAL CONSIDERATIONS AND LESSON LEARNED REGARDING THE DETERMINATION OF ACTIVE CANNABINOIDS

Dr. Revital Rapoport, *Head of Chemistry Division, Aminolab laboratories LTD., Ness Ziona, Israel*

The therapeutic characteristic of Cannabis has been known for years . However , along the last 30 years , the use of cannabis as medicine has not been rigorously tested due to production and governmental restrictions, in many countries resulting in limited clinical research to define the safety and efficacy of using cannabis to treat diseases. Now as regulation is changing , the manufacturing of cannabis products from API through the final dosage forms requires GMP conditions and accordingly , reliable fully validated analytical method to determine the assay of the active components. Aminolab laboratories has developed a HPLC method that enables to determine 8 cannabinoids. One of the main operational difficulties is the availability of reference standards. THC is the chemical responsible for most of marijuana's psychological effects, therefore the availability of THC standard is very restricted. Hence it became operationally complicated to support a growing demand of analyses and support our customers at a very challenging time frames. Hence, Aminolab decided to adopt the approach of using response factors and to calculate the Assay vs. one available non – narcotic standard- CBD. Aminolab has determined all the response factors of the cannabinoids using available reference analytical standard and compared to that of CBD. The factors relative to CBD were between the range of 0.76-1.50. However even after using these values the validation failed the strict requirements of intermediate precision (A comparison of results obtained by two different analysts on different days using different instruments) . By deep spectral and chemical evaluation it was found why the method is insufficiently robust after taking into account response factors. Corrective action has been established and the method was modified. The evaluation and the lesson learned will be covered in details along the lecture.

Biography

Dr. Rapoport has completed her Ph.D. in Biochemistry in the Hebrew University of Jerusalem in 1994. Then she joined Teva Pharmaceutical Industries analytical R&D in Israel . Since then, she has gained a large professional experience in the pharma industries. Over 23 years in Teva she fulfilled verity of rolls. As the Head of Teva API QC laboratories she supervised more than 20 QC Teva Laboratories all over the globe demanding the highest GMP standards . In 2013 she was nominated as a global site quality head of Teva API America's Operation and in 2015 nominated as a site quality head of the largest pharma site of Teva in Kfar Saba Israel. She joined Aminolab laboratories in 2017 leading a strategy of being pioneers in Cannabis tests and methods development, by implementing the pharma and Aminolab combined excellence and innovation. As the head of the chemistry division in Aminolab she fully supports , by variety of tests, the medical Cannabis industry in Israel with the growing regulatory requirements.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

DETERMINATION OF CLIDINIUM BROMIDE RELATED COMPOUND A BY MIXED MODE HPLC USING CORONA CAD DETECTOR – A CASE STUDY

Orie Rubin, *Analytical development, Global R&D, Teva Pharmaceutical Industries, Ltd., Kfar Saba, Israel (orie.rubin@teva.co.il)*
Simona Katz, *Analytical Development, R&d, Dexcel, Jerusalem, Israel*

Most of the active pharmaceutical ingredients (API), as well as their related compounds (process impurities and degradation products), contain chromophores which allow the use of UV detector in chromatographic methods. However, there are enough cases where alternative detection techniques (non-UV detectors) are required.

Similarly, we are lucky since most of LC separations are performed in well explored and developed reversed phase (RP) mode. However, there are compounds which are too polar to elute at a reasonable retention time in RP HPLC and are also incompatible with normal phase (NP) HPLC. Such compounds require alternative separation techniques, like HILIC or "Mixed Mode", which emerged during the last decade.

We present a case study, which describes method development for determination of Clidinium Bromide Related Compound A (as per USP TLC method). This development required both alternative separation method and detection technique since "Impurity A" is very polar and lacks chromophore. We provide not only the mindset while developing the method, but also challenges and obstacles which we had to overcome. One of the most challenging was the effect of excipients (which are, actually, the main part of the drug product) on system performance.

Key points of the development of a novel method using a mixed-mode HPLC combined with Corona CAD detector will be discussed, illustrated by examples and concluded with the lessons learned.

Abstract Reference & Short Personal Biography of Presenting Author

Orie Rubin is Analytical Development Team Leader at Global R&D, Teva Pharmaceuticals, Ltd. He is responsible for methods development for small molecules API and drug products in generic development and NTE (New Therapeutic Entities) at Kfar-Saba R&D site. Orie joined Teva in 2006 and worked till 2011 as a project coordinator in Generic Analytical R&D. From 2011 to 2015, Orie provided professional counselling in analytical methods, including troubleshooting, while working at Silicol Scientific Equipment, Ltd. In 2015 he returned to Teva and, since then, is involved in development of different and complex projects utilizing various analytical methods and technologies.

Orie holds a B.Sc. degree in Biochemistry from the University of Calgary, Canada and an MBA degree from the Ben Gurion University, Israel.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

METHYLERGONIVINE MALEATE: METHOD "GILDING" FOR CRITICAL IMPURITIES – ASSESSMENT OF METHOD PERFORMANCE CHARACTERISTICS

Miri Salem, *Analytical Development, Global R&D, Teva Pharmaceutical Industries, Ltd., Kfar Saba, Israel (miri.salem@teva.co.il)*

There is always a challenge when adding a new molecule to a known analytical method. Compatibility between the two is seldom immediate. Adding a molecule of unknown nature to an established method may create a real mystery…

Two critical impurities of API, which were assessed for an analytical method, exhibited inconsistent results in HPLC chromatograms, presumably caused by unexpected behavior in solutions. Partial and full degradation of the investigated molecules occurred randomly, with no evident root cause. A "third dimension" added to this "enigma", was a time factor, which contributed to the entire challenge.

Changes in peak height / area, as well as emerging additional peaks (secondary degradation?) were observed. Common and obvious reasons were evaluated and rejected, while we remained under time restraints.

Finally, our "hectic" systematic efforts during a period of one week were rewarded with revealing the source of degradation. Decarboxylation originated from reaction with the film coating used in specific vials for HPLC instrument. The suggested mechanism of this reaction is discussed and illustrated.

Abstract Reference & Short Personal Biography of Presenting Author

Miri Salem is Analytical Development Project Coordinator at Global R&D, Teva Pharmaceuticals, Ltd. She is responsible for methods development for small molecules API and drug products in generic development at Kfar-Saba R&D site. Miri joined Teva in 2011 and she is involved in development of different and complex projects applying various analytical techniques.

Miri holds a B.Sc. degree in Chemistry and M.Sc. degree in Inorganic Chemistry, both from the Hebrew University in Jerusalem, Israel.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

EXPLORING THE URINARY METABOLOME OF POLYCYSTIC KIDNEY DISEASE

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Dorien J. M. Peters, *Human Genetics, Leiden University Medical Center, Leiden, The Netherlands*
Oleg A. Mayboroda, *Center For Proteomics And Metabolomics, Leiden University Medical Center, Leiden, The Netherlands*

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder affecting 1 in 2500 individuals worldwide. In the majority of patients ADPKD is caused by a mutation in the *PKD1*-gene resulting in the formation of many fluid-filled cysts, leading to kidney enlargement, fibrosis, and progressive loss of renal function. PKD accounts to be one of the major leading causes of renal failure, in which patients around 55 years old require renal transplantation [1]. PKD has no cure, but a Vasopressin V2 receptor antagonist has been licensed as a treatment that slows down its progression. Unfortunately, this drug has several side effects, such as an abnormal excretion of urine (polyuria). Therefore, the search for alternative therapies is still ongoing and metabolic regulation in PKD is considered as one of the promising topics. Moreover, unraveling what metabolites are affected in PKD will also help understanding the mechanisms of this disease.

In previous reports we performed the analysis of kidney tissue sections of a PKD mouse model by liquid chromatography- and capillary electrophoresis-mass spectrometry [2,3], which enabled us to pinpoint a subset of metabolites affected in this disease. However, in practice, an assessment of the disease status in experimental models and patients requires a noninvasive approach based on the metabolic profiling of body fluids, mainly urine. Nuclear magnetic resonance (NMR) was chosen as the main analytical platform thanks to the quantitative nature of the technology and the reproducible data generated. Here, we applied targeted, quantitative metabolic profiling using the semi-automatic KIMBLE workflow [4]. A tamoxifen-inducible kidney-specific *Pkd1*-deletion mouse model was used. Urine samples were collected at different time points (3, 6, 9 and 11 weeks after tamoxifen administration) and concentrations of about 30 urinary metabolites were compared between PKD and wild type (control) mice. Some of these metabolites displayed a longitudinal pattern. The quantitative data generated by NMR offers a window in the insight of the underlying mechanisms of PKD.

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Abstract Reference & Short Personal Biography of Presenting Author

Elena Sánchez López (1990) obtained her PhD at the University of Alcalá (Madrid, Spain) in 2017 supervised by Dr. María Luisa Marina and Dr. Antonio L. Crego. Her thesis dealt with chiral separations and metabolomic analysis, both by means of (micro)-separative techniques coupled to mass spectrometry. During her PhD she carried out two internships in the Center of Proteomics and Metabolomics of the Leiden University Medical Center (Leiden, the Netherlands), under supervision of Dr. Oleg A. Mayboroda. There she developed analytical strategies based on capillary electrophoresis and liquid chromatography coupled to mass spectrometry to obtain the metabolic profile of 20 µm-thick sections of mouse kidney.

Her first postdoc was conducted at the University of Alcalá where she was involved in the metabolomics study of an *in vitro* model of high glucose under Dr. María Castro and Dr. María Luisa Marina's supervision. Since November 2018 she is a Marie Sklodowska-Curie postdoctoral fellow (LEaDing fellows programme) working in the Human Genetics department of the Leiden University Medical Center, supervised by Dr. Dorien Peters. Her current project involves the metabolomics study of polycystic kidney disease, a genetic disorder characterized by the development of large fluid cysts in kidneys. She has published 15 research articles and has contributed with 5 book chapters. She obtained the best young scientist oral communication award at the PBA2016 conference in Guangzhou (China).

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

INVESTIGATION OF AN UNEXPECTED DEHYDRATION BEHAVIOR AND ITS IMPACT ON MATERIAL PROPERTIES

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The selected form of a drug is a monohydrate and was found to display unusual dehydration behavior. A detailed elucidation using thermal analysis and other solid-state characterization techniques are presented to discuss the discovery, elucidation, and understanding of this phenomenon in the bulk properties, and its impact on API processing and drug product performance. The knowledge gathered from this work has implications beyond this project and may be relevant to other hydrated pharmaceutical solids.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Roxana F. Schlam received her B.Sc. in Chemistry from Hebrew University in Jerusalem, Israel and graduated with a Ph.D. in Chemistry from Brandeis University in Waltham, Massachusetts in the United States where she focused on structure/reactivity relationships in the solid state under Professor Bruce Foxman. She also did a postdoc at Purdue University in Indiana with Professors Stephen Byrn and Kenneth Morris where she applied her crystallography knowledge and learned about solid-state properties of active pharmaceutical ingredients and the pharmaceutical industry. Dr. Schlam started her career in the industry in the Solid-State Science group at Searle which became Pharmacia and then Pfizer.After five years, Dr. Schlam moved to New Jersey to work in the Materials Science and Engineering group at Bristol-Myers Squibb where she has been for almost 16 years. Dr. Schlam's research interests include the study of thermodynamics in solid-state systems and understanding bulk properties at the molecular level. Dr. Schlam holds numerous patents, scientific publications and presentations in the solid-state field of pharmaceuticals and also has extensive experience on intellectual property of crystalline forms. Dr. Schlam is also an Adjunct Full Professor at Long Island University College of Pharmacy and Health Sciences where she lectures at the graduate level an Introduction to Solids and also Polymorphism and Hydrates: Systems, Stability and Analysis.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

THE PERFORMANCE OF BORON-DOPED DIAMOND ELECTRODE IN ANIONIC SURFACTANT MEDIA FOR ENHANCING THE ADSORPTIVE STRIPPING VOLTAMMETRIC DETERMINATION OF FLUOROQUINOLONES

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Fluoroquinolones (FQs) are a class of synthetic broad-spectrum antimicrobial drugs derived from nalidixic acid, which are known for their broad spectrum of antibacterial activity against both Gram-negative and Gram-positive pathogens. Because of their widely consumption, excretion and persistence, FQs are disseminated via excrements, and can be released into the several environmental compartments. Therefore, their potential activity become may be potential risk for those target and non-target organisms. Additionally, the long-term application of FQs may cause an increase of resistant bacteria species within the treated body, which is an important threat against public health in the food chain. Thus; the determination of FQs is current important for pharmaceutical, clinical and environmental studies. The electroanalytical applications of FQs involve the use of mercury-based electrodes (due to their electroreductive behavior) and modified carbonaceous electrodes (due to their electrooxidative behavior). As far as we know, there are very few studies in the literature for their electrochemical determination at conventional bare electrodes. This may be because of the high oxidation potential of these compounds that gives poor reproducibility due to the overlapping with the discharge of the background electrolytic solution. Recently, the boron-doped diamond (BDD) as a new and excellent carbon electrode material can be used to overcome this difficulty. In keeping with the above knowledge in mind, in the present study, a mercury-free and modification-free electroanalytical methodology based on BDD electrode (except for a simple anodical pretreatment between individual measurements) is described for the quantification of two important fluoroquinolones, namely enrofloxacin and norfloxacin, which have been widely used in veterinary and human medicine, respectively. The oxidation of both compounds was irreversible and presented an adsorption controlled process. The sensitivity of the stripping voltammetric measurements was significantly improved when the anionic surfactant, sodium dodecylsulfate (SDS) was present in the electrolyte solution. Using square-wave stripping mode, a linear response was obtained for enrofloxacin determination in 0.1 M HNO3 solution containing 9×10-4 M SDS at +1.27 V (vs. Ag/AgCl) (after 60 s accumulation at open-circuit condition), and for norfloxacin in acetate buffer (pH 4.7) solution containing 7×10-4 M SDS at +1.32 V (vs. Ag/AgCl) (after 30 s at +0.1 V). Linearities were found within 7.0×10-8-2.8×10-6 M and 1.6×10-7-1.3×10-5 M, with detection limits 1.6×10-8 M and 4.1×10-8 M for enoxacin and norfloxacin, respectively. The proposed method could be used for the determination of these compounds in pharmaceutical formulations and the spiked urine samples with acceptable recoveries.

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Prof. Dr. Zühre ŞENTÜRK

She received her PhD degree in 1985 from Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University (Ankara, Turkey). She worked as visiting scientist in the laboratories of Prof. G.J. Patriarche and Prof. J.M. Kauffmann (ULB, Brussels; Belgium) in 1990 and 1996, respectively. She became full professor in 1997 in Faculty of Pharmacy, Gazi University (Ankara, Turkey). She attended Faculty of Science, Van Yuzuncu Yıl University (Van, Turkey) as a professor of Analytical Chemistry in 1999. Her research interest focuses on pharmaceutical, food, environmental and biomedical applications of electrochemical sensors (potentiometric ion-selective electrodes, modified electrodes, nanometerials).

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A TIME-CONTROLLED DELIVERY SYSTEM FOR NICOTINE USING A NICOTINE PECTIN SALT FORMULATION FOR COLON-TARGETED DELIVERY

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Local delivery of nicotine to the colon may be effective in therapy for patients suffering from ulcerative colitis. Nicotine was incorporated into a colon-specific formulation by preparing nicotine pectinate (NiP) salts from methoxylated pectin and nicotine in a water-isopropanol mixture. The water-isopropyl alcohol ratio was found to be central to the ability to form the NiP.The tablets were coated by a combination of Eudragit E (Eud.E) and calcium pectinate (CaP) particulates to obtain a delayed release corresponding to the planned time of delivery in the colon. The rate and time of onset of release of nicotine from the tablets could be controlled to provide precise delivery in the colon by optimizing the core formulation and adjusting the ratio between Eud. E and CaP, and the thickness of the film coating. The dissolution profiles of nicotine release from the coated tablets were compared to those of a commercial nicotine resinate product, Polacrilex.

The mode of binding was determined at the molecular level by nuclear magnetic resonance (NMR) and infrared spectroscopy (ATR-FTIR) to verify the interactions between the nicotine and pectin. Differential scanning calorimetry (DSC) was used to determine the thermal properties of the nicotine pectinate complexes for pectin with differing low degrees of methoxylation.

Abstract Reference & Short Personal Biography of Presenting Author

Deborah Shalev is a Professor of Chemistry and Head of the Department of Pharmaceutical Engineering at Azrieli College of Engineering Jerusalem. She did all her degrees at Tel Aviv University and then served as the liaison between academia and industry in a consortium for drug design of the Chief Scientist of Israel. She continued working with NMR as a research scientist at the Wolfson Centre for Applied Structural Biology at the Hebrew University of Jerusalem where she still holds a partial position. In 2011, Deborah joined the faculty at the Azrieli College where her interests are in spectroscopic and thermal analyses of polymeric systems for extended release.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

NANOPARTICLE FORMULATION MODALITIES: APPLYING LIGHT SCATTERING TO SCREENING AND CHARACTERIZATION

Daniel Some, *Wyatt Technology Corp., Santa Barbara, CA, USA (dsome@wyatt.com)*

From traditional small molecule drugs to vaccines, gene vectors and personalized medicine, nanoparticle modalities are increasingly of interest for enhanced and safe delivery. Characterization and quality control among the most important missions of developing any therapeutic, and particularly challenging for complex nanoparticles.

While light scattering is well known in nanoparticle research, traditional technologies such as dynamic and electrophoretic light scattering typically do not provide the level of detail needed for full characterization. Likewise they do not offer the degree of automation and throughput required to quickly bring a new nanoparticle formulation to pre-clinical and clinical trials, and then rapidly scale up the process to full production and quality control. This presentation explores three light-scattering-based technologies that overcome these hurdles: high-throughput dynamic light scattering (HT-DLS), fully-automated electrophoretic light scattering (A-ELS), and online light scattering coupled to asymmetric-flow field-flow fractionation (AF4-MALS).

A-ELS, and HT-DLS in particular, provide the usual information on colloidal size, aggregation and zeta potential familiar from traditional technologies, but enable implementation of matrix testing for formulation and process development that would not be feasible with a non-automated instrument. Several examples will be discussed, ranging from initial development phases to process optimization.

AF4-MALS is an extremely powerful tool for macromolecular and nanoparticle characterization. It combines size-based separation with detailed structural and compositional data for each size fraction. Size, shape, structure, concentration and zeta potential are determined by onlne light scattering. Composition, including encapsulation, is determined from online spectroscopy combined with light scattering as well as mass spectrometry. Fractions may be collected for further study. Examples presented include VLP-based gene vectors, liposome- and polymersome-based drug carriers.

Abstract Reference & Short Personal Biography of Presenting Author

Daniel Some is Principal Scientist at Wyatt Technology Corporation, where he explores new applications of light scattering for characterization of macromolecules and nanoparticles, in particular for the life sciences and pharmaceutical industries. He developed Wyatt's Calypso composition-gradient multi-angle light scattering (CG-MALS) system for label-free, in-solution characterization of protein-protein, protein-DNA and other macromolecular interactions. Dr. Some is a graduate of the Technion - Israel Institute of Technology, and obtained his PhD at Brown University, both in the Physics Department. He has carried out postdoctoral research at Los Alamos National Laboratory and the Weizmann Institute of Science.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

NOVEL ANALYTICAL STRATEGIES IN TRANSPLANTOLOGY – A CHANCE TO INCREASE THE POOL OF ORGANS?

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Natalia Warmuzińska, *Department of Pharmacodynamics and Molecular Pharmacology, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland*
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For most of patients with end-stage organ failure transplantation is the only life-saving procedure. Since the discovery of anti-rejection drugs the number of transplantations has increased exponentially. However, there are still tremendous problems that modern transplantology has to face: (1) severe shortage of donor grafts, and (2) lack of reliable methods of organ quality assessment prior to transplantation, just to name a few. With regards to the analytical tools, which would enable better assessment of organ quality, there are recent attempts to find new biomarkers of graft dysfunction. Early detection of organ dysfunction would increase a chance to make informed decision regarding organ suitability then prevent irreversible damage and rejection. Up to date, there are just a few reports of introducing metabolic profiling to achieve the aforementioned goal. The studies of organ quality are innumerous, primarily due to the invasiveness related to the collection of tissue sample for the analysis and lack of appropriate analytical method allowing bedside analysis.

In the present project we undertake the challenge to address the need for new analytical solution for graft quality assessment and propose to perform a series of metabolomics and lipidomics analyses using novel diagnostic protocol based on low-invasive method combining sampling, sample preparation and clean-up with metabolite extraction and liquid chromatography - high resolution mass spectrometry platform (LC-HRMS). The microextraction probe of ca. 0.2 mm was coated with 7 mm mixed-mode extraction phase. Separation of the extracts was done using reversed phase and HILIC columns and Q-Exactive Focus mass spectrometer was operated in both, positive and negative ionization mode. The sampling has been performed several times: in vivo before harvesting the kidney, in situ during organ preservation and in vivo after its transplantation. In PCA clear separation was found between clusters contacting in vivo data for living and heart-beating donor kidneys prior and after subjecting to ischemia. Also, separation of ischemia-induced and ischemia-free organs during their perfusion was observed. The statistical analysis enabled to observe changes in organ metabolic profile induced by ischemia and oxidative stress as well as influence of the preservation process on kidney obtained from heart-beating and living donors. The most pronounced changes during time of ischemia were reflected in the level of nucleosides. Among the metabolites discriminating organs originating from living and heart beating donors and exhibited preventive effect on the ischemia-reperfusion kidney was carnitine. Also, the significant differences in cysteine level between the two groups after organs reperfusion were observed. The applied preservation protocol resulted in progressive changes of pantothenic acid and methionine levels among others.
The study demonstrated that the proposed approach may be successfully used for monitoring alterations of grafts prior transplantation as well as for evaluation of organ status subjected to given preservation protocol. Further investigations will involve larger cohort to verify if the metabolites showing significance in the current study can be considered as biomarkers of kidney quality (progressing ischemia or successful recovery) and optimization of rapid method for targeted analysis on-site.

Acknowledgment: the study was supported by grant Opus UMO-2017/27/B/NZ5/01013 from National Science Centre. The authors want to thank Thermo Fisher Scientific for the access to Q-Exactive Focus orbitrap mass spectrometer.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

DRUG DISCOVERY AND DEVELOPMENT: A STRATEGY OF IN-VIVO MULTIPLE MICRODIALYSIS FOR PHARMACOKINETIC AND NEUROCHEMICAL ANALYSIS

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Based on the drug discovery and development, this topic develops a strategy of in-vivo multiple microdialysis for pharmacokinetic and neurochemical analysis. Microdialysis coupled to liquid chromatography have been used as a biological sampling technique and continuous measurement of protein-unbound analytes from experimental animal. In-vivo multiple microdialysis is applied in the preclinical studies of absorption, distribution, metabolism and excretion for the studies of pharmacokinetics, biliary excretion, enterohepatic circulation, and neurochemical analysis. Based on the passive diffusion, microdialysis probe collect biological samples governed by the concentration gradient which allows the small molecules pass through a semi-permeable membrane. According to the clinical pharmacology, only protein-unbound form of the drug molecule can be delivered to the target sites for therapeutic actions. Our hypothesis is that the multiple microdialysis probes can be implanted to multiple targets of the experimental animal and applied to preclinical pharmacokinetic and pharmacodynamics studies. The aim of study is to develop an experimental animal model coupled to microdialysis and liquid chromatographic system for the collection microdialysates from multiple target tissues such as blood, brain, muscle, liver, kidney …etc. Several research issues, such as the regional brain distribution, the portion of drug that passes through the blood-brain barrier, liver and kidney distribution can be defined by the area under the curve (AUC) ratio of brain-to-blood (AUC brain/AUC blood), liver-to-blood (AUC liver/AUC blood) and kidney-to-blood (AUC kidney/AUC blood), respectively. Furthermore, the pros and cons of microdialysis and analytical system will be discussed in the presentation, including the detailed surgical techniques in animal experiments from rat blood, liver and kidney for the analysis of protein-unbound drug. In conclusion, in-vivo multiple microdialysis system provides feasible study strategy on the experimental animal for preclinical pharmacokinetic and pharmacodynamics studies.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Tung-Hu Tsai received Ph.D. degree from Institute of Pharmacology, National Yang-Ming University, Taiwan in 1995. He then obtained a scholarship to Department of Experimental Psychology, Neuroscience Research Center, Cambridge University, England, UK, as post-doctoral research fellow. In 1997 back to Taiwan, he served as an associate professor (1996-2002) and professor (from 2002-) at National Yang-Ming University, Taipei Taiwan. Dr. Tsai is actively pursuing in the research for pharmaceutical medicine focus on the mechanisms of pharmacokinetic pathways, with a particular interest in bioavailability, intestinal absorption, lymphatic absorption, hepatobiliary excretion and barrier transportation, as this can be the link which connects pharmacokinetics and pharmacodynamics for drug discovery on natural products. He and his research team have published over 350 papers in peer-reviewed journals. Dr. Tsai has received an Award for excellence in teaching and research award at National Yang-Ming University. Dr. Tsai also serves on the editorial board for Journal of Pharmaceutical and Biomedical Analysis (2009-), Biomedical Chromatography (2015-), International Journal of Gerontology (2016-), Journal of Chromatography B (2011-). He was invited as an editor by Wiley publisher and published a book of Application of Microdialysis in Pharmaceutical Science, in 2011. He also serves as a Guest Editor for Molecules in 2016.

HYDROPHILIC INTERACTION CHROMATOGRAPHY ANALYSIS OF BIOLOGICAL COMPOUNDS

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HPLC is the most common approach to solve multiple analytical problems, because HPLC-based techniques allow for the separation of quite complicated mixtures of analytes with different physico-chemical properties. Reversed-phase liquid chromatography (RPLC) is the most popular LC separation mode. However, retention of hydrophilic compounds cannot be achieved on RPLC. This problem has been overcome by hydrophilic interaction chromatography (HILIC), which has rapidly gained popularity in recent years. HILIC can be used for a wide variety of hydrophilic compounds including biological compounds and drugs in biological fluids, pharmaceuticals, and foods. In my talk, I will describe analytical methods for biological compounds based on HPLC-fluorescence detection under HILIC conditions. Low-molecular-weight biothiols like cysteine, homocysteine, and glutathione and catechol compounds like norepinephrine and dopamine are studied as biological compounds. Application to human plasma or urine samples is also described.

Abstract Reference & Short Personal Biography of Presenting Author

Makoto Tsunoda obtained a PhD in Graduate School of Pharmaceutical Sciences, University of Tokyo in Tokyo, Japan. After graduation, he worked as a postdoctoral research fellow at Ames Laboratory, Iowa, USA. He is currently associate professor at University of Tokyo where his research interest is in on-chip liquid chromatographic separation technique and development of bioanalytical methods for biological compounds. He has over 80 peer-reviewed papers, and 3 book chapters.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

PARTICLE SIZING ANAYSIS - MYTHS AND MISCONCEPTIONS

Larry Unger, Management, *PSS, an Entegris Company, Wellington, United States (larry.unger@entegris.com)*

Particle sizing is a relatively common technique employed across a wide range of industrial and pharmaceutical applications. There exist a large number of different techniques for determining particle size, requiring the selection of a technique for any particular sample analysis. Most educational systems spend very little time educating students of the various techniques and their strengths and shortcomings. As a result, techniques chosen for any specific measurement may not be the best technique for characterizing a particular product. In fact, may measurements made today are based on methods that are not well suited for the particular application.

This presentation will provide an overview of a number of different measurement techniques, compare and contrast them, and point out their strengths and weaknesses. The emphasis will be on clarifying options available to analysists in order to achieve the best possible particle size measurements for a particular application.

With the large number of techniques available, there have been many misconceptions fostered over the years regarding the meaning of the data produced by these different methods. A review of reference materials for determining the accuracy of these methods and brief commentary on these reference materials, often referred to as standards will be provided as well, along with some comments differentiating between a standard and a reference material.

Abstract Reference & Short Personal Biography of Presenting Author

Larry is currenty EMEA Sales Channel Manager of Particle Sizing Systems, an Entegris Co. After earning a B.Sc. in Chemistry at Philadelphia College of Pharmacy and Science, Larry earned a M.A. in Chemistry from Brandeis University, A Ph.D. in Organic Chemistry, and served as a Post-Doctoral Student at University of Pennsylvania. He has been an adjunct professor at both Temple University and Florida Atlantic University Honors College. Having worked in the particle sizing industry for more than 20 years working with techniques such as Laser Diffraction, Dynamic Light Scattering, Zeta potential, SEM, Image Analysis, and Single Particle Optical Sizing, he will bring insight into the benefits and shortcomings of the various particle analysis systems, discussing the misconceptions that many users have about the accuracy and reliability of the results of these methods.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

ANALYSIS OF BOTULINUM NEUROTOXIN A (BONT/A) IN PHARMACEUTICAL PRODUCTS BASED ON PROTEOLYTIC ACTIVITY AND SIGNATURE MARKERS

Yiu-chung Wong, *Hong Kong Government Laboratory, Hong Kong, China (ycwong@govtlab.gov.hk)*

Botulinum neurotoxins (BoNT) are produced by the anaerobic bacterium *Clostridium botulinum* and are one of the most lethal known poisons (LD_{50} = 0.8 mg for a 70 kg human by inhalation). Despite its high lethal toxicity, BoNT have been used to treat spasms and other muscle problems. Most recently, BoNT serotype A (BoNT/A) gains its worldwide popularity in cosmetic surgery to prevent development of facial wrinkles. Clinical testing of BoNT/A is conventionally relied on *in vivo* mouse lethality assay in accordance with pharmacopieal and AOAC methods.

Identification of BoNT/A in pharmaceutical products by chemical means is always a challenge due to its complex structure, ultra-trace level and the interference from excipients. Howvere, to address intense public pressure to the inhumane testing, sensitive non-anaimal assays are necessary. This work reports a mass spectrometry study on BoNT/A in pharmaceutical injection samples. BoNT/A was isolated using magnetic beads immunoprecipitation, followed by on-beads digestion and was characterized by peptide mass finger printing using high-resolution tandem mass spectrometry coupled with ion mobility and Q-ToF in MS^E mode. The activity of the toxin was confirmed by its proteolytic activity towards specially designed synthetic SNAP-25 substrate. The specific cleaved peptide fragments obtained from SNAP-25 substrate correlated well with its proteolytic activity in linear range from 10 – 100 MLD_{50}/uL ($r^2 > 0.99$). Recent work on direct measurement of BoNT/A in pharmaceutical injection samples by mass spectrometry will also be reported. The study is based on ten potential signature markers, seven from the light chain and three from the heavy chain. Pharmaceutical samples that claimed to contain BoNT/A was trypsin digested under optimal conditions and its identity was examined by the presence of the concerned signiture markers. Detection limits were at 0.05 ppm level which is sensitive enough to detect registered BoNT pharmaceuticals in the market. The methods has been used to examine a few brands of BoNT/A pharmaceutical injections commonly available in the Asia Pacific region.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Wong is a senior chemist of the Government Laboratory of Hong Kong. He is interested in the measurement of trace organic and inorganic toxicants in environment, food, dietary supplement and pharmaceutical matrices. He also organized a series of of international proficiency testing programmes and developed several certified reference materials in the food and herbal medicine fields. Recently, Dr. Wong is the co-editor of the two-volume book “Toxins and Toxicants in Food” published by Wiley and the contributing chapter author of the 3rd Edition Encyclopedia of Analytical Science by Elsevier.

ACCELERATING DRUG DEVELOPMENT, QC & MANUFACTURING WITH NEW TECHNOLOGIES IN MOLECULAR SPECTROSCOPY

Dr Jan Wülfken, *Agilent Technologies, Germany*

This talk will describe newest breakthrough technologies and applications of Raman, Laser based Infrared and UV-Vis Spectroscopy in Pharma. This will include use of Raman for raw materials verification of incoming goods through unopened non-transparent container materials to streamline the drug manufacturing process and the use of transmission Raman as an alternative to UPHPLC for content uniformity and polymorph studies. Furthermore, use of a next generation IR instrument using a quantum cascade laser will be described for chemical imaging studies including analysis of tablet component distribution, polymorph distribution, salt exchange and stability studies. New breakthrough technologies for ultra-fast and productive parallel temperature based kinetic studies, protein folding, DNA melting and other temperature related UV-Vis measurements will end this overview.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Jan Wuelfken was born in Hamburg Germany and has made his PhD in biochemistry about NMR spectroscopic studies of CD4 binding peptides to inhibit the HIV infection. He is now for several years for Agilent responsible as a molecular spectroscopy product specialist for the support of the Raman, FT-IR, Fluorescence and UV-Vis/NIR business in all counties in EMEAI, where Agilent is working and supporting via distributors.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

ASSESSING THE ACTUAL UTILITY OF CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY FOR METABOLIC PROFILING OF HUMAN PLASMA

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Introduction:

Capillary electrophoresis-mass spectrometry (CE-MS) is a useful analytical technique for the profiling of highly polar and charged metabolites in biological samples. Although CE-MS has been often used for metabolic profiling of a wide range of samples in various application fields, the actual utility of this approach for biomarker discovery studies has not been properly demonstrated so far. As a significant fraction of the signals recorded in MS-based metabolic profiling studies are spurious, it is important to assess whether compounds that show up as potential biomarkers in comparative metabolic profiling studies are authentic. We have addressed this aspect in this study.

Methods:

In this study pooled human plasma samples were used, of which one set was spiked with cationic isotope-labeled metabolites at different concentration levels and denoted as “class I”, whereas another set was also spiked with cationic isotope-labeled metabolites at different concentration levels and denoted as “class II”. Blank pooled human plasma samples were used as a quality control (QC) sample to assess the performance of CE-MS over time. Cationic metabolites were analyzed at low-pH separation conditions using a fused-silica capillary. CE was coupled to MS via a conventional sheath-liquid interface. Recorded data were processed with different multivariate data analysis tools.

Results & Discussion:

A conventional CE-MS approach was used for the profiling of cationic metabolites in pretreated human plasma samples at low-pH separation conditions. The reliability of the analytical method was tested in terms of linearity, precision, accuracy and repeatability, and the method was proved to be suitable for metabolic profiling of human plasma. The examination of the metabolic features detected in the QC set by CE-MS showed that most of them had relative standard deviation (RSD) values for peak areas below 30% for an analysis carried out over two consecutive days. The obtained data was further evaluated using two different multivariate data analysis strategies. In both strategies, multivariate data analysis of the recorded metabolic profiles provided a clear distinction between class I and class II plasma samples. The metabolites primarily responsible for this classification were the isotope-labeled compounds spiked (“spiked biomarkers”) to the human plasma samples at different concentration levels. Therefore, these findings clearly indicate that our CE-MS approach can pick up the right chemical information in metabolic profiling studies used for biomarker discovery. Overall, the strategy proposed over here can be used to validate a given analytical method for comparative metabolomics studies.

Abstract Reference & Short Personal Biography of Presenting Author

Personal Biography

I obtained my MSc degree in pharmacokinetics from China Pharmaceutical Univerity in Nanjing in 2015. The main task in my master study was to develop LC-MS/MS methods for exogenous substances in various biological matrices, such as plasma, urine, and bile. My work also included in vitro drug-drug interaction study using the incubation schema of human/rat liver microsomes with classic substrates and compounds of interest.

After obtaining my master degree, I came to the Netherlands and started working in the field of metabolomics within the Leiden Academic Centre for Drug Research. Over the last three years, I dedicated my efforts in the analysis of small polar charged metabolites in biological samples using capillary electrophoresis coupled to mass spectrometry (CE-MS). The application of CE-MS in metabolomics study can provide valuable complementary information, especially when dealing with very small sample amounts. With the advancement of CE-MS interfaces, higher sensitivity could be further achieved despite very small injection volumes in practice. This technique can also serve as a great tool for the detection of polar metabolites secreted from the body after the administration of exogenous compounds and help understand how the compound is metabolized inside a living organism.

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PRECONCENTRATION AND SEPARATION SOLUTIONS FOR SIMULTANEOUS DETERMINATION OF COMPLEX PANELS OF ANALYTES IN PHARMACEUTICAL AND CLINICAL APPLICATIONS

Tomasz Bączek, *Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Hallera 107, 80-416 Gdańsk, Poland, tomasz.baczek@gumed.edu.pl*

Very often the efficient use of liquid chromatography coupled to mass spectrometry (LC-MS) capillary zone electrophoresis (CZE), like micellar electrokinetic chromatography (MEKC) or microemulsion electrokinetic chromatography (MEEKC)-based separation methods could not be sufficient without carrying out proper off-line sample preparation procedure and the choice of the most efficient on-line preconcentration technique for investigated compounds. To improve concentration detection limits, off-line sample preparation techniques, like liquid-liquid extraction (LLE), liquid-liquid microextraction (LLME), dispersive liquid-liquid microextraction (DLLME), solid-phase extraction (SPE) or solid-phase microextraction (SPME) are some of commonly applied techniques. Among on-line preconcentration methods there are relevant approaches allowing the quantification of trace amounts of compounds in pharmaceutical and biomedical samples. In here, the field-amplified sample stacking (FASS) [known also as field-enhanced sample stacking (FESS)], field-amplified sample injection (FASI) [known also as field-enhanced sample injection (FESI)] are the best recognized ones. Sweeping, micelle to solvent stacking (MSS), p-ITP (pseudo-isotachophoresis) or FESI in conjunction with sweeping (sequential stacking featuring sweeping, SSFS) are another analyte enrichment techniques. In parallel, one should remember also about the possibilities to optimize CE methods playing with hydrodynamic injection (HDI), electrokinetic injection (EKI), simultaneous EKI and HDI (SEHI) or repetitive injection (RI). Several examples of novel approaches based on variable combinations of mentioned techniques and methods to be applied for the analysis of mixtures of selected drug and endogenous substances, like e.g., LC-MS, SPME-LC-MS/MS, MEKC, SPE-MEKC, RI-FASS-CZE, DLLME-FASS-MEKC, DLLME-FASI-p-ITP, LLE-MSS-CZE, SPME-SEHI-SSFS-MEKC or ionic liquid-assisted SPME-MEKC and 3D-printed-assisted SPME-MEKC, are going to be thoroughly presented and discussed.

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CONSIDERATION OF BIOACTIVITY BY CHROMATOGRAPHIC APPROACH

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Bioactive compounds represent a diverse group of potential drug candidates due to structural diversity, lower cytotoxicity as compared to synthetic analogues, often encountered ability to overcome drug resistance towards drugs. Despite growing interest of researchers to bioactive compounds and large number of publications in the area, bioactivity data should be constantly updated, as providing appropriate bioactivity data is relevant for drug design. Therefore, it is important to develop analytical methods to answer a question: 'what is bioactivity?' [1,2].
Theoretical approach for determination of bioactivity includes structure-activity (SAR), quantitative structure-activity (QSAR), quantitative structure-retention (QSRR) relationships. Mentioned methods are based on principle that compounds with similar structure have similar activity and allow to investigate how the structure of the compounds is related to its activity. QSAR method allows to assess such relationships quantitatively as compared to SAR, while QSRR is related to chromatographic retention. Although QSRR is often applied for unravelling mechanisms of retention of analytes and prediction of retention times, this method have connection with bioactivity as well. Pioneering research work of Prof. Kaliszan [1] showed possibilities of QSRR applications except traditional ones regarding retention. Firstly, important drug parameters such as lipophilicity determined from analytes chromatographic retention behaviour can be used as descriptors for QSAR study. Secondly, important drug characteristics such as blood proteins binding and cell membrane permeability can be simulated by immobilization of human and bovine serum albumins and diacylated phosphatidylcholine on silica and silica-propyl amine (immobilized artificial membrane column) and application as stationary phases for chromatographic column. Thus, retention behaviour of analytes on such columns can extend dataset for determination of biological activity allowing to determine membrane permeability, binding to serum proteins, drug volume distribution [3,4].

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Acknowledgements

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CHIRAL STATIONARY PHASES BASED ON SUPERFICIALLY POROUS SILICA FOR ENANTIOSEPARATION OF CHIRAL DRUGS

Bezhan Chankvetadze, *Institute of Physical and Analytical Chemistry, Tbilisi State University, I. Chavchavadze Ave 1, 0179 Tbilisi, Georgia*

The advantages of superficially porous silica (SPS) over fully porous silica (FPS) for achiral and chiral separations in liquid phase have been extensively documented. These advantages include significantly higher chromatographic efficiency, a marked shift to higher optimal flow rates and limited dependence of column performance on a mobile phase linear velocity. The higher efficiency of columns made with SPS particles is probably due to the more uniform particle-size distribution of SPS particles compared to their fully porous analogues resulting in higher radial homogeneity of columns packed with SPS and to the shorter diffusion path available to analytes. These same characteristics are responsible for the limited dependence of column performance on the mobile phase linear velocity, minimizing both the eddy diffusion term (A term) and also the mass-transfer coefficient (C term) to the height equivalent to a theoretical plate (HETP) as detailed in the van Deemter equation. Realizing the minimum HETP value and maintaining it at even higher mobile phase flow rates makes SPS columns suited for high-speed separations.
Since polysaccharide esters and phenylcarbamates are recognized to be the most successful chiral selectors for the separation of enantiomers in liquid phase techniques such as high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), nano-chromatography and capillary electrochromatography (CEC), their combination with SPS seems logical for the preparation of highly efficient chiral stationary phases (CSP).
This presentation summarizes our recent studies on the preparation of polysaccharide-based chiral stationary phases (CSPs) for separation of enantiomers in HPLC [1-2], SFC, nano-LC and CEC [3]. Various effects based on superficially porous structure of silica are reported and discussed in detail.

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ANTICANCER EFFECT AND UNDERLYING METABOLIC REMODELLING OF 4'-METHOXY-1-NAPHTHYLFENOTEROL

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Pancreatic adenocarcinoma (PCA) and colorectal cancer (CRC) are considered two of the most aggressive cancers and are leading causes of cancer-related death worldwide. Despite advances in the field, resistance to chemotherapy results in poor patient survival and remains one of the most significant challenges in long-term management. We studied the novel anticancer effects of 4'-methoxy-1-naphthylfenoterol (MNF), a bi-functional agent that inhibits the pro-oncogenic G protein-coupled receptor GPR55 and activates of the β2-adrenergic receptor in xenograft mouse models of human PANC-1 PCA and CT26 CRC tumors.

Hyphenated mass spectrometry-based untargeted metabolomics approach was applied to study metabolic changes associated with the observed antitumor response of MNF. The target tumors were induced in female BALB/c mice by subcutaneous inoculation with CT26 CRC or PANC-1 PCA tumor cells. The tumor-bearing mice received daily ip injections of either vehicle, (R,R')-MNF (20 or 30 mg/kg) in CRC or (R,S')-MNF (40 mg/kg) in PCA models. The treatments significantly reduced tumor growth in each model and prolonged survival. The changes in plasma and tumor metabolomic profiles associated with the (R,R')-MNF action in CRC model were evaluated by liquid chromatography mass spectrometry (LC-MS) analysis, whereas changes related to the effect of (R,S')-MNF in PCA tumors were studied by mass spectrometry liquid chromatography (LC-MS), gas chromatography (GC-MS), and capillary electrophoresis (CE-MS). Raw data acquired was pre-processed, filtered, and corrected for signal drift. A combination of univariate and multivariate statistical analyses was used to evaluate the drug effect on CRC and PCA models. Metabolite annotation was accomplished by searching against several databases including HMDB, KEGG, Metlin, and LipidMaps metabolite databases. A metabolic-driven elucidation of the anticancer effects of (R,R')-MNF and (R,S')-MNF indicate metabolic remodelling related in particular to the class of lipids where glycerolipids and glycerophospholipids were significantly altered in both CRC and PCA models. Prominent changes with +237% increase in ophthalmic acid and +95% increase in its precursor, 2-aminobutyrate, indicated higher oxidative stress under (R,S')-MNF treatment in PCA model.

The knowledge gained in these studies advanced our understanding of the molecular mechanisms by which MNF inhibits cancer and provide a path towards to optimum use of these agents in CRC, PCA, and other aggressive cancers. The results demonstrate that untargeted metabolomics reveals information locked in metabolites that can lead to scientific breakthroughs and new clinical insights.

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SHARING PASSION FOR SEPARATION SCIENCE WITH ROMAN

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Development of rapid analytical workflows capable of providing high quality data and at the same time resulting in minimum environmental impact is garnering interest in the scientific community. Solid phase microextraction (SPME), a sample preparation tool that combines sampling and sample preparation into a single step, has demonstrated excellent performance for both qualitative and quantitative determination of a broad range of analytes in various matrices particularly when combined with GC/MS or LC/MS techniques. The additional advantage of the hyphenated techniques would be to allow convenient calibration and/or qualitative assignment. This can be facilitated by using retention times corresponding to the separation method rather than using matrix matched calibration or other approaches requiring isotopically labelled standards. Initial work demonstrated that the distribution coefficients (K) between air and SPME fiber coating can be estimated using the linear temperature-programmed retention index system (LTPRI) [1]. This alternative approach to establishing K values significantly enhances and simplifies the use of SPME for sampling and analyzing air. Also, it was determined that there is a linear relationship between log K for each hydrocarbon series and LTPRI when using aqueous matrix. The slope of the curves for all the series are the same and they are related to the partial free energy of solution for the hydrocarbon-fiber coating solution. The y-intercept of the plots is distinct for each group of hydrocarbons and related to Henry's law coefficients for each homologue series indicating that the K for a series of hydrocarbons can be estimated using literature Henry's law coefficients [2]. For complex matrices quantitative structure–retention relationship (QSRR) is a technique capable of improving the identification of analytes by predicting their retention time on a liquid chromatography column (LC) based on their properties. This approach is particularly useful when LC is coupled with a high-resolution mass spectrometry (HRMS) platform, which aids in identification of the compounds based on respective retention time and the exact mass [3]. In addition, the retention time also provides information about the distribution constants, which can be calculated using QSRR models. This approach facilitates a practical and effective method for analytical chemists working with SPME hyphenated to chromatographic separation to improve predictive confidence of identification and quantitation of small molecules in range of complex matrices.

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ANALYSIS OF TOCOPHEROLS AND TOCOTRIENOLS IN HUMAN BREAST ADIPOSE TISSUE

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Agnieszka Borsuk-De Moor, *Medical University of Gdańsk, Poland*
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Wiesław J. Kruszewski, *Medical University of Gdańsk, Poland*
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Vitamin E homologues, tocopherols and tocotrienols, have been under thorough scientific investigation for several years. In the scientific literature, there are reports about potential anticancer properties of some tocochromanols, including beneficial activity in breast cancer. As adipose tissue can represent a long-term storage of lipophilic compounds in human organism, it was used for comparative studies between women with breast cancer, healthy volunteers and women deceased by accidents. However, quantification of vitamin E in foods and plant material is extensively described in the literature, determination of these constituents in human or animal tissues still requires further research studies and improvement. Therefore, the aim of the study was development of a fast and sensitive analytical method for simultaneous determination of eight naturally occurring tocochromanols in human breast tissue. First, methods for homogenization and extraction of target analytes from breast adipose tissue, based on SPE technique were developed. Then, quantitative determination of four tocotrienols in human breast adipose tissue with the use of HPLC-MS/MS platform was evolved. Due to problematic repeatability of ionization efficiency of tocopherols in APCI fluorescence detector has been successfully applied.

Finally, the validated HPLC-FLD method was applied in quantitative determination of tocopherols and tocotrienols in breast adipose tissue samples obtained from Department of Oncological Surgery patients, women undergoing plastic surgery at the Department of Plastic Surgery and women deceased by accidents from Department of Forensic Medicine. The proposed analytical approach can facilitate verification of hypothesis concerning the potential protective role of vitamin E against breast cancer disease.

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BAYESIAN MULTILEVEL MODELS IN QUANTITATIVE STRUCTURE-RETENTION RELATIONSHIPS STUDIES

Pawel Wiczling, *Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdansk, Gdansk, Polska (wiczling@gumed.edu.pl)*

In chromatography, multilevel model is a regression model of retention time measurements, in which all chromatographic-specific parameters are themselves given a probability model. The multilevel model when combined with prior knowledge and Bayesian inference allows to quantify the uncertainty for all model parameters and predictions. In this work, the Bayesian multilevel model is applied to isocratic reversed phase high-pressure liquid chromatography data. The proposed model consists of *i*) the same deterministic equation describing the relationship between retention factor and organic modifier content, *ii*) covariance relationships relating structure of analyte and chromatographic-specific parameters through Quantitative Structure-Retention Relationships (QSRR), and *iii*) stochastic components of intra-analyte and inter-analyte variability. The model was implemented in the Stan software that provides full Bayesian inference for continuous-variable models through Markov Chain Monte Carlo methods. The fitting of retention data simultaneously for a large group of analytes allows to obtain a single model that generalizes well to other (not-tested) analytes. It also quantifies the uncertainty around predictions that is essential for decision making. e.g. during method development.

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Abstract Reference & Short Personal Biography of Presenting Author

Dr. Wiczling received his PhD in Pharmacy from Medical University of Gdańsk in 2005. Since 2003 he works at the Department of Biopharmaceutcs and Pharmacodynamics, Medical University of Gdańsk, Poland. His specialties are analytical and theoretical aspects of chromatographic techniques, development of chromatographic methods for simultaneous determination of dissociation constant and lipophilicity, modeling and simulation of drug pharmacokinetics and pharmacodynamics, and application of Bayesian inference techniques in chromatography and pharmacometrics.

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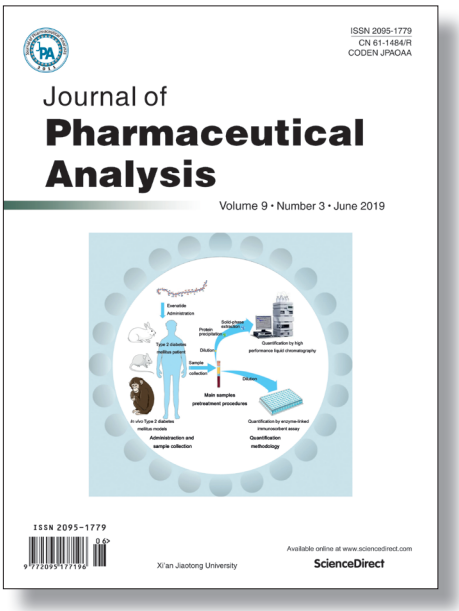

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ANALYTICAL CHARACTERIZATION AND IN VIVO ANTI-OXIDANT ACTIVITY EVALUATION OF THE POLYPHENOLIC FRACTION OF PRUNUS AVIUM L.

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Prunus avium L. (commonly known as sweet cherry) is a tree belonging to the *Rosaceae* family. It fruit is a well-known edible product, rich in nutrients and antioxidant compounds [1,2]. This fruit is considered to exert beneficial effects on human health: indeed, its consumption has been associated with the prevention of cell oxidative injury and inflammation, thanks to its free radical scavenging and anti-inflammatory activities [2]. These beneficial effects are closely related to the rich polyphenolic composition of sweet cherry fruit, which encompasses numerous chemical classes of compounds [1-3]. Therefore, sweet cherry fruit represents a source of bioactive natural compounds to be exploited in the nutraceutical field [1-3].In the light of all the above, the development of efficient analytical methods, together with the establishment of suitable extraction procedures, are crucial features in order to highlight the great nutraceutical potential of this product.

In this view, the aim of the present study was the development of a new analytical method for the comprehensive multi-component analysis of polyphenols in sweet cherry. In particular, a RP-HPLC-UV/DAD and HPLC-ESI-MSn method was successfully optimized for the qualitative and quantitative analysis of sweet cherry polyphenolic compounds. Sample preparation was based on two sequential dynamic maceration steps. The optimized analytical method was finally applied to different sweet cherry samples. Four anthocyanins were identified in sweet cherry extracts, with cyanidin-3-*O*-rutinoside being the most abundant one. Caffeoyil-quinic and cumaroyil-quinic acids were also identified as the most representative phenolic acids.

Given the high biological value of the polyphenolic fractions of sweet cherry fruit, the extracts were submitted to in vivo antioxidant assays using the *Caenorhabditis elegans* model to test their capacity to increase the worm resistance to oxidative and thermal stress.

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Short Personal Biography of Presenting Author

Dr. Virginia Brighenti graduated in 2013 in Pharmaceutical Chemistry and Technology at the Department of Life Sciences of the University of Modena and Reggio Emilia. In 2018 she got a PhD degree in Clinical and Experimental Medicine. Currently she covers a post-doctoral fellowship position in Medicinal Chemistry at the Department of Life Sciences of the University of Modena and Reggio Emilia.

The research activity of Dr. Brighenti is mainly focused on the development of innovative techniques for the extraction and analysis of bioactive natural products, and on the isolation of new bioactive compounds of natural origin with antioxidant and antiproliferative activity. Dr. Brighenti is the author of 18 papers in ISI indexed international journals, and more than 20 congress communications (oral and poster).

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CANCER TARGETED TREATMENT BY RGD-MODIFIED DIHYDROLYPOAMIDE DEHYDROGENASE

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The Reactive Oxygen Species (ROS)-dependent phototoxic effect of UV-excited titanium dioxide (TiO2), has been demonstrated in several cancer models of Photodynamic therapy. However, serious damage to the surrounding healthy tissue limits the applicability of this approach. Targeted delivery of TiO2 towards cancer cell would make PDT more selective. Cancer cells often overexpress integrin receptors (e.g. avb3) on their surface, which interact with proteins of the extra cellular matrix through RGD (Arg-Gly-Asp) recognition sites.

Studies in our lab has shown that Dihydrolipoamide dehydrogenase (DLDH) has strong TiO2-binding capabilities. Bio-engineering of DLDH with RGD moieties (DLDHRGD), generated a hybrid-conjugate nanobiocomplex (TiO2-Protein-RGD) capabilities with high affinity to the integrin expressing cancer cells. We have demonstrated that the nanobiocomplex possesses tumor-targeted and UV-excitable cytotoxicity in cutaneous melanoma cells (B16F10) while normal kidney cells (HEK293) remain unharmed.

The activity of mitochondrial dehydrogenases (such as DLDH), is often associated with elevated levels of ROS production, leading to pro-apoptotic activity. Our studies showed that DLDH possesses ROS production activity as well as DNA binding properties. We examined the cytotoxic effects of DLDHRGD and its potential use as an anti-cancer drug with melanoma (B16F10) glioblastoma (005), breast (4T1), cervical (Hela) and ovarian (Ovcar3) cancers cell lines. Normal kidney (HEK293) and cortex (NF5310) were unharmed. DLDHRGD incorporation into the cancer cells and apoptosis induction were analyzed by confocal and FACS assays.

In-vivo assay showed positive safety profile using IV, SC and IP (BALB/C or C57Bl/6 mouse strains). Treatments with DLDHRGD in subcutaneous melanoma mice model resulted in significant tumor inhibition. Currently the efficacy of DLDHRGD on Glioblastoma (murine 005 model in BALB/C) including BBB penetration are under active research.

Abstract Reference & Short Personal Biography of Presenting Author

Education and degree

- 2019 – Postdoc, Molecular Cell Biology & Biotechnology, Tel-Aviv University.
- 2014-2018 – P.hD, Molecular Microbiology & Biotechnology, Tel-Aviv University.
- 2012-2013 – M.Sc, Nanomaterials & Nanotechnology, Tel-Aviv University.
- 2008-2010 – B.Sc, Biotechnology, Bar-Ilan University.
- 2004-2005 – Practical Engineering in Biotechnology and Chemistry, AMAL College.

Publications

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

QUALITY CONTROL OF THERAPEUTIC PHOSPHOROTHIOATE OLIGONUCLEOTIDES BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY COUPLED WITH ION-MOBILITY QTOF

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Therapeutic oligonucleotides are short nucleic acids chemically synthetized. They play a major role in gene regulation and the treatment of various diseases. They target DNA, RNA, proteins, posttranslational protein modifications, carbohydrates, lipids or metabolites.

Oligonucleotides are easily in-vivo degraded and need to be modified to improve their pharmacokinetic and pharmacodynamic properties. Phosphorothioate oligonucleotides is the most dominating modification where the oxygen atom of the phosphodiester bond is replaced by a sulfur atom. This result in enhanced resistance against nucleases degradation and thus increased half-life. Currently, the FDA has approved 6 drugs but more than 180 are being tested in clinical trials. There is thus an important need for appropriate analytical techniques to ensure their quality control.

In this study, we compared the selectivity obtained with several hydrophilic interaction liquid chromatography (HILIC with diol, amide or phosphorylcholine functional groups) stationary phases on various oligonucleotides mixtures. Indeed, HILIC represents good alternative to commonly used ion-pair reversed-phase liquid chromatography for the analysis of polar compounds. Moreover, it avoids the use of ion pairing agents, which makes it more compatible with mass spectrometric detection. In this project, we investigated the coupling of HILIC with ion-mobility quadrupole time-of-flight MS detector (IM-QTOF). Ion-mobility provides a third separation dimension to mass spectrometry, as the ions will be separated based on their shape and size. Chromatographic and IMS performances were considered to assess the multidimensional efficacy of each tested system. As conclusion we can say that HILIC-IMS approach brings new potentiality in the quality control of emerging oligonucleotides.

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Short Personal Biography:
Alice Demelenne was graduated in Pharmaceutical Sciences at the University of Liege in 2015. She started a PhD thesis in the Laboratory for the Analysis of Medicines of the same university in October 2015. Her research is focused on the development of microfluidic electrodriven approaches for the analysis of pharmaceuticals and biopharmaceuticals.

ALTERNATIVE TO PROTEIN A CHROMATOGRAPHY

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A non-chromatographic, ligand-free approach for antibody (Ab) purification, having the potential to replace the gold standard technology, i.e., Protein A chromatography, will be presented. Specially designed micellar aggregates, comprising of non-ionic detergents (e.g. Tween, Brij or Triton X-100), a hydrophobic chelator (e.g. bathophenanthroline) and metal cations (e.g. Fe2+), were found to lead to high Ab recovery yields (85-90%) and purity (>95%) while preserving Ab specificity and monomeric state. Interestingly, process efficiency is tightly correlated with micellar aggregate size (>1000 nm) as determined by dynamic light scattering (DLS) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Moreover, purification is performed via three sequential filtration steps, implying that, the approach can be integrated into continuous production flows, where therapeutic grade monoclonal antibodies (mAbs) are purified at industrial-scales. Finally, the relevance of the same purification platform for IgA and IgM antibodies, will be discussed as well.

Abstract Reference & Short Personal Biography of Presenting Author

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Publications:

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

ANALYSING CELLS AND CULTURE MEDIA WITH SURFACE-ENHANCED RAMAN SCATTERING (SERS): HOW TO OVERCOME THE SERUM PROTEIN PROBLEM?

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Surface-enhanced Raman scattering (SERS) has become a valuable tool in the pharmaceutical and bioanalytical fields as it presents numerous advantages making it competitive towards other analytical tools. This vibrational spectroscopy technique is indeed specific with multiplex capabilities while requiring little sample preparation and being non-destructive. SERS is also very sensitive, benefiting from the exaltation of the Raman signal of analytes located in the vicinity of or adsorbed onto rough metallic surfaces called the SERS substrates. These substrates can be in the form of suspensions of nanoparticles or of nanostructured solid substrates, increasing the versatility of the technique.

However, a current challenge faced in biological SERS is the protein corona problem [2]. It arises from the adsorption of serum proteins onto the SERS substrates, preventing the adsorption of the analytes themselves onto the substrates. Moreover, when the SERS substrate is a suspension of nanoparticles, the protein corona stabilises the latter, resulting in a lack of aggregation.

In order to circumvent these problems, when working with a suspension of nanoparticles, the colloid can be pre-aggregated before adding the serum sample [3], therefore bypassing the stabilisation issue. On the other hand, regarding solid SERS substrates, the challenge rather concerns avoiding the adsorption of proteins onto the substrate since the nanoparticles are already set in a fixed configuration.

To that end, membranes with a low molecular weight cut-off (MWCO) have been employed. These membranes could let small molecules diffuse through them while retaining proteins of larger sizes. Indeed, the main protein component of serum is albumin, which has a molecular weight of 66 kDa [4].

Rat phaeochromocytoma PC-12 cells were selected as model to investigate the potent of these membranes. PC-12 cells mainly synthesise dopamine, a neurotransmitter of great physio-pathological importance and having a native affinity for the surface of gold nanoparticles [3]. They were cultured on inserts and their exocytosis of dopamine was followed by SERS with the help of membranes. Different analysis configurations were studied, with the SERS substrates located inside or beneath a dialysis membrane or a filter.

In conclusion, the use of dialysis or filter membranes seems extremely promising in the field of SERS bioanalyses of complex matrices.

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Abstract Reference & Short Personal Biography of Presenting Author

Elodie Dumont graduated in Pharmaceutical Sciences at the University of Liege in Belgium in 2015. She is currently a PhD student in the Laboratory of Pharmaceutical Analytical Chemistry of the Professor Philippe Hubert at the University of Liege. Her PhD thesis, funded by the Belgian National Fund for Scientific Research (F.R.S.-FNRS), is focussed on the development of SERS nanosensors for the detection of small bioactive molecules in biological matrices. She had the opportunity to attend last DA-PBA 2018 symposium in Leuven where her presentation about “A simple and easy-to-implement SERS approach overcoming the nanoparticle stabilisation by serum proteins: application to dopamine and PC-12 cells” was rewarded by the Young Scientist Prize

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

MICROBIOLOGICAL ANALYSIS AND PHYSICAL-CHEMICAL ANALYSIS: AN INTELLIGENT AND ESSENTIAL ASSOCIATION

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Quality control is the sector of the pharmaceutical industry responsible for evaluating the quality of pharmaceuticals and medicines. It is responsible for releasing or not the pharmaceutical products onto the market. For this it uses both physical-chemical and microbiological methods of analysis. Therefore, these methods must be highly reliable and represent the real quality of the medicine that will reach the patient.

On the one hand, the most widely used analytical method in pharmaceutical industries for evaluating pharmaceutical products is high performance liquid chromatography. It is an excellent physical-chemical analysis tool. On the other hand, the agar diffusion method is the most well-known microbiological method used in pharmaceutical industries for the evaluation of antimicrobial potency.

When it comes to antimicrobials, the association of physical-chemical methods and microbiological methods is fundamental. While the microbiological analysis evaluates the molecule as a whole, the physical-chemical analysis cannot always detect the real potency of the antimicrobial, because the part of the molecule responsible for the activity is not always its target. A routine analysis of quality control by high performance liquid chromatography is relatively rapid, taking about 15 minutes. The same analysis by agar diffusion lasts 24 hours.

There is an extremely viable, fast and economical alternative for the microbiological analysis called turbidimetric method. It lasts only 4 hours, uses liquid culture medium, uses less volume of material, requires fewer steps in preparation and the results are provided by a spectrophotometer. All these characteristics give the turbidimetric method advantages over the agar diffusion method.

Antimicrobial analysis should not be limited to the physical-chemical results, since the consequences of using products without adequate quality contribute to bacterial resistance, proliferation of superbugs and overload of the health system. The proposal for the evaluation of antimicrobials is to associate the physical-chemical methods, consecrated in the pharmaceutical analyzes, with the turbidimetric method, which comes as a smart reality for the current needs of laboratories and chemical-pharmaceutical industries.

Abstract Reference & Short Personal Biography of Presenting Author

My name is Ana Carolina Kogawa, I am Pharmaceutical-Biochemistry by UNESP and Master and PhD in Pharmaceutical Sciences also by UNESP. I have Black Belt-Six Sigma Training, which helped me enormously in performing activities, planning experiments, problem solving, and teamwork. Currently, I carry out post-doctoral research at UNESP in the areas of Pharmaceutical Technology and Physico-chemical and Microbiological Quality Control of Drugs and Medicines.
So far, I have 60 scientific articles published. During my Master, PhD and post-doctoral studies I participated in national and international events through presentation of poster and oral papers in London (UK), Osaka (Japan), Valencia (Spain), Rome (Italy), Lisbon (Portugal), Berlin (Germany), Paris (France), Ghent and Liege (Belgium), Cordoba and Rosario (Argentina), Cartagena de las Indias (Colombia) and several cities in Brazil. I am a reviewer of 22 international journals, which are described in the curriculum. I co-supervised and co-supervise undergraduate, master and doctoral students. My index h = 9 and Index i10 = 9.

My area of activity is the physico-chemical and microbiological quality control of drugs and medicines aimed at green analytical chemistry and everything that involves it. Our concern for quality is multidimensional. It covers the health of the analyst, the chosen reagents, the treatment of residues, the amount of residues formed, the toxicity of the reagents, the accessories used, the analysis time, the chosen technique, the equipment of protection, physical and emotional well-being, the environment and, of course, safe and effective pharmaceutical analyzes.

My projects also aim to work in a multidisciplinary way interacting in different laboratories: Quality Control and Pharmaceutical Technology; different equipment: spectrophotometer, chromatograph, dissolutor, X-ray diffractor; different methods: wet milling, complexation, spectrophotometry in the ultraviolet, visible and infrared regions, high performance liquid chromatography, microbiological turbidimetry, dissolution; and different people. I believe that this interaction is rich and my goal is always to strengthen the group with the teachings brought and lived in other laboratories with other ways of working, I believe that networking nowadays is differential and can bring great opportunities. In this way, we improve ourselves, become integrated people and contribute to world literature and benefit the community, the population, since we think in a multidimensional way focusing on the whole, the parts and, above all, the interaction between the parts of a system.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

PROFILING METABOLITES IN RAT BRAIN MICRODIALYSATES BY CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY USING DIRECT SAMPLE INJECTION

Marlien van Mever, *LACDR, Leiden University, Leiden, The Netherlands (m.van.mever@lacdr.leidenuniv.nl)*

Analysis of low-molecular-weight biomolecules in brain extracellular fluid plays an important role in the prediction, progression, and treatment outcome for various neurological diseases. Microdialysis is a powerful technique for in vivo sampling of extracellular fluid in the brain, thereby enabling the continuous measurement of neurotransmitters in freely-moving animals. However, metabolic analysis of microdialysis samples can be considered a huge analytical challenge due to limited sample amounts and low basal (nanomolar-range) concentrations of many metabolites. Capillary electrophoresis-mass spectrometry (CE-MS) emerged as a strong analytical tool for the profiling of polar and charged metabolites in volume-restricted biological samples. In this study, we have developed a CE-MS method for the profiling of amino acids and related compounds in rat brain microdialysates using direct sample injection.

CE was coupled to time-of-flight mass spectrometry (TOF-MS) employing a co-axial sheath-liquid interface and a bare fused-silica capillary. Amino acids were analyzed at low-pH separation conditions using 10% acetic acid (pH 2.2) as separation buffer. To increase the concentration sensitivity of CE-MS, an in-capillary preconcentration procedure was applied, which allowed large volume sample injections. A three-level Box-Behnken design (BBD) was used to optimize relevant CE-MS parameters for amino acids profiling in rat microdialysates.

The developed CE-MS system allowed the direct analysis of amino acids in rat brain microdialysis samples after only applying a centrifugation step. Very stable CE-MS patterns of amino acids and related compounds in microdialysis samples were obtained with a good migration-time repeatability. The optimized in-capillary preconcentration step allowed a sample injection of 105 nL (corresponding to circa 6% of the capillary volume), which resulted in detection limits down to the low nanomolar range for the amino acids. The CE-MS method provided a linear detector response for amino acids spiked into microdialysis samples over a wide concentration range, i.e. from 50 nM to 20000 nM. The influence of matrix effects on the quantification of amino acids was also investigated, and showed to be minimal for microdialysis samples. Overall, we propose here the first CE-MS method for the direct profiling of amino acids and related compounds in rat brain microdialysate samples. The the utility of this approach will be tested by analyzing a large set of rat brain microdialysate samples in a pharmacokinetic and biomarker discovery context using metabolomics.

Abstract Reference & Short Personal Biography of Presenting Author

Marlien van Mever is a PhD student at Leiden University within the group Biomedical Microscale Analytics, which is a part of the Department of the Leiden Academic Centre for Drug Research. Her project focuses mainly on the development of microscale analytical approaches for profiling metabolites in limited amounts of biological material**.** She currently focuses on brain metabolomics research using capillary electrophoresis – mass spectrometry.

A NEW CONCEPT FOR CRYSTALLIZATION OF MEMBRANE PROTEINS

Thien Van Truong, *PhD student, Department of Chemical Sciences, Ariel University, Israel*

Structure determination of membrane proteins (MPs) is currently regarded as one of the major challenges in structural biology, precluding efficient drug development due to the lack of relevant biochemical information at the atomic level. Unlike their water-soluble counterparts, in order to produce large, well-ordered crystals, MPs are generally extracted from their membranous environment with detergents and further manipulated as protein/detergent complexes (PDCs), where the target MP is enclosed in a detergent micelle.

I shall present a potentially general crystallization approach that differs conceptually from these crystallization methods as it relies on specific micellar conjugation mechanisms capable of bringing PDCs into proximity and stabilizing their nucleation centers. Consistent with the above, two micellar conjugation mechanisms, one that relies on peptide amphiphiles and the other on amphiphilic chelators, were studied. Both mechanisms were shown to concentrate target MPs without denaturing them. The use of an appropriate [amphiphilic chelator:metal] complex led to the growth of large (> 0.1mm), purple-colored, hexagon-shaped crystals of the light driven H+-pump bacteriorhodopsin (bR). Extension of this strategy to other MPs will also be discussed.

Abstract Reference & Short Personal Biography of Presenting Author

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Publications:
1. Truong et al., Crystallization of retinal proteins with amphiphiplic [metal:chelator] complexes (in preparation)
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POSTER SESSION

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

ANALYSIS OF CHONDROITIN/DERMATAN SULPHATES USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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There was developed an HPLC method for separation and quantification of underivatized chondroitin/dermatan sulphates - unsaturated disaccharides (4- and 6-sulphated disaccharide). This method is based on the separation of disaccharides by amido as well as amino-columns at acidic condition. Amido-column was core-shell particle column with amide polyol with TMS end-capping (2.6 µm particles, 150x2.1 mm) and amino column was 3-aminopropyl silica (5 µm particles, 100x3mm). There was also tested amino column modified by phenylboronate (enables separation of carbohydrates with vicinal-diols at cis-position) but only with slight improvement of separation. C18 reversed phase columns can also separate tested disaccharides but only in presence of ion-pairing agent (tetrabutyl ammonium bromide) and at lower separation efficiency. The amido column as well as the amino column enabled successful separation of 4- and 6-sulphated disaccharides at 50 mmol/L resp. 25 mmol/L phosphate buffer pH 4.25 (detection at 230 nm) at retention time less then 10 minutes. Limit of quantification was 0.5 µg/ml. Applicability of this method was demonstrated by the analysis of unsaturated disaccharides produced after the enzymatic digestion of chondroitin/dermatan sulphates of extracellular matrix gel produced from human umbilical cord.

Figure. Separation of 4-sulphated (4S) and 6-sulphated (6S) disaccharides at amino and amido column; a – standard (20 µg/mL), b - enzymatic digestion of chondroitin/dermatan sulphates of extracellular matrix gel produced from human umbilical cord, c – spiked sample (40 µg/mL + b, 1:1))

Acknowledgements

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Abstract Reference & Short Personal Biography of Presenting Author

Prof. Mikšík has a broad spectrum of professional interest: development of new methods for analysis of a wide spectrum of compounds by capillary electrophoresis and chromatography and their applications to the analysis of biological (physiological) functions of the organism. In practice he uses coupling of capillary electrophoresis and chromatography to mass spectrometry and their application to study of proteins, peptides and other physiological important compounds; analysis of steroids and their metabolites in organisms, native and modified proteins; physiology and chemistry of connective tissue and their cross-links; study of nonenzymatic posttranslational modification of proteins during ageing as well as diseases. He also analyses pigments and proteins at eggshell.

He is teaching at University of Pardubice (Czech Republic) - course “Analysis of Biological Materials” at Department of Biological and Biochemical Sciences.

He published 180 papers in the international journals with Impact factor, 9 papers in journals without Impact factor, co-editor of 1 book and 2 Special Volumes of Journal of Chromatography B, 1 Special Issue of American Journal of Analytical Chemistry, 14 chapters in books and 3 CD-ROMs about bibliography of separation techniques, 2 patents. See list at: http://analyt.natureblink.com/publikace_pdf.htm

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

NANOFIBERS AS A MODERN EXTRACTION SORBENT FOR DRUGS IN BIOLOGICAL MATRICES

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The laborious and time-consuming sample pretreatment is often required before the biological sample analysis. Restricted access materials are special type of sorbent primarily used for extraction of proteinaceous matrices. The matrix is purified from macromolecules and contained analytes are retained on sorbent at the same time. This ability of RAM is given by two types of surfaces with different hydrophilic and hydrophobic properties as well as by the porous structure of these sorbents. Placing these extraction columns into the column-switching chromatography system simplifies the analysis of the biological samples.

The nanofibers have large surface area that is able to capture the analytes. Moreover, the structure of nanofibrous materials with narrow inter-fiber spaces may exclude the macromolecules via size exclusion mechanism. Based on these properties, it was assumed that the polycaprolactone nanofibers could work as RAM. Therefore, they were tested for this purpose, although the exact mechanism of protein removal and analyte retention has not yet been clarified.

The analytes were extracted from two different matrices to prove protein removing ability of polycaprolactone nanofibers. The methods for determination of b-lactam antibiotics in bovine milk and non-steroidal anti-inflammatory drugs in human serum were developed for this purpose. Both of the matrices were injected on extraction column manually filled with nanofibers and afterwards directly separated on analytical column in column-switching chromatography system. The nanofibers provided sufficient extraction efficiency for both group of analytes. Additionally, the nanofibrous sorbent was able to remove proteins to a sufficient extent to prevent column clogging and worsening of analyte detection.

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Abstract Reference & Short Personal Biography of Presenting Author

Hedvika Raabová (25) comes from Czech Republic. Currently, she is the PhD student at the Charles University, Faculty of Pharmacy in Hradec Králové, Department of Analytical Chemistry, where she is focused on pharmaceutical analysis. She received her Master of Pharmacy degree at the same faculty two years ago. The object of her research is using of nanofibrous polymers for extraction of drugs from biological samples. She uses the HPLC systems for this purpose. She spent part of her studies at the internship in Switzerland,

where she worked in the research group of prof. Peter C. Hauser at the University of Basel. She gained experience with capillary electrophoresis and capacitive deionization on this stay.

Besides science and gaining new skills and knowledge, she is interested in travelling, sports and art. She speaks three languages – English, German and French, and would like to learn Spanish and Russian as well. In the future, she would like to work in the pharmaceutical research and development of the new drugs.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CHALLENGES IN IMPLEMENTATION OF THE METHOD FOR DETERMINATION OF AMMONIA IN SODIUM BICARBONATE

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Implementation of the ion chromatographic (IC) method for determination of Ammonia in the common excipient – Sodium Bicarbonate caused noticeable professional challenges.

Among them: contamination of diluent (water), glassware and various analytical tools with Ammonia; problems of quality of deionized water; issues with accuracy and precision and carryover, etc.

All the difficulties were timely overcome and, as a result, the method has been successfully verified and several batches of Sodium Bicarbonate – released.

Abstract Reference & Short Personal Biography of Presenting Author

Maria Zuckerman is Analytical project Coordinator in Analytical Development laboratory at Global R&D, Teva Pharmaceuticals, Ltd. She is responsible for methods development for small molecules API and drug products in generic development at Kfar-Saba R&D site. Maria joined Teva in 2012 and, since then, is involved in development of different and complex projects utilizing various analytical methods and technologies. Maria holds M.Sc degree in Chemistry from The Technion (Haifa Technological University)

Unusual Investigation Case: What Happened with Assay Reproducibility?

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Unexpected inconsistency of Assay results observed in routine duplicate testing of sample lead to deep investigation of the phenomenon.

First observation was as follows: During development and release testing, such “fluctuation” of assay results has not been observed. However, in stability testing, when running long series of injections, this variability occasionally occurred for some of samples duplicates. In most cases, reinjection of such duplicates resulted in acceptable consistency.

As an initial step, we created a protocol for systematic investigation of the problem.

Examination of structure of API molecule revealed that it can potentially create complexes with some transition metals, which, as we suspected, may be part of alloys used in the injection system of HPLC instrument. Among the metals under suspect was Molybdenum, which is a typical component of some alloys used for injection needles. This appeared to be a root cause of variability of results.

In support of this assumption, such problem has never been observed when running the testing on the instruments which use Molybdenum-free alloys for needles and other metal parts, which are defined as “biocompatible alloys”.

Abstract Reference & Short Personal Biography of Presenting Author

Akmurat Nepesov is Analytical Project Coordinator at Global R&D, Teva Pharmaceuticals, Ltd. He is responsible for methods development for small molecules API and drug products in generic development at Kfar-Saba R&D site. Akmurat joined Teva in 2007 and held numerous positions associated with analytical chemistry.

Akmurat holds a B.Sc. degree in Biology from the Hebrew University of Jerusalem, Israel

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DEVELOPMENT OF A HIGHLY SELECTIVE TWO-DIMENSIONAL HPLC-MS/MS SYSTEM FOR THE SCREENING OF INTRINSIC D-AMINO ACIDS IN HUMAN CLINICAL SAMPLES

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D-Amino acids, minor stereoisomers of chiral amino acids in living beings, have recently been discovered in mammals and several D-forms (especially, D-aspartate (Asp) and D-serine (Ser)) have been clarified to have physiological functions. These D-amino acids have also been paid attention as new biomarkers for diseases since the significant alterations of their intrinsic levels were reported. However, the amounts of D-enantiomers are extremely trace, and the determination of chiral amino acids is frequently interfered by unknown compounds even using the high selective two-dimensional (2D) HPLC or LC-MS/MS methods. Accordingly, development of the method having higher selectivity has been required for the precise screening of various intrinsic D-amino acids. Therefore, in the present study, a highly selective 2D HPLC-MS/MS system combining reversed-phase separation, enantioselective separation and detection by an MS/MS was developed to analyze a wide variety of intrinsic amino acids in human physiological fluids.

Human plasma and urine were appropriately deproteinized or diluted prior to the pre-column derivatization of the amino acids with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). As the target analytes, alanine (Ala), Asp, glutamate, leucine, lysine, methionine, phenylalanine, proline (Pro), Ser and valine were selected considering the presence of D-forms in mammals. For the development of the 2D HPLC-MS/MS system, the conditions of reversed-phase separation (1st dimension), enantioselective separation (2nd dimension) and also the MS/MS conditions were thoroughly investigated. As a result, all of the target NBD-amino acids were separated by a C18 column (KSAARP, 1.0 x 500 mm) within 450 min using aqueous solutions containing 10-28% acetonitrile (MeCN) and 0.05% trifluoroacetic acid. The amino acid fractions were automatically collected and introduced to the next dimension. All of the target enantiomers were further separated into their D and L-forms by an original enantioselective column (KSAACSP-001S, 1.5 x 250 mm) within about 30 min using the mixtures of methanol and MeCN containing formic acid as mobile phases. Using the developed system, the intrinsic amounts of chiral amino acids in human clinical samples were successfully determined without interferences. As a result, trace levels of D-Ala, D-Pro and D-Ser were observed in the plasma, and the ratios of D-enantiomers (D/(D+L) x 100) were 0.6, 0.2 and 1.6%, respectively. In the urine, all of the target D-enantiomers were detected and the ratios of D-forms were 0.7-36.7%. The %D values of target analytes were confirmed using the mobile phases of different compositions, and approximately equal values were observed. Further clinical applications are ongoing.

Abstract Reference & Short Personal Biography of Presenting Author

Chiharu ISHII graduated from Kitasato University in 2016 with a bachelor's degree in Pharmaceutical Sciences. She got her master's degree in Pharmaceutical Sciences in 2018 from Kyushu University, then she has been studying in the doctoral course of Kyushu University. From 2019, she is a Research Fellow of Japan Society for the Promotion of Science. Her research topic is the development of a reliable analytical system for trace levels of amino acid enantiomers in biological samples to discover new biomarkers.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

SIMULTANEOUS DETERMINATION OF OLIGOSACCHARIDES AND IRIDOID GLYCOSIDES IN RAT PLASMA USING HYDROPHILIC INTERACTION CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY: CHALLENGES IN BIOANALYTICAL METHOD DEVELOPMENT FOR PHARMACOKINETIC STUDY OF RADIX REHMANNIAE EXTRACT

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Quantitative bioanalysis of oligosaccharides by LC-MS/MS is challenging and rarely reported up to now. Simultaneous quantitative bioanalysis of oligosaccharides and iridoid glycosides by LC-MS/MS poses additional challenges due to their unique and different structural features. In the present study, a fast and sensitive analytical method based on hydrophilic interaction chromatography coupled with tandem mass spectrometry (HILIC-MS/MS) was developed for simultaneous determination of raffinose, manninotriose, stachyose, ajugol and catalpol in rat plasma. Multiple analytical challenges were encountered during method development, including very different retention behavior of oligosaccharides and iridoid glycosides, confusing split peaks for manninotriose, low ionization and extraction efficiency of oligosaccharides, thermal instability of catalpol and reduced column performance. The strategies to overcome these challenges were presented by optimizing chromatographic separation, mass spectrometric detection and sample preparation. The best separation was achieved on an Accucore-150-Amide-HILIC column (100 mm × 2.1 mm, 2.6 µm) at 50 °C with a gradient mobile phase containing acetonitril and 2.5 mM ammonium acetate. Ammonium adduct ions produced by positive electrospray ionization were chosen as precursor ions for multiple reaction monitoring transitions. The lower limits of quantification were 0.01-0.2 mg/mL using only 50 µL of plasma sample. The method was successfully applied to pharmacokinetic characterization of oligosaccharides and iridoid glycosides in normal and type 2 diabetic rats after intragastric administration of Radix Rehmanniae extract.

Keywords: Oligosaccarides; Iridoid glycosides; Radix Rehmanniae; Hydrophilic interaction chromatography; Tandem mass spectrometry; Pharmacokinetics

Abstract Reference & Short Personal Biography of Presenting Author

Reference

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Dr. Xin Di obtained her BS degree in Environmental Analysis from Nanjing University of Science and Technology in 1990 and her Ph.D. degree in Pharmaceutical Analysis from Shenyang Pharmaceutical University in 1995. She has been working in Shenyang Pharmaceutical University since 1995, where she was promoted to professor in 2005. She worked as visiting research scholar in Hong Kong Baptist University from 2002 to 2003 and in Oxford University from 2007 to 2008. Dr. Di serves as the director of the Key Laboratory of Drug metabolism and Pharmacokinetics of Liaoning Province and the associate editor of Journal of Shenyang Pharmaceutical University. She is also a Distinguished Professor of Liaoning Province. Dr. Di's research interests include drug metabolism and pharmacokinetics, development and applications of new analytical methodologies for pharmaceutical and biomedical purposes, and novel analytical strategies for fast screening of bioactive and toxic components from traditional Chinese medicines.

PHARMACOKINETICS OF DETERMINED BIOACTIVE COMPONENTS IN ARTEMISIA CAPILLARIS

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*Artemisia capillaris*Thunb is known as Yin-Chen in traditional herbal medicine. *Artemisia capillaris* has a wide spectrum of pharmacological properties ranging from the progress of liver dysfunction to the severe cirrhosis and cancer that could be contributed to its verified bioactive compounds. The bioactive components were found having, but not limited to, the properties of anti-oxidant, anti-inflammatory, antisteatotic, antiviral, and antitumor effects. Pharmacokinetics of bioactive compounds in *Artemisia capillaris* not only illustrated the synergic effects but also provides a guide for effective and efficient use of this herb for health professionals. The main identified constituents included scoparone, capillarisin, scopoletin, capillarin, and chlorogenic acid. The time to the maximum concentration of these compounds was reported from 21 minutes to 39 minutes with the longest half-life of 3.76 hours. These reports of pharmacokinetics support a rapid onset of its therapeutic action in various liver diseases including virus infection, cirrhosis, or cancer. In addition, the harvest time or adding of other herbs to Artemisia capillaris would largely influence the pharmacokinetics of contained bioactive compounds. This study describes the scientific evidence supporting the pharmacokinetics of *Artemisia capillaris* and its constituents synergically attribute the hepatoprotective activities.

Abstract Reference & Short Personal Biography of Presenting Author

Wan-Ling Lin is currently an attending physician in the Traditional Medicine Department of Kaohsiung Veterans General Hospital. She is rich in innovation and participates in many types of research. She obtained a master's degree from the Graduate Institute of Integrated Medicine of China Medical University. She was awarded the 2015 Outstanding Clinical Teacher in the Physician Field when she was an attending physician at Eda Hospital. She also served as a member of the Chinese Medicine and Pharmacy Committee of Eda Hospital and a member of the Committee of Traditional Chinese Medicine Clinical Trial Center of Eda Hospital. At the same time, she is also a lecturer in the Ministry of Education.

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EVALUATION OF RIFAXIMIN POTENCY BY NEW, FAST AND LOW-COST MICROBIOLOGICAL METHOD

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Rifaximin is an oral antimicrobial very used for its treatment diversity, among them hepatic encephalopathy, ulcerative colitis, irritable bowel syndrome, *Clostridium difficile*, travelers' diarrhea and acute diarrhea. For this reason, the quality control of this pharmaceutical product is essential. In this context, microbiological analytical methods are welcome for this evaluation, since they complement the physicochemical results. Thus, a new microbiological method by turbidimetry was developed and validated for the evaluation of the potency of rifaximin in tablets. The method was linear and selective over the concentration range of 50-98 $\mu g\ mL^{-1}$ with correlation coefficients 0.9976 (standard) and 0.9999 (sample), precise (intraday RSD = 4.96 and interday RSD = 3.92), exact (recovery 100.70 %) and robust against small and deliberate variations in the method using *Escherichia coli* ATCC 10536. The method can also be considered indicative of stability since it was able to evaluate the potency of the product based on rifaximin after stress conditions in acid, alkaline, neutral and photolytic media. The turbidimetric method developed, besides being adequate and reliable for the routine analysis of rifaximin in tablets, has other advantages such as speed (4 hours versus 24 hours of the traditional agar diffusion method), which allows better optimization of time, analyst activities and equipment, lower volume of material used, automated results, which allow greater confidence in the data obtained and easier handling. Therefore, this new proposal using microbiological turbidimetry can be used safely, effectively and with numerous advantages in the evaluation of rifaximin in tablets by laboratories and pharmaceutical industries around the world.

Abstract Reference & Short Personal Biography of Presenting Author

My name is Ana Carolina Kogawa, I am Pharmaceutical-Biochemistry by UNESP and Master and PhD in Pharmaceutical Sciences also by UNESP. I have Black Belt-Six Sigma Training, which helped me enormously in performing activities, planning experiments, problem solving, and teamwork. Currently, I carry out post-doctoral research at UNESP in the areas of Pharmaceutical Technology and Physico-chemical and Microbiological Quality Control of Drugs and Medicines.

So far, I have more than 60 scientific articles published. During my Master, PhD and post-doctoral studies I participated in national and international events through presentation of poster and oral papers in London (UK), Osaka (Japan), Valencia (Spain), Rome (Italy), Lisbon (Portugal), Berlin (Germany), Paris (France), Ghent and Liege (Belgium), Cordoba and Rosario (Argentina), Cartagena de las Indias (Colombia) and several cities in Brazil. I am a reviewer of 22 international journals, which are described in the curriculum. I co-supervised and co-supervise undergraduate, master and doctoral students. My index h = 10 and Index i10 = 10.

My area of activity is the physico-chemical and microbiological quality control of drugs and medicines aimed at green analytical chemistry and everything that involves it. Our concern for quality is multidimensional. It covers the health of the analyst, the chosen reagents, the treatment of residues, the amount of residues formed, the toxicity of the reagents, the accessories used, the analysis time, the chosen technique, the equipment of protection, physical and emotional well-being, the environment and, of course, safe and effective pharmaceutical analyzes.

My projects also aim to work in a multidisciplinary way interacting in different laboratories: Quality Control and Pharmaceutical Technology; different equipment: spectrophotometer, chromatograph, dissolutor, X-ray diffractor; different methods: wet milling, complexation, spectrophotometry in the ultraviolet, visible and infrared regions, high performance liquid chromatography, microbiological turbidimetry, dissolution; and different people. I believe that this interaction is rich and my goal is always to strengthen the group with the teachings brought and lived in other laboratories with other ways of working, I believe that networking nowadays is differential and can bring great opportunities. In this way, we improve ourselves, become integrated people and contribute to world literature and benefit the community, the population, since we think in a multidimensional way focusing on the whole, the parts and, above all, the interaction between the parts of a system.

NEW RIFAXIMIN SAMPLES: OBTAINING, CHARACTERIZATION, SOLUBILITY STUDY AND MICROBIOLOGICAL EVALUATION

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Rifaximin is an oral antimicrobial drug used for a wide range of ages, from adults to children, since it is indicated for the treatment of hepatic encephalopathy, travelers' diarrhea, irritable bowel syndrome, Clostridium difficile, ulcerative colitis and acute diarrhea. It is marketed as 200 mg tablets. The daily dose may be up to 800 mg (2 tablets twice a day). The success of pharmacotherapy will depend on correct fulfillment of drug administration, however it becomes difficult when the tablets are large and the doses are frequent. According to the Biopharmaceutic Classification System (BCS), rifaximin belongs to Class IV. It is both poorly soluble and poorly permeable. Thus, solubility of rifaximin was studied by the complexation to β-cyclodextrin using (i) phase solubility diagram and (ii) malaxation and (iii) decreasing particle size by wet milling. At the same time as the solubility increases, lower doses of rifaximin are possible and this facilitates patient adherence to treatment. The samples formed were characterized by spectrophotometry in the infrared region (FT-IR), differential scanning calorimetry (DSC) and X-ray diffraction (XRD) and the evaluation of their antimicrobial potencies were determined by microbiological turbidimetry. The samples obtained in all techniques were more soluble than the free drug, they presented higher thermal stability and the antimicrobial potencies of all formulations were approximately 100 %. It is fundamental to highlight that the treatment failure not only affects the quality of life of the patients, but also contributes significantly to the economic burden of the health system. This promising research opening can lead more efficient use of rifaximin in drug therapy, which is important for public health. These findings are extremely interesting, both from a technological and financial point of view.

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formed, the toxicity of the reagents, the accessories used, the analysis time, the chosen technique, the equipment of protection, physical and emotional well-being, the environment and, of course, safe and effective pharmaceutical analyzes.

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VALIDATION OF AN UV METHOD FOR THE DETERMINATION OF CIPROFLOXACIN IN COCRYSTALS

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Ciprofloxacin (CIP), the drug chosen for this study, has low aqueous solubility, which limits its bioavailability.CIP is a broad spectrum antibiotic that is used against Gram-negative and positive bacteria, as well as other microorganisms. A way to improve the solubility of CIP is through the cocrystal formation, that can be defined as a multicomponent crystal composed of active pharmaceutical ingredients and coformers, in a defined stoichiometric ratio, joined by intermolecular interactions. This work validated a UV spectrophotometric method for the determination of CIP in cocrystals, since in the literature have been reported many papers that determine ciprofloxacin hydrochloride. A stock solution containing 100 µg mL^{-1} of CIP was prepared with a 0.1 M HCl solution, using the ultrasound. Aliquots of the stock solution of ciprofloxacin were transferred to 10 mL volumetric flasks and the volume was then quenched with water. The readings were performed at a maximum wavelength of 277 nm using water as blank. This method showed linear regression in the concentration range of 2.0 to 7.0 µg mL^{-1}. The linear equation was: y = 0.1282x + 0.0056, with a correlation coefficient equal to 0.9999.The RSD was 0.14%, 0.42% and 0.46% for the intra-day, inter-day and inter-analyst precision, respectively. This method proved to be accurate since the RSD were all less than 1%. The experimental concentrations that were found were very close to the theoretical concentrations, and the recoveries were between 98% and 102%. The method was robust in relation to the changes (the absence of filtration of the stock solution, the change of the wavelength to 275 nm and the use of a solution of HCl 0.08 M), because it presented low RSD values, all lower than 1.5%. The selectivity was verified by comparing the spectra obtained for the 4.0 µg mL^{-1} solutions of pure ciprofloxacin, diluent, coformers and cocrystals, and then the recovery values were calculated. The cocrystals used were CIP-INCA (30 Hz 90 min) and CIP-NCA/ EtOH (30 Hz). The selectivity shows that there was no interference on the part of the coformer or diluent for the cocrystals synthesized with isonicotinic (INCA) and nicotinic (NCA) acids. The CIP recovery for this cocrystals were 101.23% and 99.09%, respectively. So, the developed UV method was validated and was found to be accurate, precise, specific, linear and robust. No interference from coformers were observed.

Abstract Reference & Short Personal Biography of Presenting Author

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COMPARATIVE SEROLOGICAL SCREENING OF IMMNOPROTEOMES OF HEALTHY INDIVIDUALS AND PATIENTS WITH MULTIPLE MYELOMA IN THE LONG-LASTING REMISSION

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Sabina Sevcikova, *Babak Myeloma Group, Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*

Spontaneous tumor regression is a phenomenon that has been observed throughout the history of mankind. After having been the subject of many disagreements, it is now accepted as an indisputable fact. This remarkable phenomenon has been observed in multiple myeloma patients who developed an aplastic anemia-like (AA-like) syndrome after the high-dose therapy (HDT) with autologous stem cell transplantation (ASCT). In order to provide novel insights into this phenomenon and to accelerate development of new treatment against these diseases, detail characterization of immunoproteome is an essential prelude.

In this work the sera of patients suffered from multiple myeloma currently in the long-lasting remission have been applied for detailed serological proteome analysis (SERPA) to screen its reactivity against antigens isolated from two representative cancer cell lines (HEL 92.1.7. and SKBR3). SERPA, as a tool for identification of new candidate immunoreactive antigens is a promising method in this respect. Comparative probing of the sera of patients and healthy individuals against specific antigens can be valuable for recognition of numerous reliable biomarkers associated with particular disease or specific stage of disease development. The identified candidate molecules will be essential not only for diagnostic but also prognostic or even therapeutic applications.

Abstract Reference & Short Personal Biography of Presenting Author

Zuzana Bilkova graduated in Molecular Biology and Genetics at Charles University (Prague, CZ) and currently she is a professor of Immunology and Immunochemistry at the University of Pardubice (CZ). During her twenty-year academic career, she has been named as a head of Department of Biological and Biochemical Sciences. Her scientific expertise can be summarized: surface modification and biofunctionalization of nanomaterials and their application in immunochemistry, bioaffinity chromatography and biosensing. Up to the present days, she has published more than 110 papers and gave more than 45 oral presentations at seminars, workshops, conferences.

A UNIQUE CASE OF POLYMORPHISM

Roxana Schlam, *Materials Science & Engineering, Drug Product Science & Technology, Bristol-Myers Squibb, New Brunswick, New Jersey, U.S.A. (roxana.schlam@bms.com)*

The crystal structures of the two polymorphic forms display a number of similarities. Both forms contain dense layers of molecules with extensive hydrogen bonding/electrostatic interactions, while interactions between layers are relatively weak and the regions between layers contain large void spaces. The PXRD patterns of the two polymorphs also display many similarities, but are easily distinguished by a few unique low angle peaks (5-10° in 2θ); where one form has peaks the other form has baseline and *vice versa*. In fact, there are a whole range of related patterns with variable peak heights and widths, again, mainly in the range 5-10° 2θ. Based on single crystal diffraction data and powder pattern indexing, the diversity of patterns is most likely caused by layer stacking faults. Additional studies using other characterization techniques did not show significant differences between the two forms. In the course of determining the thermodynamic relationships, it was noted that slurries containing both forms are very sluggish to convert to the more stable form. However, due to the close structural and thermodynamic relationship between the polymorphs**,** phase purity is not expected to have a significant impact on the performance of drug product.

Abstract Reference & Short Personal Biography of Presenting Author

Dr. Roxana F. Schlam received her B.Sc. in Chemistry from Hebrew University in Jerusalem, Israel and graduated with a Ph.D. in Chemistry from Brandeis University in Waltham, Massachusetts in the United States where she focused on structure/reactivity relationships in the solid state under Professor Bruce Foxman. She also did a postdoc at Purdue University in Indiana with Professors Stephen Byrn and Kenneth Morris where she applied her crystallography knowledge and learned about solid-state properties of active pharmaceutical ingredients and the pharmaceutical industry. Dr. Schlam started her career in the industry in the Solid-State Science group at Searle which became Pharmacia and then Pfizer.After five years, Dr. Schlam moved to New Jersey to work in the Materials Science and Engineering group at Bristol-Myers Squibb where she has been for almost 16 years. Dr. Schlam's research interests include the study of thermodynamics in solid-state systems and understanding bulk properties at the molecular level. Dr. Schlam holds numerous patents, scientific publications and presentations in the solid-state field of pharmaceuticals and also has extensive experience on intellectual property of crystalline forms. Dr. Schlam is also an Adjunct Full Professor at Long Island University College of Pharmacy and Health Sciences where she lectures at the graduate level an Introduction to Solids and also Polymorphism and Hydrates: Systems, Stability and Analysis.

ULTRASENSITIVE AND SELECTIVE NONENZYMATIC DETECTION OF GLUCOSE USING AUNP EMBEDDED POLYMERIC NANOSTRUCTURES

Zeynep Altintas, *Institute of Chemistry, Technical University of Berlin, Berlin, Germany (zeynep.altintas@tu-berlin.de)*

Diabetes is a chronical and serious disease affecting millions of people. The chronic complications of diabetes include end-stage renal disease, accelerated development of cardiovascular disease, limb amputations, and loss of visual acuity. These complications cause the excess morbidity and mortality in individuals with diabetes. There is currently no permanent cure for diabetes; however, treatment to reduce the symptoms and complications is possible by the aid of medication and blood sugar monitoring [1]. Molecularly imprinted polymers (MIPs) can be synthesized and used as molecular receptors for specific recognition of glucose. Nevertheless, the MIP-based sensors are often lack of desired sensitivity for the quantification of disease biomarkers in human body fluids. Hence, we embedded gold nanoparticles in MIP matrix to improve the conductivity and sensitivity of the sensor surface to measure trace amounts of glucose in human serum.

The MIPs were synthesized on gold wire by a multistep amperometry technique using o-phenylenediamine as monomer and glucose as template molecule at 1:1 molar ratio [2]. An optimum concentration of previously synthesized gold nanoparticles was added to this mixture and the polymerization reaction was performed in one step. The template molecules were then removed from the polymer network by alkaline treatment and the empty cavities, corresponding to glucose, were obtained. The MIP electrode was exposed to glucose in buffer or human serum for the rebinding studies

Electrochemical and atomic force microscopy techniques confirmed the successful fabrication of the MIP sensor under optimal conditions. The glucose rebinding studies were performed using voltammetry methods in a concentration range of 0.00125–0.32 µM. The MIP sensor without gold nanoparticles could not detect glucose even at much higher concentrations (*e.g.* 1 µM). The sensor showed a low level of cross-reaction with reference molecules, confirming the target specificity of molecular receptors.

The results of electrochemical sensor showed that we can produce ultrasensitive cavities in polymer matrix and quantify trace amounts of glucose, thanks to gold nanoparticles within the matrix. The presence of gold nanoparticles in the polymer network significantly improved the conductivity of the polymer film and led to a high sensitivity for glucose detection even in human serum.

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Abstract Reference & Short Personal Biography of Presenting Author

Dr. Zeynep Altintas is the head of the Biosensors and Receptor Development Group at the Technical University of Berlin, Germany. Her background is biomedical engineering and biotechnology. She leads an

interdisciplinary research group in the domain of optical, piezoelectric and electrochemical biosensors, bioreceptor development, and molecular modelling. Her specialization also includes but not limited to implementation of biosensors for the diagnosis of disease biomarkers, pharmaceuticals, and food and environmental pathogens. She has more than 100 publications in these fields, including a book, journal articles, book chapters, patent applications, and conference papers. She has delivered numerous invited talks at international conferences and world-renowned institutes. She has built a reputation in her areas of expertise that is recognized by several international awards to her research. She serves as an expert reviewer for EU and Wisconsin Groundwater Coordinating Council (USA) funded projects, in addition to acting as an editorial board member and a reviewer for several important journals in her areas of expertise. She is also a member of the Royal Society of Chemistry

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

CANCER BIOMARKER SENSING USING THE MOLECULAR IMPRINTS OF SELF-ASSEMBLED MONOLAYER EPITOPE BRIDGES

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Biomarkers play an important role as an indicator of a biological state or condition, such as cancer, diabetes, and cardiovascular system disorders. They are naturally occurring proteins, genes, or small molecules by which a particular physiological or pathological process can be identified. Biomarkers are also useful for the development of therapies of various diseases [1]. They are often present in body fluids at low concentrations and are embedded in a complex matrix, which makes their detection difficult. Highly sensitive bioassays could be developed using various biosensing platforms, which require target specific and high affinity (bio)receptors [1].

In this study, a novel epitope imprinting strategy is reported by employing computationally selected [2], double cysteine modified peptides as the templates and adsorbing the templates on a gold surface by means of forming self-assembled monolayer bridges, followed by electropolymerization to create a polymer network. The imprinted surface was initially designed to demonstrate specific affinity towards a short peptide (i.e. the epitope) or a target protein (i.e. neuron specific enolase) in buffer. This surface was subsequently used to measure the cancer biomarker in human serum that allows detecting 12 times lower concentration than threshold level of the biomarker. The molecular receptors exhibited a Kd <65 pM for their respective target protein and low cross-reactivity with three non-specific molecules in serum. As compared to current strategies for the epitope imprinting, for example, through traditional, vertically adsorbed or histidine modified peptides, such a molecularly tunable system based on a surface-imprinting process may provide more efficient sensing systems with desirable affinity, sensitivity and specificity in diagnostics applications. The superior features of the current method could be attributed to the combination of computationally selected epitopes and double-cysteine modified epitope template bridges, which may result in synergistic influence on improving the molecular imprinting process and assay sensitivity.

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Dr. Zeynep Altintas is the head of the Biosensors and Receptor Development Group at the Technical University of Berlin, Germany. Her background is biomedical engineering and biotechnology. She leads an interdisciplinary research group in the domain of optical, piezoelectric and electrochemical biosensors, bioreceptor development, and molecular modelling. Her specialization also includes but not limited to implementation of biosensors for the diagnosis of disease biomarkers, pharmaceuticals, and food and environmental pathogens. She has more than 100 publications in these fields, including a book, journal articles, book chapters, patent applications, and conference papers. She has delivered numerous invited talks at international conferences and world-renowned institutes. She has built a reputation in her areas of expertise that is recognized by several international awards to her research. She serves as an expert reviewer for EU and Wisconsin Groundwater Coordinating Council (USA) funded projects, in addition to acting as an editorial board member and a reviewer for several important journals in her areas of expertise. She is also a member of the Royal Society of Chemistry

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15–18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

BIOMARKER DETERMINATION USING INNOVATIVE BIOMIMETIC SENSORS

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The availability of recognition elements (antibodies, nucleic acids, aptamers or molecularly imprinted polymers) with an excellent specificity towards the target biomarkers is one of the most important requirements in disease detection using biosensors [1]. This research aims to engineer imprinted polymer based synthetic receptors for neuron specific enolase (NSE) biomarker. However, the flexible nature and larger size of proteins make their imprinting difficult and requires special care. These drawbacks can be avoided by focusing on epitope imprinting rather than entire biomolecule and the success of the MIPs may also be increased by modifying the surface epitopes [1,2].

The synthetic peptide derived from the NSE was synthesized along with its cysteine and histidine modified versions. The modified peptides were utilized as templates for molecular imprinting, which was achieved by combination of epitope- and electrochemical surface imprinting strategy. The subsequently generated imprinted cavities, characterized by atomic force microscopy and electrochemical techniques, were used for the detection of the NSE derived peptide and NSE [2]. The imprints created with cysteine (CME) and histidine modified epitopes (HME) could detect the peptide in a concentration range of 2–128 µM and 15.6 nM-128 µM, respectively. The recognition of NSE was achieved by the same imprints in a linear range of 1–64 ng mL^{-1} (CME) and 0.25-64 ng mL^{-1} (HME), respectively. The target molecules bound to the control polymer very weakly, confirming the high selectivity of the MIP cavities. Selectivity studies resulted in imprinting factors of 8.8 and 11 for the CME and HME imprints, respectively. The affinity analyses resulted in the dissociation constants of 2.31×10^{-10} M and 3×10^{-11} M for NSE recognition for corresponding epitope imprints, proving the higher affinity of histidine modified epitope imprinting toward the NSE biomarker. With regard to sensitivity, selectivity and specificity the histidine modification used for the surface imprinting turned out to be superior to the other method [2]. The fabricated sensor using histidine modified epitope MIP demonstrate a great potential for protein recognition and detection. This promising strategy can be employed not only for NSE detection, but also for a wide range protein biomarkers.

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Dr. Zeynep Altintas is the head of the Biosensors and Receptor Development Group at the Technical University of Berlin, Germany. Her background is biomedical engineering and biotechnology. She leads an interdisciplinary research group in the domain of optical, piezoelectric and electrochemical biosensors, bioreceptor development, and molecular modelling. Her specialization also includes but not limited to implementation of biosensors for the diagnosis of disease biomarkers, pharmaceuticals, and food and environmental pathogens. She has more than 100 publications in these fields, including a book, journal articles, book chapters, patent applications, and conference papers. She has delivered numerous invited talks at international conferences and world-renowned institutes. She has built a reputation in her areas of expertise that is recognized by several international awards to her research. She serves as an expert reviewer for EU and Wisconsin Groundwater Coordinating Council (USA) funded projects, in addition to acting as an editorial board member and a reviewer for several important journals in her areas of expertise. She is also a member of the Royal Society of Chemistry.

QUALITY CONTROL OF CHINESE MEDICINES

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Chinese medicines (CM) have been attracting interest and acceptance in many countries. An estimated 1.5 billion peoplenow use these preparations worldwide. This may be primarily because of the general belief that herbal drugs are without any side effect besides being cheap and effective. However, as more people are using CM products, there are increased reports on their adverse reactions. Therefore, quality control is crucial for ensuring the safety and efficacy of CM. However, different from western medicine, CM are usually used as whole plant and/or combination of several herbs, which contains more than tens to hundreds or even thousands of components. In addition, CM are usually considered that multiple constituents are responsible for the therapeutic effects. Especially, effective components in most CM are unknown, which greatly increases the difficulties of quality control. Therefore, a rational and effective quality control method for CM is helpful to improve CM. In this presentation, the key points for quality control of CM were discussed based on our research and experience.

Abstract Reference & Short Personal Biography of Presenting Author

Biography

Dr. Shao-ping Li, is a Distinguished Professor on Quality Control of Chinese Medicines and Deputy Director of the State Key Laboratory of Quality Research in Chinese Medicine, University of Macau, and Editor of Journal of Pharmaceutical and Biomedical Analysis. His research is focused on quality control and active components of Chinese medicines. As an Editor-/Associate Editor- in-Chief, he has published 4 books in USA or China. He is also the author of over 300 peer refereed journal papers and book chapters, and holder of more than 10 China and US patents.

Dr. Li is also an advisor of American Herbal Pharmacopoeia and a member of Chinese Pharmacopoeia Commission, as well as an Associate Editor of Chinese Medicine, and member of Editorial Boards of Journal of Function Foods, Journal of Food and Drug Analysis, American Journal of Chinese Medicine, International Journal of Analytical Chemistry, Molecules, Acta Pharmaceutica Sinica A/B, Journal of Pharmaceutical Analysis, Chinese Pharmaceutical Journal, Chinese Journal of Pharmaceutical Analysis, and China Journal of Chinese Materia Medica, etc. He is an Associate Chairman, the Pharmaceutical Analysis Committee of Chinese Pharmaceutical Association, the Committee of Natural Medicinal Resources /China Society of Natural Resources, the Quality and Safety Evaluation Committee, and Medicinal Mushroom Committee of China Association of Chinese Materia Medica, the Committee of Analytical Science of Chinese Medicines of Chinese Society of Chinese Medicine, etc.

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

NEW CONCEPT FOR CRYSTALLIZATION OF MEMBRANE PROTEINS

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Abstract Reference & Short Personal Biography of Presenting Author

We present a potentially general crystallization concept that relies on amphiphilic [metal:chelator] complexes capable of bringing MPs embedded in micelles (*i.e.*, Protein detergent complexes, PDCs) into proximity and further stabilizing nucleation centers. Accordingly, micelles of the non-ionic detergent OTG can be conjugated and produce oil-rich globules only when both the amphiphilic chelator and metal cations capable of binding two or more chelators in parallel, are present. These findings paved the way towards successful implementation of the strategy with the bacterial light driven H+-pump bacteriorhodopsin (bR) present in OTG\phospholipid mixed micelles. Purple globules, containing a functional (purple) and concentrated MP (*i.e.* bR) were clearly observed in the presence of the above complex and served as reservoirs for promoting nucleation and crystals growth. No crystals were observed in the absence of the metal or chelator. Moreover, cation identity and concentration seem to affect crystal size.

Short personal biography: I gradugated from the Chemistry department at Da Nang University, Vientnam. In 2017, I joined as a PhD student the Patchornik group in the Chemical Sciences department at Ariel University, Israel.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

ENGINEERED-MICELLES: ALTERNATIVE TO PROTEIN A CHROMATOGRAPHY?

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Abstract Reference & Short Personal Biography of Presenting Author

We introduce a new concept and a potentially general platform for antibody (Ab) purification that does not rely on chromatography nor specific ligands (e.g. Protein A); rather, it makes use of detergent aggregates capable of efficiently capturing Ab's while rejecting hydrophilic impurities. Captured Ab's are then extracted from the aggregates in pure form (>95%) without co-extraction of hydrophobic impurities or aggregate dissolution. The aggregates studied consist of conjugated "Engineered-micelles" built from the nonionic detergent, Tween-20; the hydrophobic metal chelator, bathophenanthroline and Fe2+ions. When tested in serum-free media with\without BSA (or HSA) as additives, human or mouse IgG's were recovered at high overall yields (74-80%). Extraction of IgG's with 7 different buffers at pH 3.8 sheds light on possible interactions between captured Ab's and their surrounding detergent matrix. Extracted Ab's preserve their secondary structure, specificity and monomeric character as determined by CD, ELISA and DLS, respectively. Possible integration of the approach within industrial scale downstream processing of therapeutic grade monoclonal Ab's, will be discussed.

Short prsonal biography: I graduated from the centre for excellence in Genomic Sciences, Madurai Kamaraj University, India. In 2017, I joined the Patchornik group at the Chemical Sciences deparment at Ariel Univeristy, Israel where we focuse on purification of soluble and membrane proteins via non-chromatographic strategies.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15–18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

AUTOMATED WORKFLOW FOR THE STUDY OF DRUG-PROTEIN INTERACTIONS BASED ON CAPILLARY ELECTROPHORESIS-FRONTAL ANALYSIS COMBINED WITH IN-CAPILLARY MIXING OF INTERACTING PARTNERS

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Biomolecular interactions are at the base of all biological events occurring in living cells. The understanding of interactions between protein-protein, protein-nucleic acids, protein-sugars, nucleic acid-nucleic acids and protein-small molecules represents a high-priority research area in the life science – *interactomics* and subsequently provides essential knowledge that may help in disease diagnosis, prognosis and therapeutics. Therefore, biomolecular interaction analyses thus do not only give fundamental insights into the biological processes in the cells but also paves the way to modify them by therapeutic molecules – drugs towards improved disease treatment that is the main goal of the research done by pharmaceutical industry in a new drug development.

An essential part of this process is a study of the plasma protein-drug binding, since according to the widely accepted *free drug hypothesis* it can limit the drug concentration available to act at the target, as well as in its final impact, drug disposition and efficacy. Quite a lot of approaches differing in their basic principle were developed over time to characterize these interactions. The capillary electrophoresis-frontal analysis (CE-FA) is together with the mobility shift affinity CE most frequently CE mode used for this purpose. Whereas in the classic CE-FA setup the sample is prepared by off-line mixing of the interaction partners in the sample vial outside the CE instrument and after short incubation period is loaded in the capillary and analysed, in this work a new methodological approach combining the CE-FA with the mixing of interacting partners directly inside the capillary has been developed. Their combination gives rise to a fully automated and versatile methodology for characterization of binding interactions besides the substantial reduction of the sample compound amounts.

The in-capillary mixing is based on transverse diffusion of laminar flow profile methodology introduced by S. Krylov et al. in 2009 using its multi-zone injection modification presented by Řeminek at al. in 2013. The apparent binding parameters of BSA for propranonol and lidocaine, drug differing both in the physico-chemical properties and binding sites on the albumin molecule, and in the binding strength were determined by on-line and off-line approaches. The values obtained by a new on-line CE-FA methodology are in agreement with values estimated by classic off-line CE-FA, as well as with literature data obtained using different techniques. According the best author knowledge the in-capillary sample mixing for the CE-FA has not been published so far.

Acknowledgements

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Abstract Reference & Short Personal Biography of Presenting Author

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Education

1993 – CSc - PhD equivalent Thesis Title "Analytical Biochemistry of Quinoproteins" Masaryk University, Faculty of Science, Department of Biochemistry

Affiliation

2007–present Full Professor, Masaryk University Brno, Faculty of Science, Department of Biochemistry

Scholarships and longer visits

1998, 2003, 2013 University of Lund, Sweden – prof. B. Matiassson, KU Leuven, Belgium – prof. A. Van Schepdael, University Bern, Swiss – prof. W. Thormann

1992–1993 Postdoctoral stay Indiana University, Bloomington, USA - prof. Novotny

Membership and activities in professional societies

- Board of Scientific Group for Chromatography and Electrophoresis, Czech Chem. Society
- Institute Council of Institute of Analytical Chemistry of the ASCR, v. v. i. Brno
- Member of Scientific Board Faculty of Science, Palacký University Olomouc.
- Member of International Editorial Board Journal od Separation Science, Wiley

Research interests in last 5 years

- Application of CE in pharmacology, clinical chemistry, enzymology and metabolomics
- Analytical biochemistry
- Metabolomics

Research publication activities

120 scientific papers in scientific journals with or proceeding abstract in WOS (all databases), 3 chapter in the book, 160 presentations at scientific conferences, 3 patents, ∑ citation cca 1127 without self-citations, H-index 20.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

THE USE OF PROFICIENCY TESTING AS A TOOL FOR A ROBUST QUALITY MANAGEMENT SYSTEM IN A PHARMACEUTICAL LAB

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Proficiency Testing (PT) is the name used by the International Standards Organization for a procedure also known as “inter-laboratory study” or “external quality assessment” or “ring test”. Proficiency testing, in simple terms, comprises a sample sent to a group of laboratories for measurement. The labs know what might be in the sample, but they don’t know exactly what is there or the concentration. Their results are compared with the known or true value and the lab is assigned a “Z” score to show how closely their result came to the target.

A major benefit of Proficiency testing is as a critical tool for quality assurance and continuous improvement. Proficiency testing performed over time can give a laboratory a true picture of their testing quality, it can help a laboratory identify and realize continual improvement opportunities and can actually help a laboratory avoid nonconforming tests.

Proficiency testing samples have specific analytes of interest, spiked in a particular matrix, in a concentration unknown to the participant. Proficiency testing samples are fully tested and characterized by the provider prior to shipment. Each Proficiency testing sample is supplied to the participant with a reporting packet which contains all of the critical information needed to perform the test such as the date range in which the test will be performed, instructions on how to report the data, any preparation or handling instructions, and the analyte ranges for the sample.

Pharmaceutical Proficiency testing samples are usually performed to demonstrate competency to Pharmacopoeia general chapters and are typically neat materials. Technology PTs are used to prove competence to a specified technology, such as titration, pH, Karl Fischer, or Residue on Ignition.

Presented here is a Proficiency testing platform and reporting results that was developed to allow for easy access to pertinent and historical data to allow the laboratory the opportunity to identify trends their testing rapidly. The laboratory’s requirements for accreditation and internal tracking were a focal point in the design of the PT system.

Abstract Reference & Short Personal Biography of Presenting Author

Nicolas J. Hauser received a B.S. degree in Chemistry from St. Cloud State University in St. Cloud, MN. He completed a PhD degree in Analytical Chemistry from University of Wyoming in Laramie, WY. His focus during graduate school was method development for proteomics using mass spectrometry. Nick joined MilliporeSigma (formerly Resource Technology Corporation) in May 2008 and began work as an analytical chemist performing R&D and QC on RTC’s vast portfolio of products. Nick is currently a Product Manager in the Reference Materials Franchise for MilliporeSigma and oversees several products used in pharmaceutical analysis as well as proficiency testing.

UPLC/Q-TOF-MS FOR METABOLIC PROFILES OF GINGER AND ITS REPRESENTATIVE PUNGENT COMPONENTS IN RATS

Zhihong Yao, *College of Pharmacy, Jinan University, Guangzhou, China (yaozhihong_jnu@163.com)*

Ginger, the dried rhizome of Zingiber officinale Roscoe (ZO), has been widely used throughout the world for centuries as a popular food homologous traditional medicine. However, its metabolic behaviors remain unclear, which entails an obstacle to further understanding of its functional components and mechanism. In this study, a four-step approach based on UPLC-Q/TOF-MS was applied to systemically reveal the metabolic profiles of ZO in vivo (Figure. 1). The results included the characterization of 92 components of ZO (57 pungent phytochemicals, 27 diarylheptanoids, and 8 others) based on the summarized fragmentation patterns and self-building chemical database. Furthermore, four abundant and characteristic pungent compounds in ZO with different structural types, including [6]-gingerol, [6]-shogaol, [6]-dehydrogingerdione and [10]-gingerol, were selected as representative components, based on the results of a preliminary analysis of ZO-related xenobiotics in vivo. Their metabolic pathways were then characterized, respectively, to explore the typical metabolic pathways of ZO. Consequently, 141 ginger-related xenobiotics were characterized, following the metabolic spots of the pungent phytochemicals were summarized (Figure. 2). These findings indicated that the in vivo effective components of ginger were mainly derived from [6]-gingerol and [6]-shogaol (nearly 60% of the xenobiotics were derived from [6]-gingerol and [6]-shogaol). Meanwhile, hydrogenation, demethylation, glucuronidation, sulfation, and thiolation were their major metabolic reactions. These results expand our knowledge about the metabolism of ginger, which will be important for discovering its in vivo functional components and the further mechanism research.

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Abstract Reference & Short Personal Biography of Presenting Author

Dr. Zhihong Yao is a full professor of College of Pharmacy, Jinan University at Guangzhou, China. Prof. Yao's research interests focus on the research of drug metabolism & pharmacokinetics, quality control method of Traditional Chinese Medicines and their products (*Allii Macrostemonis Bulbus*, *Zingiber Officinale*, Xianling Gubao Capsule, Gualou Xiebai Banxia Decoction, Danlou Tablet, etc) around the elucidation of the bioactive constituents and mechanism. The projects she had hosted mainly include 2 projects of Natural Science Foundation of China and 4 Provincial projects. In the past five years, as the first author or correspondent author, Prof. Yao has published 24 peer-reviewed papers in *J Agric Food Chem, J Ethnopharmacol, Food & Funct, J Pharm Biomed Anal, Food Chem* and other well-known international academic journals.

COMPARISON OF TWO CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY INTERFACES FOR PROTEOMIC ANALYSIS

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Untargeted bottom-up proteomic analysis aims to identify the highest number of peptides from complex protein digests. The application of this strategy to real sample might lead to the discovery of new proteic entities of biological interest. As the samples are of high complexity and that some proteins could be present at very low concentrations, efficient and sensitive instruments have to be used in order to maximize peptide identification. Nowadays, capillary electrophoresis tandem mass spectrometry (CE-MS/MS) has gained interest in proteomic analysis as it is considered as complementary to the gold standard method, namely reverse phase liquid chromatography tandem mass spectrometry (RP-LC-MS/MS).

However, the coupling of CE with MS is not straightforward. Indeed, robust interface is needed in order to conserve the high-resolution in-capillary separation while ensuring a stable spray. For this purpose, optimization of basic parameters such as BGE composition was first carried out using a simple peptide mix. Several parameters were then optimized in order to maximize the sensitivity, such as the composition of the sheath liquid, the interface position and different pre-concentration approaches (stacking, dynamic pH junction and transient isotachophoresis). Finally, transient isotachophoresis (tITP) was selected among other techniques and allowed the injection of large sample volumes without sacrificing separation efficiency.

In our study, two commercialized interfaces were compared by analysing E. coli proteome digest. The coaxial sheath liquid interface (« Triple tube », Agilent Technologies) and the nanoflow sheath liquid interface (« EMASS-II », CMP Scientific) were both coupled with an IMS-qTOF-MS. Eventually, spray stability was found to be the main strength of the triple tube interface, whereas the EMASS-II interface was found to provide higher sensitivity thanks to the reduced flow rate of the sheath liquid.

Abstract Reference & Short Personal Biography of Presenting Author

Marie Gou graduated in Pharmaceutical Sciences at the University of Liège in 2018. Since then, she has been enrolled in the Laboratory for the Analysis of Medicines under the supervision of Prof. Marianne Fillet as an assistant researcher. Currently, her research topic focuses on the development of microfluidic techniques, mainly capillary electrophoresis coupled to mass spectrometry for proteomic analysis.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

ANALYSIS OF PESTICIDE RESIDUES IN WOLFBERRY AND DIETARY EXPOSURE RISK ASSESSMENT

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OBJECTIVE To assess the levels of pesticide residues in Wolfberry and evaluate the intake exposure of pesticide residues. METHODS A total of 332 kinds of pesticides in 40 samples collected from different areas were determined and dietary intake exposure risks were assessed. Risk assessment was conducted by using deterministic approach for the pesticides with detection rate over 20%. RESULTS The analysis showed that 94% of the samples contained low levels of pesticide residues, and 85% of the samples contained five or more kinds of pesticide residues. Totally 31 pesticides were detected, most of which have low or moderate toxicity. However, carbofuran which is forbidden in Chinese herbs was detected. Risk assessment found that carbofuran had the highest acute risk and chronic risk, with risk probabilities of 89.523% and 2.000%, respectively. As the values were below 100%, it was indicated that the threat of this pesticide in Wolfberry to human health is acceptable. CONCLUSION The maximum residue limit of carbofuran in Wolfberry should be established as soon as possible. In this study,deterministic model is used to assess the risk of pesticide residues in Chinese Wolfberry, with the aim to propose an appropriate assessment model for traditional Chinese medicine and provide data for maximum residue limit.

Abstract Reference & Short Personal Biography of Presenting Author

Mr. Jiandong Yu is the deputy director of the Institute for Control of Chinese Traditional Medicine and Ethnic Medicine.He received his bachelor degree in the pharmacetical analysis,Beijing University of Traditional Chinese Medicine and Pharmacy in 1991. After graduation, he worked in Institute for Control of Chinese Traditional Medicine and Ethnic Medicine (ICCTMEM), National Institutes for Food and Drug Control (NIFDC) until now. His research has been focused on quality and safety evaluation of traditional Chinese medicine.So far, he has published 49 scientific publications.He is also a member of the Chinese Medicine Quality and Safety Professional Committee and the National Standardization Management Committee.

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QUALITY EVALUATION OF WOLFBERRY FROM DIFFERENT REGIONS IN CHINA BASED ON THE STRUCTURE, YIELD AND ANTIOXIDANT ACTIVITY OF POLYSACCHARIDES

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L. barbarum (wolfberry) has been widely cultivated in China, particularly in northwest regions.However, the fruit size and taste of *L.barbarum* from different habitats are quite different.Traditionally, only the fruit of *L.barbarum* produced in Ningxia province is recorded as an authentic herb,although the detailed mechanism responsible for this remains obscure. Polysaccharides are considered major active ingredients in *L.barbarum* which is crucial for its quality evaluation. In this study, we assessed the yield, monosaccharide composition, molecular weight, and linkage type of *L.barbarum* polysaccharides (LBPs) collected from different regions of China. The antioxidant activities of LBPs were also determined as its quality indicator. Our results showed that the similarity values of monosaccharide composition and glycoside bond fingerprints were larger than 0.9,and the *M*w of the two fractions (peaks 1–2) in LBPs were ranging from 1.36×10^6 to 2.01×10^6 (peak 1), and 6.85×10^4 to 10.30×10^4 (peak 2) which indicated that the structure of LBPs were similar. However, the yield of LBPs from Qinghai province (low atmospheric temperature, high altitude) was significantly lower ($p < 0.05$) than those collected from Xinjiang and Ningxia province,which suggested that the *L.barbarum* produced in Ningxia and Xinjiang maybe more suitable as materials for medicines and functional foods.Our data also partially explain why the *L.barbarum* produced in Ningxia is recorded as an authentic herb.Moreover, the analytical methods established in this study could be used for quality control of *L. barbarum*.

Abstract Reference & Short Personal Biography of Presenting Author

Miss Ying Wang received her Master's degree in the pharmacetical analysis, China National institute for food and drug control in 2011. After graduation, she worked in Institute for Control of Chinese Traditional Medicine and Ethnic Medicine (ICCTMEM), National Institutes for Food and Drug Control (NIFDC) until now. Her research has been focused on quality and safety evaluation of traditional Chinese medicine.So far, she has published over 20 scientific publications.

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

WASTE OF PILLS: THE HIDDEN BIN

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Inappropriate disposal of unused and expired medicines is considered a global health concern today. Action based on scientific evidence at different global and local level guarantees safer environment, saving life and money. Many countries have amended laws and regulations to address the different aspects of how, where, when and what is needed to make such disposal safe. Israel has lagged behind. According to a survey done twelve years ago recycling was a practice in only 20% of household waste in Israel, which is considered the lowest rate in westernize states. Only recently a new regulation has been passed to address this policy. HMO's are obliged to deal with some aspects of disposal, but surveys show that the public lacks sufficient understanding for the seriousness of the problem and do not act enough to handle unused and expired medicines properly. Only 14% of Israelis return unused medications to the HMO's pharmacies. Compared to Europe where 15% of the public throw household and medical waste to bins or flush it down the toilet, 84% of the Israeli do so. Very few articles appeared in medical databases commenting and researching on the Israeli experience in this field. The Israeli news-papers have addressed this phenomenon from different perspectives, trying to educate the public and recruit for action from citizen/pharmaceutical industry/ and government. Medical journalism with features of medical writing (medical communication) took the role of bringing the message to the Israeli public. Medical writers are ethically and technically skilled to effectively communicate information to the audience. In this research I review the work of medical writers, who are referred to in Israel as "reporters of health affairs". I will focus on their reports on addressing the topic of disposal of unused and expired medicines, specifically discussing their attributions in raising awareness towards this topic in Israel. I will present the knowledge on this topic, regulations, and the available research done in the field of disposal of unused and expired medicines in Israel. The aim is to map the process of writing a report on this topic and suggest ways on how to cover the gap between regulations and reality.

Abstract Reference & Short Personal Biography of Presenting Author

I am a medical writer devoted to the interest of public health. Safe environment can save lives and money. As a teacher, researcher, manager and medical writer I believe you can make it when you will know, act and express your concern of accumulating unused and expired medications. Getting rid of drugs waste can be challenging, I am here to give you the information you need to handle this properly. It is for me a first priority to channel the right word to the public. The message should reach its address to make an impact.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

MULTI-RESIDUE METHOD FOR THE DETERMINATION OF RESIDUES OF NITROIMIDAZOLES, TETRACYCLINES, SULFONAMIDES AND TRIMETHOPRIM IN HONEY BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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Nitroimidazoles, tetracyclines, sulfonamides and trimethoprim are a group of antibacterial compounds that have been applied in apiculture to prevent and control bacterial and protozoan diseases such as American foulbrood, European foulbrood and nosemosis. However, the use of antimicrobial substances in commercial beekeeping is not allowed in the European Union because there are no Maximum Residue Limits (MRLs) for these drugs in honey. Thus, illegal use of antibacterials in beekeeping could result in an accumulation of their residues in bee products, including honey, and pose a threat to human health. Therefore, to carry out official control, we have developed a multi-residue method for the determination of 12 nitroimidazoles (metronidazole, hydroxymetronidazole, dimetridazole, ronidazole, hydroxydimetridazole, ipronidazole, hydroxyipronidazole, carnidazole, ornidazole, secnidazole, ternidazole, tinidazole), 7 tetracyclines (oxytetracycline, tetracycline, chlortetracycline, doxycycline, 4-epi-oxytetracycline, 4-epi-tetracycline, 4-epi-chlortetracycline), 16 sulfonamides (sulfaguanidine, sulfacetamide, sulfathiazole, sulfadiazine, sulfapyridine, sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfameter, sulfamonomethoxine, sulfachloropyridazine, sulfadoxine, sulfamethoxazole, sulfaquinoxaline, sulfadimethoxine, sulfaphenazole) and trimethoprim in honey.

The extraction of the analytes from honey included an acid hydrolysis step with 1 M hydrochloric acid to release the sugar-bound sulfonamides followed by adjustment to pH 4 and clean-up on a Strata X cartridge using solid phase extraction (SPE). The obtained extract was evaporated to dryness, reconstituted in 0.1% formic acid and analysed by a liquid chromatography-tandem mass spectrometer (LC-MS/MS) system with positive electrospray ionization operated in MRM mode. The separation of analytes was performed on a Luna 3µ, C18(2) 100A, 150 x 2 mm analytical column using 0.1% formic acid and 0.1% formic acid in acetonitrile as mobile phases with gradient elution. The proposed multi-residue method was successfully validated according to the EU Commission Decision 2002/657/EC requirements and all validation criteria were in the required ranges. The decision limits (CCα) and detection capabilities (CCβ) for all tested analytes ranged from 2.12 to 2.98 µg/kg and from 2.13 to 3.47 µg/kg, respectively.

Acknowledgements

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Abstract Reference & Short Personal Biography of Presenting Author

Dr. Kamila Mitrowska graduated in medical analytics from the Jagiellonian University Medical College in Krakow in 2002. Since then, she has been working at the National Veterinary Research Institute (PIWet) in Pulawy, where she obtained her Ph.D. degree in veterinary sciences in 2006 and was promoted to associate professor in 2015. She was a post-doc fellow at the Institute for Reference Materials and Measurements (IRMM) in Geel from 2008 to 2010. Currently, Dr. Mitrowska is the First Deputy Head of the Department of Pharmacology and Toxicology of PIWet in Pulawy which acts as the National Reference Laboratory (NRL) for veterinary drug residues and chemical contaminants in food of animal origin. Her research is mainly focused on applications of liquid chromatography coupled to mass spectrometry especially on food safety control and pharmacokinetics of veterinary drugs.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

THE LIGAND BINDING AFFINITY OF AN ANTITUMORAL RU-COMPLEX WITH CALF-THYMUS DNA AS REVEALED BY NMR SPECTROSCOPY

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Antônio Gilberto Ferreira, *Department of Chemistry, Laboratory of Nuclear Magnetic Resonance, , Federal University of São Carlos, São Carlos, Brazil*

Metallodrugs, such as cisplatin and carboplatin, are traditional chemotherapeutics widely used, based on their strong interaction with DNA, possibly destroying its structure, but without selectivity. In this context, the search for other successful and selective metallodrugs plays an important role. Recently, a ruthenium complex, [Ru(law)(dppb)(bipy)] (law = lawsone, dppb = 1,4-bis(diphenylphosphino)butane and bipy = 2,2`-bipyridine), named as CBLAU, was prepared with this purpose. The cytotoxicity for this drug candidate against the tumor cell lines DU-145 (prostate cancer cells), MCF-7 (breast cancer cells), A549 (lung cancer cells) founds consolidated in literature as well as its potential to induce the tumor cells apoptosis. Therefore, our goal is to bring to light the most accessible interaction sites for this CBLAU Ru-complex towards to calf-thymus DNA (ct-DNA, here selected as the macromolecular target). For this purpose, advanced NMR binding-target approaches, among them Saturation Transfer Difference (STD)-NMR . The results reveal that the aromatic protons exhibit closer proximity to ct-DNA, in comparison with the other types of protons, as expected, given the aromatic character of nitrogen bases. This finding points out that this intermolecular interaction takes place through a spatial π-π stacking. The epitope map shows a shorter contact for dppb, whilst for bipy and lawsone groups this contact are longer. The epitope map obtained for the non-complexed lawsone also shows an efficient intermolecular binding to ct-DNA for the same aromatic protons, reinforcing the relevance of the π-π stacking role on this interaction. Moreover, looking at the binding efficiency, it was observed a stronger interaction (KD = 1.3 mM) for CBLAU/ct-DNA in comparison to individual lawsone/DNA (KD = 128.9 mM). Therefore, this outcome NMR results reveals, at atomic level, the binding affinity interaction for CBLAU towards ct-DNA and provides a guideline for further frontier researchers to search new metallodrugs as chemotherapeutics.

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Abstract Reference & Short Personal Biography of Presenting Author

The presenting author, Dr Tiago Venâncio, is Chemist by the University of São Paulo, Brazil, with his PhD in Chemistry at the same university. Recently, he was an academic visitor at The Univesity of Warwick, in the UK, working on high resolution solid state NMR of pharmaceuticals, at Prof. Steven Brown´s group. Currently he is an Associate Professor at Federal University of São Carlos, in Brazil. His main background is Nuclear Magnetic Resonance Spectroscopy, developing high resolution NMR applications to characterize organic and metallo-organic compounds as well as supramolecular complexes in solution and solid state. His group´s research is currently devoted to study supramolecular systems with pharmaceutical interests.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

HOW TO EXTRACT BOTH POLAR AND LIPOPHILIC COMPOUNDS FROM A SINGLE TUMOR SPECIMEN?

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Cancer tissue metabolomics is a powerful tool to acquire knowledge about molecular background and current state of the tumor. Along with better understanding of tumor pathophysiology, it is a way to find new therapeutic targets. However, tissue metabolomics has many challenges, starting from proper sampling through normalization process to reproducible extraction. Here, we tried to choose a proper method for sample preparation of gastrointestinal stromal tumor (GIST) samples. Most often GIST develops as a consequence of mutations in *KIT* gene; however, metabolic patterns during GIST development have not yet been investigated and elucidated.

To track changes in biochemical pathways in the cancer tissue, we chose to apply untargeted metabolomics with the use of LC-MS. The first stage of the study was focused on selecting the most appropriate sample preparation method. We aimed at developing a method that provides possibility of extracting both polar and lipophilic metabolites from one small GIST specimen. Tissue samples were homogenized with a 50:50 mixture (v/v) of methanol and waterin ratio 1:10 (w/v) and obtained homogenate was subjected to four different methods of extraction. They included monophasic extraction with methyl tert-butyl ether (MTBE), methanol and water, biphasic extraction with different ratios of MTBE, methanol and water as well as with dichloromethane, methanol and water. Organic extracts were analyzed with reversed phase LC-TOF-MS, while HILIC separation was used for polar extracts. Criteria for sample preparation method selection were based on the number of features detected in the extracts and reproducibility of extraction procedure.

The selected method will be further used in metabolomic fingerprinting of GIST tissue specimens, which can complement genomic studies on GIST or propose potential new therapeutic targets.

Abstract Reference & Short Personal Biography of Presenting Author

The presenting author, Szymon Macioszek graduated in Pharmaceutical Sciences at the Medical University of Gdansk in 2016. The same year, he started PhD studies under the supervision of Prof. Michal Markuszewski. His research interests focus on untargeted metabolomics with the use of liquid and gas chromatography coupled to mass spectrometry, as well as chemometric analysis of MS data.

HOW TO IDENTIFY THE STRUCTURE AND ANALYZE BIMODAL METABOLOMICS DATA?

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The key element of every data analysis in metabolomics should be the identification of the distribution of the generated data. This is important to consider if our goals are to understand the metabolomics data and to ensure that we can generate unbiased coefficients and mean values that generate reasonable predictions.

Metabolomics data generated from GC-MS or LC-MS experiments are usually non-normally distributed. Identifying metabolites with binomial distribution (recognized by the presence of two modes, each with a characteristic peak) is an important task in metabolomics data analysis as this distribution may occur either naturally from group separation, or may indicate problems with identification. Regardless of its origin a useful tool for the analysis is binomial regression which has the flexibility to fit various distributions.

The main aim of this study is to illustrate how to simply identify the structure of metabolomics data exemplified on 9 randomly selected metabolites using basic plots and how to model bimodally distributed data using a binomial regression (Bayesian Regression Models using 'Stan' – *brms* package). Also, “ranking and selection” was performed to identify metabolites with the greatest contribution for group separation.

ACKNOWLEDGEMENTS

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Abstract Reference & Short Personal Biography of Presenting Author

Marta Kordalewska graduated in Pharmaceutical Sciences at the Faculty of Pharmacy at Medical University of Gdańsk in 2013. Since then, she is a PhD student at the Department of Biopharmaceutics and Pharmacodynamics at Medical Uiversity of Gdańsk. Her research topic focuses on application of separation techniques (LC-MS, GC-MS, CE-MS) and bioinformatics in metabolomics.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

METHOD DEVELOPMENT FOR TARGETED ANALYSIS OF MODIFIED NUCLEOSIDES AND DEOXYNUCLEOSIDES IN URINE SAMPLES

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Modified nucleosides and deoxynucleosides are endogenous metabolites, products of RNA and DNA turnover. They are not metabolized and cannot be utilized for synthesis of new RNA and DNA molecules. They are excreted in unchanged form. Extended DNA and RNA turnover is observed in various diseases, including cancer. Consequently, a correlation between elevated level of modified nucleosides and deoxynucleosides in urine and pathophysiological disorders development can be expected. Increased level of this group of compounds was observed in such diseases as: hepatocellular carcinoma, breast cancer or urogenital cancer.
The aim of the study is the targeted metabolomics analysis of 11 modified nucleosides and deoxynucleosides in urine samples collected from bladder cancer patients with the use of LC-QqQ/MS technique. Since proper sample treatment influences obtained results significantly, the first task of the research covered the development of sample preparation procedure. This included optimization of separation conditions and solid-phase extraction procedure (SPE). Different chromatographic conditions were compared, including: type of stationary phase, flow rate, column temperature and gradient programme. According to SPE, differences in the sugar moiety between nucleosides and deoxynucleosides cause differences in their extraction ability and consequently difficulties with sorbent selection that allows for the extraction of nucleosides and deoxynucleosides at once. Previously, nucleosides and deoxynucleosides were more often analyzed separately. The goal was to develop method for simultaneous extraction of nucleosides and deoxynucleosides from urine matrix. Different sorbents were evaluated by their selectivity, recovery and ability to extract modified nucleosides and deoxynucleosides. Method based on selected sorbent was further optimized.

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Abstract Reference & Short Personal Biography of Presenting Author

Małgorzata Patejko is a PhD student at the Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Poland. She is doing her PhD under the supervision of Dr Danuta Siluk. Her scientific experience includes the untargeted and targeted metabolomics analysis. During her PhD thesis she mainly focuses on the quantitative analysis of modified nucleosides and deoxynucleosides in urine and plasma matrices in order to evalute the potential role of this group of compounds as a biomarker of bladder cancer development.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

SYSTEMATIC OPTIMIZATION OF ANALYTICAL PROCEDURES FOR THE UNTARGET METABOLOMICS PROFILING OF HUMAN PLASMA FOR ANALYSIS OF INSPIRATORY MUSCLE TRAINING IN HEALTHY MEN

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The untargeted metabolomic approach constitutes one of the most frequently applied method in metabolomics studies. It aims to measure the comprehensive metabolomic profles of various biological samples to discover novel biological markers as well as to gain new insights into mechanisms underlying the pathophysiology of human diseases. However, due to the diversity of physicochemical properties and concentration range of metabolites present in biological samples, the use of complementary analytical techniques is required to provide proper metabolome coverage. Among currently available analytical platforms, liquid chromatography coupled with mass spectrometry (LC-MS) allows determination of the highest number of metabolites. Given the fact that the successful application of untargeted metabolomics relies on eficient determination of the widest possible range of metabolites in biological matrices, chromatographic separation and sample preparation constitutes crucial steps in the analytical workfow since it may affect metabolite content, data quality and interpretation of any obtained results. Therefore, the scope of the present work was to study different conditions for sample preparation and chromatographic separations of human plasma from healthy men submitted to inspiratory muscle training (IMT). Inspiratory muscle training (IMT) has been extensively studied in healthy individuals and patients with chronic respiratory diseases, but the efficacy of this intervention remains controversial. Although IMT can reduce dyspnea during both performance-based and maximal incremental exercise tests in healthy subjects, the physiological mechanisms for this improvement have not been adequately studied. Serum samples from healthy males were used for method optimization in order to obtain the best resolution and detection of multiple compounds. The effect of chromatographic factors including stationay phase, temperature, pH, mobile phase compositions and sample preparations were investigated. DryLab as a traditional HPLC simulation software was used to find the best condition for separation of a wide variety of compunds. Finally, the results of optimization approach and multivariate curve resolution coupled with experimental design were obtained and compared. The optimized HILIC and RPLC analytical procedures were complementary and when combined they greatly expanded the possibilities for metabolome coverage compared with RPLC alone. We, therefore, believe that using optimized analytical procedures including analytical separation and sample preparation will enable more comprehensive untargeted metabolic studies of human body fluids and thus will be of great value for monitoring of metabolic markers and for providing insights into human physiology including IMT.

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Abstract Reference & Short Personal Biography of Presenting Author

Dr. Oliveira is a Senior Research Associate Professor at the Chemistry Department of the Federal University of São Carlos, Brazil where she supervises Senior Researchers, Graduate students, and leads a Bioanalytical Research Laboratory. She works and collaborates on projects including qualitative and quantitative analysis of small and large molecules, natural products, biologics molecules, and biomarkers.

Her main research interest includes drug metabolism, metabolomics, bioanalytical methods for small and large molecules, mass spectrometry, biomarkers assays and cutting-edge bioanalysis methods by LC-MS/MS at the intersection of Analytical Chemistry with Biology, Biochemistry and Chemistry.

Dr. Oliveira joined the National Institute of Health, National Institute on Aging (Baltimore, MD), as a Postdoctoral Research Fellow to investigate new approaches for drug discovery using on-line screening of target molecules and bioaffinity chromatography. She also worked as a Visiting Research Scientist at Quintiles Inc (Ithaca, NY) and acted as a Senior Research Investigator at Bristol-Myers Squibb (Princeton, NJ) in the Pharmaceutical Candidate Optimization Department, where she worked with MS-based assay development for exploratory biomarkers in PK/PD and toxicology studies, collaborating to Discovery and Clinical Research activities.

She has authored or co-authored over 100 journal articles, book chapters and oral/poster presentations and has served as editorial board for the Journal of Pharmaceutical and Biomedical Analysis.

TWO STATIONARY PHASES PREPARED BY NATURAL ACTIVE INGREDIENT DIOSIN AND APPLICATION IN TCM QUALITY CONTROL AND CHIRAL SEPARATION

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Considering that herbal medicine processes various medical functions and characteristic natural structures, the research on designing, developing and applying new chromatographic separation materials while taking advantage of these features has a great significance on the advance study and exploiting of the rich resource of natural products in China. This subject is also a valued topic in the field of chromatography and its relative applications.With the help of this theory, some stationary phases with ligands extracted from medicinal plants had been prepared. They are quick and highly selective for structure similar compounds. Besides, modifier or derivative natural products can be bonded to the surface of silica gel to prepare HPLC chiral stationary phases (CSP). This type of HPLC CSPs were proven to be highly efficient.

We applied the “One-pot method”, choosing the natural active ingredient dioscin, namely Polyphyllin III, an important ingredient for the synthesis of various steroid hormone drugs, bonded to the surface of silica gel to get dioscin stationary phase (D), then two derivative stationary phases were prepared, namely the phenyl isocyanate-dioscin bonded silica stationary phase (Phe-D) and the 3,5-dimethyl phenyl isocyanate-dioscin bonded silica stationary phase (DMP-D), respectively.

The application of Phe-D and DMP-D were evaluated by achiral and chiral analytes. Gongxuening capsule, a widely used traditional chinese medicine(TCM) which to treat gynecological disease and the main active ingredients are Polyphyllins. Because of the good separation ability for similar compounds, the HPLC fingerprint analysis for 10 batches of Gongxuening capsule, were conducted by Phe-D and DMP-D, respectively. Meanwhile, the Phe-D and DMP-D produced accurate results when used respectively to measure the content of Polyphyllins in the Gongxuening capsule. Amino acids were chosen as the chiral analytes and the Phe-D and DMP-D also exhibited certain resolution ability for the chiral separation of 10 α-amino acids including Lysine, Leucine, Cysteine, Arginine, Isoleucine, Threonine, Serine, Valine, Alanine and Histidine. These results indicated that the two self-made natural product stationary phases, Phe-D and DMP-D had the potential application in quality control of TCM and chiral separation as well. It offers a new application choice for natural products in China.

Keywords: self-made natural product stationary phase, dioscin, quality control of TCM, chiral separation.

Abstract Reference & Short Personal Biography of Presenting Author

Baochun Shen is a professor of pharmaceutical analysis at Kunming Medical University, Kunming, Yunnan Province, China. She received her PhD degree in analytic chemistry in Zhejiang University in 2007. She visited Georgia Institute of Technology in 2012 as a visiting scholar and visited GEORG-SIMON-OHM University of Applied Science in 2005 as a visiting student. She has been a Full Professor since 2014.

Professor Shen’s research activity essentially concerns the enantioseparation of chiral drugs and the preparation of chiral stationary phases. She selected antibiotics, diocin, binol as chiral ligand to prepare nearly 30 chiral stationary phases (CSP). These self-made CSPs showed good enantioseparation ability on

amino acids, amino alcohol, chiral quinolones, etc. Furthermore, prof. Shen focus on the preparation of materials for purification, such as molecular imprinted polymers. Professor Shen also engages in pharmacokinetics of chiral drugs. The results of her research have been published over 40 publications and obtained three authorized patents.

In recent 5 years, prof. Shen finished a youth project funded by National Natural Science Foundation of China (NSFC). She hosts a NSFC project, a Science and Technology Planning Project of Yunnan province right now.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

N-GLYCOSYLATION ANALYSIS OF THERAPEUTIC GLYCOPROTEINS BY MULTICAPILLARY GEL ELECTROPHORESIS

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As glycomics research is gaining momentum in the biopharmaceutical industry, there is an increasing need for reproducible high throughput glycoanalytical methods to monitor and characterize the N-glycosylation of therapeutic glycoproteins. Since the glycosylation pattern plays a key role in the biological activities of glycobiotherapeutics, e.g. the effector function, the anti-inflammatory properties and serum half-life can be affected by the glycosylation changes at the conserved Asn297 site of the CH2 domain of the heavy chain. So there is a demand for fast and efficient bioanalytical techniques for glycosylation analysis. In this poster we report on the simultaneous analysis of nine APTS labeled N-linked partitioned glycan libraries including high mannose, afucosyl biantennary, fucosyl biantennary and six sialylated types using multicapillary gel electrophoresis. The separation of the partitioned N-glycan libraries containing the most frequently occurring carbohydrate structures the biopharmaceuticals was presented. Based on the result, a new glucose unit (GU) database was generated. The database was then used to identify the carbohydrate structures of two high profile glycosylated therapeutic proteins, adalimumab (Humira) and etanercept (Enbrel).

Abstract Reference & Short Personal Biography of Presenting Author

Csenge Filep is a PhD student at Horvath Csaba Memorial Laboratory of Bioseparation Sciences in University of Debrecen, Hungary. She graduated with bachelor's and master's degree in biochemical engineering at the University of Debrecen. During her MSc studies, she used molecular biological methods to examine chelidonine interferes with IL-6R/STAT signaling in human uveal melanoma cells. She joined HLBS in 2018 and she is about to analyse formalin-fixed and paraffin-embedded breast cancer tissue samples by capillary electrophoresis. In addition, her work is focused on sodium dodecyl sulphate gel electrophoresis.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

POST- EXPOSURE MONITORING OF CHEMICAL WARFARE AGENTS USING DRY BLOOD SPOT SAMPLING AND LC-MS/MS ANALYSIS

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The ongoing large scale use of chemical warfare agents (CWAs) in armed conflicts and terror attacks stresses the continuous need to detect CWA markers in body fluids shortly after exposure. However, there is a real difficulty to detect G-type nerve agents in the blood since they are highly reactive (especially Sarin) and rapidly metabolize in the body. We present a simple, easy and fast method for sample preparation for minute amounts of whole blood followed by LC-MS/MS analysis for the determination of phosphonic acids and “free” G-type nerve agents.

Two novel sample preparation methods were developed in our lab. One for the determination of phosphonic acids which are the direct and immediate metabolites of organophosphorus chemical warfare agents (OP-CWAs). The second is for the determination of free G-type nerve agents by in situ derivatization on the Dry Blood Spot (DBS) paper. Both methods were implemented in Sarin in-vivo experiments. The detection limits of the phosphonic acids in whole blood were 0.3-1 ng/ml and for the free G-type nerve agents were 0.7 ng/ml in whole blood. The DBS paper was proven to be a preservative matrix, as the phosphonic acids were stable for over 35 days, and the derivatized G-type nerve agents were stable on it for 20 days. Linear response was obtained with R^2=0.99 for all compounds in the range of 3-100 ng/ml for the metabolites and 3-300 ng/ml for the free g-type nerve agents. These two methods were implemented in two Sarin in-vivo experiments with rats and mice. It was applied for the time coursed determination of isopropyl methylphosphonic acid (IMPA, Sarin hydrolysis product) and “free” GB in the blood. IMPA was successfully detected even with samples drawn 24 hours after rat exposure to $1LD_{50}$.

Abstract Reference & Short Personal Biography of Presenting Author

Lilach Yishai Aviram is a researcher at the Israel Institute for Biological Research (IIBR). She received her Ph.D. in analytical chemistry under the supervision of Prof. Grushka at the Hebrew University in Jerusalem, Israel. Her current research interests are in targeted and non-targeted analysis for the detection and identification of trace amounts of drugs and small molecules in various complex matrices by mass spectrometry methods.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

INFLUENCE OF GLYCO-OXIDATION ON HUMAN SERUM ALBUMIN STRUCTURE IN THE PRESENCE OF PALMITIC ACID

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Human serum albumin (HSA) is a main carrier protein that transports endo- and exoge-nous substances. HSA is exposed to numerous in vivo structural modifications that affect its stability, activity and lead to disorders of physiological functions. Acumulation of Ad-vanced Glycation End products (AGEs) and Advanced Oxidation Protein Products (AOPPs) cause the development of many diseases, including cardiovascular disease, Alz-heimer's disease or renal failure [1]. In diabetic patients, when hyperglycaemia and oxida-tive stress coexist, the glycation and oxidation processes are intense – mutually affecting each other they intensify their adverse effects. Structural changes of glycated and oxidized albumin are crucial in therapy planning and the nature and strength of ligand interactions with their main distributor may change during progressive disease. Binding of fatty acids (FAs) to albumin also influences its conformational changes. FAs have strong affinity to IIIA subdomain blocking the binding of drugs to this site [2]. Albumin structural modifications caused by glycation and oxidation were investigated using spectroscopic techniques. Because palmitic acid (PA) is considered to be a factor in the development of heart disease and cancer, the influence of PA on macromolecule con-formation has been assessed. Based on absorption and emission spectra and their second derivatives, glycation and oxi-dation have been found that cause the changes in the region of phenylalanine (Phe), tyro-syl (Tyrs) and tryptophanyl residues (Trp-214) of albumin. These processes alter the struc-ture of HSA binding sites, influencing the pharmacokinetics of particles (including drugs) transported by albumin. Obtained results point that PA affects the changes in polarity in the region of tryptophanyl and tyrosyl residues of macromolecule (enhances the increase in polarity for glycated and decrease in polarity for oxidized HSA). In addition, the amount of free sulfhydryl groups –SH of glycosylated, oxidized and proteins modified by the presence of palmitic acid show differences. The study also proved the increase in the amount of AGEs in the presence of palmitic acid. It is therefore justified to limit the supply of palmitic acid in patients with diabetes and to reduce the consumption of products con-taining glucose-fructose syrup. During diabetic therapy possible disorders in the associa-tion of drugs with albumin should be taken into account.

Key words: Human serum albumin (HSA), glycation, oxidation, palmitic acid.

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

EFFECT OF HUMAN SERUM ALBUMIN AGGREGATION/FIBRILLATION ON ITS TRANSPORT ABILITY

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Human serum albumin (HSA) is an important transport protein of the blood. HSA secondary and tertiary structure can be induced by modifications, i.e. glycation, oxidation, aggregation/fibrillation and aging resulting in changes of albumin functions, i.e. transport of ligands.
Sudlow's site I and II are a primary HSA binding sites. They have a very specific spatial structure allowing the binding of individual ligands. The most important markers for Sudlow's site I and II are dansyl amono acids, dGlu (dansyl L-glutamine) and dPro (dansyl L-proline), respectively. Phenylbutazone (Phb) and ketoprofen (Ket), nonsteroidal antiinflamatory drugs (NSAIDs), are also the most popular I and II binding sites markers, respectively.
A common protein secondary structure unit is the α-helix. Despite the α-helical construction, it was observed that HSA can form aggregates. HSA aggregation/fibrillation are the results of albumin molecule transformation by changes of pH and environmental temperature from one isomeric form to another (depending on the pH, HSA can occur in five isomeric forms – from E (expanded, < 3.5) to A (aged, > 9.0)). HSA forms aggregates/fibrylates that are similar to those made of in vitro amyloids at physiological and acidic pH. The aggregates/fibrylates differed in structure from HSA monomers and dimers, and the most important change was the appearance of previously absent β-structural constructs. Thioflavine T (ThT) is widely used benzothiazole dye that quantify in vitro amyloid fibrylates [1]. The phenomenon of protein aggregation/fibrillation results from the partial unfolding of the tertiary structure and from the conformational changes of the secondary structure [2]. The first stage of thermal aggregation relies on partial disorder of the native conformation. Due to these certain specific regions, such as hydrophobic sites or free
-SH groups become more exposed to new intermolecular interactions and contribute to the formation of aggregates/fibrils [3]. Protein aggregation/fibrillation would affect its transport ability leading to metabolic diseases.
The qualitative and quantitative aspects of the effect of HSA aggregation/fibrillation on transport ability have been studied employing a combination of fluorescence, UV-VIS and CD spectroscopies. Measurements were performed for freshly prepared HSA (HSAFR) and HSA incubated (HSAINC), both in the presence of Thioflavine T (ThT) for amyloid fibrylates identification, dGlu and dPro for the analysis of I and II binding sites structural changes and Phb and Ket for the study of NSAIDs association to aggregated/fibrillated albumin. It can be observed that HSA aggregation/fibrillation influences on albumin structure resulting in changes of binding sites obtained based on the decrease in association constantsc.

Acknowledgements: This work was supported by Grant KNW-1-033/N/9/O from the Medical University of Silesia, Poland.

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30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

EVIDENCE OF THE INTERACTION BETWEEN CYCLIC SILOXANES (SILICONES) AND STRATUM CORNEUM COMPONENTS

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Background

Cyclic siloxanes (silicones) are ingredients of many products applied to the skin. Considering their physicochemical properties, they may interact with the outermost layer of human skin, *stratum corneum* (SC) components. The resulting changes may affect barrier properties of this structure, thus exposing human to the increased penetration of not only medicinal substances, but also harmful or cumulative substances, causing adverse health effects.

In the context of the environmental pollution with silicones, and massive scale production [1], determining interaction type and associated risk of reducing the SC barrier is important, but not yet explored issue.

Objectives

Verification of D4, D5 and D6 impact on SC components' alterations as a result of interactions was determined by thermal analysis, DSC. To confirm obtained results, an alternative methods: ATR-FTIR and fluorescence microscopy, were used.

Methods

The research material was *ex vivo epidermis*, isolated with heat-separation technique (used in microscopy and ATR-FTIR studies) and in DSC study, *stratum corneum* isolated from epidermis by trypsinization was used.

The results of the test samples analysis (after silicones application), have been compared to the test samples (application omitted).

Results

Interactions at 2 levels of SC organization were observed:

- structural, evaluated by fluorescence microscopy and

- molecular, examined by ATR-FTIR and DSC.

In fluorescence microscopy, disorders in SC structure were observed, confirming siloxanes destructive acting.

DSC studies provided evidence of lipid conformation changes, observed as a shift in the maximum temperature of endotherms. Statistically significant changes in enthalpy values were also observed.

Except of confirming these results, the ATR-FTIR provided evidence of proteins conformation alterations, observed as shifts of individual bands' wave numbers on the spectra.

Conclusions

D4, D5 and D6 interact with SC components, disturbing it, which was confirmed by all of used research methods.

Literature:

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Abstract Reference & Short Personal Biography of Presenting Author

Dagmara Bazar obtained the Master of Pharmacy title in 2018, after graduating from Medical University of Gdańsk. From the first year of studies, she had actively participated in the organization of promoting healthy lifestyle and disease prevention events within the student association 'Young Pharmacy', later transformed into Polish Pharmaceutical Students' Association. Dagmara was also a member of the 'ISPE Industrial Pharmacy' scientific circle.

Currently, she is in second year of PhD studies at Medical University of Gdańsk and is a member of the research team, led by the Krystyna Pieńkowska, PhD., D.Sc. The subject of doctoral thesis is verification of cyclic and linear silicones' impact on the human skin structures and components. The object of her interests is also transdermal permeation and impact of xenobiotics, skin conditions and metabolism on its barrier and properties. She continually broadens the knowledge in the field of pharmaceutical sciences, especially skin care and dermal disorders treatment. Privately, art and tennis lover.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

A SYSTEMATIC APPROACH FOR PREVENTING THE LOSS OF HYDROPHOBIC PEPTIDES IN SAMPLE CONTAINERS

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Loss of proteins and peptides in the sample container before an LC-MS analysis is common, most notably at low analyte concentrations. A few methods to mitigate the problem are known, but they are effective only within limited conditions and may be incompatible with LC-MS analyses. We review the factors that affect protein and peptide losses and show how they can be reduced without compromising downstream LC-MS analyses. The sample matrix, such as the percentage of the organic solvent and the additive, the duration of storage, the analyte concentration, and the properties of the container surface were found to be the most important factors in achieving maximum recovery of challenging proteins and peptides.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

SMALL SCALE NATURAL PRODUCT ISOLATION USING UPLC WITH MASS-DIRECTED PURIFICATION

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Throughout history, the benefits of adopting natural remedies for a variety of health-related maladies has been attractive, as the plants from which these therapeutics are obtained are often readily available. Plant flavonoids, with their diverse biological properties, can be used as antiallergenics, antivirals, and anti-inflammatories, or possess heart-protective vasodilating properties. To fully understand the impact of specific flavonoids in living systems, material needed for experimental studies must often be isolated from very complex matrices. Although traditional isolations have usually been accomplished using extraction followed by chromatography with UV detection, this long and arduous process is riddled with ambiguity due to the lack of specificity in target compound identification. Tedious workup of fractions collected by UV-only detection requires time and resources. Mass detection, with its high specificity and sensitivity, readily discriminates the product of interest from complicated sample mixtures, thereby reducing downstream sample processing time. With improvements in column stationary phases and analytical instrumentation, good separations are more easily realized. The fluidically-optimized flow path of the UPLC combined with a specially-designed low dispersion fraction collector enable the mass-directed isolation of sharp, narrow product peaks. In this study, we illustrate the utility of the ACQUITY UPLC H-Class and Waters Fraction Manager-Analytical (WFM-A) Systems for the analysis and isolation of a natural product at the small scale. Fast, targeted isolation increases purification efficiency by reducing unnecessary sample handling while generating just enough product for future experiments.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

THE EFFECTS OF SFC PREPARATIVE SCALE-UP ON THROUGHPUT, PURITY AND RECOVERY OF AN IMPURITY IN AN API MIXTURE

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Scale-up of SFC analytical methods to preparative scale allows laboratories to generate purified bulk quantities of target compounds. In some laboratories, users are provided with an analytical scale method from which an isolate of a specified purity and quality must be generated within strict timelines. The success of achieving this task depends directly upon the accuracy of the scale-up procedure. In this poster we will describe the preparative scale-up of an analytical scale method for isolation of milligram (mg) to gram (g) quantities (per run) for a mixture of an API and its associated impurities. A cost and time analysis is provided after scale-up to demonstrate the relationship between column size and throughput.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

MEETING THE CHALLENGES OF IMPLEMENTING ACCURATE-MASS MASS SPECTROMETRY FOR BIOTHERAPEUTIC DEVELOPMENT IN REGULATED/NON-REGULATED ENVIRONMENT

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Despite the widespread practice of exact-mass mass spectrometry (MS) analysis in the biotheropeutics development processes, experienced MS users are typically required to manage the instrument operation, data processing, and interpretation. There are major challenges for the adoption of MS by analysts who are more familiar with optical detection methods, such as the inherent complexities of MS technology, and the lack of compliance-ready informatics platforms that are capable of converting raw MS data into product quality attribute results seamlessly. In this study, we report the development of a new high performance bench-top orthogonal acceleration time-of-flight (oa-TOF) LC-MS system with simplified and optimized operation modes to deliver automated, accurate, and reproducible mass measurements for proteins, peptides, and released glycans.

The assessments of the performance of the system were conducted under 3 major workflows, intact mass (including subunits) analysis, peptide mapping and monitoring and released glycan analysis of various biotherapeutic samples. Data acquisition, processing and reporting of the experiment results were all achieved within a single workflow-centric compliance-ready informatics platform.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

MODERNIZATION OF USP MONOGRAPHS FOR NAPHAZOLINE HYDROCHLORIDE AND PHENIRAMINE MALEATE OPHTHALMIC AND NASAL SOLUTIONS

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The United States Pharmacopeia (USP) monographs provide standardized procedures and specifications for quality testing of pharmaceutical raw materials and finished products. USP standards undergo constant revision and updating to take advantage of new developments in methodologies and technology. To provide high-quality standards, USP is modernizing current monographs for Chemical Medicines and excipients across the compendia with new technologies, incorporating safety advancements and addressing specificity for impurity testing.

In this work, we present details of the modernization of three USP drug product monographs: naphazoline hydrochloride nasal and ophthalmic solutions, and naphazoline hydrochloride and pheniramine maleate ophthalmic solution. As the current monographs lack procedures for impurities, a single LC method was developed for the analysis of the active pharmaceutical ingredients (APIs) and their related compounds. Method linearity, sensitivity, and specificity for analysis of naphazoline hydrochloride and pheniramine maleate ophthalmic and nasal solutions are demonstrated.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

STRATEGIES TO EVALUATE AND MONITOR FORCED DEGRADATION STUDIES USING A DUAL DETECTION (UV-MS) SYSTEM

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Forced degradation studies are typically performed to understand the degradation pathway of pharmaceuticals. Given the range of impurities and their chemical and physical properties, a single detection technique may not be adequate to accurately measure all of the degradants. Specifically, when ultra-violet (UV) detection is used alone, non-chromophoric species and/or co-elutions may be missed.
To address the challenges of measuring and quantifying degradants, a dual detection system consisting of a photodiode array (PDA) and a mass detector (MS) will be used to analyze a stressed drug substance. While UV is typically used to assess and measure degradants, mass spectrometry allows for detection by an orthogonal technique and provides information to aid in characterization. For example, mass detection will be used to measure any non-chromophoric degradants that may be produced. The impact of missing degradants will be assessed. In addition, orthogonal detection will also be used to illustrate the impact of co-elutions on mass balance determinations. By assessing peak purity using both MS and UV, the final separation for the API and its degradants can be optimized to ensure no co-eluting peaks. The addition of MS information, whether for non-chromophoric species or co-elutions, will allow for a more complete evaluation and more comprehensive understanding of the degradation pathway.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

EVALUATION OF AN OPEN AMBIENT IONISATION SOURCE COUPLED TO A PORTABLE MASS DETECTOR AS A TOOL FOR THE RAPID DETECTION OF UNDECLARED ACTIVE INGREDIENT(S) IN ONLINE HEALTH SUPPLEMENTS

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Dietary supplements are used by millions of consumers to improve health, maintain wellness, or to support a more challenging lifestyle. Some of these supplements address conditions that many regard as shameful, awkward or otherwise difficult to discuss with a physician, for example, sexual dysfunction or excessive weight gain.
Consumers can sometimes choose supplements because they want a 'safe' and 'natural' alternative to drugs that are contraindicated for health reasons - such as a heart condition. Almost exclusively, FDA approved erectile dysfunction medication are phosphodiesterase type 5 (PDE5) enzyme inhibitors, e.g. Sildenafil (Viagra, Pfizer). This class of drug can be fatal when taken with nitrate vasodilators like nitroglycerin.

The DART QDa System integrates Waters ACQUITY QDa Mass Detector with the DART Ion Source from IonSense to provide a rapid, sample preparation-free solution screening of uncontrolled dietary supplements. This approach generates easy to interpret mass spectral information in seconds. For this presentation we tested a variety of dietary supplements obtained online, claiming 100% herbal treatments for a variety of conditions including impotence, obesity and rheumatism. All but one of the examples were cited on the FDA website as containing undeclared pharmaceutical ingredients. We were able to detect undeclared compounds in all cases using in-source fragmentation for additional specificity.

30TH INTERNATIONAL SYMPOSIUM PBA ON PHARMACEUTICAL & BIOMEDICAL ANALYSIS

15-18 SEPTEMBER 2019, DAN PANORAMA HOTEL, TEL-AVIV, ISRAEL

DEVELOPMENT OF A SPE LC-MS/MS METHOD FOR THE BIOANALYTICAL QUANTIFICATION OF PRAMLINTIDE FROM SERUM

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Pramlintide acetate (SYMLINTM) is a synthetic analogue of the human hormone amylin developed as an adjunctive therapy for patients with type 1 and 2 diabetes. With nearing patent expiry dates, and recent research indicating a role for amylin in Alzheimer's Disease models, interest in amylin and amylin agonists is rising.

Hydrophobic peptides such as pramlintide often suffer from non-specific binding (adsorption) to any labware samples come into contact with (plates, pipette tips, etc…). This can make method development difficult as it can lead to poor recovery, loss of analyte, and poor limits of detection. This work describes optimization and development of a selective sample preparation strategy and LC-MS/MS analysis to achieve LLOQs of 25 pg/mL from 100 µL of serum.

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