

26th Young Investigators' Seminar on Analytical Chemistry
June 24 – 27, 2019, Pardubice, Czech Republic

YISAC 2019
Book of Abstracts

Edited by Radovan Metelka

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*Dedicated to Prof. Karel Vytřas (1944–2019) and Prof. Valerija Gužvanj (1975–2019),
great scientists, colleagues and friends.*

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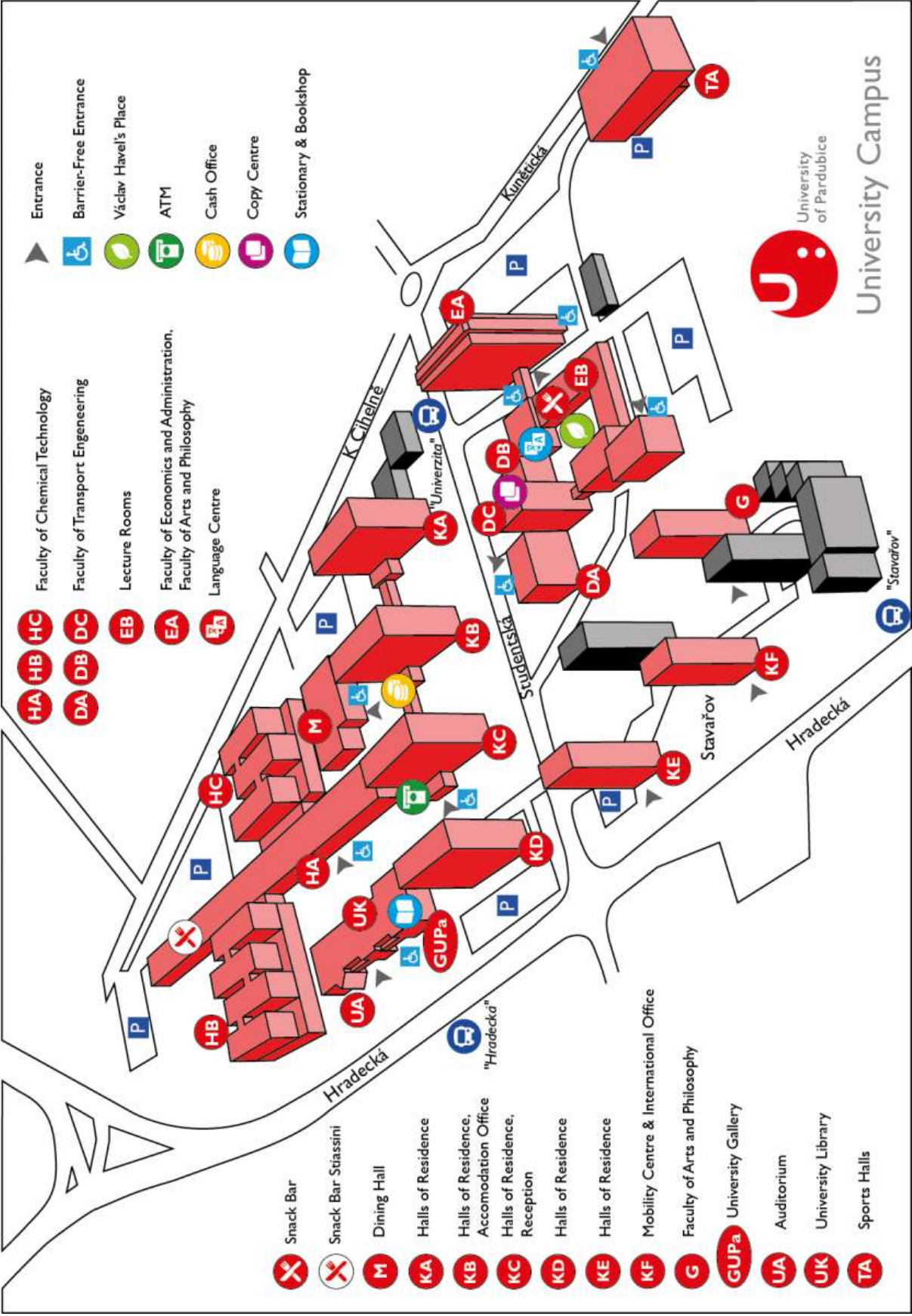
Granit Jashari

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Location and Date

The lectures of YISAC 2019 conference will take place in the CI lecture hall, located in the first floor of building HA in Faculty of Chemical Technology, University of Pardubice, Studentská 573, Pardubice, from June 24 to June 26, 2019.

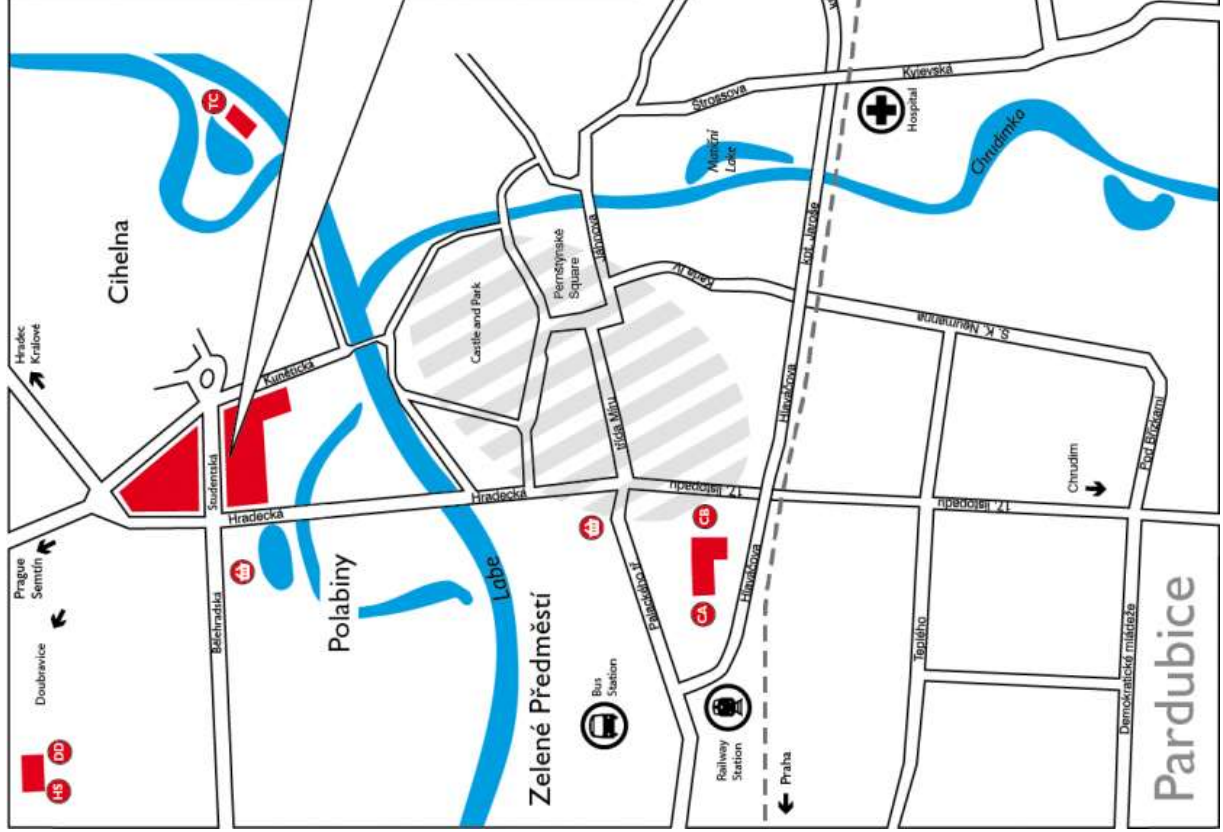
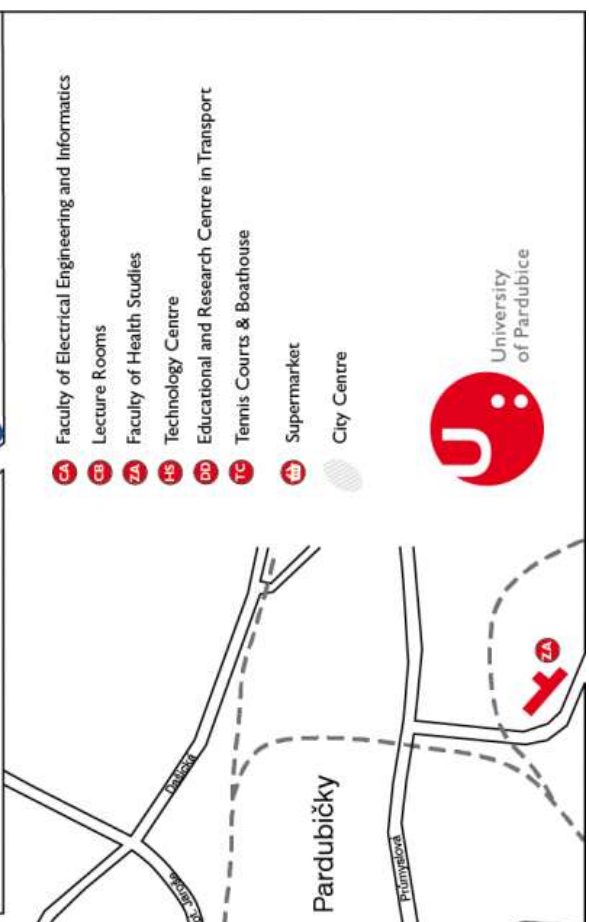
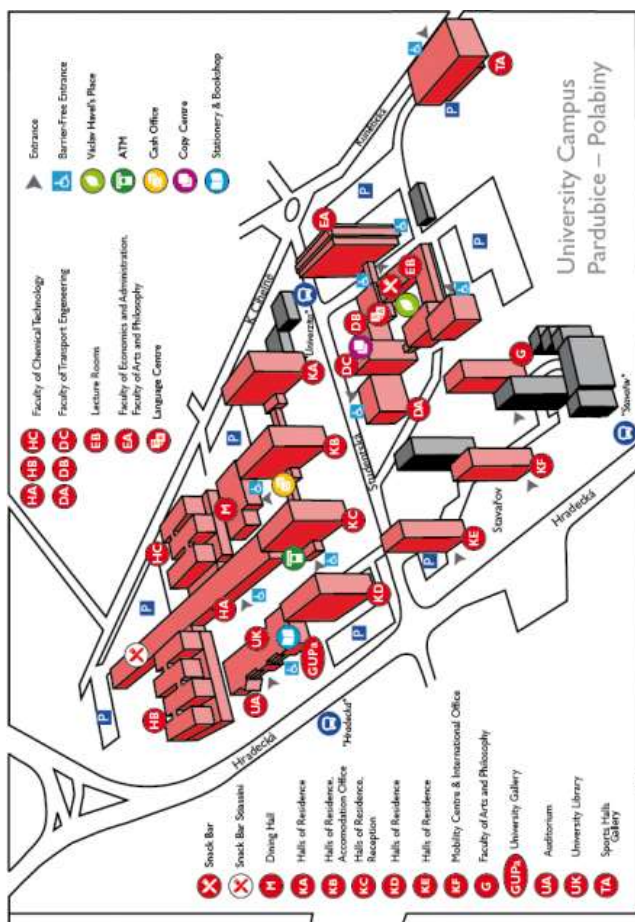
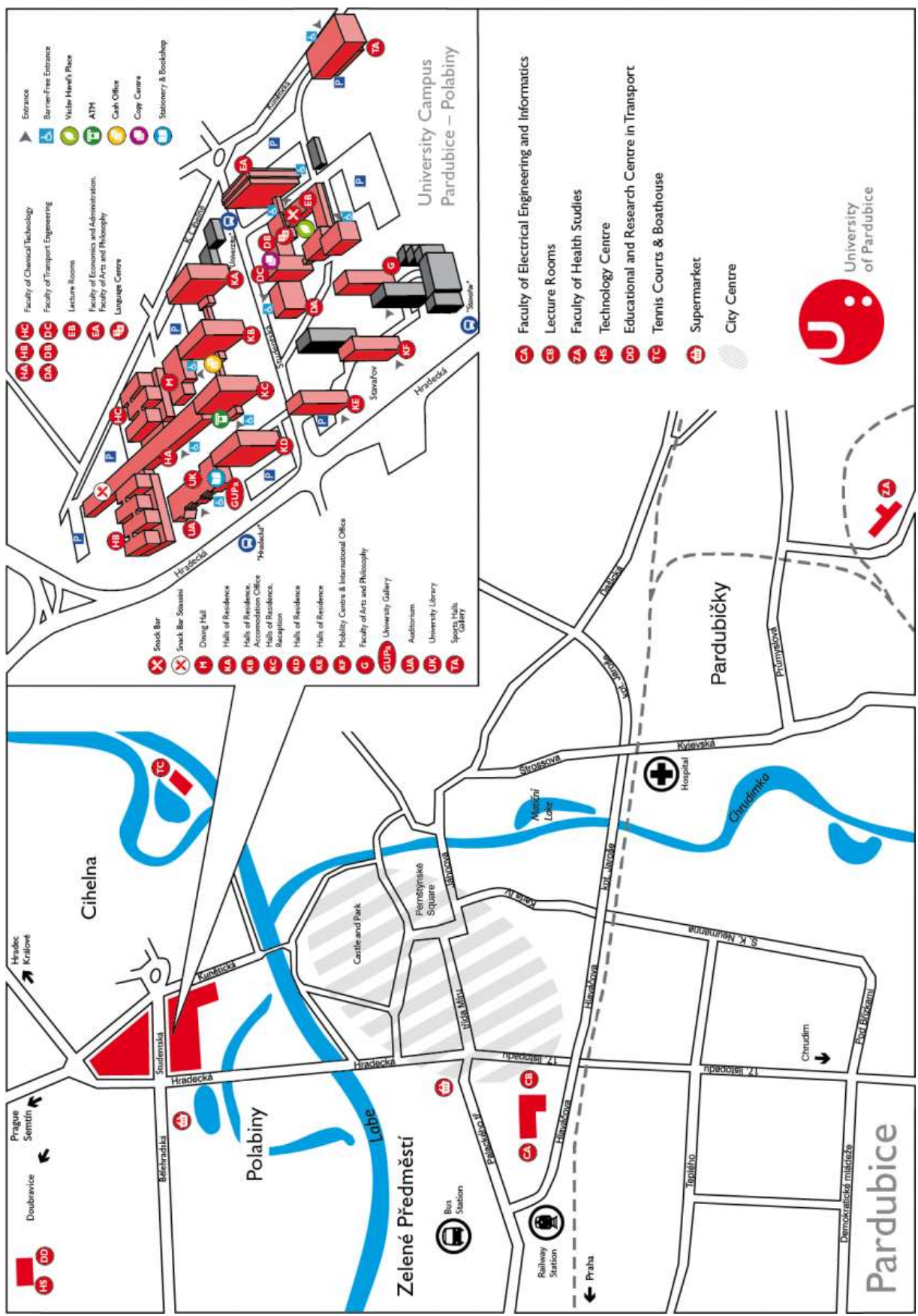


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YISAC 2019 PROGRAMME

MONDAY, JUNE 24

17:00 REGISTRATION - Entrance Hall at Faculty of Chemical Technology, Studentská 573, Pardubice (1st floor)

18:00 GET-TOGETHER PARTY - Lecture Hall CI (1st floor)

TUESDAY, JUNE 25

8:00 REGISTRATION

9:00 OPENING CEREMONY - Lecture Hall CI (1st floor)

9:15 HOMAGE to Prof. Karel Vytrás and Prof. Valerija Gužvanj

SESSION 1	Sensors I	Chairs: Łukawska A., Vlahović F.
9:45	Farag Amir Shaaban <i>Development of carbon nanotube based sensor for determination of antiarrhythmic drug propafenone in pharmaceutical and biological samples</i>	
10:05	Šekuljica Sanja <i>Comparison of two imidazolium ionic liquids modified carbon paste electrodes for trace level voltammetric determination of dopamine</i>	
10:25	Sedlar Andrea <i>Preparation of a novel membranes for ion-selective electrodes for iron determination based on iron(II) sulfide and silver sulfide</i>	

10:45 – 11:05 COFFEE BREAK

SESSION 2	Electrochemistry I	Chairs: Zrinski I., Farag A. S.
11:05	Łukawska Anna <i>Influence of constant magnetic field on electrochemical oxidation of sulfonamides.</i>	
11:25	Gričar Ema <i>Electrochemical characterization of ferrocene derivates of glyphosate and its degradation product</i>	
11:45	Jashari Granit <i>Possibilities of simultaneous voltammetric detection of tocopherols in non-aqueous media</i>	

12:05 – 14:00 LUNCH

SESSION 3 Separation Methods I Chairs: **Rijavec T., Šulc J.**

- 14:00 **Pavlin Anže**
LC-MS analysis of glycolysis metabolites using HILIC
- 14:20 **Frühbauerová Michaela**
UHPLC analysis and antioxidant activity of carob powder
- 14:40 **Doležánová Pavla**
Separation of natural dyes using two-dimensional liquid chromatography

15:00 – 15:20 COFFEE BREAK

SESSION 4 Separation Methods II Chairs: **Frühbauerová M., Pavlin A.**

- 15:20 **Warzechová Petra**
Optimization of liquid chromatographic separation of benzodiazepines
- 15:40 **Rijavec Tjaša**
Oxidative degradation of spermine and spermidine
- 16:00 **Presentation by INFINEON**
- 16:30 **Presentation by METROHM**

19:00 SUPERVISORS DINNER

WEDNESDAY, JUNE 26

SESSION 5 Separation Methods III Chairs: **Smělá D., Metarapi D.**

- 9:00 **Šulc Jakub**
Spatial distinguishing of larch wood samples using volatile compounds fingerprinting
- 9:20 **Řebíčková Kristýna**
*Comparison of chemical composition and biological properties of essential oils obtained by hydrodistillation and steam distillation of *Laurus nobilis* L.*
- 9:40 **Machová Martina**
Volatile substances released from onion
- 10:00 **Janečková Michaela**
Quality control of antidiabetic drugs using isotachopheresis

10:20 – 10:40 COFFEE BREAK

SESSION 6 Other Methods Chairs: **Janečková M., Řebíčková K.**

10:40 Vlahovič Filip

Exploring anatomy of experiment with DFT: quantitative structure-activity relationship of substituted arylazo pyridine dyes in photocatalytic reaction

11:00 Metarapi Dino

Advanced imaging of nanoparticles in biomaterials - data processing in laser ablation–single particle–ICPMS

11:20 Šťovíčková Eliška

Phosphorylation of tau protein by soluble and immobilized kinases

11:40 Smělá Denisa

Micro-RNA isolation using TiO₂ materials

12:00 – 14:00 LUNCH

SESSION 7 Sensors II Chairs: **Morawska K., Festinger N.**

14:00 Zrinski Ivana

Evaluation of total antioxidant capacity in beverages based on laccase immobilized on screen-printed carbon electrode modified with graphene nanoplatelets and gold nanoparticles

14:20 Kaczmarek Katarzyna

Electrochemical determination of clorsulon using modified with single-walled carbon nanotubes glassy carbon electrode

14:40 Djurdjic Sladjana

Enzymatic polyphenol index biosensor based on graphene nanoplatelets decorated with MnO₂ nanoparticles. Preparation, characterization and analytical application

15:00 – 15:20 COFFEE BREAK

SESSION 8 Electrochemistry II Chairs: **Djurdjic S., Kaczmarek K.**

15:20 Morawska Kamila

Application of silver amalgam film electrode to study DNA-herbicides interactions

15:40 Kołodziejczyk Karina

Electrodeposition of metals with different magnetic properties in constant magnetic field

16:00 Festinger Natalia

Voltammetric determination of mandipropamid on edge-plane pyrolytic graphite electrode

19:00 CONFERENCE DINNER

THURSDAY, JUNE 27

10:00 OPTIONAL EXCURSION - Sightseeing tour to old city of Pardubice and chateau

TUESDAY, JUNE 25
SESSION I
Sensors I

DEVELOPMENT OF CARBON NANOTUBE BASED SENSOR FOR DETERMINATION OF ANTIARRHYTHMIC DRUG PROPAFENONE IN PHARMACEUTICAL AND BIOLOGICAL SAMPLES

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A novel electroanalytical method for the determination of propafenone (PPF) in pharmaceutical dosage form and biological fluids using the glassy carbon electrode modified with NH₂-functionalized multi-walled carbon nanotubes as a sensitive sensor. Several working conditions necessary for PPF electrochemical determination using differential pulse voltammetry, such as pH, scan rate, accumulation time and potential were optimized. The results showed a significant enhancement of the peak current after modification of the electrode. The calibration curves of PPF showed a linearity from 0.1 to 10 μM, characterized by limits of quantification 0.03 μM and limit of detection 0.01 μM. The results of model analysis by the proposed method were satisfactory and statistically evaluated.

PPF is a potent and popular antiarrhythmic drug because of its β-adrenergic receptors antagonism effect, making it effective and commonly used in the treatment of supraventricular and ventricular arrhythmias [1]. Among carbon nanomaterials, carbon nanotubes are one of the most important materials which are frequently used to modify electrodes [2]. They have several advantages as electrochemical sensors, including wide potential window, large surface area, fast electron transfer rate and high electrical conductivity [3].

1. Harriet M. B., Katharine J. P., Heather D. L., Andrew F., *Drugs* **1993**, 45, 85-130.
2. Rongzhou L., Tuti M. L., Tuan T., *Electrochem. Commun.* **2018**, 86, 135-139.
3. Zhenping L., Mingliang J., Jieping C., Juan W., Xin W., Guofu Z., Albert B., Lingling S., *Sensors and Actuators B: Chemical* **2018**, 257, 1065-1075.

COMPARISON OF TWO IMIDAZOLIUM IONIC LIQUIDS MODIFIED CARBON PASTE ELECTRODES FOR TRACE LEVEL VOLTAMMETRIC DETERMINATION OF DOPAMINE

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Dopamine (DA) as one of the most significant catecholamine neurotransmitter could be determined by carbon paste electrodes (CPE) consisted of graphite powder and paraffin oil modified by imidazolium ionic liquids (ILs) such as 1-ethyl-3-methylimidazolium 1,1,2,2-tetrafluoroethanesulfonate ([emim][CHF₂CF₂SO₃]) and 1-ethyl-3-methylimidazolium thiocyanate ([emim][SCN]). The hydrophilic nature of these imidazolium ILs with different anions is proven [1, 2] and could exhibit the characteristic affinity towards DA. Voltammetric behavior of DA was investigated by means of cyclic voltammetry and direct anodic square wave voltammetry (SWV) in model systems. The obtained results indicated that the [emim][CHF₂CF₂SO₃]-CPE is more suitable for DA determination than the [emim][SCN]-CPE. Under the optimal working conditions, a linear dependences of the oxidation peak current in concentration ranges 0.16–13.46 µg mL⁻¹ and 0.02–0.28 µg mL⁻¹ of DA were received with the CPE, and [emim][CHF₂CF₂SO₃]-CPE, respectively. An evaluated limit of detection was 0.006 µg mL⁻¹ for DA oxidation peak obtained by SWV method employing the [emim][CHF₂CF₂SO₃]-CPE. The developed method was applied for controlling of the active ingredient content in DA hydrochloride injection ampoule in infusion matrices as 0.9% NaCl, 5% glucose and Ringer solution. The determined content of the target analyte was in a good agreement with declared amount in pharmaceutical preparation.

The authors acknowledge the financial support of the Ministry of Science and Technological Development of the Republic of Serbia (Project No. 172059 and 172012).

1. Červinka C., Pádua A. A. H., Fulem M., *Journal of Physical Chemistry B* **2016**, 120, 2362-2371.
2. Vataščin E., Dohnal V., *Journal of Chemical Thermodynamics* **2017**, 106, 262-275.

PREPARATION OF A NOVEL MEMBRANES FOR ION-SELECTIVE ELECTRODES FOR IRON DETERMINATION BASED ON IRON(II) SULFIDE AND SILVER SULFIDE

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Potentiometric ion determination method is very inexpensive, simple and reasonably fast method so due to that it is often applied for different cations or anions determination [1]. “Home-made” ferrous ion-selective electrode has been prepared by constructing a solid membrane disk consisting of <45 µm sized particles of silver sulphide, ferrous sulphide and PTFE. Ratio of FeS:Ag₂S:PTFE in membrane was 1:4:5. Prepared membrane was built in specially constructed electrode body made of Teflon [1, 2].

Membrane was tested to response of ferrous cations in acetic buffer (pH = 4.00). Testing were performed in laboratory prepared 0.1 mol L⁻¹ iron(II) sulphate solution and results showed linear membrane response for ferrous cations in concentration range between 0.1 mol L⁻¹ and 1.95 · 10⁻⁴ mol L⁻¹ with change of 32.3 mV per decade and detection limit of 1.45 · 10⁻⁴ mol L⁻¹.

Prepared membrane was also used for ferrous determination in real sample solution of dietary supplement which contains iron(II) sulphate as active substance in very good agreement with declared results.

1. Bralić M., Prkić A., Radić J., Pleslić I., *Int. J. Electrochem. Sci.*, **2018**, 13, 1390-1399.
2. Prkić A., Vukušić T., Mitar I., Giljanović J., Sokol V., Bošković P., Jakić M., Sedlar A., *Int. J. Electrochem. Sci.* **2019**, 14, 861-874.

TUESDAY, JUNE 25
SESSION 2
Electrochemistry I

INFLUENCE OF CONSTANT MAGNETIC FIELD ON ELECTROCHEMICAL OXIDATION OF SULFONAMIDES

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Sulfonamides are one of the oldest classes of antibiotic drugs that have been widely used in human and veterinary medicine due to their low cost, low toxicity and high efficiency in the treatment of various bacterial infections. Mechanism of sulfonamides action is based on their competition with para-aminobenzoic acid, the compound essential for the synthesis of folic acid, which is essential for the development of bacterial cells. The basic sulfonamides structure consists of benzene ring with amine group in the para position and the sulfonamide group (Fig. 1). A number of sulfonamide derivatives with different physicochemical, pharmacokinetic, pharmacodynamics properties were obtained by substitution of the hydrogen atom on the nitrogen of sulfonamide group or occasionally aromatic amino group [1, 2].

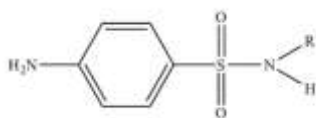


Fig.1. Base structure of sulfonamides.

Numerous studies have shown influence of the magnetic field on electrodeposition of metals, alloys, conductive polymers. There were also reported some examples of the influence of external constant magnetic field on organic reactions such as photoisomerization of isoquinoline *N*-oxide, Kabachnik-Fields reaction and the aza-Pudovik reaction [3]. The aim of these studies was to investigate the effects of constant magnetic field on electrochemical oxidation of sulfonamide compounds. The electrochemical behaviours of selected sulphonamides were studied at glassy carbon electrode (GCE) using cyclic voltammetry (CV) technique.

1. Tačić A., Nikolić V., Nikolić L., Savić I., *Advanced Technologies* **2017**, 6, 58-71.
2. Ait Lahcen A., Amine A., *Analytical Letters* **2018**, 51, 424-441.
3. Karpowicz R., et al., *Chemical Papers* **2016**, 70, 1529-1532.

ELECTROCHEMICAL CHARACTERIZATION OF FERROCENE DERIVATE OF GLYPHOSATE AND ITS DEGRADATION PRODUCT

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With glyphosate being one of the most controversial pesticides it is important to study characteristics about the compound [1]. The newly synthesized compounds between glyphosate (or its degradation product, aminomethylphosphonic acid (AMPA)) and ferrocenoyl chloride show complex electrochemical behaviour. The electron transfer process contains of one electron being transferred, however it is probably followed by chemical reaction. This can be seen on cyclic voltammogram (CV) as an increase in ΔE_{pp} value, which suggests the electrochemical process is quasi-reversible [2]. CVs obtained with AMPA or glyphosate compound do not show critical differences, but AMPA electrode process is faster. The analyte is degrading in solution, which is obvious when comparing CVs of freshly prepared solution and 4 days old solution. Using different working electrodes (AuE, MDE, BDDE and GCE) gives crucial information about the electrode processes. Merely from the peak shapes we can determine where the electrochemical process is diffusion controlled and where the reaction itself is rate the determining step. When studying different pH values' effect on electrochemical behaviour of the analyte one can conclude that the optimal pH range for studying the analyte is between 6.0 and 7.0. When studying the impact of different scan rates the Randles–Ševčík equation was used.

Additionally, thermal analysis was performed and it shows the complexity of the multiple-step degradation – in the mass spectra we can find characteristic signals for water, CO₂, nitrogen and phosphorus oxides, cyclopentadienyl ions and other reactive carbon species [3].

1. Duke S. O., *Pest. Manag. Sci.* **2018**, 74, 1027-1034.
2. Aristov N., Habekost A., *World J. Chem. Educ.* **2015**, 3, 115-119.
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POSSIBILITIES OF SIMULTANEOUS VOLTAMMETRIC DETECTION OF TOCOPHEROLS IN NON-AQUEOUS MEDIA

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Tocopherols (vitamin E; VE) are lipophilic phenolic antioxidants, naturally occurring in vegetable oils, which are widely used in pharmaceutical and cosmetic industries [1, 2]. These organic compounds are known as vitamins that organism needs in small quantities for the proper functioning of its metabolism. Hence, it is necessary to control their intake for dietary purposes and to determine their content in foodstuffs. VE protect human body against cancer, cataract and cardiovascular disease. Avitaminosis may cause circulatory disorders and affects the metabolism pathway in muscle [2-4].

An electrochemical study was performed to develop a voltammetric method suitable for simultaneous detection of tocopherols based on their anodic oxidation at various electrode materials in non-aqueous supporting electrolytes. Several working conditions, such as selection of working electrode, supporting electrolyte, effect of surfactant, water content and parameters of square-wave voltammetry, were optimized. An overlapping of corresponding peaks of alpha-, gamma- and delta- form was observed. The separation of oxidation signals was improved at optimum working conditions, but the signals were not completely resolved. It was found that this phenomenon did not cause significant changes in peak currents for constant concentrations of tocopherols in their different concentration ratios, which could be possibly utilized in analytical application. Nevertheless, use of some data processing techniques could help to improve the resolution of signals and to enable more precise analytical quantification of different tocopherols.

1. Ruperez F. J., Martin D., Barbas C., *Journal of Chromatography A* **2001**, 935, 45-69.
2. Li S. G., Xue W. T., Zhanga H., *Electroanalysis*, **2006**, 18, 2337-2342.
3. Sýs M., Švecová B., Švancara I., Metelka R., *Food Chemistry* **2017**, 229, 621-627.
4. Bakre S. M., Gadmale D. K., Toche R. B., Gaikwad V. B., *Journal of Food Science and Technology* **2015**, 52, 3093-3098.

TUESDAY, JUNE 25
SESSION 3
Separation Methods I

LC-MS ANALYSIS OF GLYCOLYSIS METABOLITES USING HILIC

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This research project is aimed at finding optimal method for separation of glycolysis metabolites using principles of Hydrophilic interaction liquid chromatography (HILIC). HILIC represents an effective separation of small polar compounds on polar stationary phases [1].

The method for separation of 11 analytes was developed. That includes several glycolysis metabolites such as glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), dihydroxyacetone phosphate (DHAP), fructose-1,2-bisphosphate (FBP), glyceraldehyde-3-phosphate (GAP), 3-phosphoglycerate (PGA), phosphoenolpyruvate (PEP), pyruvate (Pyr), lactate (Lac) and cofactor nicotinamide adenine dinucleotide (NAD) and its phosphorylated form (NADP).

For the purpose different columns were compared. We tested Xbridge Amide 3.5 µm; 3.0 × 100 mm (Waters), HILIC A 3 µm; 100 × 3.0 mm (ACE), HILIC B 3 µm; 100 × 3.0 mm (ACE) and HILIC N 3 µm; 100 × 3.0 mm (ACE) exploring a wide range of parameters such as mobile phase constitution, pH, ion strength, gradient vs. isocratic. Ion strength of the mobile phase was tested in the range from 10 to 15 mM and the pH of buffer was set to 2.1, 3.0 or 4.7. The measurements were performed at room temperature.

SRM transitions for all analytes were optimized using direct infusion, while the optimization of LC-MS method was performed on Perkin Elmer PE200 HPLC with mass spectrometer 3200QTrap (AB Sciex).

Optimal results were obtained with Xbridge Amide 3.5 µm; 3.0 × 100 mm (Waters) column where the concentration of buffer salt was 15 mM and the pH of buffer solution was 3.0.

I. Buszewski B., Noga S., *Anal. Bioanal. Chem.* **2002**, 402, 231–247.

UHPLC ANALYSIS AND ANTIOXIDANT ACTIVITY OF CAROB POWDER

Frühbauerová M.¹, Červenka L.¹, Hájek T.¹, Pouzar M.², Palarčík J.²

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Work was focused on optimization of extraction of polyphenolic compounds from carob powder after grinding on different grinders, cryogenic and vibratory (4 and 8 minutes of grinding on this grinder type). Each powder was extracted to methanol (90:10 with water (v/v), acidified by 15 µl of formic acid).

In the next step, the method for analysis of compounds in carob powder using liquid chromatography with reversed-phase (Kinetex XB-C18 100Å column, 150×2.1 mm, particle size 1.7 µm) was optimized. The mobile phase was formed by mixture of acetonitrile and deionized water with addition of formic acid (pH ~ 3.1). Gradient elution for separation was used. Diode array detector (DAD) was employed for detection ($\lambda = 270, 290$ and 320 nm). Eight compounds (vanillic acid, ferulic acid, cinnamic acid, quercitrin, luteolin, naringenin, apigenin and chrysoeriol) were found in all samples. The highest amount of these compounds was detected in extract from powder after cryogenic grinding.

Determination of antioxidant activity of carob extracts by DPPH and ABTS method was part of this work too. Results were reported as Trolox equivalent (TE). For extract from carob powder after cryogenic grinding, 15.60 ± 0.87 mg TE/g of powder (DPPH method) and 28.58 ± 2.92 mg TE/g of powder (ABTS method) were found. Regarding the extract of carob powder (vibratory grinding for 4 min), 9.06 ± 0.60 mg TE/g of powder (DPPH method) and 17.31 ± 1.28 mg TE/g of powder (ABTS method) were determined. Preparation of carob powder by vibratory grinding for a longer period of time (i.e. 8 min) resulted in the increase of antioxidant activity in terms of DPPH (13.21 ± 0.63 mg TE/g of powder) and ABTS methods (22.82 ± 1.77 mg TE/g of powder) in comparison with vibratory grinding for 4 min. All results are shown as arithmetic mean with confidence interval (level of significance 0.05).

SEPARATION OF NATURAL DYES USING TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY

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This work is focused on optimization of separation of natural food dyes and natural antioxidants by liquid chromatography. We analysed porphyrin dyes that belong to the group of tetrapyrrole dyes, consisting of four pyrrole rings bonded by methine bridges. The antioxidants used were flavonoids and phenolic acids typically present in plant material. Both groups of dyes represent complex mixtures, which are not easy to analyse using conventional liquid chromatography.

For separation of more complex samples, two-dimensional liquid chromatography (2D LC) can be applied, utilising two different chromatographic processes. The sample is separated on the first column, collected in the eluate fractions and further separated on the second column containing different stationary phase. The method can provide higher resolution with respect to the conventional LC, but the issues of dilution and compatibility of mobile phases should be avoided.

In present work, the purity of available dye standards was verified using liquid chromatography coupled with mass spectrometry. To realize the two-dimensional liquid chromatography, the retention behaviour of the compounds in reversed-phase (RP) and hydrophilic interaction mode (HILIC) was studied utilizing orthogonal separation mechanisms suitable for 2D LC. We have studied retention using various stationary phases (RP: octadecyl silica gel and biphenyl, HILIC: silica gel) and mobile phase additives (ammonium acetate, ammonium formate, formic acid and acetic acid). We have evaluated effect of sample solvent on separation using different mixtures of acetonitrile and water. In 2D LC mode, we have tested different gradient profiles, loop sizes and switching times for separation of standards of porphyrin dyes, chlorophyllin sample and mixture of antioxidant standards.

TUESDAY, JUNE 25
SESSION 4
Separation Methods II

OPTIMIZATION OF LIQUID CHROMATOGRAPHIC SEPARATION OF BENZODIAZEPINES

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Benzodiazepines are well known group of chemical substances, which are the most popular psychotropic pharmaceuticals commonly used for therapeutics, treatment and care for lot of psychic diseases. Benzodiazepines are used for treating of anxiety, insomnia, muscle relaxation, panic attacks or for depression [1]. Function of benzodiazepines is connected with neurotransmitters (dopamine, serotonin and gamma-aminobutyric acid), which are commonly presented in human brain. These neurotransmitters are responsible for communication between brain cells and can have either tranquilizing or excitatory effects. Benzodiazepines enhance the effect of the GABA, which add to the calming effect already produced by the human body and keep the brain in a more tranquilized state.

In our research, we have optimized isocratic separation of selected benzodiazepines using window-diagram approach. We used six standards of benzodiazepines (phenazepam, pyrazolam, flubromazepam, meclonazepam, diazepam and diclazepam). These standards were separated with reversed-phase chromatography with UV detection and the identity of the compounds was verified using mass spectrometry. For separation, several stationary phases were tested including C18 phases (Luna Omega and Ascentis Express columns), phenyl-hexyl phase and biphenyl phase. The composition of mobile phase was optimized using acetonitrile and methanol in mixture with water containing additives (acetic acid and phosphate buffer). Optimal conditions obtained using window-diagram approach were directly applicable as initial composition of mobile phase for gradient elution analyses.

I. Tomková J., Švindroch M., *Journal of Separation Science* **2017**, *40*, 1855-2080.

OXIDATIVE DEGRADATION OF SPERMINE AND SPERMIDINE

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Latest research indicates that polyamines are important biological molecules as they have beneficial effects associated with health and ageing [1, 2]. As polyamines transform at oxidative conditions in food products, degradation of some polyamines was investigated, including the identification of degradation products. This is an important aspect influencing quantitative determination of individual polyamines.

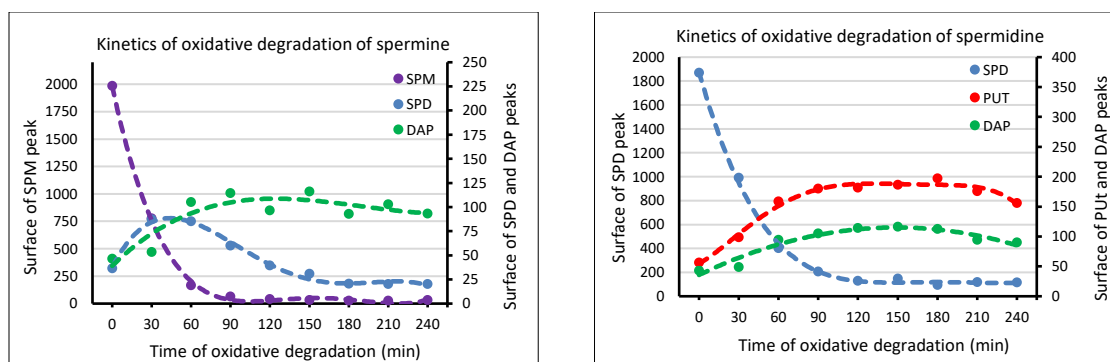


Fig. 1. Kinetic of degradation of spermine (left) and spermidine (right) with 1.8 mM KMnO_4 at 40 °C by HPLC-FLD. The oxidation products were already present in the solution at $t = 0$ min.

Initially the method for quantitative determination of biogenic amines agmatine, tryptamine, β -phenethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine in the concentration range 0.1 – 1.0 mg L^{-1} was established using a HPLC-FLD system after the derivatisation of amines with dansyl chloride. Further, oxidative degradation of spermine and spermidine was investigated with H_2O_2 and KMnO_4 . With both oxidants degradation products were observed. The major degradation products of 0.34 mM spermidine were putrescine and 1,3-diaminopropane, and of 0.25 mM spermine were spermidine and 1,3-diaminopropane, which were identified with LC/MS/MS after derivatisation. Oxidation with 1.8 mM KMnO_4 solution was carried out at 40 °C, where degradation kinetics were observed (Fig. 1). The concentration of the products was high enough for quantification with a fluorescent detector.

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WEDNESDAY, JUNE 26
SESSION 5
Separation Methods III

SPATIAL DISTINGUISHING OF LARCH WOOD SAMPLES USING VOLATILE COMPOUNDS FINGERPRINTING

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Optimized headspace solid-phase microextraction combined with gas chromatography with flame ionization detector (HS-SPME-GC-FID) method was used to analyse volatile organic compounds (VOC) in multiple samples from Central Europe and Siberia. Different composition and content of VOC was found and used as a volatile compounds fingerprint. To characterized fingerprints the retention indices (RI) were calculated for each compound. Obtained RI was statistically evaluated by multivariate regression with reduction of dimensionality – orthogonal projections to latent structure (OPLS). It was able to distinguish correct origin of all 82 larch wood samples. Hence, analysis of VOC fingerprints using HS-SPME-GC-FID in combination with OPLS presents useful tool for distinguishing between wood of European larch and Siberian larch.

COMPARISON OF CHEMICAL COMPOSITION AND BIOLOGICAL PROPERTIES OF ESSENTIAL OILS OBTAINED BY HYDRODISTILLATION AND STEAM DISTILLATION OF *LAURUS NOBILIS* L.

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The purpose of this study was to compare the yield, chemical composition and antimicrobial and antioxidant properties of essential oils isolated from leaves of *Laurus nobilis* L. by two different distillation methods. The essential oils isolated by hydrodistillation (HD) and steam distillation (SD) were analyzed by gas chromatography coupled to mass spectrometry (GC-MS) and gas chromatography with flame ionization detector (GC-FID). Hydrodistillation produced a yield of 0.95 ± 0.06 % which is slightly higher than yield obtained by steam distillation 0.79 ± 0.07 %. Seventy three compounds in the bay leaves oil obtained by steam distillation were identified while in essential oil obtained by hydrodistillation only fifty four compounds were identified. The antioxidant activity was evaluated by the DPPH radical method. Antimicrobial activity of obtained essential oils was evaluated by disc diffusion method in comparison with several chosen antimicrobials. The antimicrobial activity was tested on five microorganisms - *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Candida albicans*. In general, oils produced by steam distillation had higher antimicrobial and antioxidant activities than hydrodistillation extracts. It seems that hydrodistillation is better for higher yield while steam distillation is better to use for more quality oils with stronger biological properties.

VOLATILE SUBSTANCES RELEASED FROM ONION

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Plants belonging to the genus *Allium* are known for their high content of organosulfur compounds, which give them typical flavor and aroma released during their processing, but also a lot of biologically important effects, especially antimicrobial. The volatile compounds are released enzymatically after rupture of the plant cells. The released volatile organosulfur compounds of onion and their monitoring in time was done by using headspace solid phase microextraction coupled to gas chromatography with mass spectrometry (HS-SPME/GC-MS). It was identified 19 sulfur compounds with different course of release. The main released compounds were thiopropanal S-oxide, methylprop(en)yl disulphide and prop(en)yl trisulphide. Antimicrobial activity of onion juice was tested on 8 microorganisms using disc diffusion and well diffusion method. Completely resistant microorganisms were *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The largest inhibitory zones showed *Candida albicans*.

QUALITY CONTROL OF ANTIDIABETIC DRUGS USING ISOTACHOPHORESIS

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In this contribution determination of chosen antidiabetics is described. There are several types of antidiabetics, but drugs based of biguanide are the most used and they are first-line medication for the treatment of type 2 diabetes mellitus. Because of this fact biguanides (metformin, buformin, phenformin) were chosen as analytes of our interest.

Due to analytes structure, cationic mode of isotachopheresis was used. Firstly, optimization of separation, including mainly selection of electrolyte system, was done. Use of leading electrolyte consisting of 0.01 mol L⁻¹ potassium acetate with acetic acid for adjusting pH to 5.0 and 0.01 mol L⁻¹ acetic acid as terminating electrolyte was the best choice for successful separation (Fig. 1). Detection was done by two detectors – conductometric and spectrophotometric (230 nm). Under these conditions calibration curves and analytical parameters of developed method were determined as follow: repeatability from 3.1 to 5.2 %, recovery from 90.6 to 108.0 %, LOD for metformin 4.2 µg L⁻¹, for buformin 6.2 µg L⁻¹ and for phenformin 4.4 µg L⁻¹, LOQ for metformin 12.6 µg L⁻¹, for buformin 18.6 µg L⁻¹ and for phenformin 13.2 µg L⁻¹.

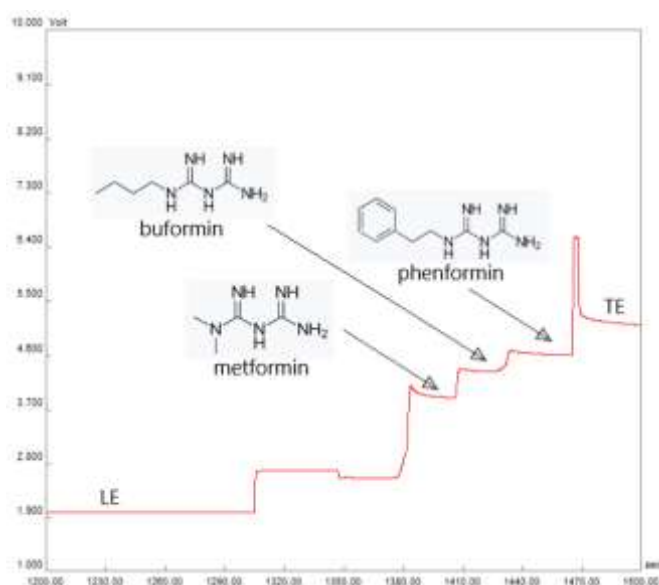


Fig. 1. Isotachopheretic separation of biguanide antidiabetics - LE: 0.01 mol L⁻¹ potassium acetate with acetic acid (pH 5.0), TE: 0.01 mol L⁻¹ acetic acid, conductometric detection.

WEDNESDAY, JUNE 26

SESSION 6

Other Methods

EXPLORING ANATOMY OF EXPERIMENT WITH DFT: QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP OF SUBSTITUTED ARYLAZO PYRIDINE DYES IN PHOTOCATALYTIC REACTION

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A series of arylazo pyridone dyes was synthesized by changing the type of the substituent group in the diazo moiety, ranging from strong electron-donating to strong electron-withdrawing groups. The structural and electronic properties of the investigated dyes was calculated at the M062X/6-31+G(d,p) level of theory. The observed good linear correlations between atomic charges and Hammett σ_p constants provided a basis to discuss the transmission of electronic substituent effects through a dye framework. The reactivity of synthesized dyes was tested through their decolorization efficiency in TiO₂ photocatalytic system (Degussa P-25). Quantitative structure-activity relationship analysis revealed a strong correlation between reactivity of investigated dyes and Hammett substituent constants. The reaction was facilitated by electron-withdrawing groups, and retarded by electron-donating ones. Quantum mechanical calculations were used in order to describe the mechanism of the photocatalytic oxidation reactions of investigated dyes and interpret their reactivity within the framework of the Density Functional Theory (DFT). According to DFT based reactivity descriptors, i.e. Fukui functions and local softness, the active site moves from azo nitrogen atom linked to benzene ring to pyridone carbon atom linked to azo bond, going from dyes with electron-donating groups to dyes with electron-withdrawing groups [1].

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ADVANCED IMAGING OF NANOPARTICLES IN BIOMATERIALS - DATA PROCESSING IN LASER ABLATION–SINGLE PARTICLE– ICPMS

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Characterization of nanoparticles (NPs) has in the past decade increased in importance with increasing introduction of NPs in fields ranging from biology and medicine to energy production and consumer goods [1]. Depending on the sample analyzed, many methods are available, one of them being single particle-inductively coupled plasma mass spectrometry (SP-ICPMS) [2]. While ICPMS is a highly sensitive technique, SP-ICPMS is inherently limited with its sample introduction method which generally allows only NPs to be analyzed in a solution, thus losing the spatial distribution information of the analyte in the sample. In a recent paper we have shown that determining the size of gold nanoparticles (AuNPs) in solid samples is possible without losing spatial distribution information using laser ablation-SP-ICPMS [3]. However, unlike in solution SP-ICPMS where the NP signal can be differentiated from the dissolved signal of the analyzed species by simple dilution of the analyzed sample, this is not possible with solid samples and laser ablation-SP-ICPMS. Therefore, considerable efforts have to be devoted to developing an algorithm capable of differentiating the NP signal from the background or dissolved signal of the analyzed species. This presentation will focus on the fundamentals of the technique for localized NP analysis in biomaterials, i.e. retrieve both the NP number concentration and the NP size.

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PHOSPHORYLATION OF TAU PROTEIN BY SOLUBLE AND IMMOBILIZED KINASES

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Phosphorylation is a significant post-translational modification of proteins. Phosphorylation is catalyzed by kinases and is based on the transfer of the phosphate group from adenosine triphosphate to the hydroxyl group of serine, threonine or tyrosine [1]. Phosphorylation is involved in the regulation of protein functions, intercellular communication, cell proliferation, differentiation and apoptosis [2].

Tau protein is a diagnostically significant phosphoprotein with 85 potential phosphorylation sites [3]. Tau protein is phosphorylated at 18 phosphorylation sites under physiological conditions. In the case of Alzheimer's disease, tau protein is hyperphosphorylated [4].

The aim of our study is to prepare phosphorylated protein standards for detection and quantification of hyperphosphorylated tau in cerebrospinal fluid of patients with Alzheimer's disease. Recombinant tau protein was phosphorylated by soluble and immobilized kinases and final products of catalyzed reaction were evaluated by MALDI-MS. The profits provided by immobilized form of kinases have been verified and discussed in presentation.

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MICRO-RNA ISOLATION USING TiO₂ MATERIALS

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Micro-RNAs (miRNAs) are endogenous, short (~23 nucleotides), non-coding molecules of RNA. They partake in regulation of gene expression and various biological processes. Expression of miRNAs differs for healthy and pathological conditions. Therefore, miRNAs are considered very promising diagnostic or prognostic biomarkers for several diseases, i.e. carcinogenesis [1]. Isolation and detection of miRNAs are difficult due to their small sizes, low concentrations (in fM) and high homology in sequences. Commonly used methods for miRNA isolation are phenol-chloroform extraction and solid-phase extraction [2].

It was described, that materials based on TiO₂ have an affinity to nucleic acids thanks to their strong interactions with phosphate backbone. Nucleic acids are negatively charged and charge of TiO₂ under acidic conditions is strong positive. In addition, it was suggested, that the adsorption of DNA by TiO₂ is caused by interaction between DNA and hydroxyl groups on the surface of TiO₂ [3]. Similar principles apply for TiO₂ interactions with RNA [4].

In our work, we tested two different materials based on TiO₂ to determine their applicability for miRNA isolation - 10 μm TiO₂ microspheres (Titansphere (TiO) - GL Sciences) and TiO₂ nanotubes coated with Fe₃O₄ (TiO₂NTs@Fe₃O₄NPs, CEMNAT). Those were tested on the mixture of genomic RNA pre-isolated from Jurkat cells (T-lymphoblast cell line) spiked with oligo RNA standard based on hsa-miR-18a-3p (Generi Biotech). We optimized binding and elution conditions for extraction of short RNAs from this mixture containing wide range of RNA sizes.

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WEDNESDAY, JUNE 26
SESSION 7
Sensors II

EVALUATION OF TOTAL ANTIOXIDANT CAPACITY IN BEVERAGES BASED ON LACCASE IMMOBILIZED ON SCREEN-PRINTED CARBON ELECTRODE MODIFIED WITH GRAPHENE NANOPLATELETS AND GOLD NANOPARTICLES

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A simple and highly sensitive electrochemical biosensor based on laccase immobilized onto a gold nanoparticle/graphene nanoplatelet-modified screen-printed carbon electrode (AuNP/GNPI/SPCE) was developed for the determination of hydroquinone (HQ) and other polyphenolic compounds. The biosensor shows excellent electrocatalytic activity towards oxidation of hydroquinone at a potential of -0.05 V (vs Ag/AgCl, 3M KCl) in phosphate buffer as supporting electrolyte (0.1 M, pH 7.0) using hydrodynamic amperometry.

Analytical characteristics uncover that the AuNP/GNPI/SPCE comprises a wide linear range for the dependence of the signal on hydroquinone concentrations from 4 to 120 μ M with a detection limit (3σ) of 1.5 μ M. The repeatability (5 measurements, 100 μ M hydroquinone) is ± 2 % and the reproducibility (5 biosensors, 100 μ M hydroquinone) is ± 3 %. Interference studies of most common compounds with the determination of hydroquinone demonstrated negligible effects.

Finally, the biosensor and the proposed analytical method were applied to the determination of total antioxidant capacity (TAOC) in wine, sirup and pharmaceutical drugs based on Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and hydroquinone equivalents. The results were evaluated by using their calibration curves which were satisfactory and agreed well with the results obtained by the reference method Trolox Equivalent Antioxidant Capacity assay (TEAC-Assay) [1].

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ELECTROCHEMICAL DETERMINATION OF CLORSULON USING MODIFIED WITH SINGLE-WALLED CARBON NANOTUBES GLASSY CARBON ELECTRODE

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Clorsulon (Clo) (4-amino-6-(trichlorovinyl)-1,3-benzenedisulfonamide) is one of the representative of the veterinary drugs. Clo is mainly used to treat cattle, as a medicine against liver fluke (*Fasciola hepatica* and *Fasciola gigantica*) [1]. Furthermore, many research has proved that this drug exhibits a broad spectrum of activity against mature flukes, roundworms and flatworms [2]. In addition to many advantages that distinguish this veterinary medicine, it is commonly known about the contemporary problem is the pollution of the environment with antibiotics, as is mentioned in all types of European Union reports.

The purpose of the work was application of the modified with single-walled carbon nanotubes (SWCNTs) glassy carbon electrode (GCE) in determination of veterinary drug – clorsulon (Clo). The comprehensive microscopic and electrochemical characterizations of the unmodified and the modified GCE were performed by atomic force microscopy (AFM), electrochemical impedance spectroscopy (EIS), and cyclic voltammetry (CV). The prepared sensitive sensor was applied in the square-wave voltammetric determination (SWV) of the veterinary drug clorsulon (Clo). It is worth to stress that so far there has not been information about voltammetric studies of the compound (there are only few reports in the literature concerning chromatographic determinations of Clo). The drawn up method was applied also in the voltammetric determination of Clo in milk samples. The electrode mechanism of Clo oxidation was also studied.

Authors acknowledge financial support obtained from the University of Lodz, Poland (Grant No. B1711100001602.02) and CEEPUS III network (CIII-CZ-0212-11-1718-M-109220).

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ENZYMATIC POLYPHENOL INDEX BIOSENSOR BASED ON GRAPHENE NANOPATELETS DECORATED WITH MnO₂ NANOPARTICLES. PREPARATION, CHARACTERIZATION AND ANALYTICAL APPLICATION

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A nanocomposite formed from graphene nanoplatelets (GNP) and manganese oxide (MnO₂) nanoparticles (GNP/MnO₂) was proposed as a novel and suitable support for enzyme immobilisation. The performances of screen-printed carbon electrodes (SPCEs) was highly improved after modification with GNP/MnO₂ (SPCE/GNP/MnO₂). The polyphenol index biosensor was prepared by surface modification of SPCE/GNP/MnO₂ with drop coating of the laccase (from *Trametes Versicolor*) and Nafion[®].

All electrochemical measurements were carried out in acetate buffer, pH=4.60. The developed laccase biosensor shows fast and reliable amperometric response toward caffeic acid, as model compound, at operating potential of +0.40 V (vs. Ag/AgCl), with a linear range from 5 μmol L⁻¹ to 2.75 mmol L⁻¹ ($r^2 = 0.9997$), with detection limit of 2.38 μmol L⁻¹. Moreover, effects of possible interfering compounds were investigated.

The developed procedure was successfully applied for the determination of total polyphenol content in red and white wine samples. In order to validate the proposed method, the polyphenol content in wine samples, under optimized parameters, was determined using a glassy carbon electrode. Recovery tests (95.7-97.5%) shows satisfactory accuracy and precision of the developed method, concluding that proposed construction of biosensor can offer fast, stable and reproducible determination of the polyphenol index.

WEDNESDAY, JUNE 26
SESSION 8
Electrochemistry II

APPLICATION OF SILVER AMALGAM FILM ELECTRODE TO STUDY DNA-HERBICIDES INTERACTIONS

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Silver amalgam film electrode (AMFE) was designed by research group from Cracow, Poland. Very small consumption of silver amalgam make this electrode environmentally friendly and consistent with green chemistry principles [1]. Excellent properties of AMFE have been successfully utilized in the determinations of wide group of compounds, such as drugs, pesticides etc. [2].

There are many biologically active organic compounds possessing toxic side effects or/and being potentially dangerous for health. Herbicides are specific type of pesticides used in agricultural and forest ecosystems to reduce the growth rate of weeds. Herbicides belonging to the group of aromatic nitro compound are extensively used in the selective control of weeds because of their chemical stability, broad spectrum of function, and relatively low direct toxicity [1, 2]. Examples of such compounds are profluralin and lactofen. Profluralin is toxic to honeybees and fish, while lactofen doesn't exhibit any kind of toxicity towards bees, it exhibits rather undesirable effects to fish [1, 3]. Thus, the study of interaction between mention herbicides and DNA is an important field of chemical research and can also provide deep insight into mechanisms of these interactions.

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ELECTRODEPOSITION OF METALS WITH DIFFERENT MAGNETIC PROPERTIES IN CONSTANT MAGNETIC FIELD

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A constant magnetic field applied during metal electrodeposition may affect the process in two ways – it changes the electrolysis process and the morphology of obtained coatings. Electrodeposition of metals may occur faster under a constant magnetic field. The surface of metal coatings obtained in the presence of a magnetic field may be smoother and tougher than those obtained without the influence of the magnetic field [1].

Metals can be classified according to their magnetic properties – there are ferromagnetic, paramagnetic and diamagnetic metals. The nature and the intensity of changes caused by a constant magnetic field may depend on the magnetic properties of electrodeposited metal [2].

The aim of our study was to investigate how the constant magnetic field affects the electrodeposition process of ferromagnet, paramagnet, and diamagnet. The metals were deposited from their salts solutions on gold disc electrode under the influence of constant magnetic field up to 1000 mT and without magnetic field (for comparison purposes). We used two different directions of the magnetic field induction vector – parallel and perpendicular to the surface of the working electrode. To investigate the electrochemical changes, we used cyclic voltammetry and electrodeposition at the constant potential.

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VOLTAMMETRIC DETERMINATION OF MANDIPROPAMID ON EDGE-PLANE PYROLYTIC GRAPHITE ELECTRODE

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Mandipropamid (MAN) is a highly efficient fungicide against fungi causing potato and tomato late blight (*Phytophthora infestans*), grape downy mildew (*Oomycete viticola*) and cucumber downy mildew (*Pseudoperonospora cubensis*). Mandipropamid is highly effective in preventing spore germination, but also inhibits sporulation and mycelium growth. Mandipropamid, quickly adsorbs on the waxy surface of the plant, causing rain-proof properties and long-lasting barrier to fungal diseases [1]. MAN belongs to the class of carboxylic acid amides (Fig. 1.). The goal of this research was to develop electrochemical procedure for MAN determination.

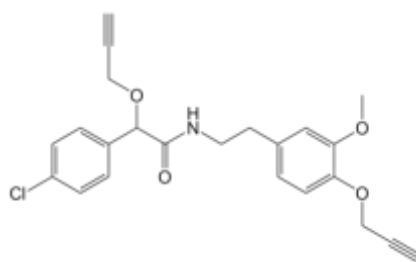


Fig. 1. Structure of mandipropamid.

In this studies edge-plane pyrolytic graphite electrode (EPPGE) was used as a working electrode. EPPGE is fabricated from highly ordered pyrolytic graphite (HOPG) and has many applications in voltammetry [3]. The method of quantitative determination of MAN was developed on the basis of MAN oxidation. The limit of detection and limit of quantification was calculated and was equal to $7.83 \cdot 10^{-8} \text{ mol L}^{-1}$ and $2.61 \cdot 10^{-7} \text{ mol L}^{-1}$, respectively. The developed method was used to determine this pesticide in spiked river water samples.

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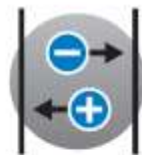
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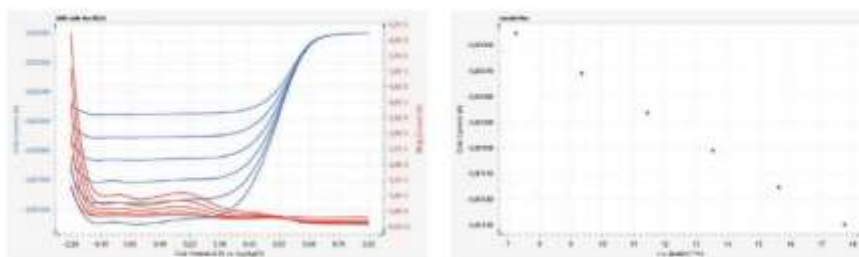
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References

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- **AN-EC-11** – Investigation of intermediates in the electrodeposition of copper using the Autolab RRDE
- **AN-EC-14** – Oxygen reduction reaction with the rotating ring disk electrode

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- Four-electrode setup (Pt disk/Pt ring)
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- Ring – The potential is fixed at a value where the product or intermediate will react

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- The researcher specifies a fixed potential value at which the data is chosen for these plots.
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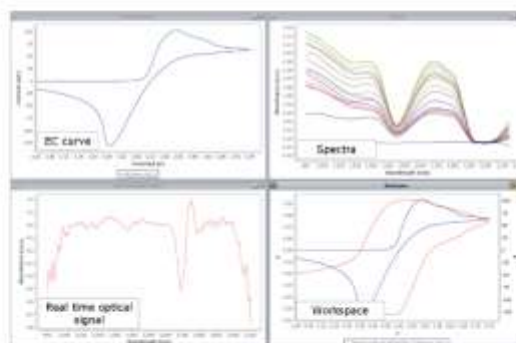
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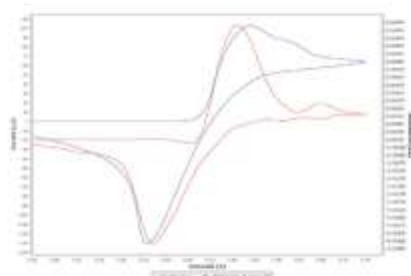
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Weight	2500g
Light Source	
Wavelength range	360-2500 nm Tungsten halogen
Spectrometer	
Detector	InGaAs photodiode array 256 pixels TE cooled
Wavelength range	900- 2200 nm
Integration time	1 ms to 2s
Optical resolution	~ 17 nm FWHM
Signal to Noise Resolution	10000:1 (at 10 ms integration time)
Fiber optic connector	SMA 905
(Bi)potentiostat/Galvanostat	
Operating modes	BiPotentiostat, Potentiostat, Galvanostat
DC-potential range	±4V
Maximum measurable current	±40 mA
Current ranges (potentiostat)	±1 nA to ±10 mA (8 ranges)
Applied Potential Resolution	1 mV
Measured Current Resolution	0.025 % of current range
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Since its founding in 1950, the Faculty of Chemical Technology, University of Pardubice, has become a renowned institution of higher education in a variety of chemistry related areas. At present, the Faculty provides courses leading to Bachelor, Master and Doctoral degrees.

The backbone of the study programme is represented by the two-level five-year Master degree course in Chemistry and Technical Chemistry. The students of the first level cover the basic areas of technical chemistry such as general, inorganic, organic and analytical chemistry; mathematics; physics; physical chemistry; computing; chemical engineering supplemented by the study of toxicology; ecology; and technically oriented foreign language training. The follow-up course extends the first level and students' knowledge. The students can also participate in a scientific research at the Faculty departments or gain the first experience in the leading companies in the country or abroad. The offered specialisations are: Inorganic and Bioinorganic Chemistry, Inorganic Technology, Chemical Technology of Paper and Pulp, Chemical Engineering, Material Engineering, Environment Protection, Organic Chemistry, Economics and Management of Chemistry and Foodstuff Industries, Process Control, Technical Physical and Analytical Chemistry, Technology of Organic Specialities, Technology of Polymers Manufacturing and Processing, Theory and Technology of Explosives, Fibres and Textile Chemistry, Environmental Engineering. The second level is concluded by the defence of the diploma thesis and the Final State Examination leading to the Master degree (Ing.).

Another five-year course in Chemistry and Technology of Foodstuffs offers the study specialisation in Evaluation and Analysis of Foodstuffs. Beside the subjects of technical chemistry and language training, the first study level comprises general and foodstuffs microbiology, biochemistry, and the basis of food-processing technologies. The advanced programme offers subjects such as food analysis and enables the students to become acquainted with the standard methods of food assessment, trace analysis of the food chain using modern instrumental methods, and computing.

Master study course in the Special Chemical and Biological Programmes consists in the first study level of the specialisation in Clinical Biology and Chemistry. The course comprises - besides the chemistry-oriented subjects and mathematics - biostatistics, physics, computing, general and clinical biochemistry, general biology, physiology, general microbiology, genetics, immunology, toxicology and medical information technology. The course leads to the Bachelor degree (Bc.) and is followed by the second level course in Analysis of Biological Materials extending the students' knowledge of clinical biology and chemistry and leading to the Magistr (Master - Mgr.) degree.

The Faculty as the only one in the Czech Republic offers higher education in the field of Graphic Arts, both in the Bachelor, and Master degree courses. Graduates of the three-year Bachelor programme are knowledgeable in printing techniques and materials; they are able to use computer techniques for pre-printing operations, electronic publishing and multimedia communication. Acquired knowledge can be extended in the follow-up two-year Master course that prepares students for both managing positions in the large companies, and private entrepreneurship, too.

The three-year Bachelor study programme in Chemical and Process Engineering specialising in Chemical Process Control is also taught on the basis of special needs of the chemical industry. The graduates assert themselves as a middle company management and technical workers in plants of chemical, pharmaceutical and food industry. They can also continue their study in some of the two-year Master courses in Chemistry and Technical Chemistry.

After the Master courses, the successful graduates can continue their studies in the three-year postgraduate Doctoral courses leading to the PhD. degree. The Doctoral study programmes, which are closely connected to Chemistry and Technical Chemistry courses, progressively extend the gained knowledge and put a stress on the independent research activities. It is common that a part of the Doctoral studies can be carried out at one of the well-known universities abroad in close co-operation with the Faculty and its research laboratories. The scientific activities of the Faculty aim at both elementary and technological research. The postgraduate Doctoral programmes are consequently focused on the following fields: Inorganic Chemistry, Organic Chemistry, Analytical Chemistry, Physical Chemistry, Inorganic Technology, Organic Technology, Technology of Polymers, Chemistry and Technology of Inorganic Materials, Chemical Engineering, Technical Cybernetics, Economics and Management and Applied and Landscape Ecology.

A number of successful technical projects were completed in co-operation with the industrial sector and a wide range of scientific papers was published and met with an excellent scientific response. The growing reputation of the Faculty is also indicated by many international conferences and congresses held at the University every year. The Joint Laboratory of Solid State Chemistry of the Academy of Sciences of the Czech Republic and the University of Pardubice, the Research Centre of New and Perspective Inorganic Compounds and Materials, as well as the shared laboratories at other research institutions and the Pardubice Hospital also contribute to the high reputation of the Faculty.

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