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BUILD YOUR OWN CAPILLARY ELECTROPHORESIS INSTRUMENT WORKSHOP

BOOK OF ABSTRACTS

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Introduction to Capillary Electrophoresis: Build your own CE instrument

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Abstract

Open source paradigm is becoming widely accepted in scientific communities and open source hardware is finding its steady place in chemistry research. Capillary electrophoresis (CE) is still underused as a separation technique but it offers unique flexibility, low-cost, and high efficiency. CE is relatively easy to build and the separation systems based on CE are simpler and open source alternatives could provide similar performances than significantly more expensive commercial instruments. In general a CE system is composed of three major parts. These include (i) the separation capillary, (ii) the high voltage power supply (HVPS) – this is the driving element common in all separations and replaces for instance the high pressure pumps in HPLC and (iii) a detector with appropriate data acquisition supporting electronics.

The system comprises also other parts, such as buffer vials, platinum or stainless steel electrodes, capillary flushing manifold, injection parts or thermostatting device; each research laboratory using their own designs.

In this introductory talk the main building block of a CE system will be discussed with the emphasis on their open source availability. Also some basic principles of electrophoretic separation mechanisms, general consideration for selection of the background electrolyte suitable with the detector of choice, aspects of injection mechanisms etc. will be briefly discussed as an introduction to other lectures that will later provide deeper insights into various parts of the CE instrumentation.

Acknowledgements

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Research Plan of the Institute of Analytical Chemistry of the ASCR, v. v. i. (RVO:68081715).

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UV/Vis- and Conductivity Detectors for Capillary Electrophoresis

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Abstract

Capillary electrophoresis is a very powerful analytical method, but by relying on manual flushing and siphoning injection it is possible to build simple and inexpensive instruments. However, even the most basic instruments require a reliable detection system.

Commercial benchtop instruments for the laboratory are usually fitted with monochromator based UV/Vis-absorption detectors. Capacitively coupled contactless conductivity detectors (C^4D) are available from 3rd party suppliers for retrofitting to benchtop instruments.

However, neither of these commercial products are well suited for low cost or battery operated portable instruments.

An alternative are UV-detectors based on light-emitting diodes (LEDs) which have in recent years become available for the deep UV-range down to 235 nm. By combining an LED, which has a narrow emission bandwidth, with a photodiode monochromators are not necessary and compact detectors with low power consumption can be built.

C^4Ds may also be constructed relatively easily in the laboratory. The measurement principle is fully electronic and the only mechanical part required is a simple measuring cells.

An introduction to both approaches will be given as well as a discussion of the critical points to consider when building either type of detector.

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An open C⁴D for capillary electrophoresis

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Abstract

Over the last 20 years, capacitively coupled contactless conductivity detection (C^4D) evolved from a purely academic approach for detection in capillary electrophoresis (CE) into a commercially-available device. Thanks to its simplicity, robustness, and wide use, C^4D spread to other fields like liquid chromatography, microchip, and impedance imaging. The simplicity was apparent since the first version,[1] which allowed it to be moved to an open hardware project, named openC^4D, 10 years ago.[2] The newest version (github.com/claudimir-lago/openC4D) is a 6.5-cm^3 box containing the electronics for excitation of the detection cell, as well as transimpedance amplifier, rectifier, low-pass filter, and 22-bit analog-to-digital converter (ADC). Such a high-resolution ADC allows detecting small peaks even when a high-conductivity background electrolyte is used. The serial output of the ADC allows openC^4D to be connected to any microcontroller inside a CE instrument. Moreover, it can be used with any computer having an USB port by using an Arduino-based microcontroller and a Java-based frontend, also available. We have used openC^4D in our lab-made CE instruments[3] as well as in commercial ones. It has been used also in an unmanned vehicle[4] and in combination with mass spectrometry.[5,6] This versatility is granted by the open-protocol Serine, which allows the communication with microcontrollers in a heterogeneous medium. Basically, this protocol works by exchange of simple character strings through virtually any serial channel (github.com/claudimir-lago/Serine-Protocol).

Acknowledgements

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3D printed LEDIF detector for CE

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Abstract

The attempts for assembling lab-built fluorescence detection system for capillary electrophoresis are quite often due to (1) high price of commercially available equipment, and (2) relatively wide range of commercially available optical elements.

In this presentation, common approaches of fluorescence detection will be introduced, (orthogonal, epifocal, confocal, fiber-based, Fig. 1) in terms of their pros and cons together with practical aspects of their assembling. Part of the talk will be devoted to excitation sources, detectors and other important optical elements.

Sources of fluorescence background and noise will be also discussed.

Last part of the theoretical section will be addressed to possibilities of optical and optomechanical components purchase.

As applications, lab-built 3Dprinted LED-and laser-based fluorescence detection systems[1] (Fig. 2) and fiber-based LIF detector for commercial CE devices will be presented.

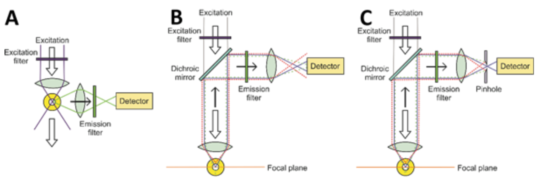

Fig. 1: Schematic principle of various fluorescence arrangements. Orthogonal (A), epifocal (B) and confocal (C) arrangement.

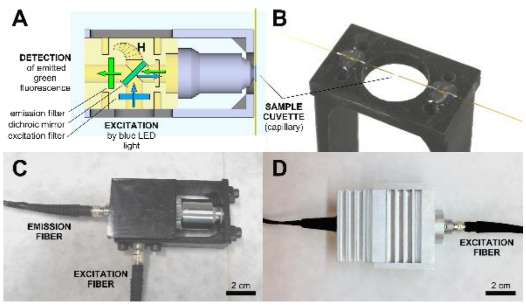

Fig. 2: 3D printed fluorescence detection head scheme (A). Detail of the capillary guide: sample cuvette (B). The printed and assembled detection system including the capillary holder with mounted fused silica capillary (C). The LED source in an aluminum housing (D).

Acknowledgements

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Using portable CE instruments for *in-situ* determining of banned compounds.

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Abstract

Building portable CE instruments seems to be a straightforward and easy task. However, the usefulness and the real value of the particular design will be revealed only in the practical situations, on the site.

Based on the personal experience we report on the potential of a portable capillary electrophoresis (CE) instrument, coupled either to C^4D or deep UV fluorescence detector, for the determination of degradation products of chemical warfare agents (CWA), post-blast explosive residues in various matrices and illegal drugs in oral fluids. Suitable protocols for all mentioned analytes have been developed that enable the determination of target compounds with high confidence and in many situations with LODs that are lower than regulations require. In many cases portable CE instruments performs better (in terms of false positives/negatives) than existing rapid tests used by the law enforcement agencies now.

Practical work with the portable CE instrument at the site, at the CWA and in the explosives test fields and at police stations by electronic music festivals contributed significantly to the understanding of the needs and problems of implementing the outcome of an academic research into the real life. Our experience suggests that a portable CE instrument in the hands of an experienced chemist meets the needs of law enforcement agencies. In the cases when the official screening method was able to detect the presence of illegal drugs in the suspect samples, the coincidence rate was higher than 80 %. However, the agencies expect to use instruments that are as convenient and rapid as present alcohol testers. This is especially urgent for the roadside drug testing. We will discuss various ways of developing an instrument that is sufficiently user-friendly and robust to be operated by any law enforcement officer. Such an instrument would integrate suitable sample preparation procedures/protocols and the CE analysis into one smart device.

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Instrumentino – A practical approach for fast setup, control and monitoring of experimental CE instruments

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Abstract

During scientific research in the field of capillary electrophoresis (CE), many expert groups faced the need to build and control their purpose made experimental set-ups. Depending on the complexity of the experiments, a wide variety of different hardware from different vendors must be combined to an integrated composition. On the one hand from the fluid side to the other in an appropriate electronic way. The most common hardware interface these days is a USB connector, but also RS232/485 and analog interfaces are prevalent. To automate the experiments for autonomous operation the use of a personal computer is reasonable and plays the role of a mediator between hardware and software control. The most common interface on a PC, to enable hardware communication, is only a USB port. For analog and digital input and output, additional hardware is required to meet this challenge. In the scientific community a new trend emerged in recent years controlling self-made experiments with the "Arduino" open-source hardware platform [1,2]. The core of an Arduino board is a microcontroller on a standardized circuit board that connects to different components of experimental systems to enable monitoring and control abilities. The widespread acceptance of the Arduino platform stems from the corresponding integrated development environment for easy programming compiling and uploading code to the microcontroller. Countless code examples and straightforward support is available through the constantly growing user community.

The drawback of using the Arduino platform is the limited ability to interactively control and monitor processes. The control code is stored inside the microcontroller and can only be modified by uploading a recompiled version. To overcome this limitation the research group of Peter C. Hauser (University of Basel, Department of Chemistry) identified the need for an easy-to-use and adaptable graphical user interface (GUI) to control purpose-made experimental systems and developed the Instrumentino software[3,4]. Instrumentino is a user-friendly open source Python[5] framework and released under GNU General Public License. Downloading and also contributing is possible at the GitHub repository[6]

One part of Instrumentino is the Controlino sketch (controlino.ino) that needs to be uploaded on the Arduino. This sketch enables a textual master / slave communication between Instrumentino and the Arduino board over a USB connection. The complete communication overview is shown in Figure 1.

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for interaction and debugging. For example to set the digital pin 2 on the Arduino to high state, define pin 2 as output pin with the command "*set D2 out*" followed by "*write D2 digi high*". When successful the Arduino response is "*Done!*". The so far implemented command strings can be found in [3].

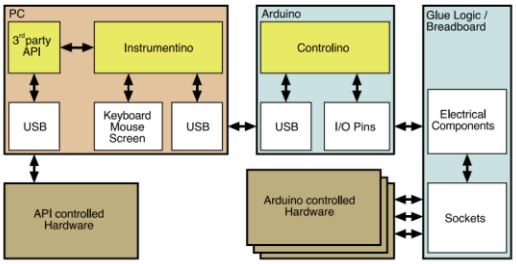

Figure 1: Data flow using instrumentino in purpose-made experimantal CE setups (reprinted with permission from I.J. Koenka)

The Instrumentino Python code generates the user front-end for communication with Controlino and acts as the master in the defined communication protocol. During initial setup, the user needs to configure the system description file in Python, which keeps all controlled system components and their connection ports to the Arduino microcontroller (e.g. solenoid valve, connected to Arduino on pin 5 to switch it on and off). All system actions can also be defined here, which can then be executed in the GUI (e.g. close the valve, wait X seconds and then open it again). The GUI features three sections to gain full control and monitoring over the corresponding hardware (see Figure 2). Far left is the section for direct manual control of components (e.g. setting voltage, pressure…). In the middle section, the user can define and save methods out of system actions and run them as sequences for a defined number of repetitions. The log panel on the right side enables logging of all system actions with execution timestamp. The log-file is stored locally and acts as a lab journal to document conducted experiments with all settings and parameters. The second mode of the panel is the signal-log, a timeline graph that draws all measured signals during method execution.

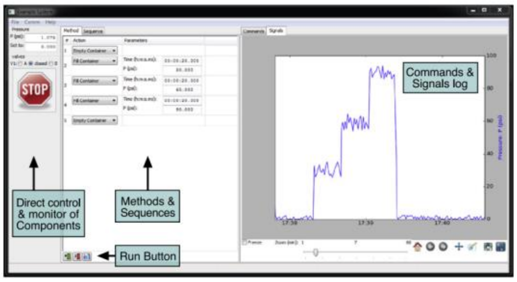

Figure 2: Instrumentino user interface (GUI) to control and monitor CE experiments (reprinted with permission from I.J. Koenka)

Acknowledgements

Special thanks to Israel Joel Koenka (University of Basel, Department of Chemistry) the inventor of this excellent software, also for his professional support during the implementation of Instrumentino at TU Wien.

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Open source data acquisition systems

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Abstract

Data acquisition (DAQ) is one of the last steps of signal processing in any analytical technique. In CE, the signal is typically recorded with moderate data sampling frequency (5 to 20 Hz) but for fast CE separations a higher sampling frequency (e.g., 100 Hz) may be required. This depends on the peak efficiency and separation speed. The DAQ aims to transform the measured signal (voltage, current, or any other measured electrical or physical property) into digital numeric values that can be further processed. Researchers building their own instrumentation with an autonomous DAQ system often face the question whether to buy a more expensive, but ready to use, "black box" type DAQ device available from a wide selection of vendors or to build their own, using the open source hardware/software knowledge base. In this lecture the principles of DAQ, aspects of bit-resolution and signal quality will be discussed. Some of the commercially available DAQ devices will be presented to give an overview what is available in the market. Other significant part of the talk will be devoted to the in-house built DAQ devices that are based on the open source paradigm.

Acknowledgements

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How to promote OpenCE instruments using the COST networking tools

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Abstract

The aim of this "roundtable discussion" is to discuss how to promote Open CE instruments to academia SMEs and society using the tools available within the COST Action. The discussion should also focus on how researchers that will get involved will benefit from their time (scientific output, networking).

The goals and tools of the PortASAP COST Actions (CA 16215) will also be briefly presented.[1]

Acknowledgements

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Method Development in CE using computer tools

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Abstract

The theories of movement of charged species in solutions stem from fundamental physico-chemical laws, which form an inherently nonlinear mathematical model. Its direct numerical solution (simulation) gives a complete picture about behavior of the electrophoretic systems in the electric field. Another approach is formulation of the approximate linear model. The linear model reveals that any solution of electrolytes possesses a set of certain characteristics – eigenmobilities, which play a substantial role when the electrolyte solution is used as the background electrolyte in electrophoresis.

Both the nonlinear and linear model of electromigration are implemented in two computer programs we developed, Simul[1] and PeakMaster[2], respectively. Both of them serve for method development in CE.

Simul helps to understand what takes place during the electrophoretic run. Specifically, it can be used for (i) optimizing analytes' stacking to obtain initial preconcentration, (ii) inspecting unusual peak broadening, and (iii) simulation of isotachophoresis.

PeakMaster serves rather for computer design of background electrolytes for capillary zone electrophoresis with optimized properties to reach (i) more sensitive detection, (ii) higher efficiency of separation, and (iii) better selectivity of separation. It calculates several parameters of the background electrolyte: pH, ionic strength, conductivity, buffer capacity. Specifically, it calculates system eigenmobilities, which gives information about number, positions and shapes of system peaks. For separated analytes it calculates effective mobility, extent of electromigration dispersion, and response in direct, indirect, and conductivity detection.

Acknowledgements

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Application of Capillary Electrophoresis with Contactless Conductivity Detection in Analysis of Clinical Samples.

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Abstract

It is advantageous to analyse small volumes of clinical samples in the range 10 – 50 µM by capillary electrophoresis (CE) performed in capillaries with thin internal diameters. In addition to high separation effectivity, short separation time and minimum requirements on the sample volume for analysis, CE enables direct determination of metabolites in their native biochemical forms. This eliminates the need to use time-demanding derivatisation of the individual metabolites, which is difficult to perform in such small volumes. In addition, the introduction of contactless conductivity detectors (C^4D) into CE avoids problems with the minimal ability of most metabolites to absorb electromagnetic radiation in the UV-VIS spectral region. C^4D is a universal detection technique whose detection sensitivity is not directly dependent on the structure of the analyte. The C^4D response can be greatly optimised by the varying composition of the background electrolyte.

A set of CE/C^4D methods for clinical analysis of i) whole spectrum of biogenic amino acids in different kind of body fluids; ii) a rapid determination of branched chain amino acids in human blood for metabolic research; iii) determination of spectrum of low molecular weight organic acids in urine for monitoring of the inborn metabolic fault; iv) the sensitive determination of neurotransmitters in microdialysates of periaqueductal gray matter for pharmacological treatment of pain; v) the rapid and sensitive monitoring of the oral antidiabetic drug metformin in human urine and blood; will be demonstrated and discussed.

Acknowledgements

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Application of CE in forensic analysis

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Abstract

For nearly 40 years, capillary electrophoresis (CE) has been found as a interesting, efficient and cost-effective tool for the investigation and separation of an enormous number of substances. Since its very beginning, the applications of CE in forensic analysis have become widely used by many forensic experts and researchers over the world[1]. Presently, the fundamental capabilities of CE, so important in case of criminal investigation, such as its high separation power, low-cost chiral analysis, or hyphenation with the mass spectrometry are quite commonly used, while other remain to be further explored, particularly those involving on-site analysis at the crime scene (for instance, with the use of chip electrophoresis or portable CE instruments).

In this presentation, the use of capillary electrophoresis in forensic analysis is reviewed, focusing on achievements accomplished at the Laboratory for Forensic Chemistry at Jagiellonian University in Kraków. Particularly, the applications of CE in the forensic toxicology, the questioned documents examination, and the investigation of psychoactive or toxic ornamental plants have been elaborated. Additionally, the use of CE-based techniques for investigation of physicochemical parameters of designer drugs has been also pointed out as the way to learn more about those dangerous substances. Finally, some potential development of CE techniques and methods with their application in forensic analysis has been also proposed and discussed.

Acknowledgements

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Challenges for portable analyzers space.

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Abstract

"Is there life in space or not?" There is definitely no clear answer to this question.

First issue to solve is definitely what to look for? If concentrate on the Earth – like life form then definitely liquid water and organic molecules to reflect the needs of biotic reactions[1]. For example, Creamer et al.[2] propose to focus on amino acids that occur in high abundance in both abiotic (meteorites) and biotic (E. coli protein mass) samples: Ala, Asp, Glu, Gly, His, Leu, Ser, and Val.

Second question to answer is which analytical tools to use? Existing techniques for extraterrestrial life search in space include various spectroscopic methods and GC-MS (on-site). Regrettably, when it comes to analyzing Earth – like concentrations of polar organic molecules in planetary samples in situ sampling techniques are preferable over optical ones. Stalport et al.[3] demonstrated that hydrated minerals and oxides present in Martian samples react rapidly with derivatization agent, making in situ derivatization and subsequent detection of amino acids and carboxylic acids by GC/MS on board of Mars rover Curiosity problematic.

Over the years as alternative for gas phase analysis, the liquid phase analytical techniques have been discussed. Namely, capillary (CE) or microchip (ME) electrophoresis can face the challenges of biosignature molecules analysis in extreme environmental conditions[4] like various temperatures, radiation, microgravity and complicated planetary sample matrix.

The final topic to clarify is where to look for life? According to the ESA's COSMIC VISION 2015-2025: PLANETS AND LIFE in chapter: "Life and habitability in the Solar System" - the main interest should be focused on the Mars and icy Jupiter's moon Europa.

In my presentation, I will make a short overview of the stated above issues and formulate the main challenges that portable analyzers of the liquids should address.

Acknowledgements

I would like to acknowledge my colleagues from Jet Propulsion Laboratory, NASA, namely Petr Willis, Jessica Creamer, Maria F. Mora and Aaron Noell for wonderful experience and cooperation. Additionally I would like to express gratitude to Fulbright Scholar Program (Tallinn).

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