FACULTY OF PHARMACY
IN HRADEC KRÁLOVÉ
Charles University

STARSS
Specialized Team for Advanced
Research on Separation Science

FA&CE 2018 Hradec Kralove, June 25-27, 2018

Polish-Czech Symposium

(Flow Analysis & Capillary Electrophoresis)

in cooperation with STARSS

Book of Abstracts

This conference is supported by the STARSS project

(Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by the European Union.

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION,
YOUTH AND SPORTS

Flow analysis determination of arsenate using on-line extraction and gas-diffusion membrane-based separation

Vera R.[a,b], Zhang Y.[b], Fontàs C.[b], Almeida M.I.G.S.[a], Anticó E.[b], Cattrall R.W.[a], Kolev S.D.[a]

[a] *School of Chemistry, The University of Melbourne, Victoria 3010, Australia*

[b] *Chemistry Department, University of Girona, C/ Maria Aurèlia Capmany, 69, 17003 Girona, Spain.*

e-mail: s.kolev@unimelb.edu.au

A novel flow analysis (FA) system, incorporating on-line extraction and gas-diffusion cells, was developed for the trace determination of arsenate (As(V)) in drinking water. A polymer inclusion membrane (PIM), composed of poly(vinylidenefluoride-co -hexafluoropropylene) as the base polymer and Aliquat 336 as the extractant (carrier), was used for the on-line extractive preconcentration and separation of arsenate. The PIM separated arsenate from the sample, propelled for a predetermined period of time (stop-flow time) through the donor channel of the extraction cell, into the static acceptor solution located in the acceptor channel of the cell. The acceptor stream was re-started after the stop-flow time and arsenate was first reduced to arsenite (As(III)) by merging the acceptor stream with a reductant reagent stream. This was followed by arsine (AsH_3) generation using sodium borohydride. Arsine diffused across the hydrophobic microporous membrane of the gas-diffusion cell of the FA system into a potassium permanganate stream causing partial discoloration of the latter. The decrease in absorbance of the potassium permanganate stream was monitored spectrophotometrically at 528 nm. Under optimal conditions, the FA system is characterized by a limit of detection of 3 μg L^{-1} As(V), sampling rate of 2.8 h^{-1} and repeatability of 1.8% (n=5, 25 μg L^{-1} As(V)) and 2.8% (n=5, 50 μg L^{-1} As(V)). The newly developed FA method was successfully applied to the determination of arsenate in drinking water samples in the μg L^{-1} concentration range.

AutoAnalysis:

A software package for laboratory automation

Victor Cerdà[1,2]

[1] *Department of, University of the Balearic Islands. 07122 Palma de Mallorca Spain*

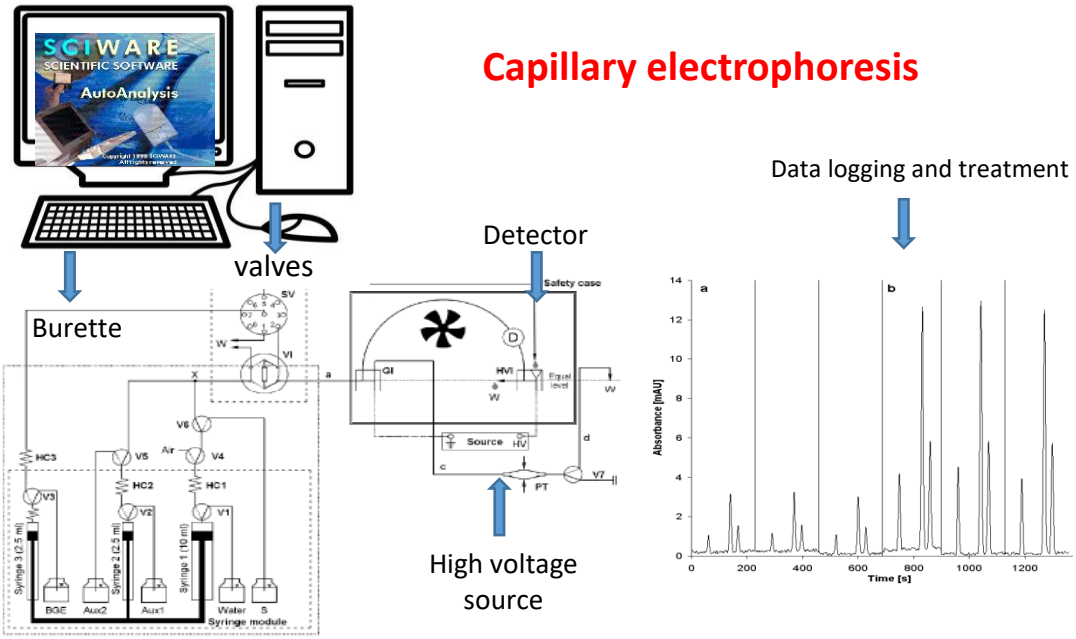
[2]*Sciware Systems, S.L. Pi 37, 07193 Bunyola. Spain*

E-mail: victor.cerda@uib.es

Flow techniques have been developed in several stages. In a first step (SFA, FIA) they were manually applied and data were represented in a paper recorder. In a second stage, with the advance of electronics and the appearance of personal computers, the situation changed completely. Thus, by means of the computer it was possible to control the liquid drivers, injection and selection valves, detectors and other devices of the system, besides being able to make the acquisition and processing of data in a more comfortable and automatic way. However, this introduced serious of difficulties in the development of new flow systems, given the inexperience of analytical chemists in connecting computers to instrumentation.

This problem has been resolved over time through the appearance of software adapted to the different flow techniques

This contribution intendeds to give a vision about the AutoAnalysis program [1], emphasizing its versatility, easy handling and possibility of application in the vast majority of flow techniques. It will also describe how to combine the different flow techniques with the large equipment of the laboratory (CG / MS, ICP-AES, ICP-MS, EC, etc.) [2]



[1] E.Becerra, A.Cladera and V.Cerdà. Design of a very versatile software program for automating analytical methods. Lab. Robotics & Automation, 11 (1999) 131-140.

[2] Piyawan Phansi, Kaewta Danchana, and Victor Cerdà. Hyphenation of flow analysis with spectrometric techniques. Applied Spectroscopy Reviews, doi.org/10.1080/05704928.2018.1446975

Capillary electrophoresis coupled with mass spectrometry: an efficient alternative for the determination of contaminant residues in foods

Moreno-González D., Lara F.J., Tejada-Casado C., Gámiz-Gracia L., del Olmo Iruela M.,

García-Campaña A.M,

Department of Analytical Chemistry, Faculty of Sciences, University of Granada,

Campus Fuentenueva s/n, E-18071, Granada, Spain

e-mail: *amgarcia@ugr.es*

The global concern about food safety has led to the development of a legal framework to control residues (e.g. pesticides and veterinary drugs), being mandatory the establishment of analytical methods achieving satisfactory performance in terms of sensitivity, identification and sample throughput. Multiresidue methods based on chromatographic techniques have been extensively applied, while sample preparation has evolved through the development of environmentally friendly procedures according to Green Chemistry. In this way, capillary electrophoresis (CE) coupled with mass spectrometry (MS) emerges as an efficient alternative, due to the development of preconcentration strategies and sensitive detectors to overcome its main limitation, the lack of sensitivity inherent to the use of small sample volume.

In this communication, we show the potential of CE-MS for the monitoring of residues of important compounds widely used in agriculture and animal husbandry, such as antibiotics, anthelmintics and pesticides. Different proposals are applied to increase sensitivity and efficiency, involving stacking techniques [1], in-line analyte concentrators [2] or the use of volatile surfactants, such as ammonium perfluorooctanoate, compatible with MS in order to apply micellar electrokinetic chromatography (MEKC) [3]. In addition, CE-MS is an excellent choice for the analysis of highly polar compounds [4], avoiding some of the drawbacks in their determination by liquid chromatography (LC), such as low retention factors or poor peak shapes. The combination of CE with off-line miniaturized sample treatments or the use of selective methodologies based on molecularly imprinted polymers make the proposed methods useful for routine analysis. The methods have been validated, demonstrating their satisfactory sensitivity and accuracy for the analysis of real samples.

Acknowledgements: MINECO (AGL2015-70708-R). FJL is grateful to the Special Research Program of the University of Granada and CTC to the grant from Project P12-AGR-1647.

[1] Tejada-Casado C., Moreno-González D., Lara F.J., García-Campaña A.M., del Olmo-Iruela M. J. Chromatogr. A 1490 (2017) 212-219.

[2] Moreno-González D., Lara F.J., Gámiz-Gracia L., García-Campaña A.M., J. Chromatogr. A 1360 (2014) 1-8.

[3] Moreno-González D., Gámiz-Gracia L., Bosque-Sendra J.M., García-Campaña A.M., J. Chromatogr. A 1247 (2012) 26-34.

[4] Moreno-González D., Lara F.J., Jurgovská N., Gámiz-Gracia L., García-Campaña A.M. Anal. Chim. Acta 891 (2015) 321-328.

New Advances in Comprehensive Glycosylation Analysis of Biopharmaceuticals

Guttman A.

Horváth Csaba Laboratory of Bioseparation Sciences, University of Debrecen,

Nagyerdei 98, Debrecen, Hungary

e-mail: *guttmanandras@med.unideb.hu*

Full characterization of the N-glycosylation moieties of biopharmaceuticals is of high importance, especially when glycovariants may impact the biological effect. Well over half of the new generation protein therapeutics are monoclonal antibodies, in which the attached oligosaccharides not only affect their physicochemical properties and stability, but also their receptor binding activity, circulating half-life and last but not least, their immunogenicity. Therefore, high performance glycoanalytical techniques are of great demand for N-glycosylation analysis of therapeutic antibodies, especially during clone selection, process development and lot release [1]. Analysis of complex carbohydrates is a very challenging task due to the lack of their chromophore / fluorophore activity and, in many instances, easily ionizable groups, necessitating derivatization before electric field mediated analysis. Full N-glycosylation characterization may also require sequencing with consecutive exoglycosidase digestion steps, followed by capillary electrophoresis analysis [2]. In this presentation, the state of the art of liquid phase separation methods will be conferred for comprehensive structural elucidation of protein N-glycosylation using capillary electrophoresis and its combination with mass spectrometry (CESI-MS). Assisted by the emerging field of glycoinformatics, assignment of the identity of the separated glycan structures will be demonstrated by using the recently introduced GUcal software [3].

[1] Guttman A., LC.GC Magazine 30 (2012) 412-421.

[2] Szigeti M., Guttman A., Nature Scientific Reports 7 (2017) 11663

[3] Jarvas G., Szigeti M., Chapman JD., Guttman A., Anal. Chem. 88 (2016) 11364–11367

CE Enantioseparations and Application to the Determination of the Stereoisomeric Purity of Drugs

Scriba, G. K. E.

Friedrich Schiller University Jena, Department of Pharmaceutical/Medicinal Chemistry, Philosophenweg 14, 07743 Jena, Germany

E-mail: *gerhard.scriba@uni-jena.de*

The importance of the stereochemistry of pharmaceutical drugs is well recognized as stereoisomers often differ in their pharmacological, toxicological and/or pharmacokinetic profile. Consequently, powerful analytical techniques are required in drug development and quality control allowing the accurate and sensitive determination of the stereoisomeric purity of synthetic drugs, natural products or pharmaceutical formulations. Apart from HPLC, 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, i.e. the formation of transient diastereomeric complexes between analyte enantiomers and the chiral selector (also referred to as the thermodynamic or chromatographic enantioselective mechanism) as well as the motility of these complexes (electrophoretic enantioselective mechanism). Both principles can cooperate or counteract each other.

The presentation will discuss the effects of analyte complexation and mobility of the analyte-selector complexes on enantioseparations in CE. The application of design of experiments (DoE) in method development for chiral drugs such as dextromethorphan and dexmedetomidine will be addressed.

Sequential injection analysis as a monitoring tool

Sklenářová H.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University,

Akademika Heyrovského 1203, Hradec Králové 500 05, Czech Republic

e-mail: *sklenarova@faf.cuni.cz*

Sequential injection analysis (SIA) was applied for sample handling in the field of the process monitoring when long-term processes were monitored in pre-defined intervals for description of detailed kinetic profiles. The kinetic profiles were built of data from on-line measurement in the SIA system using spectrophotometric or fluorimetric detection. To compare the profiles for repeated testing, three units were connected to a single SIA system. Time sequence of sampling from the respective unit was pre-set to get the comparable data points.

Applications of long-term monitoring were described for a dissolution testing of a solid pharmaceutical formulation (sustained release tablets), a liberation of an active substance from semi-solid formulations (gel and ointments) [1], and permeation studies of interaction of exogenic compounds with cell membrane transporters [2]. For liberation and permeation testing thermostated Franz cell units were applied. Donor compartment with gel/ointment sample placed on the membrane/pig skin or insert with cell monolayer was in a contact with acceptor liquid inside the Franz cell. The sampling was adjusted from the close circuit with the donor liquid circulated by means of additional peristaltic pump. T-junctions adjacent to the ports of the selection valve were used as sampling points.

The advantages of manipulation with solutions in the SIA system was applied to build fully automated flow system for monitoring of tests lasted 2-6 hours without a need of manual intervention.

Financial support of the project STARSS reg. No.: CZ.02.1.01/0.0/0.0/15_003/0000465 co-funded by ERDF is gratefully acknowledge.

[1] Klimundová J., Mervartová K., Sklenářová H., Solich P., Polášek M, Anal. Chim. Acta 576-574 (2006) 366-370.

[2] Zelená L., Marques S., Segundo M., Miró M., Pávek P., Sklenářová H., Solich P., Anal. Bioanal. Chem. 408 (2016) 971-981.

Direct-injection detectors in flow analysis

Kalinowski S., Koronkiewicz S.

Department of Chemistry, University of Warmia and Mazury in Olsztyn

Plac Łódzki 4, 10-928 Olsztyn, Poland

e-mail: kalinow@uwm.edu.pl

Introduction of solenoid pulse micropumps allows for construction of a new kind of flow analytical systems. The main elements of such systems are detectors with direct injection of sample and reagents into the chamber, which plays the role of| both the reaction and the detection chamber. The solutions are injected simultaneously in counter-current into the chamber. It results in immediate mixing of the solutions. Volume of the chamber should be larger than total volume of the injected liquids. Whole product of reaction remains inside the chamber during formation of an analytical signal. Time of analysis is determined mainly by kinetics of the chemical reaction. For fast reactions it is possible to complete the analytical signal in time shorter than 1 s, and a whole analytical cycle can be shortened to a few seconds, including a stage of cleaning the reaction-detection chamber. The second advantage of these detectors is very low consumption of solutions. Pulse micropumps have fixed volume of a single serving of solution. Typical pump volumes are: 10, 20 and 50 µl. For simple analytical systems, without pretreatment of the sample, total consumption of solutions for a single analysis is lower than 1 ml.

We constructed several kinds of detectors – single- and double-beam photometric, two kinds of chemiluminometric and potentiometric [1-7]. The photometric and chemiluminometric detectors do not require reaching the homogeneity of the mixture inside the reaction-detection chamber before reading the analytical signal. As a result, the signal can be read in short time. Depending on the kind of the detector, the volume of the reaction-detection chamber was between 60 and 280 µl. Another advantage of the detectors is very simple calibration of an analytical signal. It can be done in one cycle, with analysis of sample. Direct-injection detectors allow for construction of an automatic flow analytical systems with low power consumption. They can be applied in portable analysers and in monitoring stations, supplied by solar and wind energy.

[1] Koronkiewicz S., Kalinowski S., Talanta 86 (2011) 436-441.
[2] Koronkiewicz S., Kalinowski S., Talanta 96 (2012) 68-74.
[3] Koronkiewicz S., Kalinowski S., Talanta 133 (2015) 112-119.
[4] Nalewajko-Sieliwoniuk E., Iwanowicz M., Kalinowski S., Kojło A., Anal.Chim. Acta 911 (2016) 82-91.
[5] Kalinowski S., Koronkiewicz S., Sensors Actuators A 258 (2017) 146-155.
[6] Vakh C., Koronkiewicz S, Kalinowski S., Moskvin L., Bulatov A., Talanta 167 (2017) 725-732.
[7] Koronkiewicz S., Trifescu M., Smoczynski L., Ratnaweera H., Kalinowski S., Environ. Monit. Assess 190 (2018) 1-12.

Looking critically upon the youngest member(s) of the flow technique family

Burkhard Horstkotte[1], Manuel Miró[1,2], Petr Solich[1]

1 Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Akademika Heyrovského 1203, Hradec Králové 500 05, Czech Republic

2 FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Carreterra de Valldemossa km7,5, 07122 Palma de Mallorca, Spain

e-mail: horstkob@faf.cuni.cz

An overview is given on the transition from earlier laboratory automation towards today's applications of analytical flow approaches [1,2] and recent trends in of flow methodologies.

For this, a short overview is given on the conceptual differences of flow techniques with focus to recently developed and complementary techniques Lab-On-Valve [3] and, in particular, Lab-In-Syringe [4,5].

In the second, application niches and contributions of flow techniques to past and modern analytical chemistry are highlighted such as the development of sample pretreatment approaches, their potential for in-situ/on-site monitoring of environmental compartments or technical processes, ability of miniaturization of laboratory chemistry, the unique advantages for kinetic determinations, and the benefits of online-coupling flow methodologies with spectrometric and separative detection techniques.

Finally, a critical comparison to alternative approaches for automation including autosampler and robotic systems is given as well as an outlook on future developments including 3D prototyping and on future applications and the specific needs for improvements of flow techniques.

Acknowledgements: This work was supported by the project EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF. B. Horstkotte further acknowledges the financial support by the Czech Science Foundation by Project No. 301/17/05409S. M. Miró acknowledges financial support from the Spanish State Research Agency (AEI) through projects CTM2014-56628-C3-3-R (MINECO/AEI/FEDER, UE), CTM2017-84763-C3-3-R (MINECO/AEI/FEDER, UE), and CTM2014-61553-EXP (MINECO/AEI/FEDER, UE). We dedicate this article to the first-generation inventors and researchers of flow techniques.

[1] Zagatto E.A.G., van Standen J.F., N. Maniasso, Stefan R.I., Marshall G.D., Pure Appl. Chem. 74 (2002) 585-592.

[2] Trojanowicz M, Kołacińska K. Analyst 141 (2016) 2085-2139.

[3] Prabhu GRD, Urban PL., TrAC 88 (2017) 41-52.

[4] Růžička J., Analyst 125 (2000) 1053-1060.

[5] Alexovic M, Horstkotte B, Solich P, Sabo J. TrAC 86 (2017) 39-55.

[6] Hárendarčíková L, Baron D, Šebestová A, Rozsypal J, Petr J. Talanta 2017;174:285-288.

Investigation of acid dissociation constants and octanol-water partition coefficients of synthetic cathinones using capillary electrophoresis

Woźniakiewicz M.[1], Nowak P.[1], Gołąb M.[1], Adamowicz P.[2], Kała M.[2], Kościelniak P.[1]

1) Laboratory for Forensic Chemistry, Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Kraków, Gronostajowa 2, 30-387 Kraków, Poland

2) Institute of Forensic Research, Kraków, Westerpaltte 9, 31-033 Kraków, Poland

e-mail: *michal.wozniakiewicz@uj.edu.pl*

The abuse of designer drugs is one of the major problems of the modern society. Those substances are recreationally used by people across the Europe leading to serious intoxications, and often, fatal deceases. Among them cathinone derivatives are quite commonly met. They are still weakly characterized and the knowledge on their acidity and lipophilicity is limited and incomplete. In this work we report on the determination of pK_a and logP values for six cathinone derivatives: 2-methylmethcathinone, 3-methylmethcathinone, 4-methylmethcathinone, α-pyrrolidinovalerophenone, methylenedioxypyrovalerone and ephedrone. For that purpose we employed three capillary electrophoresis-based techniques: capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC).

In case of the pK_a determination two methodologically different CZE approaches have been compared. The standard methodology relied on measuring electrophoretic mobility across the broad pH range and fitting the sigmoidal function. The obtained pK_a values were in the range 8.59 – 9.10 [1].The alternative two-values (TVM) and one-value (OVM) methods [2], have been used herein for the first time to the cationic molecules. TVM enables estimation of pK_a using only two electrophoretic mobility values, referring to the total and partial ionization of molecules. OVM requires only a single measurement because mobility of totally ionized form could be predicted. As a whole, the analytical potential of the TVM/OVM approach seems to be huge and invaluable for fast pK_a screening/estimation or investigation of pK_a shifts induced by supramolecular interactions.

The lipophilicity determination was performed using two methodologies: MEKC and MEEKC. In both approaches, a model of retention factors (log k) of standard substances vs. their know logP values has been built, which enabled the estimation of logP of the investigated cathinones. The obtained logP values were between 1.5 to 3.3 with satisfactory agreement between both CE-based methods. However, they were different than those predicted theoretically by I-Lab/ACDLab software. This difference has been further investigated by the LC-MS method. In this experiment, the separation was performed on a C18 column in isocratic conditions and at low pH, when hydrophobic interaction played the main role in the separation mechanism. As a result, the recorded elution order of analytes was in the perfect agreement with the determined logP values.

[1] Wozniakiewicz M., Nowak P.M., Gołąb M., Adamowicz P., Kała M., Kościelniak P., Talanta 180 (2018) 193-198.

[2] Nowak, P.M., Woźniakiewicz, M., Piwowarska, M., Kościelniak, P., J Chromatogr A (2016) 149-157.

Flow analysis methods for sample extraction and separation

Chocholouš P., Šatínský D., Sklenářová H., Solich P.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University,

Akademika Heyrovského 1203, Hradec Králové 500 05, Czech Republic

e-mail: petr.chocholous@faf.cuni.cz

Flow Injection Analysis (FIA) and Sequential Injection Analysis (SIA) represent flow analysis methods used for the execution of various analytical tasks. Key features of the methods are automation, miniaturization, precision, easy operation, and longtime durability within a closed environment of manifold. The development of flow methods results either in "automated batch process" (classical methods) or "flow process" (on-line methods) with a broad potential in analytical chemistry [1].

Different approaches are used for partially or fully automated sample analysis using flow analysis methods. Sample pre-treatment is typically carried out via on-line solid phase extraction using reusable (Micro-Extraction by Packed Sorbent or particle packed) and disposable columns (Lab-On-Valve Bead Injection), respectively. Following chromatographic separation is achieved using short (30 or 50 mm of length) commercial monolithic column or fused-core particle packed column. However, the flow analysis enables carrying out multiple steps of analysis such as sample collecting, sample handling, continuous detection, and their on-line hyphenation within the analysis. Mutual compatibility of the steps is critical for high method performance. Also, the flow analysis methods can be hyphenated with other methods including HPLC, UHPLC, GC, and MS.

Typical characteristics of flow analysis-based methods, examples of various application [2,3], together with further development and opportunities will be presented.

This work was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

[1] Růžička J. Flow injection analysis, www.flowinjectiontutorial.com

[2] Grand, M.M., Chocholouš, P., Růžička, J., Solich, P., Measures, C.I. Anal Chim Acta 923 (2016) 45-54.

[3] Šrámková I., Chocholouš P., Sklenářová H., Šatínský D. Talanta 143 (2015) 132-137.

Monolithic stationary phases with layered chemistries for capillary electrochromatographic (CEC) separations of basic proteins and peptides at neutral pH

Frantisek Svec

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University,

Akademika Heyrovského 1203, Hradec Králové 500 05, Czech Republic

e-mail: svecfr@faf.cuni.cz

CEC is similar to capillary electrophoresis with the difference that the capillary is filled with a stationary phase. Several years ago, we have developed UV photoinitiated polymerization method for the preparation of generic monolithic in capillaries. The advantage of this approach carried out at room temperature is its speed and the ease of tailoring the pore size within a broad range via a simple modification of the composition of the polymerization mixture. The next step that leads to the stationary phases for CEC is photografting of pore surface of this generic monolith with chains of functional polymers. This technique is a very powerful tool enabling precise control of surface chemistry. For example, our novel monolithic columns for the rapid CEC separations of basic peptides and proteins at neutral pH were prepared by grafting the pore surface in two consecutive steps: In the first step, an ionizable monomer was grafted from the pore surface of the generic monolith while the second grafting step involved a non-ionizable, typically a hydrophobic monomer. This "two layer" grafting approach is advantageous for the preparation of highly efficient CEC columns for the desired separations since the charged functionalities, required for generation of electroosmotic flow are shielded from non-specific interactions with the analytes by an outer neutral polymer layer that also acts as the actual chromatographic stationary phase. The significant benefit of this approach compared to "classical" stationary phases for CEC is that each layer is formed independently thus enabling separate control of both EOF and retention properties of the column.

This work was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

CE-CE-MS to run compounds out of "MS-incompatible" electrolytes

Jooß K., Montealegre, C., Römer, J., Hühner, J., Neuberger S., Neusüß C.

Department of Chemistry, Aalen University

Beethovenstraße 1, 73430 Aalen, Germany

e-mail: *kevin.jooss@hs-aalen.de; christian.neusuess@hs-aalen.de*

Electromigration separation techniques require specific electrolyte compounds to achieve adequate selectivity and efficiency. These compounds, including the electrolyte itself, ampholytes, polymeric compounds, surfactants, etc. are often non-volatile. Thus, these electrolyte systems are frequently associated with high electrospray interference, impeding direct coupling to mass spectrometry (MS).

Recently, we have introduced a two-dimensional CE system applying a mechanical valve in order to couple the 1st dimension (ESI-interfering) to CZE-MS in a heart-cut approach. A mechanical 4-port valve with low-dead-volume capillary connections serves as a fully electric isolated interface transferring low volumes (4 – 20 nl) into the capillary of the 2nd dimension (CZE-MS). There, the analytes of interest are separated from co-transferred interfering constituents of the 1st dimension using an MS-compatible BGE [1].

Here, we present recent results of several applications utilizing this CE-CE-MS system: Applying a highly ESI-interfering BGE as 1st dimension (CZE-UV), it was possible to separate and characterize ascorbic acid (AA), acetylsalicylic acid (ASA) and related degradation product. For the first time, mono- and diacetylated AA could be identified as major degradation products of AA in the presence of ASA [2]. CIEF-CZE-MS enables the sensitive characterization of several proteins in a multi-heart-cut approach, including hemoglobin and its glycated form (ΔpI = 0.037) [3]. This system was expanded to iCIEF-CZE-MS for the characterization of intact charge variants of monoclonal antibodies (mAbs). Due to the high injection volume, it was possible to achieve mass spectrometric information of a minor basic variant of Trastuzumab [4]. Furthermore, a CZE-CZE-MS setup using a generic ε-aminocaproic acid (EACA) based buffer system as 1st dimension was applied for mAb characterization. Interference-free, highly precise mass data (deviation 0.4 – 0.8 Da) of intact charge variants of Trastuzumab were achieved, allowing the unequivocal determination of a deamidated antibody impurity in an on-line approach [5]. The coupling of SDS-capillary sieving electrophoresis ("CE-SDS") to CZE-MS (CSE-CZE-MS) is of major interest in the context of protein characterization. The co-injection of complexing agents and organic solvents has been incorporated in the 2nd dimension [6]. In this way, the CSE-CZE-MS approach allows, for the first time, the mass characterization of impurities of antibodies separated by CE-SDS.

All these examples demonstrate the versatility of this CE-CE-MS approach. This is of special interest for the MS-coupling of generic and validated methods, utilizing ESI-interfering electrolytes as frequently applied in the pharmaceutical context.

[1] Kohl F., Montealegre C., Neusüß C., Electrophoresis 37 (2016), 954–958.

[2] Neuberger S. *et al.*, Anal. Bioanal. Chem. 408 (2016), 8701-8712

[3] Hühner, J., Neusüß, C., Anal. Bioanal. Chem. 408 (2016), 4055-4061

[4] Montealegre C., Neusüß C., Electrophoresis (2018), DOI: 10.1002/elps.201800013

[5] Jooß K. *et al.*, Anal. Bioanal. Chem. 409 (2017), 6057-6067

[6] Sanchez-Hernandez L. *et al.*, Electrophoresis 38 (2017), 1044-1052

Advances and trends in software for electrophoresis: from data processing to computer modelling

Pavel Dubský

The group of Electrophoretic and Chromatographic Separation Methods, Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Hlavova 8, 128 43, Prague 2, Czech Republic

e-mail: *dubsky@natur.cuni.cz*

Simul, PeakMaster and CEval form a trio of publicly available software tools [1] that are traditionally developed in our research group. In this talk, we summarise recent progress in software development and demonstrate a potential of this triad for solving various scientific problems.

Simul, introduced in late 1980's, is one of the first simulators of capillary electrophoresis. It can handle any electrophoretic process from classical capillary zone electrophoresis (CZE), through isotachophoresis (ITP) or isoelectric focusing (IEF) to various preconcentration effects. The computational core is now being completely redesigned resulting in much faster and more robust simulations. This, as we hope, will make Simul a true assistant in everyday method optimizations. The former computational core was able to solve only acid-base equilibria. Complexation equilibria is newly implemented in full extent, which enables users of Simul to model affinity capillary electrophoresis (ACE) and sweeping techniques.

Among all the electrophoretic modes, CZE has the leading role in routine analysis. PeakMaster is thus dedicated to CZE. It calculates BGE properties (pH, buffer capacity, conductivity etc.) and discloses an occurrence of system peaks. The computational libraries used in Simul for the ACE mode are also newly available in PeakMaster. This allows for calculations of effective mobilities of analytes, shifts in system peak positions and pH-shifts in the BGE due to complexation.

CEval, the newest member of the family, is designed for easy data evaluations in CZE and ACE. It performs automated HVL fit, a feature essential for reading correct migration times in CZE that is however not provided by CE manufacturers. Automated evaluation of ACE data along with their advanced statistical processing is included for obtaining complexation constants and mobilities of complexes.

Financial support by the Czech Science Foundation grant no. 15-18424Y is acknowledged.

[1] https://echmet.natur.cuni.cz/software/download

Flow system and analytical strategies for immunoprecipitation measurements

Strzelak K.

Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland

e-mail: kamil.strzelak@chem.uw.edu.pl

In this contribution, the construction of flow analysis system (based on the idea of MultiCommutated Flow Analysis technique) with optoelectronic detection and analytical methodologies for immunochemical determination of proteins will be shown.

At the beginning, the basis of antigen-antibody complex formation and factors affecting such a process will be discussed. All presented measurements will be carried out with the use of the model protein - human serum albumin. Subsequently, the immunoprecipitation methodology will be presented under flow analysis conditions, focusing on the construction of both detection (nephelometric Paired Emitter Detector Diode) and MCFA systems. In the case of PEDD, the comparison between two developed detection cells will be discussed. In turn, the operation of MCFA system including particular dilution module required for quantitative analysis due to the specific characteristic of immunoprecipitation measurements will be described. The key point of this presentation will be the discussion over analytical strategies of immunological determination of low- and high-concentrated proteins, referring to ranges of proteins concentration in human sera.

Acknowledgement

The research was supported by the Polish National Science Centre (project SONATA no. 2016/21/D/ST4/00924)

Paper-based device for simultaneous determination of calcium and phosphate ions

Fiedoruk-Pogrebniak M.

Faculty of Chemistry, University of Warsaw,

Pasteura 1, 02-093 Warsaw, Poland

e-mail: *mfiedoruk@chem.uw.edu.pl*

Both calcium and phosphate ions play important roles in the human body and are necessary for its proper functioning [1]. An elevated level of phosphate ions could consequently lead to states threatening health and life of a human. Indirectly, calcium ions level could be related to the concentration of phosphate ions in body fluids. As it was pointed out [2] the concentration of one of them could not be estimated on the basis of the other easily. For this reason, the determination of both calcium and phosphate ions should be detected simultaneously.

Nowadays, carrying out reactions on the surface of the paper is very popular in view of its availability, low cost and easiness both of fabrication and usage. The simple paper-based microfluidic flow systems (named *Lab on Paper*, LOP) have been constructed by applying hydrophobic substances to design channels for reagents flow [3].

In this contribution, the idea of construction of a small, portable and low-cost device for the simultaneous calcium and phosphate ions determination is presented. Both reactions and fluorometric detections carried out using paired light emitting diodes occur in the paper matrix. Such fluorometric detectors have been used in conventional measurements under flow conditions [2] as well as for analysis performed in the paper matrix [4]. The developed microfluidic analytical system was applied for the Ca^{2+} and PO_4^{3-} determination in real samples such as serum as well as blood.

[1] Burtis C.A., Ashwood E.R., Bruns D.E., Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, fourth ed., Elsevier Health Sciences, London, 2012.

[2] Fiedoruk-Pogrebniak M., Koncki R., Talanta 144 (2015) 184–188.

[3] Carrilho E., Martinez A.W., Whitesides G.M., Anal. Chem. 81 (2009) 7091-7095.

[4] Fiedoruk-Pogrebniak M., Granica M., Koncki R., Talanta 178 (2018) 31–36.

Acknowledgments

These investigations were supported by the Polish National Science Centre (Project OPUS NCN 2014/13/B/ST4/04528).

In-line carbon nanofiber reinforced hollow fiber-mediated liquid phase microextraction using a 3D printed extraction platform as a front end to liquid chromatography for automatic sample preparation and analysis: A proof of concept

Worawit C.[a], Cocovi-Solberg D.J.[b], Varanusupakul P.[a] Miró M.[b]

[a] *Chemical Approaches for Food Applications Research Group, Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Rd., Pathumwan, Bangkok 10330, Thailand*

[b] *FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Carretera de Valldemossa, km 7.5, E-07122 Palma de Mallorca, Spainepartment of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University,*

e-mail: david.cocovi@uib.es

A novel concept for automation of nanostructured hollow-fiber supported microextraction, combining the principles of liquid-phase microextraction (LPME) and sorbent microextraction synergically, using mesofluidic platforms is proposed herein for the first time, and demonstrated with the determination of acidic drugs (namely, ketoprofen, ibuprofen, diclofenac and naproxen) in urine as a proof-of-concept applicability. Dispersed carbon nanofibers (CNF) are immobilized in the pores of a single-stranded polypropylene hollow fiber (CNF@HF) membrane, which is thereafter accommodated in a stereolithographic 3D-printed extraction chamber without glued components for ease of assembly. The analytical method involves continuous-flow extraction of the acidic drugs from a flowing stream donor (pH 1.7) into an alkaline stagnant acceptor (20 mmol L−1 NaOH) containing 10% MeOH (v/v) across a dihexyl ether impregnated CNF@HF membrane. The flow setup features entire automation of the microextraction process including regeneration of the organic film and on-line injection of the analyte-laden acceptor phase after downstream neutralization into a liquid chromatograph (LC) for reversed-phase core-shell column-based separation. Using a 12-cm long CNF@HF and a sample volume of 6.4 mL, linear dynamic ranges of ketoprofen, naproxen, diclofenac and ibuprofen, taken as models of non-steroidal anti-inflammatory drugs, spanned from ca. 5–15 µg L−1 to 500 µg L−1 with enhancement factors of 43–97 (against a direct injection of 10 µL standards into LC), and limits of detection from 1.6 to 4.3 µg L−1. Relative recoveries in real urine samples ranged from 97% to 105%, thus demonstrating the reliability of the automatic CNF@HF-LPME method for in-line matrix clean-up and determination of drugs in urine at therapeutically relevant concentrations.

Open-source hardware 3D-printed flow analysis platform for hemodialysis monitoring

Michalec M.[1,2], Tymecki Ł. [1]

[1],*Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland*
[2] *MISMaP College, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland*

e-mail: mmichalec@chem.uw.edu.pl

The Open-source hardware (OSH) movement aims at encouraging the sharing of hardware projects and designs. The core of the OSH philosophy is to give an unlimited, free of charge and free of contribution designs and hardware to the community.

Presented flow analysis platform is complete, prototype design consisting of all necessary elements: the flow manifold, miniaturized pumps and valves, programmable controller and an optical detector. All prototype elements of this design are accessible under an Open license. The manifold is a 3D-printed mesofluidic chip, which is responsible for the logic of flow analysis tasks. The chip was printed with use of widely available Fused Deposition Modelling (FDM) technique from biodegradable polylactide (PLA). The optical detector was 3D printed as a part of manifold and consist of easily available electroluminescent diodes (LED's) used as both light emitters and detectors. The analytical signal from detector diode is registered with use of uncomplicated analog-digital voltage reader, which the central element is a one-plate Arduino-compatible microcontroller. The same microcontroller is also the programming and electrical interface for the flow analysis system.

The utility of the OSH-3D-printed Flow Analysis platform was verified in real case scenario by on-site and on-line bloodless monitoring of hemodialysis therapy [1-3]. With the use of this open design and altering the optoelectronic detector, it is possible to construct flow analysis monitors of phosphate [1], creatinine [2] and urea [3] removal.

In principle, elements of the presented system can be freely composed to achieve demanded results. The microcontroller is programmable with use of Open-source programming language via the Open compiler. The chip design can be altered for specific purposes with the use of the 3D-modelling and signal is collected using both Open-source software.

This work was supported by the Polish National Science Centre; project PRELUDIUM NCN no. 2017/25/N/NZ5/01556. MM kindly acknowledges financial support from the Ministry of Science and Higher Education in Poland.

[1] Michalec, M., Fiedoruk-Pogrebniak, M., Matuszkiewicz-Rowińska, J., Tymecki, Ł., Koncki, R., J. Pharm. Biomed. Anal. 126, (2016) 9–13.

[2] Michalec, M., Tymecki, Ł. Koncki, R., J. Pharm. Biomed. Anal. 128, (2016) 28–34.

[3] Michalec, M., Granica, M., Bzura, J., Koncki, R., Matuszkiewicz-Rowińska, J., Tymecki, Ł., Sens. Actuators, B 226, (2016) 563–569.

Automated flow manifold dedicated to electrochemical analysis based on the capacitance measurements

Wieczorek M., Madej M., Starzec K., Knihnicki P., Telk A., Kochana J., Kościelniak P.

Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Krakow, ul. Gronostajowa 2, 30-387 Kraków, Poland

e-mail: *marcin.wieczorek@uj.edu.pl*

The automated flow manifold for realization of full analytical procedure of potassium determination based on the capacitance measurements has been presented [1]. A novel method for electrochemical determination of potassium is based on an innovative capacitance-to-frequency conversion method and exploits self-assembled thiol monolayers (SATMs) deposited on the surface of gold electrodes [1,2]. The terminal functional groups on the dielectric monolayer interacts selectively with an analyte, changing the thickness of the layer depending on the amount of potassium and the applied voltage, resulting in a change in the registered dielectric capacitance. However, as the designed SATMs are not completely specific large additive interference effects appear. Therefore, a new calibration approach based on the Chemical H-point Standard Addition Method (C-HPSAM), allowing both specific (proportional) and unspecific (constant) interference effects caused by sodium ions to be corrected, was developed and applied [1]. The proposed method was employed for potassium determination in highly mineralized water, juice and pharmaceutical samples without any special pretreatment.

In order to improve the analytical procedure, to make it faster and automated, the dedicated flow system was designed. The great advantage of using the proposed flow system is the possibility of performing whole analytical procedure, i.e. cleaning of the electrodes (SATMs removing process), adsorption process of SATMs and measurement signals in the calibration stage, without pulling out of the system the working electrode on each step. It makes the analytical procedure much easier and shorter. Furthermore, the volumes of each solutions needed for analysis were significantly reduced which corresponds to the principle of a green analytical chemistry.

Acknowledgments

The study was supported by Polish National Science Centre, Project 2013/11/B/ST4/00864.

[1] Wieczorek M., Madej M., Starzec K, Knihnicki P., Telk A, Kochana J. and Kościelniak P. Talanta 186 (2018) 183-191.

[2] Wieczorek M., Kochana J., Knihnicki P., Wapiennik K., Kościelniak P., Talanta 166 (2017) 383-390.

Photometric determination of iron(II) in pharmaceutical formulations using double-beam direct-injection detector integrated with multi-pumping flow system

Koronkiewicz S., Kalinowski S.

Department of Chemistry, University of Warmia and Mazury in Olsztyn,

Pl. Łódzki 4 10-727 Olsztyn, Poland

e-mail: stankor@uwm.edu.pl

Iron plays an important role in cellular processes such as respiration, synthesis of DNA and RNA, electron transport, and regulation of gene expression. Iron deficiencies leading to anemia are one of the world's most common nutritional diseases. Supplementation of diet is carried out by administering iron (II) preparations.

The aim of the research was to develop a new, automated method for the determination of iron (II) in pharmaceutical preparations. The latest version of the direct injection detector (DID) photometric detector was used in the research. In detectors of this type, the sample and reagents are injected using solenoid micropumps, in countercurrent, directly into the reaction-detection chamber. There the analytical reaction takes place and the analytical signal is read. In photometric DID detectors, both single and double beam, a system of paired LEDs was used, which was dedicated to the appropriate determination [1,2]. In the double-beam DID system, the detector's detector diodes were connected to the inputs of a LOG101 logarithm amplifier (Burr Brown, USA), which made it possible to obtain an output signal linearly dependent on absorbance.

Iron (II) was determined using the 1,10-phenentroline reaction (λ_{max} = 512 nm). The calibration graph was linear in the range from 1 to 30 mg L^{-1}. The detection limit of the method was 0.5 mg L^{-1}. The throughput of the method was 45 samples/hour. The repeatability of the method expressed as the relative standard deviation was 2% (n = 10). The method was characterized by low consumption of reagents and samples (20 μL each) and a small amount of wastewater produced (about 1 mL for one analysis).

The developed flow system was used for determination of Fe (II) in Chela-ferr biocomplex, Hemofer Prolongatum and Sorbifer Durules formulations. The obtained results were compared with the results obtained using the classic UV-Vis method (stationary conditions). T-student test indicated that the differences between the results obtained by the proposed method and the reference method were statistically insignificant at 95 % confidence level. The recovery of the developed method ranged from 93 to 107 %. The flow system discussed worked in a very stable manner and was insensitive to bubbles appearing in the system. A high level of automation and low reagent consumption were obtained. The developed system can be recommended for determinations in automatic mode, in accordance with the principles of Green Analytical Chemistry.

[1] Koronkiewicz S., Kalinowski S., Talanta 2011, 86 (2011) 436-441.

[2] Kalinowski S., Koronkiewicz S., Sensors & Actuators A, 258 (2017) 146-155.

Development of methods for two-component analysis using flow techniques

Paluch J., Wieczorek M., Kozak J.

Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Krakow,

Gronostajowa 2, 30 - 387 Krakow, Poland

e-mail: *justyna.paluch@doctoral.uj.edu.pl*

Flow techniques are attractive tools for developing novel procedures of two-component analysis because of their instrumental flexibility, the capabilities of controlling a sample dispersion and good reproducibility of results. Two-component analysis can be performed using flow systems of various configurations. The analytes can be determined with the use of e.g. dedicated modules, special kinds or various detectors, separation techniques, chemometric treatment of the registered data, or applying the approaches based on the use of various parameters of a peak registered in a flow-based mode. The systems offer the possibilities of determining analytes sequentially i.e. one after the other or simultaneously.

The aim of the presented study was to develop the simple methods for two-component analysis with the use of flow analysis systems with spectrophotometric detection. The proposed methods are based on three approaches. The first method employs an instrumental modification with a module for solid-phase extraction. The extraction was performed in-line, in a sequential-injection system with a column packed with Chelex 100, developed for a simultaneous determination of zinc and copper. The next two methods are based on the use of selected analytical signals of a single "complex peak" registered with the use of a developed sequential-injection system. Because of the mutual influence of analytes on their signals, two-component calibration was applied, in which four standard solutions, each containing both analytes on two concentration levels, were prepared according to 2^2 factorial design. The approach was employed for the determination of calcium and magnesium as well as of iron(II) and iron(III). The last of the presented methods was developed to perform speciation analysis, but it can be also applied to determine two different analytes. The method relies on simultaneous application of two calibration methods for the determination of different forms of an analyte. One of them is determined in extrapolative way, whereas the second – using the same calibration plot – in interpolative way, after its oxidation or reduction. The method was verified on the examples of simultaneous determination of iron(II) and iron(III), and of chromium(III) and chromium(VI) using Lab-In-Syringe or SIA systems, respectively.

The developed methods were verified with the use of synthetic samples and various CRM materials, and their analytical usefulness was proved by applying them to analysis of various natural samples of water and wastewater.

Sequential injection analysis and 3D-printing in screening of extraction properties of nanofibers

I. H. Šrámková, N. Štefčáková, B. Horstkotte, D. Šatínský

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University,

Akademika Heyrovského 1203, Hradec Králové 500 05, Czech Republic

e-mail: *sramkovai@faf.cuni.cz*

Polymeric electrospun nanofibers (NF) are one of the most innovative materials with versatile applicability. A large surface-to-volume ratio and a large number of binding sites make them a suitable medium to be used in medicine, e.g. for drug delivery, or as scaffolding in bioengineering [1]. Lately, due to these properties, NF have been gaining interest for application in analytical chemistry, particularly as sorbents for solid phase extraction (SPE) [2].

Sequential injection analysis (SIA) [3] is a flow technique enabling feasible sample treatment and flow manipulation. It was employed in numerous automated methods comprising sample pretreatment, both liquid phase- and solid phase-based. Sorptive materials in SIA-automated SPE are typically used in a column format, which is often related to backpressure problems and issues of column preparation.

Here, the extraction properties of polyvinylidene fluoride, polyethylene, polyamide, polystyrene, polycaprolactone and polyacrylonitrile NF were tested in a SIA system. Two formats, namely use as column filling or as a sheet, were examined.

For the latter one, a specially designed 3D-printed holder was used to accommodate a disk-shaped NF sheet with the advantages of lesser amount of NF needed and lower backpressure in comparison to the column format. The extraction characteristics were evaluated with model analytes of different physical-chemical properties including bisphenols, non-steroidal anti-inflammatory drugs, and steroidal compounds.

The suitability of using NF for SPE will be discussed. The advantages and flaws of using them either in a column or membrane format will be outlined.

Acknowledgements: This work was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF. The authors also acknowledge the support of the Czech Science Foundation through project No. 17-08738S.

[1] Wen, L., Xie, J., Current Pharmaceutical Design 21 (2015) 21 1944-1959.

[2] Płotka-Wasylka, J., Marć M., Szczepańska N., Namieśnik J., Current Reviews in AnalyticalChemistry 47 (2017) 373-383.

[3] Růžička J., Marshall G., Sequential injection: a new concept for chemical sensors, process analysis and laboratory assays, Talanta 237 (1990) 329-343.

Organic-silica sol-gel monoliths for applications in capillary liquid chromatography

Zajíčkova Z.

Department of Physical Sciences, Barry University,

11300 NE 2nd Ave, Miami Shores, FL 33161, USA

e-mail: *zzajickova@barry.edu*

Organic-silica sol-gel monoliths prepared solely from 3-(methacryloyloxy)propyltrimethoxysilane (MPTMS) attracted much attention in the early 2000s especially due to the possibility for fast *in situ* preparation via photopolymerization. The appeal of the MPTMS monomer precursor is that it contains both, organic (methacrylate) as well as silica-based (alkoxysilane) functionalities. In acid catalyzed conditions, methoxy groups of the methoxysilane undergo simultaneous hydrolysis and polycondensation forming silanols and siloxane chains. Subsequently, methacrylate functionalities undergo thermally or UV light-initiated free-radical polymerization [1, 2]. We have compared chromatographic performance of thermally (TSG) and photo-polymerized sol-gel (PSG) monoliths in the reversed-phase mode in respect to column efficiency, methylene and steric selectivity, and extent of hydrogen bonding [3]. Slightly more hydrophobic surfaces with reduced number of residual silanols and comparable steric selectivity and column efficiency (111 000 plates/m for alkylbenzenes) were found for thermally polymerized sol-gel monoliths. Overall, photopolymerization processes have many advantages over thermal polymerization. They are faster, can be stopped at selected times and monoliths can be formed selectively in certain segments of the capillary. However, better batch-to-batch repeatability was observed using thermally polymerized conditions. Recently, we have explored the feasibility of single-pot approach towards preparation of C18 and fluorinated TSG monoliths by the addition of an appropriate monomer ditectly into a polymerization mixture. Strong affinity towards alkylbenzenes, better retention and resolution for planar and non-planar analytes were displayed on C18 columns. On the other hand, fluorinated stationary phases were shown to provide enhanced retention and selectivity towards fluorinated compounds. In conclusion, the single-pot approach proved to be viable for modifying the surface chemistry of monolithic stationary phases.

[1] Dulay M. T., Quirino P. J., Bennett B. D., Kato M., Zare R. N., Anal. Chem. 73 (2001) 3921-3926.

[2] Weed A. M. K., Dvornik J., Stefancin J. J., Gyapong A. A., Svec F., Zajickova Z., J. Sep. Sci. 36 (2013) 270-278.

[3] Gharbharan D., Britsch D., Soto G., Weed A-M. K., Svec F., Zajickova Z., J. Chromatogr. A 1408 (2015) 101-107.

Sensitive micellar electrokinetic chromatography-tandem mass spectrometry using sheathless porous-tip interfacing for the determination of carbamate pesticides

[1,2]Moreno-González D., [1]Gámiz-Gracia L., [3]Haselberg, R., [4]de Jong G. J, [3]Somsen, G.W., [1]García-Campaña A.M

[1]*Department of Analytical Chemistry, Faculty of Sciences, University of Granada*

[2] *Specialized Team for Advanced Research on Separation Science (STARSS), Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic*

[3] *AIMMS Division of Biomolecular Analysis, Department of Chemistry and Pharmaceutical Sciences, Faculty of Sciences, VU University Amsterdam, The Netherlands*

[4]*Biomolecular Analysis, Utrecht University, The Netherlands*

e-mail: *morenogd@faf.cuni.cz*

The on-line coupling of micellar electrokinetic chromatography and mass spectrometry (MEKC-MS) is often hampered by incompatibility problems leading to reduced separation performance and unfavorable limits of detection (LODs). Here we propose a new selective and highly sensitive MEKC-MS/MS method employing a sheathless porous-tip interface in combination with a micellar phase comprised of semi-volatile surfactant molecules. Carbamate pesticides (CRBs) were selected as representative model compounds being neutral toxic pollutants potentially present at trace levels in environmental water samples. A background electrolyte of 75 mM perfluorooctanoic acid adjusted to pH 9.0 with ammonium hydroxide allowed efficient separation of 15 CRBs and appeared fully compatible with electrospray ionization (ESI)-MS. Interfacing parameters, such as the distance between the capillary tip and mass spectrometer inlet, ESI voltage, and dry gas temperature and flow were optimized in order to attain good spray stability and high analyte signal-to-noise ratios. For CRBs the LODs ranged from 0.2 to 3.9 ng L^{-1} (13 nL injected, i.e., 2% of capillary volume), representing an improvement for certain CRBs of more than 300-fold when compared with conventional sheath-liquid interfacing [1,2]. Good linearity ($R^2 > 0.99$) and satisfactory reproducibility were obtained for all CRBs with interday RSD values for peak area and migration time of 4.0–11.3% and below 1.5%, respectively. Analysis of spiked mineral water showed that the new MEKC-MS/MS method allows selective and quantitative determination of CRB concentrations below the maximum residue limit of 100 ng L^{-1} without the need for sample preconcentration.

Acknowledgements: DMG thanks the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

[1] Moreno-González D., Gámiz-Gracia L., Bosque-Sendra J.M., García-Campaña A.M., J. Chromatogr. A 1247 (2012) 26-34.

[2] Moreno-González D., Haselbergb R., Gámiz-Gracia L., García-Campaña A.M, de Jong G.J., Somsen G.W., J. Chromatogr. A 1524 (2017) 283-289.

Capillary zone electrophoresis of synthetic and biological nanoparticles under dynamic coating conditions

Szymon Dziomba[1], Krzesimir Ciura[2], Martyna Franczuk[3], Michalina Filipiak[3], Krzysztof Waleron[3], Bruna Correia[1], Paulina Sawicka[1], Bartosz Wielgomas[1]

Department of Toxicology, Medical University of Gdansk, 107 Hallera Avenue, Gdansk, Poland

Department of Physical Chemistry, Medical University of Gdansk, 107 Hallera Avenue, Gdansk, Poland

Department of Pharmaceutical Microbiology, Medical University of Gdansk, 107 Hallera Avenue, Gdansk, Poland

e-mail: szymon.dziomba@gumed.edu.pl

Capillary electrophoresis (CE) has recently gained an attention as an alternative technique for nanoparticles (NPs) characterization and quality assessment. Taking into account rapid development of nanotechnology, the utility of CE for NPs analysis became a widely discussed issue in analytical science world [1].

In our research we focused on various parameters affecting NPs stability during capillary zone electrophoresis (CZE). The influence of background electrolyte (BGE) composition was found to play predominant role. Using gold NPs as model analytes it was shown that BGE components can provide steric stability to particles during CE run [2]. The finding enabled to perform isotachophoresis (ITP) of gold NPs under buffering conditions. In a view of BGE composition, a correlation between particles stability during CZE and ITP was found.

The acquired knowledge was utilized in biological NPs analysis. The outer membrane vesicles (OMVs) isolates from *Pectobacterium sp.*, obtained with ultracentrifugation and filtration, were submitted to CE analysis. Similar behavior of biological and synthetic NPs during CE was observed. The data enabled to conclude on particles stability during storage, particles size, aggregation process as well as quality and abundance of obtained isolates. Additionally, the off-line analysis of CZE fractions with transmission electron microscopy (TEM) proved the identity of recorded signals in CE.

The findings indicate great potential of CE in NPs analysis. Among the advantages relatively short analysis time and quantitative information should be mentioned. Moreover, combination of CE with TEM creates a comprehensive tool for NPs analysis.

This work was supported by the National Science Centre of Poland (grant number 2016/21/D/ST4/03727).

[1] Chetwynd A., Guggenheim E., Briffa S., Thorn J., Lynch I., Valsami-Jones E., Nanomaterials 8 (2018) 99 – 127.

[2] Dziomba S., Ciura K., Kocialkowska P., Prahl A., Wielgomas B., Journal of Chromatography A 1550 (2018) 63 – 67.

The application of capillary electrophoresis to inks examination

Król M.

Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Krakow,

Gronostajowa 2, Kraków 30-387, Poland

e-mail: *malgorzata.p.krol@uj.edu.pl*

Capillary electrophoresis (CE) techniques have already been established as promising, effective and economical tools for the separation of different kind of coloring matter, e.g.: writing, stamp pad and inkjet printing inks. Methods enabling reliable ink analyses have been developed since automated CE instruments became commercially available. Inks are mixtures of a wide variety of substances that exhibit a lot of different physicochemical properties. Moreover, inks designed for particular purpose, for instance, for use in writing or printing instruments yield quite dissimilar composition. That is why, the forensic applications of CE methods for questioned document examination constantly constitute a growing research field. CE provides not only high resolution of mentioned complicated mixtures but also requires a minimal amount of sample what is essential from forensic expert point of view. Many aspects of CE and related technologies are currently undergoing rapid development, e.g. different modes of CE (CZE, MEKC, NACE, NAMEKC), different stacking processes and capillary coatings, and various kind of detectors with their different advantages (PDA, LIF, C4D, and MS). However, there are still many types of applications which should be further explored, particularly those involving microfluidic chip electrophoresis enabling on-site analysis during crime scene investigation.

Chiral separation of substituted cathinones by cyclodextrin-assisted capillary electrophoresis and examination of changes in the acid-base properties induced by cyclodextrins

Nowak P., Olesek K., Woźniakiewicz M., Kościelniak P.

Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Kraków, Gronostajowa St. 2, 30-387 Kraków, Poland

e-mail: *nowakp@chemia.uj.edu.pl*

Ephedrone, mephedrone and metaphedrone are chiral toxicologically-important cathinone derivatives. They are popular on illegal market due to the various psychostimulant and hallucinogenic symptoms observed after consumption. This raises the need for developing new analytical tools for their discrimination. Their simultaneous chiral separation is important for the toxicological and forensic analysis, however it is challenging owing to the minor structural differences restricted to the presence/position of one methyl group.

Herein we present the first analytical method allowing to enantio-separate these three important cathinone derivatives, with an excellent resolution between all enantiomers. We have discovered that 2-hydroxyethyl-β-cyclodextrin, unreported so far for separation of this groups of molecules, is a very efficient chiral selector of these drugs, better than seven other cyclodextrins. The method was calibrated and validated, pointing out that the use of internal standard improves an overall precision, and that electrophoretic mobility ratio is the best qualitative parameter. The further analytical potential of this method was also shown, by adding some other cathinone-related drugs to the sample, maintaining a good resolution between them.

In the second part we show a simple and effective method for examining the possible changes in the acid-base properties (pK_a values), induced by complexation with chiral selector molecules. The method was used to test six various cathinones and eight cyclodextrins. It has turned out that the formation of host-guest inclusion complex with 2-hydroxyethyl-β-cyclodextrin significantly increases the acidity of α-pyrrolidinovalerophenone (α-PVP) and methylenedioxypyrovalerone (MDPV), the maximal drop of pK_a value reaches 1.5 pH unit. The effect was analyzed from a thermodynamic point of view, and explained by a dominant contribution of the deprotonation-promoting changes of enthalpy. Finally, we demonstrated that this effect is enantioselective and may pose a basis for chiral separation of these two important drugs, independent from the commonly discussed affinity-related mechanism which is far less effective in this case.

The results of our study may be of interest for developing new enantioseparation methods, and for extending a scarce knowledge on the mechanisms involved in the supramolecular acidity modification.

Acknowledgements

National Science Centre, Poland, Preludium, 2015/17/N/ST4/03792, for financial support.

Flow analysis system for urease activity determination

Bzura J.

Laboratory of Basic Aspects of Analytical Chemistry, Faculty of Chemistry, University of Warsaw,

Pasteura 1, 02-093 Warsaw, Poland

e-mail: *jbzura@chem.uw.edu.pl*

Urease (urea amidohydrolase, EC 3.5.1.5), which was first isolated from jack beans (Canavalia ensiformis) by Sumner in 1926 is widely distributed in the evolutionary hierarchy [1]. This enzyme catalyzes the hydrolysis of urea and converts it into ammonia and carbon dioxide. These gaeous species stay in protolytic equilibrium with the ionic species. These reactions causes changes in pH as well as in conductivity. Urease is present in some plants seeds like beans, soybeans and peas.or molds, yeasts and numerous species of bacteria including *Helicobacter pylori, Proteus vulgaris, Klebsiella pneumonia*, etc. [2,3]. Urease is one of the most widely measured soil enzyme activities [4]. Several techniques are available to assess urease activity, including based on determination of ammonium ions release or increase in pH.

A mechanical, multicommuatated flow analysis system (MCFA) based on solenoid micropumps and microvalves have been developed for photometric determination of urease activity. The system is based on developed flow through detector dedicated for selective photometric detection of ammonia formed in the course of enzymatic hydrolysis of urea. The detector has been constructed using the ordinary light emitting diodes (LEDs), implementing the concept of Paired Emitter Detector Diode (PEDD) [5]. The utility of developed flow analysis system for practical bioanalytical uses will be demonstrated.

Acknowledgements

These studies are support by the Polish National Science Centre

(Project OPUS NCN 2014/13/B/ST4/04528)

[1] Sumner J.B., Journal of Biological Chemistry 69 (1926) 453-441

[2] Mobley H.L.T., Hausinger R.P., Microbiological Reviews 53 (1989) 85-108.

[3] Michael A.M., Mohamed M.N., Food Analytical Methods 8 (2015) 2482–2486.

[4] Sinsabaugh R.L., Reynolds H., Long T.M., Soil Biology & Biochemistry 32 (2000) 2095-2097

[5] Kołacińska K., Koncki R., Analytical Sciences 30 (2014) 1019-1022

Enzyme activity assays in flow analysis format using optoelectronic detector of nitrophenol

Skoczek J.

Department of Chemistry, University of Warsaw,

Pasteura 1, 02-093 Warszawa, Poland

e-mail: jskoczek@wch.uw.edu.pl

Accurate determination of the catalytic activity of the enzymes is of great importance due to their numerous biochemical, clinical and environmental applications. For this purpose, the use of chromogenic substrates of the enzymatic reaction is often recommended. Among available, derivatives of nitrophenol seem to constitute a convenient platform for enzyme activity assays development.

Deeply yellow nitrophenol formed in the course of enzymatic reactions strongly absorbs electromagnetic radiation of 400 nm wavelength, therefore could be easily detected even by visible photometry. It has been proven that the optical detection might be performed with a pair of light-emitting diodes [1]. Such optoelectronic detectors in the form of flow-through devices are robust and efficient and are a way to simplify and lowering of the cost of manifolds for flow analysis. Recently multicommutated systems based on such detector for alkaline phosphatase assays [2], as well as for simultaneous determination of acid and alkaline phosphatase activity [3] in human serum, have been developed.

In this contribution results of recent investigations on flow analysis systems based on nitrophenol detection that are suitable for determination of the enzymatic activity of some other hydrolases will be presented. Moreover, the utility of such bioanalytical systems for the assays of oxidoreductases activity is going to be put forward.

[1] Tymecki Ł., Brodacka, L., Rozum B., Koncki R. *Analyst*, 134 (2009) 1333-1337

[2] Strzelak K., Koncki R., Tymecki Ł., *Talanta* 96 (2012) 127-131.

[3] Tymecki Ł., Strzelak K., Koncki R., *Anal. Chim. Acta* 797 (2013) 57-63.

Different strategies for determination of redox species in paper-based analytical devices.

Granica M., Tymecki Ł.

Faculty of Chemistry, University of Warsaw,1 Pasteura St., 02-093 Warsaw, Poland

e-mail: *mgranica@chem.uw.edu.pl*

The simple, fast and reliable detection of the redox species has importance for example in the matter of enzyme modified microfluidic paper-based analytical devices (μPAD). The universal and easy to operate platform for hydrogen peroxide determination, generated during oxidase catalyzed reactions, can provide the possibility to expand the usefulness of this kind of analytical equipment to a wide range of the complex analytes.

Despite the fact that paper-based analytical devices offer many advantages like cost-effectiveness, low reagent consumption or spontaneous flow they still suffer from utilizing different optical accessory for signal reading and its interpretation. For fast signal readout, the distance based measurements may be applied [1]. During the sample flow along the microfluidic channel, the analyte is reacting with the reagent, deposited in the paper matrix. The color development occurs until the analyte is consumed, and the signal quantification is accomplished by measuring the length (mm) of the colored zone. On the other hand, the detection can be performed in flow analysis conditions in the paper channel with PEDD detector, as an idea of the reusable paper system [2].

In this work, the simple paper-based microfluidic devices in the form of the straight channel were prepared using wax printing technique [3]. Paper channel was covered with Prussian Blue (PB) sensor layer which shows Redox sensitivity. The reversible reduction/ oxidation process of the PB into Prussian White (PW) are manifested with a rapid change of the compound's color. Therefore PW can be used as a sensor for hydrogen peroxide. Moreover, PB shows the sensitivity for pH and for this reason it could be used for the construction of hydrolase-based sensors (urea sensor) [4].

The Prussian Blue deposition process, the μPAD system optimization and the calibration curves obtained using standard solutions will be presented.

This research was supported by University of Warsaw DSM project 501-D112-86-DSM-115 100

[1] D.M. Cate, W. Dungchai, J.C. Cunningham, J. Volckens, C.S. Henry, Lab Chip. 13 (2013) 2397.

[2] M. Granica, M. Fiedoruk-Pogrebniak, R. Koncki, Ł. Tymecki, Sensors Actuators, B Chem. 257 (2018) 16–22.

[3] E. Carrilho, A.W. Martinez, G.M. Whitesides, Anal. Chem. 81 (2009) 1–5.

[4] R. Koncki, T. Lenarczuk, A. Radomska, S. Głąb, Analyst. 126 (2001) 1080–1085.

The application of micellar electrokinetic chromatography capillary in analysis of red lipsticks

Gładysz M., Król M., Szczurek K., Kościelniak P.

Laboratory for Forensic Chemistry, Department of Analytical Chemistry, Faculty of Chemistry,

Jagiellonian University in Krakow, 2 Gronostajowa St., 30-387 Krakow, Poland

e-mail: *marta.gladysz@doctoral.uj.edu.pl*

For the forensic point of view, lipstick samples left on various surfaces (tissue papers, cigarette butts, drinking cups etc.) may be constitute indirect proof linking a suspect with the victim or crime scene. This cosmetic can be a valuable forensic evidence in many investigations, especially in rape, sexual assault or burglary. Consequently, there is a need to develop analytical methods making possible the examination of such traces.

The main aim of this research was the application of the micellar elektrokinetic chromatography (MECK) to develop a method enabling the discrimination of red lipstick samples with very similar hue.

Firstly, the separation using dyes occurring in lipsticks and prohibited in their production was expanded. The various buffers and different concentrations of sodium dodecyl sulphate (SDS) were investigated and the best results were obtained using the 25 mM borate buffer at basic pH 9.0 enriched with 80 mM SDS. Then, the developed method was evaluated determining: the stability of background electrolyte (BGE) (10h) and sample (5h) as well as the repeatability of migration time of dyes (RSD_{tM}) measured in different days (0.001 – 0.029), using different capillaries (0.002 – 0.022) and two different CE instruments (0.003 – 0.016).

Secondly, testing different extraction agents (organic solvents, diluted or not diluted BGE, Brij 35 and Triton 114 in various pH and concentration) the ultrasound assisted extraction (UAE) method of compounds occurring in red lipstick samples was developed. The two the most promising extraction agents, BGE and 0.1% w/v Triton 114 in pH 10, were further investigated. Two factors Doehlert experimental design (temperature and duration time) combined with a surface response methodology, employing numbers of peaks as the crucial response was used. It should be empathized that extraction process was investigated using six different lipstick samples in order to develop an optimal versatile extraction method. The satisfactory results for all investigated lipstick samples were obtained in following conditions: extraction agent – BGE, time – 22 min and temperature – 45ºC. The entire process (preparation of sample, extraction and separation) was also evaluated in terms of both precision and stability of the obtained extracts analyzing migration time and height of peaks.

The developed UAE/MECK method was utilized for red lipsticks differentiation achieving high value of discrimination power. Moreover, some dyes, which was used during the development of separation method, were successfully identified in lipstick samples using the in-lab build UV-VIS spectra library.

Acknowledgement

Authors gratefully thanks Inglot Sp. z. o. o for donating the dyes and lipstick samples using during investigated research.

The gradient ratio flow-injection technique used for elimination of additive interference effects

Dębosz M., Wieczorek M., Kościelniak P.

Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Kraków, Gronostajowa 2, 30-387 Krakow, Poland

e-mail: marek.debosz@doctoral.uj.edu.pl

Flow injection techniques offer numerous calibration procedures, which can be utilized during the determination of analytes in unknown and complex matrices. Compared to the traditional calibration procedures they are usually more effective (in particular, making possible to prepare a set of standard solutions from a single stock solution) and fully automated. Moreover, the registered signals are the source of a large amount of information, which is the basis of numerous gradient calibration methods [1]. One of the example is the Gradient Ratio-Standard Addition Method (GR-SAM) [2] allowing a set of calibration graphs with different slopes to be obtained based on two injection peaks produced only.

In the present work, the GR-SAM method has been adapted to the implementation of Chemical H-Point Standard Addition Method [3] in order to determine accurately the analytes in the samples containing components that cause the additive interference effect. The procedure is based on the generation of a continuous change of chemical conditions (e.g., pH environment). As a consequence, several calibration curves can be constructed, all crossing in the so-called H-point, indicating both the accurate result and the additive interference effect.

The applicability of the proposed approach was verified by spectrophotometric determination of paracetamol in medicaments and of ascorbic acid in juices and soft drinks [4]. The analytical results were able to be obtained in very short time (around several dozen seconds) on the basis of twelve calibration curves and with the use of only two calibration solutions. In both cases, the developed calibration procedure allows the analytical results to be obtained with relative error not exceeding 6%.

The developed method is readily applicable to the analysis of real samples with complex and unknown matrices. As it is additionally effective, low-cost and green, it can be considered as a helpful analytical tool.

[1] Kościelniak P., Kozak J.: Anal. Chimica Acta 460 (2002), 235–245.

[2] Kościelniak P., Anal. Chimica Acta 367 (1998), 101-110.

[3] Wieczorek M., Rengevicova S., Świt P., Woźniakiewicz A., Kozak J., Kościelniak P.: Talanta 170 (2017), 165-172.

[4] Wieczorek M., Dębosz M., Świt P., Woźniakiewicz A., Kościelniak P., Talanta 186 (2018) 215-220.

Histamine determination using potentiometric detection coupled to sequential injection analysis

Ana Rita Pereira, Célia Gomes Amorim, Alberto Araújo, Maria da Conceição Montenegro

LAQV/REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy in University of Porto

R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal

email: up201305740@ff.up.pt

Histamine is a biogenic amine that results from enzymatic decarboxylation of the amino acid histidine. It is synthetized and released from many cells (mast cells, basophils, platelets, histaminergic neurons, etc.) [1]. A strongly potential therapeutic exploitation in allergy, inflammation, autoimmune disorders and cancer has been reported in preclinical data for histamine [2]. It is the most important inflammatory mediator during an allergic reaction and plays a significant role in anaphylaxis cases [3]. Furthermore, histamine is regarded as one of the most important biomarkers for quality control during the food production and transportation [4].

Different methods have been applied into determination of histamine, such as GC, HPLC, capillary electrophoresis and biochemical assays [5]. However, potentiometry have been described as an alternative due to being simpler, faster, portable and cheaper than the other analytical methods referred above [4].

In the present work, the histamine sensor is optimized by using different membrane polymers, ionophores, solvent mediators, as well the presence of anionic additive and the multiwalled carbon nanotube (MWCNTs). The sensor with the best analytical response is composed of 1.0% (w/w) of cucurbit[6]uril, 66.8% (w/w) of 2-nitrophenyl octyl ether, 29.8% (w/w) of polyvinylchloride, 0.3% (w/w) of potassium tetrakis(4-chlorophenyl) borate and 2.0% (w/w) of MWCNT. The histamine sensor´s performance is characterized by a slope of 30.9±1.2 mV dec^{-1}, a detection limit of (3.01±0.61) $\times 10^{-7}$ mol L^{-1} and a lower limit of linear range of (2.99±0.00) $\times 10^{-7}$ mol L^{-1}.

Sequential injection with this sensor, gives rise to similar response characteristics when volume of 195µL was propelled at a flow-rate of 30 µL s^{-1}.

The optimal system will be applied to the analysis of real samples (biological fluids). Due to the complexity of the matrix, different pre-treatments are under study by using different extraction processes.

[1] L. Maintz and N. Novak, *American Journal of Clinical Nutrition* **2007**, *85*, 1185-1196.
[2] R. Cacabelos, C. Torrellas, L. Fernandez-Novoa and F. Lopez-Munoz, *Mediators of Inflammation* **2016**.
[3] R. Cecchi, *Legal Medicine* **2016**, *22*, 75-81.
[4] C. G. Amorim, R. C. Souza, A. N. Araujo, M. Montenegro and V. L. Silva, *Food Chemistry* **2010**, *122*, 871-876.
[5] A. Onal, *Food Chemistry* **2007**, *103*, 1475-1486.

Sample stacking mode in MEKC as a powerful toll used for decrease of questioned document destruction

Gładysz M., Król M., Woźniakiewicz M., Kościelniak P.

Laboratory for Forensic Chemistry, Department of Analytical Chemistry, Faculty of Chemistry,

Jagiellonian University in Krakow, 2 Gronostajowa St., 30-387 Krakow, Poland

e-mail: *marta.gladysz@doctoral.uj.edu.pl*

Questioned document examination is a broad field in forensic sciences, which is focused on comparison of inks components from writing instrumentation. Despite the fact that non-destructive analytical techniques are the most recommended in forensic protocol, many publications (for instance [1-3]) report huge potential of capillary electrophoresis (CE) technique in forensic document examination.

Consequently, the aim of this research was focused on the comparison of on-line sample concentration techniques (sample stacking modes) applied in the micellar electrokinetic chromatography (MECK) in order to enhance the detection of components of stamp pad inks so that destruction of analysed document could be significantly reduced. In particular, the normal stacking mode/field amplified sample stacking (NSM/FASS), high-salt concentration, sweeping, electrokinetic injection + FASS, and the field enhanced sample injection (FESI) were taken into consideration. At first, a mixtures of four red dyes and a mixture of four violet/blue dyes frequently met in stamp pad inks were examined. As a result, in case of red dyes two environments: ACN/1 $mg \cdot mL^{-1}$ aqueous NaCl (95:5, v/v) and 10-fold water dilution of BGE preceded by water plug (6 s, 0.7 psi) injection were chosen as the most promising ones for further examination. Regarding the blue/violet dyes, the 100-fold water dilution of BGE was selected as giving the best increase of height of the peaks.

Ultimately, nine stamp pad inks in three colors (red, blue and violet) produced by different manufacturers were examined under the optimal conditions. Only 5 dots cut-out from inked paper (O.D. 0.45 mm) were sampled. Almost all examined samples were possible to be differentiated basing on the obtained electrophoretic profiles.

In conclusion, the developed sample stacking mode of MECK–based method was found as a useful tool in the differentiation of stamp pad inks for the purposes of forensic questioned document analysis.

[1] Cruces-Blanco C., Gámiz-Gracia L., García-Campaña A.M., TrAC, 26 (2007) 215–226.

[2] Tagliaro F., Bortolotti F., Electrophoresis, 27 (2006) 231–243.

[3] Zlotnicki J.A., Smith F.P., J. Chromatogr. B, 733 (1999) 265–272.

Development of the new method for drug detection based on the conjunction of Dried Blood Spot method and Capillary Electrophoresis

Świądro M.[1], Wietecha-Posłuszny R.[1], Kościelniak P.[1]

[1]*Laboratory for Forensic Chemistry, Department of Analytical Chemistry,*

Jagiellonian University in Krakow, 2 Gronostajowa St., 30-060 Krakow, Poland

e-mail: *mg.swiadro@op.pl*

Analytical toxicology deals with various problems like screening, confirmation, identification and quantification of foreign compounds in different biological matrices. Blood is the most popular material especially for the determination of xenobiotics. The blood samples are prepared using various methods, including the Dried Blood Spot (DBS) method. This technique is becoming more and more popular among scientists. It offers a simple and practical means of blood collection, alternative to traditional methods. It is mainly used in toxicology, HIV testing, therapeutic drug monitoring, clinical pharmacokinetic screening, and neonatal screening. The DBS technique has many advantages being a non-invasive way of sampling and requiring a small amount of a blood sample for analysis. Moreover, the samples (DBS carts) can be easily transported and stored at a room temperature. On the other hand, haematocrit effect has a significant influence on blood sample that the risk of sample contamination is high [1,2].

For the evaluation of the DBS method, the most important features are the selection of the appropriate DBS card and optimization of the extraction process. There are many possibilities of analysis of obtained extracts. Nowadays, the capillary electrophoresis coupled with mass spectrometry (CE-MS) is usually used for monitoring various psychoactive compounds in biological materials, which is a crucial area in clinical, toxicology and forensic science [2,3].

The purpose of this research was to develop a new approach to sample preparation of biological material based on the DBS method in order to identify and determine psychoactive drugs - diazepam, estazolam and lorazepam - in human blood. A blood sample was spotted on the FTA Classic cards, then dried and 6-mm discs were punched. The following extraction methods were investigated: microwave-assisted extraction (MAE) and ultrasonification extraction (UAE). The various extraction solvents: MeOH:ACN, (1:l, v/v) and n-hexane:isoamyl alcohol (99:1, v/v), their volume as well as different duration of extraction were investigated in order to achieve the highest efficiency of conducted process. The obtained extracts were analysed by using CE-TOF-MS method, previously developed in our laboratory [3].

Literature

[1] Venzon Antunes M., Feiffer Charao M., Linden R.; Clin. Biochem. 49 (2016).

[2] Lee H., Park Y., Jo J., In S., Park Y., Kim E., Pyo J., Choe S.; Forensic Sci. Int. 255 (2015).

[3] Woźniakiewicz A., Wietecha-Posłuszny R., Woźniakiewicz M., Bryczek E., Kościelniak P., J. Pharmaceut. Biomed. 111 (2015).

Acknowledgement

The authors gratefully acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16

Development of Flow Injection and Sequential Injection Methods for the Determination of N-Acetyl-L-Cysteine Ethyl Ester (NACET) Generating Chromogenic Copper(I)Ln Complexes With Different Ligands

Lea Kukoc-Modun [a,*], Dimitrios Tsikas [b], Tomislav Kraljević [a,c], Maja Biocic [a], Njegomir Radić [a] and Darko Modun[d]

[a] *Department of Analytical Chemistry, Faculty of Chemistry and Technology, University of Split, Ruđera Boškovića 35, 21000 Split, Croatia*

[b] *Center of Pharmacology and Toxicology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany*

[c] *Department of Chemistry, Faculty of Science and Education, University of Mostar, Matice hrvatske bb, 88 000 Mostar, Bosnia & Hercegovina*

[d] *Department of Pharmacy, University of Split School of Medicine, Šoltanska 2, 21000 Split, Croatia*

e-mail: *lkmodun@gmail.com*

New simple, sensitive and robust flow injection and sequential injection methods for the determination of a novel lipophilic thiol compound, *N*-acetyl-L-cysteine ethyl ester (NACET), have been developed, validated and compared. NACET is the ethyl ester of NAC, it has advantageous pharmacokinetics and is a potential drug and antioxidant. The proposed methods are based on the reduction of Cu(II)-ligand complex to Cu(I)-ligand complex with the analyte. Studied ligands were neocuproine, bicinchoninic acid (BCA) and bathocuproine disulfonic acid (BCS). The flow injection system consisted of an Ismatec IPC eight-channel peristaltic pump, Rheodyne low pressure Teflon six port rotary valve and three-line manifold with one reaction coil. For the sequential injection measurements Cheminert® M50 pump (VICI Valco), a syringe-free stepper motor driven pump, was used for liquid handling. A 10-port selection valve model C25-3180D with a multiposition actuator control module EMHCA-CE (VICI Valco) was used in conjunction with the M series pump. The signal was continuously monitored at 458, 562 and 483 nm for the reactions of NACET with neocuproine, BCA and BCS, respectively, using a Shimadzu UV-1601, UV/Vis spectrophotometer equipped with a flow through cell. Optimization of manifold parameters and experimental conditions were carried out by means of univariate method. Developed flow injection methods based on the reaction systems with neocuproine, BCA and BSC were linear in the concentration ranges:4.0×10^{-6} - 2.0×10^{-4}, 2.0×10^{-6} - 1.0×10^{-4} and 6.0×10^{-7} - 1.2×10^{-4}, respectively. The linear ranges of sequential injection methods were: 2.0×10^{-6} - 2.0×10^{-4}, 2.0×10^{-6} - 1.0×10^{-4} and 2.0×10^{-6} - 1.2×10^{-4}, respectively. The analytical performance of the methods, in terms of accuracy and precision, was established. The analytical frequency was 70 samples per hour for all proposed methods.

3D-printed flow-through PEDD optosensor with dithizone membrane. Proof of concept.

Pokrzywnicka M.[1], Michalec M.[2]

[1)] *Faculty of Chemistry, University of Wroclaw, F. Joliot –Curie 14, 50-383 Wroclaw, Poland*
[2)] *Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland*

e-mail: *marta.pokrzywnicka@chem.uni.wroc.pl*

Optosensors show many advantages besides conventional optical methods. They allow reagent-less, selective detection of desired analytes. They meet the assumptions of Green Chemistry and significantly simplifies measurement procedures. However, they reveal the real potential when coupled with flow analysis systems.

Construction of the most of flow-through optosensors is based on optical fibers. However, there are also examples of the optical sensors operating according to paired emitter-detector diode (PEDD) concept [1]. Unfortunately, these smart devices require a labor-intensive and rather detailed manufacturing process, and they are dedicated to specific assay.

Dithizone (1,5-diphenylthiocarbazone), in various pH, form a colored complexes with many metal cations (eg. Cu^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Fe^{2+}, Bi^{3+}, Ag^{+} [2]). The ligand is insoluble in water and has strong sorption properties. Such characteristics make dithizone an interesting component for ion selective optomembrane dedicated for simultaneous determination of several analytes. However, the optosensors described until now were applied only for the determination of one or maximally two cations [3]. The full analytical potential of this solution was not explored so far. Meanwhile properly constructed flow analysis system, that allows both, controlled change of reaction conditions and reproducibility of the measurements can enable this membrane feature.

In this contribution, we present a more straightforward procedure to prepare a flow-through PEDD optosensor. Instead of manufacturing of very tiny elements for the membrane casing, a 3D-printing was employed. The sensor casing was made of biodegradable polylactide (PLA), and fluidic channels were printed of elastic material (thermoplastic polyurethane, tPU) with use of the most available and economic Fused Filament Fabrication. Then, the device was equipped with the dithizone-based optosensing membrane and dedicated LEDs. The elementary parameters of newly constructed optosensor and the potential of future analytical application are presented.

[1] Cocovi-Solberg D.J., Miro M., Cerda V., Pokrzywnicka M., Tymecki Ł., Koncki R., Talanta 96 (2012) 113-120.

[2] Burger K., Organic Reagents in Metal Analysis: International Series of Monographs in Analytical Chemistry, Pergamon, Oxford, 1973, 120.

[3] Alberti G., Re S., Chiara Tivelli A.M., Biesuz R., Analyst 141 (2016) 6140-6148.

Flow injection method with chemiluminescence detection for the determination of silver nanoparticles

Nalewajko-Sieliwoniuk E., Malejko J., Topczewska A., Kojło A., Godlewska-Żyłkiewicz B.

Department of Analytical Chemistry, Institute of Chemistry, University of Bialystok, K. Ciołkowskiego 1K, 15-245 Białystok, Poland

e-mail: *nedyta@uwb.edu.pl*

The new flow injection chemiluminescent (FI-CL) method for determination of silver nanoparticles (AgNPs) has been proposed. The study of various chemiluminescence systems, based on: luminol, $KMnO_4$, Mn(IV), $K_3Fe(CN)_6$, Ce(IV) and lucigenin have been investigated in terms of their applicability to the trace detection of AgNPs. Among the fourteen CL systems tested, Mn(IV)-formaldehyde-hexametaphosphate was chosen as the optimal for the determination of AgNPs. The FI-CL method based on enhancing effect of AgNPs on Mn(IV)-formaldehyde-hexametaphosphate chemiluminescence was developed. It was found that the sensitivity of the proposed method depends on the size of AgNPs as well as the type of nanoparticles stabilizer (sodium citrate, polyvinyl pyrrolidinone (PVP), polyethylene glycol (PEG)). Under the optimized experimental conditions, the detection limits for AgNPs with nominal diameters of 10 nm, 40 nm and 100 nm stabilized by sodium citrate were in the range 0.3 – 2.9 μg mL^{-1}, AgNPs with nominal diameters of 40 nm, 60 nm and 100 nm stabilized by PEG were in the range 2.7 – 13.3 μg mL^{-1}, AgNPs with nominal diameters of 20 nm and 75 nm stabilized by PVP were 20.2 μg mL^{-1} and 24.9 μg mL^{-1}, respectively. The developed method is simple, offers high precision (RSD ≤ 3.3%) and high sampling rate (up to 180 samples per hour). The accuracy of the FI-CL method was confirmed by analysis of NIST reference material RM 8017 containing PVP-coated AgNPs with nominal diameter of 75 nm. The method was successfully applied to the trace determination of AgNPs in tap and natural mineral water samples.

Acknowledgement

This work was supported by the Polish National Science Centre (DEC-2014/15/B/ST4/04641).

Versatile flow system for titration

Kozak J.[1], Paluch J.[1], Wieczorek M.[1], Kozak M.[2], Kościelniak P.[1]

[1]*Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Kraków, Gronostajowa 2, Kraków 30 387, Poland*

[2]*Oil and Gas Institute – National Research Institute in Kraków, Lubicz 25 A, Kraków 31 503, Poland*

e-mail: *j.kozak@uj.edu.pl*

Versatile flow system for implementing both tracer-monitored titration [1] and titration based on titrant dilution factor is presented.

In the tracer-monitored titration [1] an additional substance (a tracer) is introduced into a titrant (or a sample [2]) solution to estimate the instant titrant and sample volumetric fractions. This way the titration process can be performed without the need for volume, mass or peak width measurements. The approach was applied to spectrophotometric flow titrations involving variations of the sample and titrant flow rates or concentration gradients established along the sample zone [3]. The strategy requires simultaneous monitoring of two absorbing species, the indicator of titration and the dye tracer. The limitation of its application can be the difficulty in selecting a tracer to avoid the influence of other components on its signal.

In titration based on the titrant dilution factor, defined volumes of the sample and titrant are introduced simultaneously into the system in the form of monosegments and merged together to form a new monosegment for which a signal in a form of short plateau is obtained. In the subsequent monosegments the sample to titrant volume ratio is changed, maintaining a constant the total volume. As the sample and titrant volumes in each monosegment are known, the degree of titrant dilution in the monosegment corresponding to the end point of titration can be calculated.

The above modes of titration were implemented using the developed flow system and titration procedure. The advantage of applying the developed system is the possibility of separate titrating and diluting the analyte, and tracer, respectively. The methods were verified on the examples of complexometric (iron(II), iron(III)) and acid-base (hydrochloric, phosphoric, acetic, citric and tartaric acids) titrations, and applied to the determination of iron(II) and iron(III) in water samples collected from artesian wells, and the total acidity in beverages. The results were compared with those obtained using the ICP OES method and potentiometric titration, respectively.

Acknowledgements

The research was performer in the frame of Project OPUS 12 nr 2016/23/B/ST4/00789 funded by MNiSW.

[1] Martz T.R., Dickson A.G., DeGrandpre M.D., Anal. Chem., 78 (2006) 1817-1826.

[2] DeGrandpre M.D., Martz T.R., Hart R.D., Elison D.M., Zhang A., Bahnson A.G., Anal. Chem., 83 (2011) 9217-9220.

[3] Sasaki M.K., Rocha D.L., Roch F.R.P., Zagatto E.A.G., Anal. Chim. Acta 902 (2016) 123-128.

Chromatographic method for monitoring of dipyrone and diclofenac in water samples

Fernandes S. R.[1,2], Barreiros L.[1,2], Springer V.[3], Avena M.[3], Segundo M. A.[1]

[1]*LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal*

[2]*Núcleo de Investigação e Intervenção em Farmácia (NIIF), Centro de Investigação em Saúde e Ambiente (CISA), Escola Superior de Saúde, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 400, 4200-072 Porto, Portugal*

[3]*INQUISUR (CONICET-UNS), Departamento de Química, Universidad Nacional del Sur, Av. Alem 1253, B8000CPB Bahía Blanca, Argentina*

e-mail: saraferns@sapo.pt

Pharmaceutical products are recognized as a group of emerging environmental contaminants. The chronic exposure to these substances represents potential risks to the ecosystems and human health [1]. Therefore, their monitoring and removal is of utmost importance. Non-steroidal anti-inflammatory drugs (NSAIDs), are classified as a class B drug by the European Council Decision 2002/657/EC and are frequently used in human and veterinary medicine for the treatment of different conditions, namely fever (antipyretic), pain (analgesic), and inflammation (anti-inflammatory) [2]. This group includes numerous substances such as dipyrone (DIP) and diclofenac (DC) that are commonly used for human health care and have been detected in the environment at concentrations ranging from nanograms to milligrams per liter [1,3]. In this work, a high performance liquid chromatography method was developed for the simultaneous determination of DIP and DC, targeting its application to monitor the efficiency of adsorption into magnetic nanoparticles as water treatment strategy.

For the optimization of the chromatographic method, the composition of the mobile phase, the flow rate, and the injection volume were evaluated. Thus, it was possible to separate the two analytes by performing a gradient elution and using a reversed-phase C18 monolithic column (100 mm x 4.6 mm i.d.). A diode array detector, set at 254 and 279 nm, was employed for DIP and DC detection, respectively. Futhermore, the developed chromatographic method was validated for specificity, linearity, working range, accuracy, intra- and inter-day precision, detection and quantification limits, and stability in accordance with European Medicines Agency and International Conference on Harmonisation guidelines, showing its applicability to monitor the adsorption of DIP and DC onto nanoparticles.

Acknowledgements: This work received financial support from FEDER funds POCI/01/0145/FEDER/007265 and National Funds (FCT/MEC, Fundação para a Ciência e a Tecnologia and Ministério da Educação e Ciência) under the Partnership Agreement PT2020 UID/QUI/50006/2013. S. R. Fernandes and L. Barreiros thank FCT and POCH (Programa Operacional Capital Humano) for their grants (SFRH/BD/130948/2017 SFRH/BPD/89668/2012). This work also received financial support from CONICET and Universidad Nacional del Sur (Argentina).

[1] Li W.C., Environmental Pollution 187 (2014) 193-201.

[2] Gallo P., Fabbrocino S., DowlingG., Salini M., Fiori M., Perrettad G., Serpea L., Journal of Chromatography A, 1217 (2010) 2832–2839.

[3] Lonappan L., Brar S.K., Das, R.K., Verma, M., Surampalli, R.Y., Environment International 96 (2016) 127–138.

Immunopurification of nanoparticles under lab-on-valve format

Marques S.S.[1], Ramos I.I.[1], Barreiros L.[1], Lima S.A.C.[1], Reis S.[1], Domingues M.R.[2], Segundo M.A[1]

[1]*LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal*

[2]*QOPNA, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.*

e-mail*: micf09213@ff.up.pt*

Nanotechnology-based drug delivery systems hold promise for the treatment of several diseases due to their potential to improve active ingredients' delivery to target tissues, increasing therapeutic safety [1]. This led to an increase in nanoparticles´ (NPs) production, namely resorting to surface decoration for targeting improvement. However, the translation of NPs from "bench to bedside" depends on their efficient purification and characterization [2]. In fact, the removal of unloaded molecules from the final formulation without compromising NPs integrity and physicochemical properties is mandatory and yet quite challenging. In this context, this work aims at the implementation of an automated miniaturized method using the lab-on-valve (LOV) platform for the purification of PEGylated polymeric NPs loaded with methotrexate (MTX). The micro-bead injection spectroscopy (μBIS) technique [3] was implemented through the assembly of a micro-column of beads coupled to anti-PEG IgG inside the LOV detection unit, followed by the confirmation of coupling using an antibody labelled with horseradish peroxidase towards anti-PEG antibody and passage of 3,3′,5,5′-tetramethylbenzidine substrate. Later on, the real-time monitoring of NPs retention in the microbeads column with simultaneous elution of free MTX was pursued.

Different coupling strategies (protein G, protein A, NHS chemistry) were exploited using Sepharose as solid support. The interaction of the secondary antibody with the solid supports was evaluated in order to select the most suitable coupling chemistry for further assays. The interaction of free MTX with functionalized beads was also exploited. MTX elution was observed for ≥ 20 μg/mL levels confirming that this compound was not retained in the functionalized solid support.

Acknowledgements: S. S. Marques acknowledges FCT (Fundação para a Ciência e a Tecnologia) for the PhD grant (PD/BD/128398/2017) supported through the FCT PhD Programmes and by Programa Operacional Capital Humano (POCH) (Doctoral Programme on Cellular and Molecular Biotechnology Applied to Health Sciences (BiotechHealth), reference PD/00016/2012). I. I. Ramos and L. Barreiros thank FCT and POCH for their grants (SFRH/BD/97540/2013 and SFRH/BPD/89668/2012). S. A. C. Lima acknowledges funding from FEDER - Operational Competitiveness and Internationalization Program (COMPETE 2020) through project NORTE-01-0145-FEDER-000011. Authors also acknowledge financial support from The European Union (FEDER funds POCI/01/0145/FEDER/007265) and National Funds (FCT/MEC) under the Partnership Agreement PT2020 UID/QUI/50006/2013 and FCT Grant UID/QUI/00062/2013.

[1] Parveen S., Misra R., Sahoo, S. K., Nanomedicine-Nanotechnology Biology and Medicine 8 (2012) 147-166.

[2] Lopez-Serrano A., Olivas R.M., Landaluze J.S., Camara C., Analytical Methods 6 (2014) 38-56.

[3] Gutzman Y, Carroll A.D., Ruzicka J., Analyst. 131 (2006) 809–15.

Novel automatic in-vitro methods for on-line assessment of the gastrointestinal bioaccessibility of micronutrients from food commodities

María Rosende, David J. Cocovi-Solberg, Manuel Miró

FI-TRACE group, Department of Chemistry, University of the Balearic Islands,

Carretera de Valldemossa km 7,5; E-07122 Palma de Mallorca, Spain

e-mail: *maria.rosende@uib.es*

The mere occurrence of nutrients in foodstuff does not guarantee by its own their availability by the human body after ingestion. Recently, in-vivo methods to assess the actual pools of nutrients that are released into the human gastrointestinal (GI) tract and are available for intestinal absorption are become ethically controversial, time-consuming, cost expensive and require specialized and trained personnel. Therefore, in-vitro bioaccessibility methods mimicking the human gastrointestinal tract have been proposed instead.

Two different automatic on-line methods based on flow approaches for the in-vitro assessment of the gastrointestinal bioaccessibility of micronutrients (viz., Cu, Fe and Mn) from seeds are presented.

In the first, an automatic batch-wise biomimetic testing incorporating real-time monitoring of the human digestion process based on the Unified Bioaccessibility Method [1] is proposed as a front end to inductively coupled plasma optical emission spectrometry (ICP OES) for rapid assessment of oral bioaccessible fractions of micronutrients. A fully automated flow analyzer is designed to foster in-line filtration of gastrointestinal extracts at predefined times (≤15 min) followed by on-line multi-elemental analysis of bioaccessible Cu, Fe and Mn, in well-defined volumes of extracts of soybean and genetically modified seeds.

In the second, an automatic flow-through dynamic extraction method is proposed for in-vitro exploration, with high temporal resolution, of the transit of the chyme from the gastric to the duodenal compartments using a physiologically base extraction test in fed conditions, so-called Versantvoort's method [2]. To this end, the simulated intestinal and bile biofluid (added to the gastric phase) is successively pumped at 1.0 mL min^{-1} through a large bore column (maintained at 37.0 ± 2.0 °C) initially loaded with a weighed amount of linseed (250 mg) using a PVDF filter membrane (5.0 μm pore size) for retaining of the particulate matter and in-line filtration of the extracts that are on-line injected into ICP OES.

[1] M. A. Herrera, M. Rosende, M. A. Z. Arruda, M. Miró, On-line coupling of physiologically relevant bioaccessibility testing to inductively coupled plasma spectrometry: Proof of concept for fast assessment of gastrointestinal bioaccessibility of micronutrients from soybeans, Anal. Chim. Acta 939 (2016) 1-9.

[2] L. A. Souza, M. Rosende, M. G A. Korn, M. Miró, Flow-through dynamic extraction system for exploring chyme bioaccessibility in food commodities, Accepted manuscript Anal. Chim. Acta, accepted.

Assessment of the distribution of emerging contaminants across the cellular membrane using a very simple unmaned fluidic system

Miquel Oliver, Marc Roca Jiménez, David J. Cocovi-Solberg, Manuel Miró

FI-TRACE group, Department of Analytical Chemistry, University of the Balearic Islands.

Carretera de Valldemossa km 7.5, 07122 Palma de Mallorca, Illes Balears, Spain.

email: miguel.oliver@uib.es

Membranotropic effects of different emerging contaminants, ECs, that is, interaction with lipid bilayers, can be investigated in-vitro on the basis of generalized polarization (GP) measurements using soy lecithin unilamellar lipid vesicles as biological membrane surrogates [1] and Prodan and Laurdan as membrane fluorescent probes. Different molecules feature varied times and depths of permeation into the liposomal membrane, so the conditions for probe and contaminant stabilization have to be optimized beforehand.

Herein we propose an automatic and intelligent closed-loop fluidic system to monitor permeation kinetics of different membrane probes and ECs into monodispersed liposomes using generalized polarization as a feedback measurement. The fluid driver consists merely of a Tecan Xcalibur syringe pump with a 9-position head valve furnished with a 500 µL syringe and a 5-mL glass mixing chamber. The dual (440 and 490 nm) fluorescence measurements are acquired with a Jasco FP-4025 flow fluorometer furnished with a custom made analog-to-digital converter and are fed at real time into a user-fiendly software, called CocoSoft [2]. The computerized method encompasses the fluidic manipulations as well as the fluorescence monitoring in order to integrate both in a smart control for unsupervised analysis of permeation of ECs with varying physicochemical characteristics into liposomes of different lipid composition. GP values are compared before and after adding a contaminant. When the software determines that there are no significant GP variations and thus a steady-state equilibrium of the molecule in the membrane is reached, the system initiates a cleaning procedure and starts a new assay automatically, making replicates or changing the probe, the contaminant or the liposome type.

Future work will involve setting on-line temperature-controlled reactions using a Peltier device to study the permeation kinetics across varying temperatures so as to afford GP plots under and above lipid transition temperature.

[1] M. Oliver, A. Bauza, A. Frontera, M, Miró, Environ. Sci. Technol., 2016, 50: 7135–7143.

[2] D. J. Cocovi-Solberg, M. Miró, Anal Bioanal Chem 2015, 407:6227-6233

Automatic determination of free and total sulfites in wines

Danchana K, Cerdà V.

Laboratory of Environmental Analytical Chemistry, University of the Balearic Islands, Carretera Valldemossa km 7.5, E-07122 Palma de Mallorca, Spain

e-mail: kaewtadanchana@hotmail.com

A conductometric system provided with a multipumping module [1] and a gas-diffusion cell has been developed in order to determine free and total SO_2 in wine. The developed method has 2 different parts to determine both kind of SO_2 using the same system. For free SO_2, sulfite will turn to H_2SO_4 by using acidification and diffusing into H_2O_2 in an acceptor channel [2, 3]. On the other hand, to determine total SO_2, the sample was previously hydrolyzed by mixing the sample with NaOH and heated at 70 degrees Celsius in order to break the bond of combine SO_2 [2, 3]. Free and total SO_2 were determined in the range of 5-50 ppm and 20-150 ppm with a sample throughput of 13 and 12 h^{-1}, respectively. The calibration curve of free and total SO_2 were in the range of ΔG (mS min^{-1}) = 0.3363[Na_2SO_3] – 1.0216, r^2=0.997 and ΔG (mS min^{-1}) = 0.0628[Na_2SO_3] – 0.5811, r^2=0.997. The proposed automatic method is simple and easy to apply for the determination of SO_2 in wines using simple reagents.

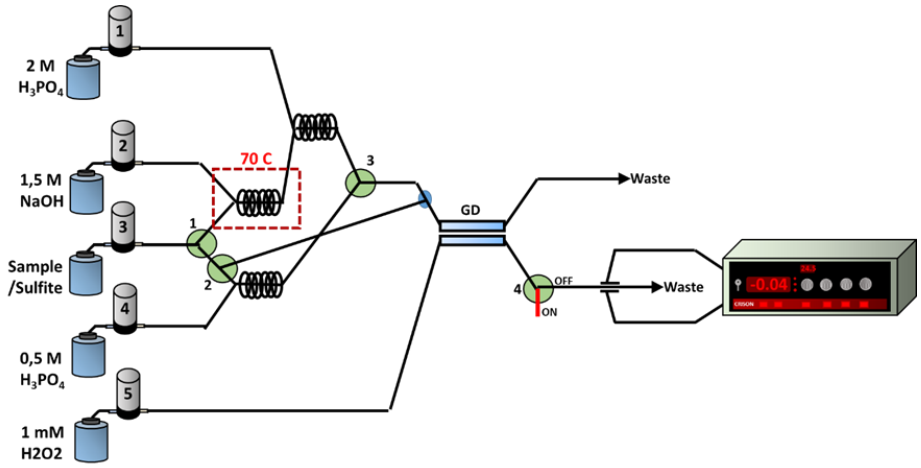


Figure1. The manifold of the autometric determination of sulfite in wines, GD is Gas-diffusion cell**.**

[1] C. Henríquez, B. Horstkotte, V. Cerdà., Talanta (2014), 118, 186–194.

[2] S.M. Oliveira, T.I. M. S. Lopes, I.V. Toth, A.O.S.S. Rangel., J. Agric. Food Chem. (2009), 57, 3415–3422.

[3] S. Mcload., Anal. Chim. Acta (1992), 266, 107-112.

Capillary Electrophoresis Enantioseparations with Cyclodextrins as Chiral Selectors - Choice of Separation Conditions

Jáč P.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University,

Akademika Heyrovského 1203, Hradec Králové 500 05, Czech Republic

e-mail: jac_p9aa@faf.cuni.cz

Chirality and chiral recognition are of crucial importance for living systems and therefore these phenomena are intensively studied since the discovery of molecular chirality by Louis Pasteur in 1848 [1]. To describe and understand chirality and chiral recognition, analytical techniques such as nuclear magnetic resonance, circular dichroism and chiral separation methods are employed [2].

Capillary electrophoresis (CE) is nowadays well established and respected analytical technique allowing separation of various analytes ranging from small inorganic ions to large biomolecules. Chiral separations belong to the main application and research domains of CE. The benefits of CE such as high flexibility of the method as well as small consumption of reagents and samples are of big importance in the development of enantioselective CE methods. In chiral CE, the addition of one or more chiral selectors to the background electrolyte represents more flexible approach compared to the employment of chiral stationary phase in chromatographic separations (where the chiral selector is attached to the backbone structure of the stationary phase by covalent bonds). Cyclodextrins (CD) belong to the most frequently used chiral selectors in electrodriven enantioseparations as documented by numerous publications appearing in past three decades. The separation of enantiomers is attained by the formation of transient diastereomeric complexes between the enantiomers and the respective CD. The individual enantiomers are resolved during the separation run if the association constants of the diastereomeric complexes between the individual enantiomers (selectands) and selector are different or if the electrophoretic mobilities of these complexes differ. Since the addition of cyclodextrin brings another equilibrium (i.e., guest-host complexation) into the separation process, this technique is frequently called as electrokinetic chromatography (EKC) [2, 3]. Different separation modes can be applied depending on the structure and charge of a chiral analyte as well as on the structural characteristics of a cyclodextrin employed [3].

The basic considerations regarding the development of a chiral capillary electrophoretic method employing cyclodextrins as chiral selectors will be presented. The separation modes discussed will be exemplified by chiral separation of 1,1´-binaphthyl-2,2´-diyl hydrogen phosphate and ofloxacin with native β-cyclodextrin and sulfated-β-cyclodextrin, respectively. The reversal of enantiomer migration order will be shown as well [3]. Finally, an application example of validated enantioselective CE assay of impurity profiling of escitalopram in bulk drug and dosage forms will be presented [4].

[1] Gal J., Helvetica Chimica Acta 96 (2013) 1617-1657.

[2] Scriba G.K.E., Chromatographia 75 (2012) 815-838.

[3] Scriba G.K.E., Jáč P., Methods in Molecular Biology, Humana Press, 970, 2013, 271-287.

[4] Sungthong B., Jáč P., Scriba G.K.E., J. Pharm. Biomed. Anal. 46 (2008) 959-965.