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Szanowni Państwo,

Katedra i Zakład Farmacji Fizycznej Wydziału Nauk Farmaceutycznych w Sosnowcu Śląskiego Uniwersytetu Medycznego w Katowicach ma zaszczyt zaprosić na **V Seminarium Ogólnoakademickie pn. „Metody fizykochemiczne w badaniach naukowych”. Patronem tegorocznej edycji jest Jego Magnificencja Rektor Śląskiego Uniwersytetu Medycznego w Katowicach Pan Prof. dr hab. n. med. Tomasz Szczepański oraz Prezydent Miasta Katowice Dr Marcin Krupa.**

Metody fizykochemiczne stanowią kluczowy element w badaniach naukowych. Są niezbędnym narzędziem pozwalającym na pozyskiwanie wartościowych danych a ich analiza, poprzez wnikliwą dyskusję, prowadzi do uzyskania niezbędnej wiedzy dotyczącej prawidłowego funkcjonowania organizmów żywych. **Celem przedsięwzięcia jest popularyzacja nauki poprzez prezentację interesujących badań naukowych prowadzonych przy użyciu różnych metod fizykochemicznych z zakresu farmacji, biologii, medycyny i nauk pokrewnych**.

Podobnie jak w latach ubiegłych, Seminarium jest dedykowane pracownikom naukowym, młodym naukowcom, doktorantom, magistrantom, członkom kół naukowych i wszystkim pasjonatom nauki. Podczas Seminarium będziecie Państwo mogli wysłuchać ciekawych wykładów zaproszonych gości oraz wymienić się doświadczeniami i wiedzą na temat metod fizykochemicznych stosowanych aktualnie w nauce poprzez przedstawienie rezultatów swoich badań.

Zapewniamy miłą atmosferę w gronie naukowców z różnych dziedzin, mając nadzieję, iż liczne zainteresowanie przyczyni się do uczynienia naszego Seminarium spotkaniem cyklicznym.

W imieniu Katedry i Zakładu Farmacji Fizycznej
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CONFERENCE PROGRAMME*

08.00 – 09.00	Registration
09.00 – 09.15	***Opening ceremony***
09.15 – 12.15	***Speakers presentations*, part I****
09.15 – 10.00	**"Glycosylated Sulfonylureas Anti-Diabetic Activity: Molecular Mechanisms and Their Effects to Improve Blood Sugar Levels"** Head of Praxis, Industry and Community Engagement, Department of Biotechnology, Faculty of Applied Sciences, UCSI University Kuala Lumpur (South Wing) – *Ass Prof. Dr Patrick Nwabueze Okechukwu*
10.00 – 10.45	„**New thiosemicarbazide derivatives with promising biological activity**" Department of Organic Chemistry, Collegium Pharmaceuticum, Medical University of Lublin – *Prof. Monika Wujec*
10.45 – 11.30	**„Bioanalytical challenges in developing LC-MS/MS methods for the determining cefazolin and ondansetron for testing their pharmacokinetics"** Department of Physical Pharmacy and Pharmacokinetics, Collegium Pharmaceuticum, Karol Marcinkowski's Medical University in Poznań – *Prof. Franciszek Główka*
11.30 – 12.15	**„Practical approach for bioanalysis of anti-tuberculosis drugs using the UPLC-MS/MS method"** Department of Physical Pharmacy and Pharmacokinetics, Collegium Pharmaceuticum, Karol Marcinkowski's Medical University in Poznań – *Marta Karaźniewicz – Łada, PhD, DSc, Professor of Karol Marcinkowski's Medical University*
12.15 – 12.45	**Group Photo**, Coffee break
12.45 – 13.30	Poster session, best presentation competition
13.30 – 14.00	Oral session**,** best presentation competition *cont.*
14.00 – 15.45	***Speakers presentations*, part II** **
14.00 – 14.45	„**A novel approach to therapeutic monitoring of drug concentration and distribution in tissues using chemical biopsy – an overview and perspectives**" Department of Pharmacodynamics and Molecular Pharmacology, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń – *Barbara Bojko, PhD, DSc, Professor of Nicolaus Copernicus University*
14.45 – 15.15	„**Innovative ion sources in mass spectrometry**" Waters Sp. z o.o., Warszawa –*Maria Jacewicz, MSc*
15.15 – 15.45	„**Spectrofluorimetry from yesterday untill today**" ABL&E JASCO Polska Sp. z o.o, Kraków – *Mirosław Danch, PhD*
15.45 – 16.00	***Award ceremony, Closing Ceremony***

*** the Organizer reserves the right to make minor changes to the Conference program; ** Due to limited time, some of the speeches were submitted as online presentations, https://seminariumogolnoakademickie.sum.edu.pl/wystapienia-on**

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L_1 GLYCOSYLATED SULFONYLUREAS ANTI-DIABETIC ACTIVITY: MOLECULAR MECHANISMS AND THEIR EFFECTS TO IMPROVE BLOOD SUGAR LEVELS

Patrick Nwabueze Okechukwu[1]*, Ghadeer A. R. Y. Suaifan[2], Abbirami Balachandran[1]
Morak-Młodawska Beata[3], Jeleń Małgorzata[3]

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Normally, skeletal muscle accounts for 70-80% of insulin-stimulated glucose uptake in the postprandial hyperglycemia state. Consequently, abnormalities in glucose uptake by skeletal muscle or insulin resistance (IR) are deemed as initial metabolic defects in the pathogenesis of type 2 diabetes mellitus (T2DM). Globally, T2DM is growing in exponential proportion. The majority of T2DM patients are treated with sulfonylureas in combination with other drugs to improve insulin sensitivity. Glycosylated sulfonylureas (GS) (sulfonylurea-glucosamine analogues) are modified analogues of sulfonylurea, they exist in two forms acetylated and deacetylated glycosylated sulfonylureas (AGS and DGS) and have been previously reported to possess antidiabetic activity. The aim of this study was to evaluate the in vitro and in vivo antioxidant and antidiabetic potential of GS and its impact on the insulin signalling pathway at the molecular level. To create an in vitro model, insulin resistance was established utilizing a high insulin-glucose approach in differentiated L6 muscle cells from Rattus norvegicus. Female Sprague-Dawley rats were induced T2DM by intraperitoneal injection of 50 mg/kg of streptozotocin to induce T2DM. After insulin resistance/T2DM induction, compounds under investigation and standard medicines (metformin and glimepiride) were tested. For In vivo test, plasma blood glucose levels and body weight of the rats were measured weekly for 8 weeks to evaluate the antidiabetic activity of the tested drug. post treatment rats were sacrificed, and blood samples were drawn to conduct the biochemical analyses while the organs (liver and pancreas) and smooth muscle tissues were extracted to conduct the antioxidant and gene expression studies. The differential expression of PI3K, IRS-1, PKC, AKT2, and GLUT4 genes involved in the insulin signalling pathway was evaluated using qPCR. GS analogues were able to replenish the β-cell regeneration in the pancreas, show antioxidant activity and prevent hepatocellular damage, HbA1c reduction, and relieve cardiovascular and renal complications associated with T2DM in vivo. The gene expressions of IRS1, PI3KCα, PKCα, AKT2 and GLUT4 was upregulated in tested compound in vitro and in vivo. The findings of this study revealed that these compounds possess strong antihyperglycemic activity through the PI3K/Akt pathway in STZ-induced diabetic rats.

Keywords: glycosylated sulfonylurea; antioxidants; T2DM; PI3K/GLUT4 gene expression

Acknowledgements: The authors would like to acknowledge UCSI University and Medical University of Silesia for providing the fund and facility for this research.

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[2] Okechukwu PN, Sharma M, Balachandran A, Małgorzata J, Beata M-M, MP. Comparative Studies of Palmatine with Metformin and Glimepiride on the Modulation of Insulin Dependent Signaling Pathway In Vitro, In Vivo & Ex Vivo, Pharmaceuticals, 2022, 15, 1317.

L_2 NEW THIOSEMICARBAZIDE DERIVATIVES WITH PROMISING BIOLOGICAL ACTIVITY

Monika Wujec

Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Lublin

Over the years, several new medicinal substances have been introduced into the treatment of diseases caused by bacteria and parasites. Unfortunately, due to the production of numerous defense mechanisms by microorganisms and parasites, they still pose a serious threat to humanity around the world. Therefore, laboratories all over the world are still working on finding new, effective methods of pharmacotherapy. Thiosemicarbazides showed many biological activities, additionally are often an initial compound for the synthesis of new active substances. On the other hand, the trifluoromethyl group is known to possess multidirectional activity. Hence our research work aimed to synthesize new thiosemicarbazide derivatives with 3-trifluoromethylphenyl substituent and to determine their nematocidal and antibacterial activity. The first stage of the research was to obtain seven new compounds, including six linear compounds and for comparison one cyclic derivative. The potential probabilities of biological activity of the newly obtained derivatives were then estimated using the PASS software. Afterward, studies were carried out to determine the nematicidal potential of the compounds with the use of nematodes of the genus *Rhabditis* sp. Next, new compounds were tested for antibacterial activity using the ACCT standard strains. To determine the lack of cytotoxicity, tests were performed on cell lines. All tested compounds do not show cytotoxic effects in the tested concentration range. Additionally, an antioxidant activity test was performed. The conducted research proved the anthelmintic and antibacterial potential of the newly obtained compounds with no cytotoxic effect. The most effective were linear and cyclic derivatives with a 3-chlorophenyl substituent; in the case of anthelmintic activity, higher efficacy was demonstrated than the therapeutics used in the current treatment.
In the case of compounds that are subjected to biological tests, their purity is an extremely important aspect. Often, the commonly used spectral methods are not sufficient to determine whether we are dealing with the right molecule. In the case of compounds containing fluorine atoms in their structure, ^{19}F NMR is also helpful.

Keywords: synthesis, biological activity, spectral analysis

L_3

BIOANALYTICAL CHALLENGES IN DEVELOPING LC-MS/MS METHODS FOR DETERMINING CEFAZOLIN AND ONDANSETRON FOR TESTING THEIR PHARMACOKINETICS

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Pharmacokinetics is an essential tool in Therapeutic Drug Monitoring (TDM). Sensitive methods for analyzing changes in drug concentrations in body fluids allow for the determination of appropriately low concentrations of drugs and, on this basis, the development of pharmacokinetic parameters, and then the determination of an individual drug dose [1]. The challenge is to determine drugs in complex biological matrices, such as adipose tissue, or in those in which drugs occur in low concentrations, such as cerebrospinal fluid.

The aim of the presentation is to show the steps of developing two LC-MS/MS methods. The first one concerned the determination of cefazolin in adipose tissue, an effective antibiotic widely used in the prevention of surgical site infections (SSIs), also in obese patients in whom the pharmacokinetics of this drug may be changed. A second method was developed for determination of ondansetron, an antiemetic drug that is currently being investigated for use in the treatment of neuropathic pain. Insufficient effectiveness of the drug may be caused by obtaining low drug concentrations in the cerebrospinal fluid due to increased expression of P-glycoprotein. Therefore, a sensitive LC-MS/MS method was developed to assess drug pharmacokinetics in plasma and cerebrospinal fluid, in which attention was paid to elute the analyte from the chromatographic column.

Research results and conclusions. In the developed LC-MS/MS method for the determination of cefazolin in adipose tissue, Captiva EMR Lipid plates were used to clean samples before analysis. They have been shown to effectively remove lipids from the biological matrix, which reduced the matrix effect. This allowed to use the rat adipose tissue as a surrogate matrix to human adipose tissue. The validated method was successfully used in a pilot study of cefazolin pharmacokinetics in patients with an average BMI of 31.2 kg/m^2 [2]. In the LC-MS/MS method for the determination of ondansetron, a significant sample carry-over turned out to be a challenge. A W-shaped mobile phase gradient was used to elute ondansetron from the column, which ensured the effective cleaning of the autosampler, reducing carry-over to acceptable levels and enabling repeatable assay results. The drug concentrations after intravenous administration of 4–16 mg of ondansetron to healthy volunteers ranged from 1.2 to 235.9 ng/ml in plasma, and from 0.02 to11.9 ng/ml in cerebrospinal fluid. The developed method met the validation criteria and can be used in pharmacokinetic studies of ondansetron in the human CNS [3]. Both LC-MS/MS methods have been validated according to FDA and EMA criteria.

Keywords: bioanalysis, LC-MS/MS, cerebrospinal fluid, adipose tissue, validation, pharmacokinetics

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L_4

PRACTICAL APPROACH FOR BIOANALYSIS OF ANTI-TUBERCULOSIS DRUGS USING THE UPLC-MS/MS METHOD

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Tuberculosis remains a significant cause of illness and death in developed countries, and also a major contributor to antimicrobial resistance [1]. Drug-resistant TB treatment involves a 2-month intensive phase characterized by the administration of four key anti-TB drugs: rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (ETH) [2]. Implementing therapeutic drug monitoring (TDM) for TB drugs in routine clinical practice requires understanding the limited stability of anti-TB agents. The aim of the study was to develop and validate the UPLC-MS/MS method useful for TDM of first-line anti-tubercular agents in plasma and urine. Since the analyzed TB drugs differ in polarity, we evaluated several analytical columns and mobile phase compositions with different additives to obtain the best chromatographic separation of the analytes in a reasonable time. Finally, chromatographic separation was achieved on a Kinetex Polar C18 column (2.6 μm; 150 x 3 mm) with a mobile phase consisting of 5mM ammonium acetate and acetonitrile, both containing 0.1% formic acid, in gradient elution. The analytes were detected using a positive ionization mode by multiple reaction monitoring. The method was successfully validated based on FDA guidance regarding selectivity, linearity and LLOQ, precision and accuracy, matrix effect, carry-over. In plasma, the LLOQ for RIF and its degradation products was 0.1 μg/mL, 0.05 μg/mL for INH, and 0.2 μg/mL for PZA. In urine, the LLOQ for INH, PZA, ETH, and RIF was 0.5 μg/mL, and for RIF's metabolites and degradation products, it was 0.1 μg/mL. Furthermore, we performed the extended stability study of the TB drugs which included quantifying RIF's metabolites/degradation products: 25-desacetylrifampicin, rifampicin quinone and 3-formyl-rifampicin, under various pH and temperature conditions mimicking the sample collection process in warm and hot countries. Based on the stability study, we proposed specific recommendations for handling plasma and urine samples.

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L_5 A NOVEL APPROACH TO THERAPEUTIC MONITORING OF DRUG CONCENTRATION AND DISTRIBUTION IN TISSUES USING CHEMICAL BIOPSY – AN OVERVIEW AND PERSPECTIVES

Barbara Bojko[1,2], Nikita Looby [1], Anna Roszkowska[1,4], Mariola Olkowicz [1,3], Miao Yu[1]
Bogumiła Kupcewicz[5], Pedro Reck dos Santos[6], Mauricio Pipkin[6], Khaled Ramadan[6]
Shaf Keshavjee[6], Thomas K. Waddell[6], German Gomez-Ríos[1], Marcos Tascon[1]
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Therapeutic drug monitoring is an integrated part of pharmacotherapy. Particularly, drugs with narrow therapeutic windows require strict control of their concentration to ensure safe and effective treatment. For practical reasons, the sample of choice is plasma, serum, or whole blood. However, the blood concentration does not provide direct information about the distribution of the therapeutic agent in the body. Moreover, there are applications, like in vivo lung perfusion (IVLP), which make blood drug measurement impossible because of the procedure's setup. The aforementioned IVLP is an innovative technique developed to enable the local, in situ administration of high-dose chemotherapy to isolated lungs to treat metastatic lung cancer. To optimize the appropriate dosage regimen (e.g. concentration and perfusion time) in the experimental porcine model, it is required to determine the level of the target drugs multiple times in different lobes of the lungs. Conventional sample preparation methods based on sample removal followed by homogenization and solvent extraction are highly invasive, considering the length of the entire procedure. A similar issue applies to drug monitoring during patient treatment, where personalized therapy is a key element of success. Therefore, to fill this gap in the analytical aspect of the strategy, chemical biopsy or in vivo solid phase microextraction (SPME) has been proposed as a sampling and extraction tool. A probe of the size of an acupuncture needle coated with a sorbent of biocompatible morphology (Bio-SPME) has been evaluated preclinically and clinically for *in vivo* lung tissue extraction and determination of doxorubicin and its key metabolites in one study as well as of drugs in FOLFLOX therapy in the other. In addition, SPME also extracted various endogenous molecules, thus providing a real-time snapshot of the physiology of the cells, which might assist in the tailoring of personalized treatment strategy.
Future perspectives on the strategy in view of the current modifications of technology and messages taken from up-to-date studies will be discussed.

L_6 INNOVATIVE ION SOURCES IN MASS SPECTROMETRY

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Liquid chromatography combined with mass spectrometry most often uses the electrospray ionization technique (Electrospray, ESI), which involves spraying liquid at atmospheric pressure from a capillary to which high voltage is applied. Introduced in 1984, this ionization has gained enormous popularity due to its universality and ease of use. However, alternative ionization techniques are still being developed, both in combination with chromatographs and in the form of direct techniques. They increase the research potential by offering higher analysis sensitivity, shorter experiment time, reduction of the sample preparation step or the ability to image the sample surface.
An example of innovation is the APGC source, which makes it possible to adapt the LCMS spectrometer to work with a gas chromatograph. Unlike the electron ionization (EI) technique commonly used in GCMS systems, this solution offers soft ionization of the analyzed substances, with a mechanism similar to atmospheric pressure chemical ionization (APCI). The use of this source gives higher sensitivity and better selectivity compared to EI.
An extension of ESI ionization is the UniSpray source, which increases the sensitivity of spectrometers by increasing the degree of solvent desolvation in the produced spray.
Recently, direct techniques have become popular at work with a mass spectrometer, without chromatography.
This possibility is provided by the DESI source (desorption electrospray ionization), which allows for conducting imaging experiments from the sample surface, as well as the simple and compact RADIAN ASAP system, for quick confirmatory analyses.

Keywords: LCMS, ESI, UniSpray, DESI, APGC

L_7 SPECTROFLUORIMETRY FROM YESTERDAY UNTILL TODAY

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Spectrofluorimetry is one of the commonly used molecular spectroscopy research techniques, which has found its place in many modern analytical laboratories: chemical, biochemical and other. Although it is one of the oldest instrumental methods used in chemical analysis [1], its importance in routine analyzes cannot be overestimated. Therefore, device manufacturers not only constantly develop the designs of spectrofluorimeters themselves, but also expand the selection of additional measurement accessories related to the devices and increasing their operational properties.
The use of additional accessories of various designs, mounted in the optical path of the measuring device, significantly increases the functional capabilities of spectrofluorimeters. Depending on the accessories used, the following can be carried out: measurements in small sample volumes (on the order of tenths of a microliter), measurements with high sensitivity and selectivity, quantum efficiency measurements, and many other important ones. Measurements carried out in this way are characterized by high repeatability and accuracy and a wide dynamic range [2].
The aim of our presentation is to present to the listeners the latest technical solutions in the field of spectrofluorimetry.

Keywords: spectrofluorimetry, spectrofluorimetry, chemical metrology

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L_8*

TF–SPME AS A TOOL FOR TARGETED ANALYSIS OF STEROID HORMONES IN FISH – A SHORT SUMMARY OF OWN RESEARCH

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***all research presented in the paper were conducted during a postdoctoral fellowship at Department of Chemistry, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada*

Steroid hormones (SH) are crucial for various physiological processes in vertebrates, including fish. These hormones are synthesized primarily in the gonads, adrenal glands and placenta, and they play key roles in regulating reproduction, differentiation, development and metabolism.
The main object of the study was to develop an efficient and sensitive analytical method using TF-SPME-LC–MS/MS for targeted analysis of specific endogenous steroid hormones in the plasma of wild white sucker fish, where the concentrations of these hormones were relatively low. The sampling was conducted in the Athabasca River, which is a major river in Alberta, Canada. The Athabasca River is known for its importance in the context of the Alberta oil sands industry, as it runs through the center of the oil sands region. All experiments involving white sucker fish plasma were conducted in compliance with protocols approved by the institutional Animal Care Committee, in the Chemistry Lab Facility at the University of Waterloo.
Based on the obtained data it can be concluded that TF-SPME-LC-MS/MS method is sensitive, accurate and precise for the analysis of cortisol, testosterone, progesterone, E1, E2, and EE2 in biological samples such as white sucker fish plasma. The results of the study indicated that thin-film solid-phase microextraction (TF-SPME) was effective for the analysis of compounds with a polarity range between 1.28 and 4.31. This range includes steroid hormones (SH) with diverse physicochemical properties.
TF-SPME combines simplicity, sensitivity, robustness and versatility, making it a powerful tool for the extraction and analysis of targeted analytes in diverse sample matrices. Its advantages lead it to widespread application in various fields, including environmental monitoring, food analysis, pharmaceutical research and clinical diagnostics.

Keywords: TF-SPME-LC–MS/MS, steroid hormones, fish

Acknowledgements: I would like to thank Janusz Pawliszyn and Barbara Bojko for their critical review, Vincent Bessonneau for his help in discussing the results, Jennifer Ings, Mark McMaster , Leslie Bragg and Mark R. Servos for kindly providing the samples and for their help in the biological field

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L_9*

THE USE OF SOLID PHASE MICROEXTRACTION IN PHARMACOLOGICAL APPLICATION. A SHORT REVIEW

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Traditional sample preparation methods, such as liquid-liquid extraction (LLE) or solid-phase extraction (SPE), often have significant problems due to the matrix effects. This is the result of significant co-extraction of matrix components, which leads to errors.
Solid-phase microextraction (SPME) is an innovative technique for extracting low-molecular-weight compounds (less than 1.500 Da) from highly complex matrices, including biological matrices. The technique shortens analysis time and reduces the use of organic solvents. In addition, it eliminates pre-analytical and analytical errors. SPME has many advantages compared to alternative extraction methods. In particular, it has minimal environmental impact, allows sampling and pre-concentration of analytes in a single step. Due to the SPME technique, simultaneous extraction of multiple analytes is possible.
Numerous literature data confirm the wide range of SPME applications and its potential as a future technique in various scientific fields, particularly in pharmaceutical sciences. The application of the SPME technique in pharmaceutical research may contribute to the development of personalized therapies, which could result in a possible reducing of side effects and the occurrence of multidrug resistance. Summarizing the significant number of successful *in vitro* and *in vivo* studies, it can be expected in the near future that the SPME technique can be implemented into routine testing of human biological material.

Keywords: solid phase microextraction, pharmacological application, monitored therapy

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L_10* THE USE OF CARBON NANOTUBES AS SORPTION COATINGS – WHY AND WHERE?

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Nanotechnology is a set of synthesis methods and study of particles in dimension from 1 to 100 nm. According to their size, nanoparticles (NPs) are characterized by different properties, compared with molecules. Based on the previous definition, nanomaterials (NMs) are defined as a material that consist of at least 50% of the nanoparticles. Compared to other materials used in Solid Phase Microextrction (SPME) coatings, nanomaterials offer significantly higher extraction efficiency and performance. Due to their excellent physical and chemical properties, NMs have become a great topic of interest in term of SPME coatings.

Carbon nanomaterials can interact with molecules through non-covalent forces such as hydrogen bonding, π-π stacking, electrostatic forces, van der Waals forces and hydrophobic interactions. Moreover, carbon nanotubes (CNTs) are the most commonly used carbon nanomaterials for sample preparation. In the literature there is a lot of information about the use of CNTs (single-walled carbon nanotubes SWCNTs and multi-walled carbon nanotubes MWCNTs), which are derivatives of fullerene structures, to coat the extraction fiber. Carbon nanotubes have many disadvantages as well as advantages. Despite the possibility of modifying their surface with functional groups, which increases their sorption capacity, they can also be contaminated after synthesis.

This work aims to introduce CNTs as an element of sorption fiber and to present a literature review as a samples of their practical application.

Keywords: carbon nanotubes, SPME

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L_11*

QUININE (QI) AND NEW SYNTHETIC QUINOLINE DERIVATIVES (QUI) AS POTENTIAL NOVEL HSA'S ANTIOXIDANT ACTIVITY MODULATORS

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Quinine (Qi) is an antimalarial drug with a very long history of use. It is applied for protozoan infections of the species *Plasmodium falciparum* resistant to chloroquine. Qi, due to its chemical structure, belongs to the quinoline derivatives [1]. Quinoline derivatives constitute a large group of compounds with many applications in medicine. They have been shown to have antiprotozoal, antimicrobial, antidiabetic, antiviral and antineoplastic activities [2]. For this reason, the synthesis of new compounds belonging to this group may be beneficial for the pharmaceutical industry and patients.
The aim of the lecture is to present the quinine (Qi) and newly synthesized quinoline derivatives (Qui) as modulators of endogenous antioxidants activity. Qi has low antioxidant activity, but it may have a synergistic effect on the HSA`s antioxidant potential [1]. In turn, selected, synthetic quinoline derivatives (Qui1-3) may have different antioxidant activity against different free radicals. This may be due to the presence of different types of substituents (methyl and hydroxyl group) and their location in the Qui`s molecules. There was no significant effect of Qi and Qui1-3 on the secondary and tertiary structure of HSA [1,3], and even slight changes in protein`s conformation can contribute to modify the exposure of individual amino acid residues [1,3]. It has also been demonstrated that interactions between Qi, Qui1-3 and HSA may increase the availability of Cys-34 residue.
Based on the presented brief review it can be concluded that synthetic quinoline derivatives have great prospects not only as potential therapeutic substances but also as modulators of the activity of endogenous metabolic processes, especially related to antioxidant activity.

Keywords: antioxidants, free radicals, quinoline derivatives

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L_12* STRUCTURAL MODIFICATION INDUCED BY GLYCATION OF ALBUMIN IN THE PRESENCE OF PALMITIC ACID – SPECTROSCOPIC STUDIES

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The process of non-enzymatic glycation, which represents a significant posttranslational modification of human serum albumin (HSA), is visible during the persistent state of hyperglycemia. It might have an impact on albumin's stability, activity, physical and chemical properties, and physiological functions [1]. Reactive glycation products, known as Advanced Glycation End-Products (AGEs), are formed during the final stages of the glycation process. AGEs comprise a heterogeneous mixture of compounds that can contribute to various organ and tissue disorders [2]. The goal of this research is to answer the following: (i) how glycation of defatted human serum albumin by glucose–fructose syrup (GSP) altered its tertiary structure; (ii) whether palmitic acid (PA) affects the in vitro glycation process and causes conformational changes of glycated albumin, which is a protein that simulates diabetes in the organism; and (iii) whether palmitic acid inhibits the formation of AGEs. Therefore, to show differences in the tertiary structure of macromolecules, the absorption and emission fluorescence spectra, as well as their second derivatives, excitation fluorescence and synchronous spectra, Red-Edge Excitation Shift (REES effect), and the degree of modification of sulfhydryl groups of defatted, unglycated serum albumin (HSA), HSA glycated with glucose–fructose syrup (gHSA), and HSA glycated with the syrup in the presence of palmitic acid (PA) with molar ratios of PA:gHSA 1.5:1 and 3:1 have been investigated.
Findings of the research: (i) glycation of defatted HSA by GFS, as a glycating agent, causes conformational changes in the entire protein structure, particularly in subdomains IIA and IIIA, IB, and IIB, where one tryptophanyl (Trp-214) residue and 17 tyrosyl (Tyr) residues are located, respectively; as a consequence of glycation reactions, the binding properties of albumin may be changed, even if they happen at a distance from a binding site; (ii) PA, at ratios of 1.5:1 and 3:1 with glycated albumin, influences the in vitro glycation process and induces conformational changes in gHSA. These changes are evident as alterations in the hydrophobicity environment of Tyr and Trp-214 residues, along with a significant decrease in the mobility of the Trp-214 environment; (iii) in the PA:gHSA samples at ratios of 1.5:1 and 3:1, PA does not inhibit the formation of AGEs.

Keywords: glycation; AGEs; glucose-fructose syrup; palmitic acid; tertiary structure of HSA; spectroscopic analysis

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O_1 ANALYSIS OF THE IRON STATE IN FOOD AND PHARMACEUTICALS: MÖSSBAUER SPECTROSCOPY STUDY

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Iron plays a very important role in the living systems. It is an integral part of many proteins and enzymes and a mineral that works with other substances to create hemoglobin. Iron deficiency is one of the best-known forms of nutritional disorders [1]. It occurs when there is a negative balance between iron absorption and iron requirements and losses. Iron deficiency is caused not only by iron-deficient diets but also by the low iron bioavailability of the diet. Pregnant women, infants, young children, and adolescents have higher iron requirements and thus have a greater risk of developing its deficiency [2]. Iron deficiency causes anemia and other pathological changes in the body.
Iron is an ingredient of many food products, so with a bit of willingness, there is no problem in providing the body with the right amount of it. Most iron is found in meat, cereal products, vegetables, and fruit. However, in the case of iron deficiency anemia, we often choose oral supplementation in addition to modifying the diet. What is important is that absorption of iron from food or pharmaceutical products requires recognition of the chemical form of the iron by gut receptors. Therefore, knowledge of the iron valence state is fundamental because it may be related to the effect and toxicity of pharmaceutical products. The most sensitive technique for the analysis of the iron state is Mössbauer spectroscopy (nuclear resonance technique). This technique is very sensitive to the local atomic structure, its local deformation, and atomic or lattice defects when treating the Fe nucleus as a probe of its local surroundings. Based on the results of this method, we obtain information about the iron oxidation states, the iron local microenvironments, the iron magnetic states, and the relative fractions of iron-bearing components.
In this work, we analyze the iron state and quality of selected iron-containing vitamins and dietary supplements but also some in food products of animal and plant origin (e.g., beef liver, duck liver, beef spleen, cumin). ^{57}Fe hyperfine parameters of the studied pharmaceuticals indicate that major ferrous compounds exist, as indicated by the manufacturer. However, Mössbauer spectra of these products demonstrated the presence of additional ferrous and ferric components, probably related to impurities or a partially modified main component. The food products contained Iron, mainly in a ferric state.

Keywords: iron state, pharmaceutical products, food, Mössbauer spectroscopy

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O_2 STRUCTURAL CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF NOVEL PLANT-DERIVED COMPOUNDS

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The plant kingdom represents an enormous reservoir of compounds with various bioactivities, including antioxidant potential. Demand for such plant-derived substances has increased because they are perceived as natural and safe for applications in numerous industries such as cosmetics, food, agriculture, and pharmaceuticals [1-3]. This study aimed to analyse the selected plant materials for the presence of new bioactive compounds and the characterization of newly found substances in terms of their chemical structure and antioxidant activity.
During HPLC analysis of extracts of numerous plant species, we found in the chromatograms of baobab (*Adansonia digitata*) seed oil and lemon (*Citrus limon*) seed several new fluorescent compounds that have not been found in any other plant extract investigated so far. After preparative isolation of these substances, their structural characteristics were elucidated by physicochemical analyses, such as nuclear magnetic resonance spectroscopy, ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry, gas chromatography, and fluorescence spectroscopy. These methods allowed the identification of these substances as a series of *N*-acylserotonins. For selected newly found *N*-acylserotonins, a functional analysis was performed in terms of their antioxidant activity. The experiments were carried out in model systems (liposomes) as the ability to inhibition of lipid peroxidation and *in vitro*, through the reaction with DPPH radical. Acylserotonins showed pronounced inhibition of membrane lipid peroxidation in liposomes and strong DPPH radical scavenging capacity.
In conclusion, we identified a novel class of plant-derived compounds, *N*-acylserotonins. We also proved that these substances possess high antioxidant activity and could be a new natural antioxidants. Nevertheless, *N*-acylserotonins might have other potential bioactivities and application values, which are worthy of being further studied.

Keywords: antioxidant activity, *N*-acylserotonins, baobab seed oil, lemon seeds

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O_3

DELVING INTO PLANT THERMAL DYNAMICS: A NON-INVASIVE APPROACH

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Non-invasive approaches are of paramount importance in plant research, providing invaluable insights into the physiological responses of plants to various stressors. Thermal imaging, a non-invasive method, has emerged as a powerful tool for studying plant responses to environmental stimuli such as excessive light, salinity, and nanoparticle exposure. This technique enables the real-time monitoring of temperature dynamics and stomatal behavior, facilitating a comprehensive understanding of plant stress responses. Thermal imaging technique was employed to investigate the response of *Arabidopsis thaliana* to common environmental stressors, including excessive light [1], NaCl treatment [2], and titanium dioxide nanoparticle (TiNPs) exposure [3]. The study revealed distinct thermal kinetics in response to these stressors, along with significant alterations observed in stomatal conductance, transpiration rate, and chloroplast morphology. In salt-treated plants exposed to high light, an increase in rosette temperature was observed, and the thermal time constants were shortened, indicating an enhanced response to stress compared to untreated plants. In turn, exposure to TiNPs further modified the thermal response, resulting in the deceleration of thermal processes in TiNP-treated plants and influencing chloroplast nanomechanical properties. These findings underscore the utility of thermal imaging as a non-destructive and sensitive tool for studying the dynamics of plant responses to various environmental factors.

Keywords: *A.thaliana*, dynamic thermal imaging, environmental stress factors, thermal kinetics

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O_4 ANALYSIS OF THE MOLECULAR STRUCTURE OF O-QUINOLINE DERIVATIVES OF 1,4-NAPHTOQUINONE

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The 1,4-naphthoquinone scaffold occur in many natural compound that exhibit wide spectrum of biological activity such as anticancer, antibacterial, antiviral, and antimalarial [1-3]. The clinical used of many compounds with this moiety is limited due to their low bioavailability and toxicity [3]. Modification of the 1,4-naphthoquinone scaffold at the C2 and C3 positions allow to obtain a novel compounds with better activity and bioavailability. Quinoline has been demonstrated to possess various biological effects, with its notable anticancer property standing out [1,2]. These diverse properties make quinoline a valuable consideration in the quest for new drug discoveries, as well as potential substituent group in research for molecules with better biological properties.
The aim of the presented study was synthesis and structural characteristics of the O-quinoline derivatives of 1,4-naphtoquinone.
The structure of new derivatives was analyzed by one-dimensional NMR (Nuclear Magnetic Resonance) spectra: ^{1}H and ^{13}C NMR, as well as two-dimensional techniques, including HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Correlation). The analysis of 2D NMR spectra is necessary to determine the regioisomerism of compound.
The presented work is a valuable source of information on the structure of O-quinoline derivatives of 1,4-naphtoquinone The methods and conclusions described in this work can be used for further research on these compounds and their derivatives.

Keywords: 1,4-naphthoquinone, Spectroscopy, Molecular Structure, NMR

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P_1 OXIDATIVE STABILITY OF OIL-IN-WATER EMULSIONS CONTAINING VITAMIN E NANOCARRIERS

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We have investigated the effect of α-tocopherol incorporated into different nanocarriers on the oxidative stability of oil in water emulsion. The antioxidant activity of free and encapsulated α-tocopherol was analysed in a 2,2-diphenyl-1-picrylhydrazyl (DPPH) reaction. Apart from α-tocopherol micelles, the samples showed similar antioxidant properties. The number of primary oxidation products (conjugated dienes and lipid peroxides) in the emulsion with vitamin E liposomes and niosomes was lower than in the emulsion with micelles. During storage period, the lipid peroxides gradually increased, whereas in emulsion with no vitamin E carriers remained constant. The content of the conjugated dienes increased at the beginning, and after 14 days at the end of testing time it remained stable in both types of emulsions. Our results might suggest that vitamin E when incorporated into carriers exhibits lower antioxidant activity. The results obtained could be due to the better solubility of vitamin E in lipid droplets and thus the lower availability for the interfacial area, which is thought to be the place of the most pronounced lipid peroxidation.

P_2 NEW 6,8-DISUBSTITUTED QUINOBENZOTHIAZINES - SYNTHESIS AND SPECTROSCOPIC STUDIES

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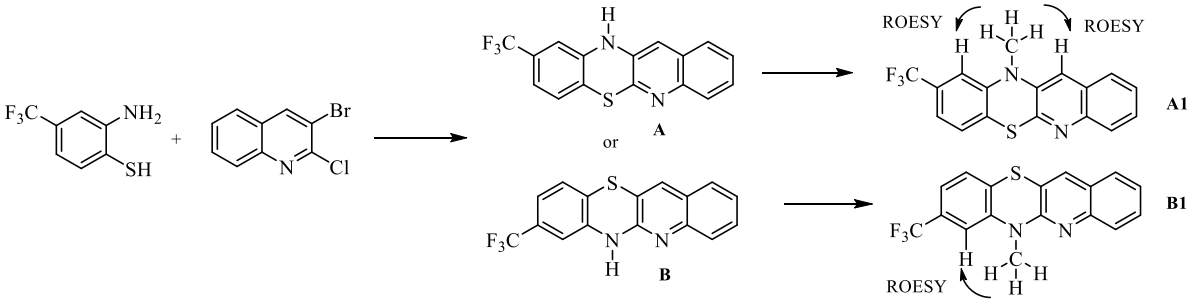
Phenothiazines are a large group of bioactive heterocyclic scaffolds containing sulphur and nitrogen, that have been well known for years as antipsychotics and antihistamines. Currently, these are classic phenothiazines that are used as good targets for drug repurposing, and at the same time their new analogues are being synthesized. Substituting one or two benzene rings with quinoline rings leads to obtaining quinobenzothiazines or diquinothiazines – azaphenothiaznines with various directions of biological activity [1]. One method of azaphenothiazine synthesis is the reaction of o-aminobenzenethiols with o-disubstituted azines. These reactions can proceed via the Ullmann cyclization or via the Smiles rearrangement. Therefore, it is necessary to correctly determine the structure of the products of these reactions [2].

The aim of this study is the synthesis and structural analysis of 6*H*-8-trifluoromethyl-[1,4]quinobenzothiazine as a substrate for the synthesis of new bioactive analogues of triflupromazine, trifluoperazine and fluphenazine.

For the synthesis of 6*H*-8-trifluoromethylquinobenzothiazine, 2-amino-4-trifluoromethylbenzenethiol and 3-bromo-2-chloroquinoline were used. This reaction can lead to a quinobenzothiazine with structure **A** or **B**. The obtained quinobenzothiazine was converted into a methyl derivative (**A1** or **B1**) and analyzed using spectroscopic methods.



The analysis of ^{1}H NMR proton signals in the benzene ring in the trifluoromethyl derivatives showed that the multiplicity of signals resulting from the coupling of the protons at the ortho (Jo = 6-10 Hz) and meta positions (Jm = 1-3 Hz) was useful. In order to further confirm the assumed structure of quino[3,2-b]benzothiazines (**B1**) and exclude the reaction to a quino[2,3-b]benzothiazine-type derivative (**A1**), the ROESY experiment was performed. To fully document the structure of **B1** derivative, a ^{13}C NMR spectrum and two-dimensional HSQC and HMBC spectra were also studied. Therefore, the products were identified as 8-trifluorometylquino[3,2-b]benzo[1,4]thiazines.

Keywords: azaphenothiazine, phenothiazine, Smiles rearrangement, synthesis, spectroscopic methods

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P_3 2D NMR TECHNIQUE - NOESY IN STRUCTURAL STUDIES OF SELECTED HETEROCYCLICAL SYSTEMS

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One of the main challenges of modern medicine is the search for new bioactive substances to combat lifestyle diseases. Research is being conducted on completely new chemical substances whose direction of action is related to new molecular targets, as well as structural modifications of known drugs, called leading structures, are carried out in order to improve their affinity for the molecular target [1]. The common denominator of both strategies is the precise determination of the structure of chemical compounds using NMR spectroscopy [2]. The aim of this project was to demonstrate the usefulness of two-dimensional NOESY (*Nuclear Overhauser Effect Spectroscopy*) spectra in detecting the spatial proximity of neighboring hydrogen atoms in new heterocyclic compounds. Spectral studies were performed on two new dipyridothiazine derivatives obtained at the Department of Organic Chemistry, Medical University of Silesia, Katowice. The compounds were examined in a solution of deuterated chloroform using a Bruker 600 MHz NMR spectroscope. In the first stage, standard ^{1}H NMR spectra and correlation COSY spectra (*Correlated Spectroscopy*) were performed, followed by NOESY experiments. The conducted research confirmed the validity of NOESY analyzes in identifying new molecules with biological potential.

Keywords: NOESY spectra, NMR, dipyridothiazines

Acknowledgements: Research was supported by grants PCN-1-041K/2/F, BNW-2-015/N/3/F (Medical University of Silesia in Katowice).

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P_4 TWO-DIMENSIONAL NMR TECHNIQUE - NOESY IN STRUCTURAL STUDIES OF SELECTED HETEROCYCLICAL SYSTEMS

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One of the main challenges of modern medicine is the search for new bioactive substances to combat lifestyle diseases. Research is being conducted on completely new chemical substances whose direction of action is related to new molecular targets, as well as structural modifications of known drugs, called leading structures, are carried out in order to improve their affinity for the molecular target [1]. The common denominator of both strategies is the precise determination of the structure of chemical compounds using NMR spectroscopy [2]. The aim of this project was to demonstrate the usefulness of two-dimensional NOESY (*Nuclear Overhauser Effect Spectroscopy*) spectra in detecting the spatial proximity of neighboring hydrogen atoms in new heterocyclic compounds. Spectral studies were performed on two new dipyridothiazine derivatives obtained at the Department of Organic Chemistry, Medical University of Silesia, Katowice. The compounds were examined in a solution of deuterated chloroform using a Bruker 600 MHz NMR spectroscope. In the first stage, standard ^{1}H NMR spectra and correlation COSY spectra (*Correlated Spectroscopy*) were performed, followed by NOESY experiments. The conducted research confirmed the validity of NOESY analyzes in identifying new molecules with biological potential.

Keywords: NOESY spectra, NMR, dipyridothiazines

Acknowledgements: Research was supported by grants PCN-1-041K/2/F, BNW-2-015/N/3/F (Medical University of Silesia in Katowice).

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P_5 ASSESSMENT OF THE SENSITIVITY OF MICROORGANISMS TO ANTIMICROBIAL DRUGS USING THE MARA TEST PROCEDURE

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Modern analytical chemistry can detect environmental drug contamination at the nanogram-per-liter level. However, assessing the actual effects of drugs on the environment requires systematic monitoring of many exposed ecosystems. It is extremely difficult to detect incidental contaminants or substances degrading or dispersing quickly. Moreover even the short-term presence of antimicrobial drugs in the environment can result in a long-term reduction in the sensitivity of microorganisms to these drugs.

The aim of our study was to evaluate the sensitivity of microorganisms from different aquatic ecosystems to antimicrobial drugs. The research methodology was based on the commercial chronic toxicity MARA test [1]. The procedure takes advantage of the ability of micoorganisms to reduce 2,3,5-triphenyltetrazolium chloride. The reaction results in the formation of a pink-red dye form with color intensity proportional to the activity of tested microorganisms. Incubation was carried out on 96-well microtiter plates inoculated with samples taken from the selected aquatic ecosystems. Addition of drugs to the medium used for incubation of microorganisms allowed to assess their sensitivity to the applied drug. The resulting image was scanned and analyzed digitally (NCIMB Ltd, Scotland). The microbial toxic concentration (MTC) values were used as a measure of microbial sensitivity [2].

A high positive correlation was found between the degree of pollution of the studied waters (expressed as total organic carbon (TOC) and chemical oxygen demand (COD)) and the determined MTC values. A significant reduction in the sensitivity of microorganisms in the model ecosystem exposed to anti-infective drugs was confirmed. However, we also found that the obtained results were vulnerable to the incubation conditions and the volume and preparation method of inoculum.

Keywords: antimicrobial drugs, sensitivity of microorganisms, chronic toxicity test, drug resistance.

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P_6

LIPOPHILICITY OF NOVEL DERIVATIVES OF 2,7-DIAZAPHENOTHIAZINE WITH POTENTIAL ANTICANCER PROPERTIES

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Lipophilicity is one of the most important physicochemical properties used to predict the biological activity of drug substances. It plays an important role in determining the ADMET parameters of potential drugs. In addition, it helps predict the ability of molecules to bind to plasma proteins and receptors at the site of drug action. Lipophilicity is an important physicochemical descriptor used to look for correlations between a chemical compound's structure and properties and biological activity (QSAR) [1,2].
The Department of Organic Chemistry, Medical University of Silesia has been conducting research on the synthesis of biological active heterocyclic systems as well as pharmacokinetic properties for many years. In continuation of our search in the area of anticancer active dipyridothiazines [3,4], we have obtained new 2,7-diazaphenothiazine derivatives in the form of dimer systems for which promising anticancer activities have been determined.
The next stage and the goal of our project was to determine the lipophilicity parameters and ADME parameters for the newly obtained compounds. The tests were performed using *in silico* methods and experimental reversed phase thin layer chromatography RP-TLC, TRIS buffer environment with pH 7.4 and using a standard curve. An attempt was made to correlate the obtained biological results with pharmacokinetic parameters.

Keywords: lipophilicity, diazaphenothiazines, anticancer activity

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P_7 3,7-DINITRO-10*H*-PHENOTHIAZINE 5-OXIDE: STRUCTURAL, SPECTROSCOPIC & COMPUTATIONAL STUDIES

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In recent years, push-pull chromophores based on oxidized 10*H*-phenothiazine (PTZ) have been extensively studied for their potential applications in OLEDs, photovoltaic devices, data storage, sensors and bioimaging [1]. However, among many elaborate directions in which 10*H*-phenothiazine 5-oxide and 5,5-dioxide have been modified and studied, the introduction of nitro groups and nitrated moieties seem to be generally avoided. No wonder – NO_2 substituent often yields non-emissive compounds and is regarded as a fluorescence quencher [2].
In this work [3], we have synthesized 3,7-dinitro-10*H*-phenothiazine 5-oxide (DPO), a compound originally prepared and described by A. Bernthsen and H. Sattler in 1885 [4], and then virtually forgotten by the scientific community compared to the parent PTZ. A straight-forward procedure efficiently yielded pure DPO with no need for purification by column chromatography. 3,7-Dinitro-10*H*-phenothiazine 5-oxide crystallizes to a monoclinic system with the oxygen atom from the -SO group disordered over two positions. The UV-VIS absorption spectrum of DPO shows a large bathochromic shift of λ_{max} compared to PTZ 5-oxide. The hydrogen atom at the N10 position is easily removed upon the addition of a base, which effects changes in NMR and UV-VIS absorption spectra. Moreover, DPO exhibits fluorescence with an emission band at 647 nm in DMSO and a Stokes shift value of 122 nm. The obtained results were interpreted based on the DFT and TDDFT computations.
3,7-Dinitro-10*H*-phenothiazine 5-oxide falls within the definition of a photoluminescent push-pull chromophore despite its affiliation with nitroaromatic compounds. This unusual activity stems from the unique electronic properties of PTZ. Nevertheless, by the example of DPO, we intend to emphasize a holistic approach to the design of new photoluminescent molecules and to press on the research on phenothiazine-based chromophores.

Keywords: 10*H*-phenothiazine, nitration, photoluminescence, halochromism, DFT

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P_8 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AS A TOOL IN THE STRUCTURAL ANALYSIS OF NEW LOMEFLOXACIN DERIVATIVES

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Fluoroquinolones are a group of synthetic antibiotics widely used in the treatment of bacterial infections of the urogenital, respiratory, and digestive tracts. Their action is based on inhibition of the enzymes involved in DNA replication – DNA gyrases and topoisomerases [1]. In addition to their antibacterial activity, the data in the literature indicate the anticancer potential of fluoroquinolones resulting from their ability to inhibit cell cycle progression and induce apoptosis of cancer cells [2,3]. The aim of this work was to design and synthesise selected floroquinolone derivatives containing a 1,4-substituted triazole system with antiproliferative activity.
The second-generation difluorinated quinolone antibiotic lomefloxacin was selected as the main substrate. In the first stage of the work, 30 lomefloxacin derivatives selected from own library of compounds were docked. As a result, compounds showing greater affinity for the molecular target, i.e. topoisomerase I, than the reference camtothecin were selected. In the next stage of work, selected compounds were synthesised. A propargyl group was introduced into the lomefloxacin pharmacophore system at selected positions. A 1,2,3-triazole system was introduced using a copper (I) ion-catalysed azide-alkyne cycloaddition protocol (CuAAC). In the reaction of the cycloaddition of azides to alkynes, regioisomeric 1,4- and 1,5-disubstituted triazoles are formed. When copper(I) ions were used as catalyst, only 1,4-disubstituted regioisomers were expected to be obtained [4]. To confirm the structure of the newly obtained compounds, the ^{1}H NMR, HSQC, and HMBC nuclear magnetic resonance techniques were used, with particular emphasis on the ^{13}C NMR technique. In proton nuclear resonance spectra, differences in the chemical shifts of the proton located in the 1,4- and 1,5-triazole ring do not allow a clear distinction between regioisomers. However, significant differences in the chemical shifts of carbon (^{13}C) atoms are noticeable in the ^{13}C NMR spectra. Therefore, this technique plays a particularly important role in the structural analysis of disubstituted 1,2,3-triazoles.
In the next stage, the obtained lomefloxacin derivatives will be submitted for cytotoxicity tests against selected cancer cell lines.

Keywords: lomefloxacin derivatives, synthesis, CuAAC, NMR spectra analysis

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P_9

ANALYSIS OF THE CHEMICAL COMPOSITION OF PROPOLIS DEPENDING ON ITS ORIGIN AND MEDICAL USE

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Propolis, also known as bee glue, is a natural product produced by honey bees (*Apis melifera*) from plant resins. Propolis has been known for its medicinal properties since ancient times and has recently become the object of interest again among researchers due to its potential healing properties [1].
Studies have shown that propolis has various activities such as antibacterial, anti-inflammatory, antiviral, anticancer and antioxidant [2]. The composition of propolis is complex and varied and, as a natural product, it may change the content of active substances depending on the location of the apiary or wild nest, season, weather, or feeding method. It has been shown that some components of propolis may have anticancer effects by inhibiting the growth of cancer cells, inducing apoptosis (programmed cell death) and blocking inflammatory processes that may contribute to the development of cancer [3].
The work is a review of research methods on propolis, focusing on its biologically active ingredients and methods of their determination. The initial stage of the analysis of propolis components is extraction, usually with 70% ethanol, aimed at isolating plant secondary metabolites. Identification of individual chemical compounds found in propolis is a complex process. It usually requires a combination of various complementary analytical techniques, such as chromatography (GC, HPLC) and mass spectrometry (MS). The most frequently used are couplings of mass spectrometers with certain types of chromatographs, such as a gas chromatograph (GC-MS) or a liquid chromatograph (LC-MS) [4,5]. Nuclear magnetic resonance (NMR) is commonly used in the analysis and identification of natural products, including propolis. Two-dimensional experiments (HMBC, HSQC, COSY) enable confirmation of the structure of individual components [5].

Keywords: propolis, mass spectrometry, biological activity

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P_10 MICROCALORIMETRIC ANALYSIS OF THE INTERACTION BETWEEN 9-HYDROXY-5-METHYL-12(H)-CHINO[3,4-B][1,4]BENZOTHIAZINE (SALT4) AND HUMAN SERUM ALBUMIN. PRELIMINARY STUDIES

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The nano Isothermal Titration Calorimetry (nanoITC) is a technique which allows to determine the energy effect of ligand-macromolecule interaction based on thermal measurements. The main aim of the study was the microcalorimetric analysis of the interaction between the chloride-5-hydroxy-12(H)-chino[3,4-b]-1,4-benzothiazine (Salt4) and human serum albumin (HSA). Salt4 is a newly synthesized substance with potential anticancer activity. Salt4 has been tested in vitro against the HCT116 and LLC tumor cell lines [1]. The IC_{50} concentration was obtained IC_{50} 6.7±5 μg/mL for HCT116 cells line and 7.2±2.8 μg/mL for LLC cells line. Doxorubicin was used as a reference substance. The project is a continuation of studies concerning the analysis of the interaction between Salt4 and main carrier proteins. Based on the experimental measurements, the association constant (K_a=(4.75±1.15)×10^6 L/mol), stoichiometry (n=3.71±0.0) and thermodynamic parameters (ΔH=107.6±15.3 kcal/mol, ΔS=381.4±64.8 cal/moLK, ΔG=-6.1±4.0 kcal/mol) calculated for Salt4-HSA complex were determined.
Salt4 binds strongly to HSA. The binding reaction is endothermic (ΔH>0) and spontaneous (ΔG<0). The dominant bonds in the formation of Salt4-HSA complex are hydrogen bonds (ΔH>0, ΔS>0) [2].
NanoITC is a very useful tool for analyzing the interaction between chloride-5-hydroxy-12(H)-chino[3,4-b]-1,4-benzothiazine and human serum albumin and it provides a number of valuable data on the ligand-protein complex formation. Through this, it can be complementary technique for spectroscopic techniques.

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Keywords: chloride-5-hydroxy-12(H)-chino[3,4-b]-1,4-benzothiazine, nanoITC, thermodynamics parameters

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P_11 TOPOLOGICAL INDICES OF SELECTED ANTIVIRAL DRUGS

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One of the most important factors determining the activity and properties of a molecule is its structural construction. QSAR and QSPR (Quantitative Structure-Activity/Property Relationships) studies are concerned with observing the effect of a molecule's structure on activity and the effect of a molecule's structure on its properties. Through these studies, the process of designing new molecules with potentially beneficial effects is facilitated. It is possible to study the activity, pharmacokinetic properties, pharmacodynamic properties, and ADME/T profile regarding absorption, distribution, metabolism, elimination, and toxicity of a molecule at an early stage - even before synthesis [1].
Topological indices are a mathematical representation of the structure of a molecule based on graph theory. It consists in representing a molecule in the form of a hydrogen-free graph, in which the vertices are atoms, while the edges are bonds. The construction of such a graph allows scientists to calculate topological indices according to their authors' formulas. The values of these indices are then correlated with other values such as lipophilicity parameters and more. Thanks to these mathematical methods, it is possible to predict many parameters of substances, which reduces the time and cost of synthesizing many compounds [2].
The aim of this study was to calculate the selected topological indices based on distance matrix (Wiener W index, Rouvray-Carfford R index, Pyka indices A, ${}^{0}B$, ${}^{1}B$) and neighboring matrix (Randić indices ${}^{0}\chi$, ${}^{1}\chi$, ${}^{0}\chi^{v}$, ${}^{1}\chi^{v}$, Gutman indices M, M^{v}) of two antiviral drugs such as darunavir and ritonavir. The mentioned drugs were chosen because of their great importance in medicine during three last years. They were effective substances against the SARS-CoV-2 pandemic, among others [3].
The newly calculated topological indices allowed us to propose new equations enabling the prediction of the lipophilicity parameter ($logP_x$) of both compounds. This will make it possible to develop new derivatives of antiviral drugs with the desired activity and physicochemical properties, thus in the design of new antiviral drugs.

Keywords: topological indices; antiviral drugs; QSPR; ADME/T

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P_12

SIMULTANEOUS ANALYSIS OF BICALUTAMIDE AND FEBUXOSTAT USING TLC WITH DENSITOMETRY

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Thin Layer Chromatography (TLC) combined with densitometry is a simple and relatively economical technique for analyzing chemical compounds. The small amounts of samples needed and the ability to test many compounds simultaneously are other big advantages of TLC. The discussed technique can be successfully used in quality control of many medicinal substances, such as bicalutamide and febuxostat, for example [1].
The purpose of this study was to develop rapid and effective method for the simultaneous determination of febuxostat and bicalutamide using TLC method combined with densitometry. The drugs under study were chosen because of their relevance in the treatment of common co-occurring diseases such as gout and prostate cancer [2,3].
For the development of new methods for analysis of bicalutamide and febuxostat by TLC combined with densitometry, chromatographic plates precoated with different adsorbents such as silica gel 60 F_{254}, RP18F_{254}, RP8F_{254}, RP2F_{254} and a mixture of silica gel 60 and kieselguhr F_{254} were tested as stationary phases. Mixtures of acetonitrile and water, n-hexane and propan-2-ol, chloroform and methanol, as well as a mixture of three components: n-hexane, ethyl acetate and glacial acetic acid were used as mobile phases.
Values of R_f (retardation factor) for bicalutamide and febuxostat were calculated for each of the tested systems, and other important separation parameters such as ΔR_f, R_s, R_f^{α} and α determining the correctness of separation of the two substances were calculated. Of all the systems tested, the best separation was observed with the mobile phase of n-hexane/propan-2-ol mixture in a volume ratio of 28/22 (v/v) and the stationary phase silica gel 60 F_{254} chromatographic plates ($\Delta R_f = 0.56$).
The developed method of bicalutamide and febuxostat presented in this work are characterized by beneficial results regarding separation parameters such as ΔR_f, R_s, R_f^{α} and α. The obtained results proved to be very promising and can be successfully applied in routine controls of matrices containing the tested substances side simultaneous.

Keywords: TLC; analysis; separation; bicalutamide; febuxostat

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P_13 EXTRACTION METHODS AND IDENTIFICATION TECHNIQUES USED IN THE BETULIN ISOLATION PROCESS

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Betulin is a pentacyclic triterpenoid of the lupane type. It is commonly found in many species of birch, such as *Betula verrucosa*, *B. pendula*, *B. pubescens*, *B. alba*, as well as other plant species such as *Alnus subcordata*, *Ziziphus jujube*, *Atractylis carduus*, *Plumeria obtuse* and *Triadenum japonicum* [1]. However, it is usually obtained from the outer part of birch bark, which is waste from wood processing [2]. This compound has many properties that may potentially find uses in pharmacy and cosmetology [3].The aim of our work is to compare the most commonly used betulin isolation methods and the solvents used in terms of their effectiveness in the extraction process. Due to the structural analogues of betulin present in the extracts, the selection of appropriate methods for its identification is also an important issue.The purest betulin (99.8%) was obtained by purifying the dry residue of ethanolic birch bark extract with benzene and chloroform. The disadvantage of both of these solvents is their toxicity, but they can be recovered by distillation, minimizing their impact on the environment [4]. A less environmentally harmful method uses sodium hydroxide to hydrolyze birch bark, followed by extraction with isopropanol (yield 43.1% of bark dry weight). However, the method is time consuming [5]. The most effective method of obtaining high yields of betulin from birch bark is the microwave method, in which water vapor enlarges the capillaries in the bark, which facilitates access of the solvent to the ingredients contained therein [2]. Other known methods include maceration, pressurized liquid extraction, ultrasonic extraction, supercritical fluid extraction, DES extraction, extraction after esterification, as well as a sublimation method [4,6].There are many methods of isolating betulin from plant material. Each of them has its advantages and disadvantages. The selection of the appropriate method depends on our expectations regarding the purity and amount of the obtained product. Budget, researcher and environmental safety also need to be considered [2].

Keywords: extraction, betulin, microwave, solvents, triterpenoides

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P_14 THE USE OF INORGANIC PIGMENTS AS PHOTOCATALYSTS

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The high costs of many modern wastewater treatment technologies encourage scientists to look for cheap solutions. An effective method for removing anthropogenic pollutants from wastewater is the photocatalytic process that uses, among others, titanium dioxide (TiO_2) as a catalyst.
The aim of the research was to assess the photocatalytic activity of pigments for the degradation of dyes. Azo dyes (Acid Orange 7 and Basic Orange 66) were selected as model compounds due to their harmful effects on aquatic organisms. Three types of white pigments based on TiO_2 and commonly used in the industry were used in the experiments, i.e. nanoparticle anatase (designated as PK 20A), rutile-anatase white (FS), pure anatase (AV-01 SF). The reference catalyst was commercial TiO_2 P25. Acidic, neutral and alkaline dye solutions with added water-insoluble pigments were irradiated with UVB radiation. At set intervals, aliquots were taken and, after centrifugation, the remaining dye content was measured using the spectrophotometric method.
It was found that the FS pigment did not show photocatalytic activity. The two remaining pigments (PK 20A and AV-01 SF) showed photocatalytic activity, although lower than that of TiO_2 P25. Moreover, the pH of the environment had a significant impact on the rate of dye degradation; the process was fastest in alkaline solutions. The results of these studies indicate that industrial TiO_2-based pigments that contain only anatase can be used as a cheap photocatalyst in the degradation process of azo dyes.

P_15 ELEMENTS AS POTENTIAL MARKERS OF ASSOCIATION GEOGRAPHICAL GREEN COFFEES

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Green coffee is a seed harvested from the coffee fruit that has not been roasted [1]. It is a rich source of compounds with antioxidant and anti-radical properties [2,3]. The total element content in green coffee is approximately 5% (m/m), which includes toxic and essential elements. The content of elements depends on the place where the coffee beans come from, the type of soil and the variety of coffee, and the differences in their content depend on the environmental conditions of the growing area and the genetic properties of the plant. Therefore, differences in the content of elements in green coffee samples from different regions of the world are possible [4]. The elements found in coffee are P, Cu, Mn, Ca, Mg, K, Sr, Ba, Fe, Na, Zn [5], Pb, Cd. The research material included green coffee samples from 10 countries: Colombia, Brazil, Peru, Costa Rica, Ethiopia, Kenya, Rwanda, India, Indonesia and Papua New Guinea, differing in variety and processing method. Quantitative measurements of the samples were performed using the FAAS technique after wet mineralization supported by microwave radiation. The conducted research shows that Fe is the element with the highest concentration found in the tested samples. Its content in a sample of Colombian coffee is the highest among all. However, Cd and Pb occur in the tested coffee samples in trace amounts or do not occur at all. The highest Mn content was observed for samples from Rwanda, Kenya, Costa Rica and Colombia, and the lowest for Ethiopia and Peru, below 30 μg/g. For the rest of the countries, the Mn concentration was similar. According to the literature [5], the percentage of Mn in green coffee samples should be higher for Arabica. However, the percentage of P and Cu should be higher for robusta. The Cu content was the highest for samples from India, the rest had similar contents. Also for Zn, the contents in individual samples were similar. The green coffee samples belonged to one species (Arabica), therefore the concentrations of individual elements do not differ significantly. Such research should be continued based on Robusta coffee samples. If there was a difference in the content of elements between species, there would be hope to use the analyzed elements as potential markers of the geographical affiliation of coffee samples.

Keywords: metals, AAS, green coffee

Acknowledgements: Work carried out as part of the Excellence Initiative - Research University program, a project implemented with secondary school students

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P_16 PHOSPHONIUM SALT-BASED STRATEGY FOR THE SYNTHESIS OF BIO-ACTIVE SCAFFOLDS

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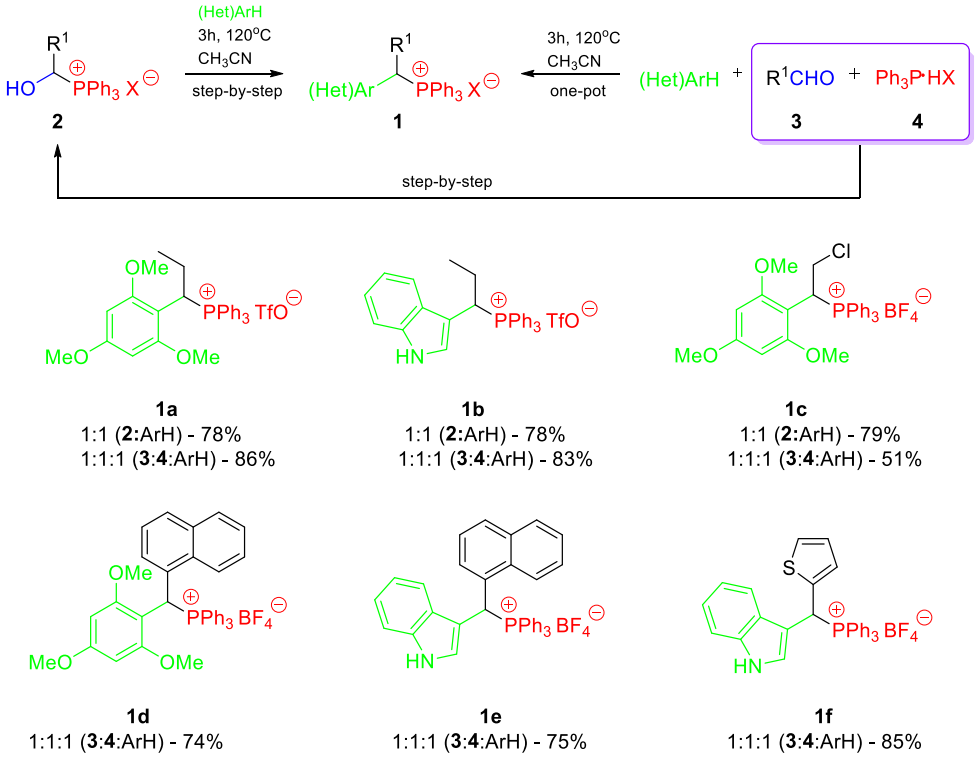
Phosphonium salts are becoming more and more popular in organic synthesis. They can be used as substrates in Wittig reactions, Friedel-Crafts reactions, or chemo- and regioselective couplings (such as amination, arylation, or thiolation). In this study, an efficient and convenient procedure was developed for the synthesis of 1-arylalkylphosphonium salts, important building blocks for the formation of bio-active scaffolds.[1] New products were obtained with good yields using a step-by-step or one-pot protocol.[2] The course of the reaction (mechanism, optimization of conditions) was monitored using NMR methods (^{1}H and ^{31}P NMR).



Scheme 1. Synthesis of 1-arylalkylphosphonium salts.

Keywords: phosphonium salts, synthesis, building blocks, NMR

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P_17

NMR SPECTROSCOPY AS A USEFUL TOOL FOR DETERMINATION OF THE CONFIGURATION AT THE C=C DOUBLE BOND OF α,β-DEHYDRO-α-AMINOPHOSPHONATES

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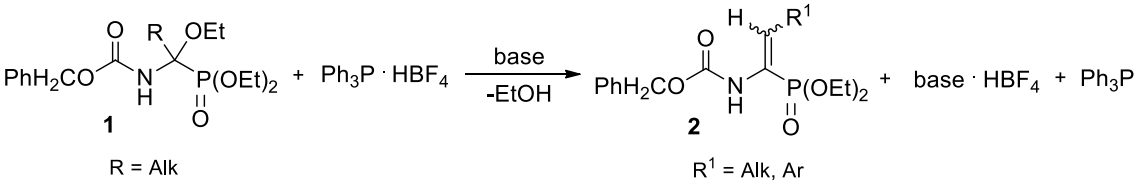
α,β-Dehydro-α-aminophosphonic acids can be considered as the phosphorus analogs of the well-known α,β-dehydro-α-amino acids. Similarly, as for the asymmetric synthesis of α-amino acids with the use of α,β-dehydro-α-amino acids, the stereoselective catalytic hydrogenation of α,β-dehydro-α-aminophosphonic acids and their derivatives is one of the most general methods for the asymmetric synthesis of α-aminophosphonic acids. The latter compounds, as structural analogs and mimetics of α-amino acids, display a broad spectrum of biological activity, and thus their stereoselective synthesis has currently attracted significant interest [1].

A characteristic structural feature of α,β-dehydro-α-aminophosphonic acids is the presence of a double bond between the C_α carbon atom in the main chain and the C_β atom of the side chain of the phosphorylated α-amino acid analog. This leads to a potential *E*/*Z* isomerism. It is worth noting that the *E*-isomers of dehydroaminophosphonates are almost 100-fold more reactive than their *Z*-configuration counterparts. This difference is crucial in the subsequent use of these compounds for enantioselective hydrogenation to obtain biologically active phosphorus analogs of α-amino acids [2].

The NMR spectroscopy technique is a very convenient and useful tool used by our research team to determine the configuration at the C=C double bond of new models of dehydrophosphonates **2** synthesized from the corresponding α-ethoxyphosphonates **1**. We do this by comparing the appropriate coupling constants, i.e. between the hydrogen atom and phosphorus ($^3J_{HP}$) and between the carbon atom of the R^1-substituent and phosphorus ($^3J_{CP}$) with the values available in the literature for *E*/*Z* isomers [1,3,4].



Keywords: α,β-dehydro-α-aminophosphonates, α-aminophosphonates, enantioselective hydrogenation, NMR spectroscopy, *E*/*Z* isomerism

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P_18

GENERAL METHOD FOR THE SYNTHESIS OF 1-ALKOXYALKYLPHOSPHONIUM SALTS – NMR STUDIES

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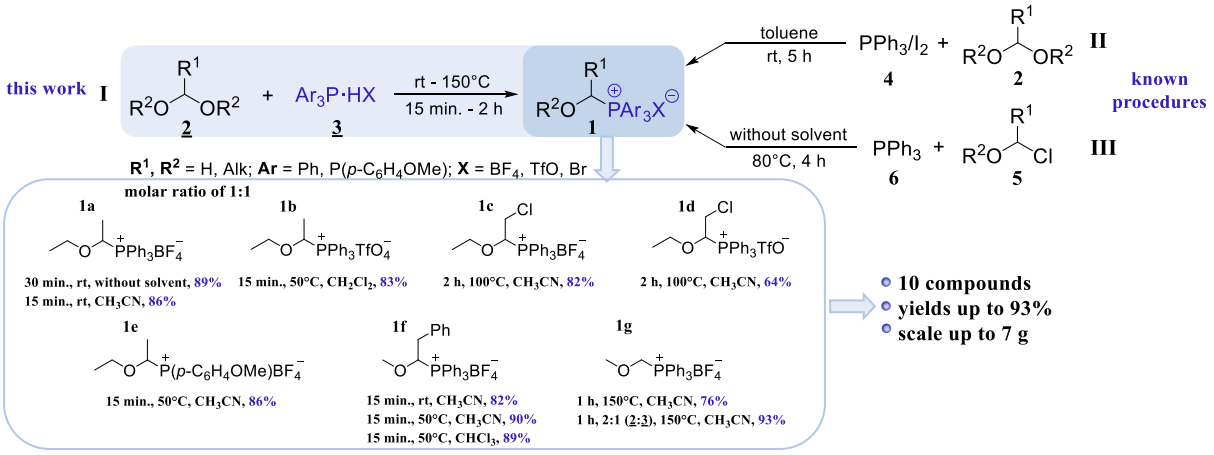
1-Alkoxyalkylphosphonium salts **1** belong to a specific class of *O,P*⁺-acetals, which are characterized by the presence of a P⁺-C-O bond. *O,P*⁺-acetals are mainly used as ylide precursors in Wittig reactions, but also as substrates in Friedel-Crafts reactions, or chemo- and regioselective couplings (amination, arylation, thiolation).[1] Among the most notable methods for the synthesis of 1 alkoxyalkylphosphonium salts described in the literature are those employing acetals and a triphenylphosphine-iodide complex (**II**), or 1-chloro-1-alkoxyalkanes and triphenylphosphine (**III**). Another example with limited applicability is based on the acid-catalyzed addition of triphenylphosphine to the double bond of enolic ethers.[2,3] Recently, we have developed a novel, efficient, one-step procedure (**I**) for the synthesis of 1-alkoxyalkylphosphonium salts **1** utilizing acetals and phosphonium salts.[4] We used NMR methods (^{1}H and ^{31}P NMR) to control the course of the reaction and elucidate the structure of the compounds obtained.



Scheme 1. Synthesis of 1-alkoxyalkylphosphonium salts **1**.

Keywords: phosphonium salts, acetals, synthesis, NMR

References

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P_19

SYNTHESIS AND SPECTROSCOPIC PROPERTIES OF PHOSPHONYL-PHOSPHINOYL ANALOGS OF 1-AMINO-1,1 BISPHOSPHONATES

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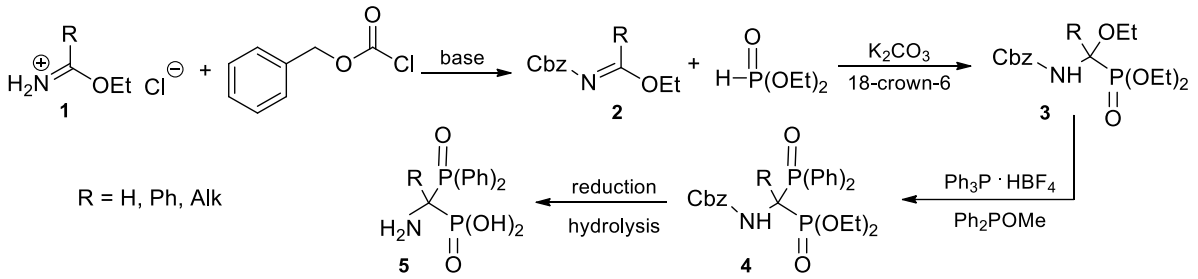
Last year, a procedure was developed for the preparation of phosphonyl-phosphinoyl analogs of 1-amino-1,1-bisphosphonates from α-functionalized derivatives of phosphorus analogs of protein and non-protein α-amino acids. The synthesis route involved the acylation of ethyl imidate hydrochlorides **1**, resulting in ethyl *N*-acylimidates **2**, followed by nucleophilic addition under PTC conditions using diethyl phosphite as a nucleophile to give α-ethoxyaminophosphonates **3**. The final step of the protocol was performed using a *one-pot* method involving α-ethoxy derivatives of phosphorus analogs of α-amino acids **3**, triphenylphosphonium tetrafluoroborate and methyl diphenylphosphinite as a nucleophile resulting in phosphonyl-phosphinoyl analogs of 1-amino-1,1-bisphosphonates **4** [1].
Currently, studies are being conducted to extend the applicability of this synthesis method to the leucine model (R = *i*-Bu), as well as subject compounds **4** to catalytic reduction to remove the protective benzyloxycarbonyl group (Cbz) and subsequent hydrolysis to obtain other compounds with the skeleton of P-C-P, that is diphenylphosphorylphosphonic acids derivatives **5**.
Phosphonyl-phosphinoyl analogs are an interesting subclass of α-aminobisphosphonates. However, their medical potential has not yet been fully explored. Due to their unsymmetrical structure, they have a lower affinity for hydroxyapatite than their symmetrical analogs and can be used to treat non-skeleton diseases [1,2].
It is worth mentioning that NMR spectroscopy is a useful and convenient technique that can be used to determine the spectroscopic properties of compounds obtained at each stage of the synthetic pathway. It is also used to monitor the progress of the reaction by the presence of characteristic signals in the ^{1}H-, ^{13}C, and ^{31}P-NMR spectra.



Keywords: 1-amino-1,1-bisphosphonates, unsymmetric bisphosphoric derivatives, α-ethoxyaminophosphonates, NMR spectroscopy

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P_20 INVESTIGATION OF THE PHYSICOCHEMICAL PROPERTIES OF PERYLENEBISIMIDES

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Nanographenes play a significant role in the modern world of electronic devices due to their unique thermal, emission, and electrochemical properties [1]. Graphene has been known and used for some time – because of its unique advantage of high electron mobility at room temperature. On the other hand, it has a zero energy gap between the valence band and the bandgap, preventing its use in some electronic technologies [2]. However/Importantly, functionalized molecular nanographenes equipped with functional groups can help solve this problem.
From the organic chemistry point of view of, molecular nanographene is a polycyclic aromatic hydrocarbon (PAH). This applies not only to PAHs that consist only of carbon atoms but also to those whose skeleton also contains heteroatoms. Generally, there are two methods of nanographene synthesis: bottom-down and bottom-up. The first one involves obtaining smaller fragments from larger ones through their selective destruction (molecular cutting) [3]. The bottom-up method involves expanding smaller structures (in a controlled manner) until the expected polyaromatic systems are obtained [4]. The essential element is selecting the appropriate starting structure and synthetic method, which significantly affect the properties of the obtained products - the research focused on PAHs whose skeleton is based on a perylene core, synthesized using the "bottom-up" method.
The research began with synthesizing a nanographene precursor, i.e., cis-dibenzoperylenebisimide (cis-DBPDI), through dimerization of the starting anthracene substrate using classical chemical synthesis and electrochemistry. Then, cis-DBPDI was subjected to further functionalization via cycloaddition and tandem cycloaddition-cycloisomerization of acetylenes and butadiynes into its bay region. Several pi-extended derivatives were obtained, for which the following were performed: NMR, HRMS, and physicochemical measurements: UV-Vis spectroscopy, photoluminescence, and electrochemistry. Structural studies allowed us to observe the lack of aromatization (via thermal dehydrogenation) of the obtained cycloadducts. It is an unexpected novelty in the acetylenes and arynes [4 + 2] cycloaddition to the perylene core bay region. On the other hand, other studies allowed us to confirm the expansion of the pi-electron structure and observe the unique properties represented by each structure.

Keywords: bisimides, perylenobisimdes, electrochemistry, photoluminescence

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P_21 SURFACE AND STRUCTURAL STUDIES OF TIALV ALLOYS MODIFIED WITH COATINGS CONTAINING BN NANOPARTICLES OBTAINED BY EPD METHOD

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The growing demand for various types of biomaterials necessitates development of new, advanced technologies to improve their mechanical and biological properties. While bone implantation remains a widely utilized technique in human orthopedics, an increasing number of veterinary surgeons decide to employ different types of implants during orthopedic treatment procedures [1]. Placing an implant in the tissues of a living organism takes a risk of adverse reactions such as different kind of bacterial infections [2], collagen formation or even necrosis and complete implant rejection [3]. To avoid mentioned complications authors propose a solution that might protect both implant's surrounding tissues as well as implant surface. Recent studies indicate that Boron Nitride and its derivatives offer significant potential for biomedical applications and are increasingly being investigated as biologically active additives. The aim of this study was to assess surface and structural properties of Chitosan/BNNPs EPD coatings deposited onto TiAlV substrates. Authors focused on physical-chemical studies such as SEM observations, XRD analysis, surface roughness and wettability assessment. SEM observations of deposited coatings confirmed the formation of a homogeneous layer of BN nanoparticles for each tested concentrations. XRD analysis confirmed the composition of the deposited coatings, which included a crystalline BN phase and amorphous chitosan throughout its whole thickness. Surface roughness of deposited coatings significantly increased with the increase of BNNPs concentrations. Deposition of Chitosan/BNNPs coatings changed surface wettability. The findings of this study lead to the conclusion that depositing homogeneous EPD coatings consisting of BN nanoparticles and chitosan alters the surface, physical, and chemical properties of implant surfaces to varying degrees. Depending on the implant's intended application, these alterations may be deemed favorable. The applied coatings exhibit notable potential for subsequent biological testing, particularly concerning their utilization in veterinary medicine.

Keywords: BN nanoparticles, Electrophoretic deposition, Surface and Structural Properties

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P_22 HYBRID SOL–GEL COATINGS DOPED WITH SiO_2 AND TiN NANOPARTICLES APPLIED ON TIATNIUM ALLOY: SURFACE AND STRUCTURAL PROPERTIES

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Titanium (Ti) or Ti alloys reveals suitable mechanical properties and high biocompatibility as an osseointegrative implant materials [1]. Although Ti alloys has many benefits, it also has certain drawbacks. One of the major downside is poor wear resistance [2]. The wreckage from wear and corrosion might react with bone tissues, leading to implant failure. Therefore, it is necessary to improve bioactivity and the tribological properties and prevent corrosion of titanium alloys through surface modification [1,2]. One of the prospective and dynamic trend in this field is the application of various protective layers [1]. Any of the forms applying coatings is the sol-gel technique which shows great potential as a means of corrosion protection allows the incorporation of antimicrobial ingredients into a pure silica matrix [2]. In this research, we have fabricated a TiNNPs/SiO2 coating for titanium implants by sol – gel method. The aim of this work was to investigate how deposited layers influence the microstructure and surface properties.
The Ti alloy layer modification changed the surface properties, such as wettability, surface energy and roughness. The coatings took on the morphology of the substrate. The thickness of the coatings are influenced by withdrawal speed and the method of substrate preparation. When analyzing the surface roughness, we observed that the modification of the TiAlV alloy with the sol–gel layers significantly rudimentary the samples surface smoothness. The increase in the Ra and Rq parameters was observed for all tested layers in relation to the base alloy. The tests also confirmed an increase contact angle values. SEM observations of the layers revealed that titanium nitride particles were not distributed homogeneously. Further research should focus on improving the homogeneity of the obtained layers.

Keywords: sol – gel layers, TiN nanoparticles, surface properties

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P_23 METHOTREXATE IN ACUTE LYMPHOBLASTIC LEUKEMIA TREATMENT – AN OVERVIEW

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Methotrexate (MTX), a structural analog of folic acid, plays a significant role in the treatment of cancer and autoimmune diseases. MTX significantly affects the metabolism of cancer cells by inhibiting an enzyme involved in folate metabolism, leading to a deficiency of this component and inducing cells death. MTX during the treatment of acute lymphoblastic leukemia requires monitoring of MTX concentration in human serum. Ongoing research on the mechanism of action and new applications of MTX gives hope for further progress in therapy. The advantages of MTX include low costs, safety of use and proven effectiveness make it highly valued by medical professionals. These studies provide an overview of the current knowledge regarding the applications of MTX as an anticancer drug in the treatment of childhood acute lymphoblastic leukemia. Pharmacokinetics, pharmacodynamics, mechanism of action, and the role in treatment of acute lymphoblastic leukemia are discussed. Moreover, the studies present promising perspectives including new methods for the determination of MTX concentration in patients' serum.

Keywords: methotrexate, acute lymphoblastic leukemia, anticancer properties, foliate acid

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P_24

ASSESSMENT OF THE ANTICANCER EFFICACY OF DOXYCYCLINE AGAINST AMELANOTIC MELANOMA CELLS IN AUTOPHAGY-INHIBITED MODEL

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Melanoma is a type of malignant tumor that originates from melanocytes. In recent years, the incidence of melanoma has increased rapidly around the world and constitutes a serious public health problem [1]. Currently, many drugs are being tested for their anticancer effects on melanoma cells. One of the drugs is doxycycline (DOX). Previous research has shown, among others: the ability of DOX to induce apoptosis and inhibit the proliferation of amelanotic melanoma cells [2]. Unfortunately, the effect was observed at relatively high drug concentrations, which could be due to the induction of autophagy.

Autophagy is a process in which damaged organelles and cell molecules are degraded in lysosomes, undergoing a type of biochemical recycling. Therefore, this process may affect cell survival and proliferation. It may also affect the effectiveness of anticancer therapy. Currently, some substances can inhibit autophagy, including 3-methyladenine (3-MA) [3].

This study aimed to evaluate the effect of autophagy inhibition on the antitumor activity of doxycycline against C32 amelanotic melanoma cells. Assessment of melanoma cell number, viability, and cell cycle was performed using the NucleoCounter® NC-3000™ imaging cytometer. The tested cultures were analyzed after 48-hour incubation with doxycycline at a concentration of 100 μM, 150 μM, 200 μM, and 5 mM 3-methyladenine.

The obtained results showed that 3-methyladenine significantly inhibits the proliferation of melanoma cells and slightly reduces cell viability. In turn, simultaneous exposure of C32 cells to 3-MA and doxycycline decreased cell viability in proportion to the DOX concentration. Additionally, the combination therapy disrupted the cell cycle and significantly reduced cell numbers in the melanoma cell populations tested. The conducted research shows that inhibiting autophagy may limit the development of amelanotic melanoma and increase the effectiveness of anticancer therapy with doxycycline.

Keywords: autophagy, melanoma, doxycycline, 3-methyladenine, cell cycle

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P_25 FORMATION AND BIOLOGICAL CHARACTERISTICS OF OXIDE – POLYMER HYBRID LAYERS ON TITANIUM IMPLANTS

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Development of oxide – polymer hybrid layers on a surface of titanium veterinary implants is very promising method of surface treatment and drug delivery system [1]. The aforementioned layer contains ceramic oxide layer which is then covered in thin film of biopolymer with drug (in this case clindamycin) loaded into the polymer layer deposited on implant surface. Using hybrid layers loaded with drugs it is possible to lower required dose of the drug to hinder the probability of infection in surrounding tissue where the implant was implemented.
Main techniques in achieving oxide – polymer layer are: PEO (plasma electrolytic oxidation) technique, which allows to obtain highly porous surface allowing to maximally develop surface area which helps in osseointegration between implant and surrounding bone tissue [2], and dip-coating used to cover oxide layer with biopolymer and drug mixture. The morphology and chemical composition of the modified implant surface were analyzed. To determine drug antibacterial properties microbiological tests with S. aureus ATCC25923 bacteria were performed. For cytocompatibility analysis the two tests were performed: first where Alamar Blue dye reduction test was applied, and second one, where cytometer measurements were used to analyze live and dead cells.
Obtained results confirmed that clindamycin is effective in prohibiting growth of bacteria in rather low concentrations, simultaneously being non – cytotoxic towards cells.

Keywords: PEO, hybrid, coating, oxide, polymer

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P_26

INFLUENCE OF NITI SURFACE TREATMENT ON ITS PHYSICOCHEMICAL PROPERTIES

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Modification of metal surfaces has a significant impact on improving their physicochemical properties. Proper preparation of the metal surface is crucial before its application. In bioengineering, this is particularly important. A properly prepared material, when introduced into the body as an implant, should easily integrate with the tissue or, conversely, depending on the application [1].
Plasma electrolytic oxidation (PEO) and electrochemical polishing (EP) allow the achievement of extremely different surfaces. PEO allow to obtain pores of various sizes, shapes, and distributions form on the sample surface, enhancing the tissue integration process with the implant [2]. On the other hand, electropolishing, by partially etching the top layer, results in a smooth surface resembling a mirror. Lack of irregularities does not favor osteointegration, making these elements easy to remove from the body [3].
Research involves PEO and EP of NiTi alloy. Surface properties resulting from modification were compared using physicochemical methods. The conducted studies include images obtained through scanning electron microscopy with EDX, Raman spectroscopy, XPS analysis. The surfaces obtained as a result of plasma electrochemical oxidation are porous, while electropolishing smoothed the surfaces, as can be seen in **Fig. 1**. Properly chosen surface treatment guarantees that the properties of the biomaterial are customized for the intended application.

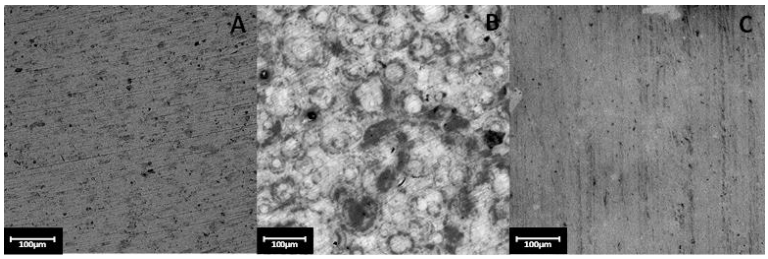

Figure 1 SEM images of NiTi alloy surface after grinding (A), PEO (B), electropolishing (C)

Keywords: plasma electrochemical oxidation, electropolishing, nitinol

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P_27 DETERMINATION OF TAURINE IN FOOD SUPPLEMENTS

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Food supplements are products that complement the human diet. They represent sources of nutrients or other substances with a nutritional or physiological effect. There are many substances that can be found in food supplements: vitamins, minerals, amino acids, fatty acids, dietary fiber, lutein, probiotics and prebiotics, and products of plant origin. Food supplements may be found in many forms, e.g. pills, tablets, capsules, liquids in measured doses. They can be also divided according to their compositions and purpose [1]. As a review of the literature shows, the consumption of these products has been increasing for several years, and this trend will be probably continued [2,3]. One of the ingredients of food supplements is taurine (2-aminoethanesulfonic acid). It is a biogenic, non-protein amino acids, which in comparison to protein amino acids does not have an acidic carboxyl group, but sulfonic group [4]. The concentration of taurine is the highest in newborns and drastically decreases with human age. Taurine has an important function, among others, in the development of the central nervous system and it prevents different diseases (e.g. Alzheimer's disease). Therefore it is ingredient of many food supplements consumed for support nervous system and concentration [5,6]. In view of the above, the aim of study was elaboration of an analytical method for determination of taurine in commercially available food supplements. The first step was extraction of taurine from samples using solid phase extraction technique (SPE). Then spectrophotometric technique was used for determination of this amino acid based on ninhydrin reaction in acidic conditions. Selected validation parameters were determined for the method: limit of detection (LOD), limit of quantification (LOQ) and recovery of analyte. Results show that elaborated SPE-UV-Vis method may be used for determination of taurine in food supplements.

Keywords: taurine, food supplements, SPE, UV-Vis spectrophotometry

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P_28 CALORIMETRIC, STRUCTURAL, AND DIELECTRIC STUDIES ON ACTIVE SUBSTANCES FORMING PLASTIC CRYSTAL PHASES

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Plastic crystals (PCs) have recently attracted considerable interest from many researchers due to their unique physicochemical properties and potential applications, for example, in pharmacy, magnetics, or optoelectronics. Among the various materials forming plastic-crystalline phases, adamantane (ADM) and its derivatives are particularly interesting.

The main purpose of the presented research was to determine the nature of the phase transitions in two ADM derivatives: 1-adamantylamine (1-NH_2-ADM), a drug known as 'amantadine', commonly used to treat Parkinson's disease and its symptoms, and 3-amino-1-adamantanol (3-NH_2-1-OH-ADM), which is a substrate to synthesize vildagliptin - an anti-diabetic drug. In our studies, three experimental techniques, differential scanning calorimetry (DSC), X-ray diffraction (XRD), and broadband dielectric spectroscopy (BDS) enabled us to obtain an in-depth analysis of thermal, structural, and dielectric properties of the two examined compounds. Calorimetric measurements revealed three characteristic endothermic peaks in the thermograms of both ADM derivatives. Further XRD studies showed that in 1-NH_2-ADM, these thermal events correspond to the transitions between various PC phases (PC(I), PC(II), PC(III), PC(IV)), while in 3-NH_2-1-OH-ADM – to the transition between the ordinary crystal (OC) and PC(I) phase, as well as PC(I)-PC(II) and PC(II)-PC(III) transitions. Especially interesting were the outcomes of dielectric investigations, which showed noticeable changes in the frequency dependencies of the imaginary (ε'') and real (ε') parts of the complex dielectric permittivity that occurred around temperatures of the characteristic peaks detected by the calorimetry for both substances. One can also mention that time-dependent dielectric measurements carried out for 1-NH_2-ADM and 3-NH_2-1-OH-ADM indicated the kinetic nature of the first phase transition observed in the thermograms collected during the initial heating of the samples.

The presented results can have important implications for predicting i) phase transitions between different PC phases in amantadine during the pharmaceutical production, as well as ii) the OC-PC and two PC-PC transitions in 3-NH_2-1-OH-ADM during vildagliptin synthesis. This knowledge, in turn, may help to obtain formulations of both mentioned drugs with well-defined physicochemical properties.

Keywords: amantadine, 3-amino-1-adamantanol, vildagliptin, phase transition, plastic crystal

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P_29 STUDIES ON THE MOLECULAR DYNAMICS AT HIGH PRESSURES AS A KEY TO IDENTIFY THE SUB-ROUSE MODE IN PMMS

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In this paper, we have discussed the results of high-pressure dielectric studies carried out for poly(mercaptopropyl)methylsiloxane (PMMS) characterized by chain backbone of alternate silicon and oxygen atoms substituted by a polar pendant group terminated with the thiol/sulfanyl (-SH) moiety. We are strongly convinced that an increasing steric hindrance induced by the presence of an alkyl pendant chain terminated by H-bonding moiety might help us to better understand puzzling dynamics of siloxane-based materials and properties of associating polymers, a new generation of systems intentionally designed to reveal noncovalent interactions, i.e., H-bonding, metal–ligand bonding, π−π interactions, ionic bonding, and host−guest interactions. This targeted modification is aimed to produce novel macromolecules characterized by structural reversibility leading to the unique and most-needed features, such as recyclebility, degradability, stimuliresponsivity, self-healing etc.
PMMS exhibits two dielectric relaxation processes observed above the glass transition temperature related most likely to either the mobility within self-assemblies or the sub-Rouse mode (α'-slower process) and segmental (α -faster process) dynamics, whereas mechanical measurements revealed only the presence of terminal and segmental relaxations. In order to distinguish the origin of the dielectric α'-slower process, further high-pressure experiments were performed. These experiments allowed us to find out that the additional (α') relaxation process, slower than the segmental mode detected in dielectric loss spectra of this polymer, mimics the behaviour of the normal mode at varying thermodynamic conditions. Herein, one can list a few similarities, such as a constant time scale separation between τ_α and $\tau_{\alpha'}$ during compression, as well as activation volumes comparable to those determined for the segmental motions. Obtained results, together with earlier mechanical data, clearly indicated that the α'-process in PMMS is the sub-Rouse mode. Therefore, apart from PMPS, this polymer is a second polysiloxane, for which such kind of mobility was identified in dielectric loss spectra. Moreover, we also found that the pressure coefficient of the glass transition determined for PMMS is much smaller with respect to PMPS of similar M_n and other polymers investigated in the literature. This outcome, although quite surprising considering the flexible nature of the Si-O connections, is surely connected to the presence of thiol moiety in side groups attached to each monomer. Even though specific interactions formed by this moiety are regarded as one of the weakest of all known, they have a strong impact on the dynamics of segmental and sub-Rouse mode in PMMS. The data presented in this paper clearly emphasize an important role of high-pressure experiments in elucidation of the real nature of additional motions detected in the loss spectra. Furthermore, they seem to be a key element to identify the sub-Rouse mode, which provides information about cooperative larger distance chain motions.

Keywords: Insulators, Molecular dynamics, Polarity, Polymers, Silicones

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P_30 THE IMPACT OF PVP TOPOLOGY ON THE CRYSTALLIZATION OF METRONIDAZOLE – THERMAL, STRUCTURAL AND SPECTROSCOPIC STUDIES

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Amorphous Solid Dispersions (ASDs) are homogenous mixtures of active pharmaceutical ingredients (APIs) with various excipients, including both low- and high-molecular-weight (LMW and HMW) substances. The combination of APIs with different auxiliary compounds aims to improve their properties, such as solubility, bioavailability, as well as inhibit the recrystallization from the amorphous state. Certainly, HMW substances, specifically polymers, have recently garnered significant research attention as effective additives for ASDs. Undoubtedly, one of the most commonly used polymers in binary formulations is polyvinylpyrrolidone (PVP). In the literature, the impact of molecular weights of commercially available linear PVP on the stability of amorphous APIs is widely discussed. However, the influence of dispersity, topology, or microstructure of this polymer on the properties of drugs is completely overlooked, which constitutes an interesting research gap.
Hence, we tested two self-synthesized polymers (linear PVP (*lin*PVP) and three-arm star-shaped PVP (*star*PVP)), and a commercial sample (PVP K30) with similar molecular weights but different topologies as inhibitors of crystallization of metronidazole (MTZ) - a pharmaceutical with a very strong tendency to crystallize. We prepared a series of binary mixtures (BMs) of MTZ with PVPs in various weight ratios (75:25, 60:40, 50:50 *w*/*w*), which were thoroughly examined using the following techniques: Fourier Transform Infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD). Interestingly, during the preparation of BMs, better miscibility of MTZ with *star*PVP (even up to 70 wt% of the polymer) was observed. As indicated by FTIR measurements, the reason for this phenomenon may be various interactions occurring between the macromolecule and the API, depending on the type of polymer matrix used. It was shown that there are dipole-dipole interactions between linear PVPs and MTZ, whereas hydrogen bonding occurs between the *star*PVP and the API. In turn, DSC and XRD studies indicated that in MTZ-PVP 75:25 and 50:50 *w*/*w* mixtures, linear PVPs are more effective inhibitors of MTZ recrystallization compared to *star*PVP. Moreover, non-isothermal calorimetric data revealed differences in the activation barrier for API crystallization depending on the topology of the polymer and its content in BMs. Importantly, long-term XRD studies showed that in the ASD with the highest possible content of *star*PVP (30:70 *w*/*w*), the recrystallization of API from the amorphous form is most effectively suppressed. In this system, the onset of MTZ crystallization was observed after more than a month, while for the BMs with linear polymers (50:50 *w*/*w*), the initiation of this process was noticed within 3 days. Thus, the presented results touch upon a significant aspect of the impact of PVP polymer topology on the crystallization process of APIs.

Keywords: metronidazole; polyvinylpyrrolidone; topology; star-shaped polymer; binary mixtures

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P_31 THE EFFECT OF A DEPOSITION METHOD ON THE APPLICABILITY OF REDUCED GRAPHENE OXIDE FOR ELECTROCHEMICAL BIOSENSORS

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Reduced graphene oxide (rGO), a derivative of graphene, is a versatile material possessing high electrical conductivity and good mechanical properties. For this reason, rGO can be utilized in various applications including the design of electrochemical biosensors. rGO is typically formed by the reduction of graphene oxide (GO) via the use of either chemical or electrochemical route. In our study, we aimed to explore how different approaches of the formation of rGO affect its characteristics, and to identify the most effective method for producing rGO, particularly for its application in the fabrication of electrochemical biosensors. GO was deposited on the surface of glassy carbon electrodes *via* either electrodeposition, drop casting, or self-assembly technique, and reduced into rGO using an electrochemical approach. The study examined the electrochemical characteristics of the material, including its electroactivity, electroactive area, and the catalytic efficiency in redox reactions, with the use of a cyclic voltammetry. Electrical properties of rGO were assessed using electrochemical impedance spectroscopy. Raman spectroscopy was employed to investigate the structural composition of rGO.
Our studies revealed a significant effect of the parameters of fabrication of rGO on its performance. Among these methods, it was a drop-casting technique that yielded a material with superior electrochemical attributes, in terms of high electroactive area and electrocatalytic activity. Conversely, rGO fabricated through a self-assembly process exhibited the least structural imperfections in the graphene lattice. Electrodeposition technique, on the other hand, resulted in rGO with enhanced capacitance compared to those produced by self-assembly and drop-casting, underscoring its suitability for use in the development of electrochemical biosensors.

Keywords: biosensor; graphene oxide; reduction; electrochemical detection

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P_32 EVALUATION OF APPLICABILITY OF NEAR INFRARED SPECTROSCOPY TO DIRECT DETERMINATION OF COMPOSITION OF BINARY ELUENT MIXTURES USED IN COLUMN CHROMATOGRAPHY

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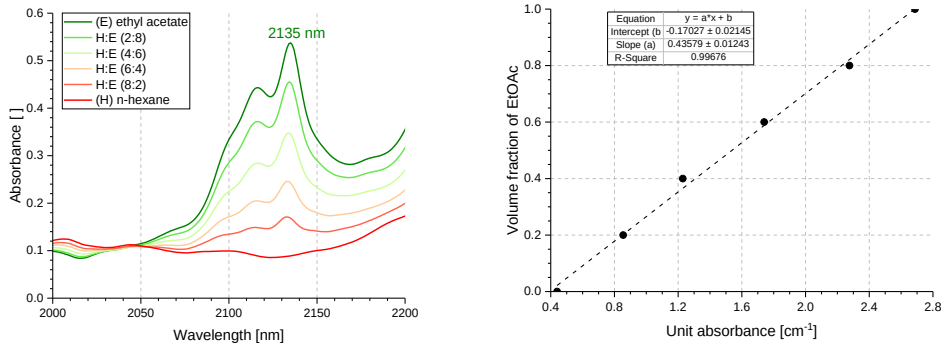
Column chromatography is a widely used technique for the purification of organic compounds and separation of their mixtures. This method, however, generates large amounts of environmentally harmful spent eluent liquid waste, requiring special treatment. Spent eluent mixtures could be recycled, but separating them into individual components, followed by re-blending, entails energy- and labour-intensive distillation process. Composition of an eluent is crucial to its eluotropic properties. Direct re-use of recovered eluent mixtures is hindered by their unspecified composition, arising from inevitable losses of the more volatile components during rotary-evaporator isothermal vacuum distillation. Quick and easy method of determining the composition would enable recycling of eluent mixtures, after adjusting their composition to the desired eluotropic properties.
Organic solvents exhibit characteristic absorption bands in the near infrared (NIR) region which can be used for their quantitative analysis [1]. In this study, near-infrared spectra of n-hexane, ethyl acetate, petroleum ether, toluene, and dichloromethane were recorded, along with the spectra of their most common binary mixtures at various volumetric ratios. For each solvent, characteristic bands were identified, and calibration curves were plotted for selected binary mixtures. The relationship between the volumetric fraction of solvent and unit absorptivity was found to follow Beer-Lambert's law, giving a linear relationship that enables spectroscopic identification of the composition of solvent mixtures. Our study demonstrates that NIR spectroscopy can serve as a quicker and cheaper alternative to more elaborate and time-consuming methods, such as ^{1}HNMR spectroscopy or gas chromatography.



Figures 1, 2. Collection of NIR spectra and calibration curve of ethyl acetate/n-hexane binary mixture

Keywords: Near-Infrared, spectroscopy, chromatography, eluent, solvent recovery

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P_33 NEW APPLICATIONS OF ELECTROANALYTICAL METHODS: POTENTIOMETRY

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The electroanalytical methods are a class of measurement techniques that involve examining an analyte by measuring the potential in volts and/or current in amps, in an electrochemical cell containing the analyte. One of the electroanalytical methods is jest potentiometry measures the potential, usually to determine the concentration of a solution. In this technique, the potential between two electrodes (indicated and reference) is measured using a high-impedance voltmeter [1,2]. The use of a high impedance voltmeter ensures that current flow is negligible. Hence the system is in equilibrium. This method was developed to use also ion-selective membrane electrodes, which have many advantages such as simple design, reasonable selectivity, fast response time, and can be used for colored, and cloudy solutions. Moreover, it is possible to connect them to automated and computerized systems. Ion-selective electrodes are routinely used in clinical laboratories to determine various concentrations of ions in blood samples. Every year, millions of Ca^{2+} or K^{+} measurements are performed in clinics using e.g. a calcium or potassium electrode [3]. Ion-selective electrodes are also regularly used in environmental analysis, for example in sewage treatment plants to monitor nitrate levels. And also in quality control of food, cosmetic, or medicinal products [3,4].
The purpose of the study is to introduce new applications of potentiometric methods. These new applications in potentiometers concern for example its use in measurements to evaluate the reversibility of adsorption on titanium dioxide of divalent ions from a solution [5] and others, such as progress in the development and improvement of reference electrodes, and discoveries in the domain of solid-contact ion-selective electrodes, and analytical applications for potentiometric sensors.

Keywords: potentiometry, titration, ion-selective electrode, sensing

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APPLICATIONS OF ELECTROANALYTICAL METHODS: CONDUCTIVITY

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One of the broadly applicable electroanalytical methods is conductometry. This method uses the ability of electrolyte solutions to conduct electricity and adopts Ohm's law for calculations. The origins of this method were made in 1869 by physicist Friedrich Kohlrausch, who investigated the conductive properties of electrolytes (specific conductance, law of Kohlrausch). Electrolytes can conduct electricity in solution, which can be measured by using the Wheatstone bridge and standard cell with a width of 1cm and read in the form of aliquots of units of conductivity – simens per meter (S/m) [1,2]. Conductivity meters are commonly used to assess the ionic content in solutions [3,4]. The study aimed to show the applications of conductivity measurement in natural sciences.
The conductivity is used in monitoring the performance of water purification systems routinely in many industrial and environmental applications [4]. The advantages of this method are simplicity, short measurement time, cheapness, and reliability in assessing the ion content in the solution. This method also uses titration to determine different electrolytes, including medicines [1-4].
Current research on electrolyte solution conductivity is based on the concentration-temperature dependence and the influence of the number of free ions and their mobility, as well as studies of electrolyte solutions of medium and high concentrations in binary solvents and organic solvents [5]. The application of conductivity tests not only in pure solvents but also in solvent mixtures may be of value from the point of view of their use in multi-component body fluid systems.

Keywords: conductivity, electrolytes, titration, binary systems, organic solvents

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P_35

NANOITC STUDY OF 6-ACETYLAMINOBUTYL-9-CHLOROQUINO[3,2-B]BENZO[1,4]THIAZINE AND AN ATTEMPT TO ENCAPSULATION INTO HUMAN SERUM ALBUMIN NANOPARTICLES

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Study of 6-acetylaminobutyl-9-chloroquino[3,2-b]benzo[1,4]thiazine (QBT) is azaphenothiazine derivative which has shown effectiveness in suppress contact sensitivity to oxazolone and possibility to inhibition chemically induced psoriatic changes in mice [1]. Nanoparticles serve as carriers of therapeutic substances and their use enables the development of the most optimal therapy [2]. The nano Isothermal Titration Calorimetry (nanoITC) allows to investigate the thermodynamic parameters and ligand interaction with proteins [3]. The aim of the study was to characterize the interaction between azaphenothiazine derivative (QBT) and human serum albumin (HSA) using nanoITC and encapsulation of QBT into HSA nanoparticles.

Calorimetric measurements were conducted using nanoITC calorimeter (TA Instruments, New Castle, USA). QBT (concentration: $1 \cdot 10^{-3}$ mol·dm^{-3}, syringe volume: 50 μl) was titrated into HSA at injection volume: 2.38 μl (concentration: $3 \cdot 10^{-5}$ mol·dm^{-3}, cell volume: 300 μl) with constant stirring at rate 300 rpm. QBT:HSA molar ratio was from 0.25:1 to 5.47:1. Encapsulation of the drug into human serum albumin nanoparticles was performed using the desolvation method. Quantitative measurement of unbounded QBT was determined at λ_{max} 267 nm with the use of UV-vis spectrophotometer (JASCO V-760, Hachioji, Tokyo, Japan).

Based on the experimental measurements, the association constant ($Ka=(0.12\pm1.51)\cdot10^{7}$ l·mol^{-1}), stoichiometry ($n=2.29\pm0.15$) and thermodynamic parameters ($\Delta H=-23.09\pm0.36$ kcal·mol^{-1}, $\Delta S=-45.1\pm96.2$ cal·mol^{-1}·K^{-1}, $\Delta G=-9.66\pm0.07$ kcal·mol^{-1}) of QBT-HSA complex were determined. Verification of encapsulation efficiency (EE) showed 88%.

The nanoITC can be a useful technique for the analysis of the interaction between QBT and HSA. QBT has bound with HSA at two classes of binding sites. The complexation reaction was exothermic ($\Delta H<0$), spontaneously ($\Delta G<0$) and the van der Waals and hydrophobic bonds were the dominant that stabilizing the complex ($\Delta H<0$, $\Delta S<0$). EE value has shown its possibility to incorporate into HSA nanoparticles with high efficiency.

Keywords: NanoITC, Nanotechnology, Azaphenotiazine

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P_36 THE USE OF IMMUNOTHERAPY, NANOPARTICLES AND GENE THERAPY IN THE TREATMENT OF HODGKI'S LYMPHOMA

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Hodgkin's lymphoma (HL) is a malignant disease of the lymphatic system characterized by the presence of giant neoplastic Reed-Sternberg cells with a multinucleated cells and mononuclear Hodgkin cells that induce non-neoplastic proliferation and infiltration of other lymphocytes, monocytes, histiocytes and macrophages. Although, most of the cases of Hodgkin's disease are cured with the first line of therapy, for example chemotherapy or radiotherapy, relapsed/refractory HL remains a major clinical obstacle and is fatal for patients who are not candidates for autologous stem cell transplantation or relapse after treatment [1].

The aim of the study was to consider, based on the literature, currently used or at an advanced stage of research anticancer therapies without the need to include pharmacotherapy in the treatment of HL. The work outlines proposed approaches used to treat HL, such as immunotherapy, CAR-T therapy and nanotechnology. The latest immunotherapy research proves the beneficial therapeutic effects of interacting drugs: brentuximab, vedotin and nivolumab [2,3]. CAR-T cell therapy can also show promising results, but combining it with other methods such as: lymphodepleting chemotherapy shows greater effectiveness of treatment [4].

The formulation of curcumin in solid lipid nanoparticles (SLN) or d-α-tocopheryl poly-ethylene glycol succinate nanoparticles (TPGS) have been investigated. Curcumin formulated in SLN and in TPGS resulted in higher plasma curcumin levels in mice. In HL cells in culture, curcumin reduced the expression of relevant anti-inflammatory cytokines (IL-6 and TNF-α) in a concentration-dependent manner. Moreover, when administered in combination with bleomycin, doxorubicin and vinblastine, curcumin showed additive growth inhibitory effects [5].

The cited studies suggest that classical chemotherapy, despite its effectiveness, may be replaced in the future by more advanced forms of drugs and biotechnology solutions.

Keywords: Hodgkin's lymphoma, immunotherapy, CAR-T, nanotechnology

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APPLICATIONS OF ANALYTICAL CHEMISTRY IN PHARMACEUTICAL SCIENCES

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The main goal of analytical chemistry is the determination of the chemical composition of matter and the identification of substances in their quality and quantity. Analytical chemistry is a part of chemistry that makes significant contributions to many fields of science, particularly the pharmaceutical sciences, when solving problems with newly synthesized biologically active substances or known drugs needs to use the knowledge of chemistry, instrumentation, computers, and statistics [1,2]. Assuring the safety and quality of pharmaceuticals, and water in their preparation is the duty of pharmacists [3,4]. The pharmaceutical industry relies primarily on analytical techniques to develop, produce, and bring to market safe and effective medicines. The work aimed to new knowledge about applications of analytical chemistry in pharmaceutical sciences.
Pharmaceutical analytical chemistry in the pharmaceutical industry is responsible for ensuring the constant quality of medicines and meeting the requirements set by institutions registering medicines around the world. From the point of view of ensuring the quality and safety of various products, from pharmaceuticals to diet supplement products, herbs or cosmetics analytical chemistry is a crucial technique used in this field. Various analytical techniques are widely used in the pharmaceutical industry to test for the purity and potency of drugs and in the detection of contaminants or adulteration. The titration techniques recommended by the Pharmacopoeia are selected depending on the type of substance being determined and used mainly through staff controlling drug quality [5]. To ensure the quality of pharmaceutical products and meet legal requirements, modern techniques and analytical methods are used.

Keywords: analytical chemistry, titration, quality, quantity, instrumentations

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P_38 PREPARATION AND STUDY OF NANOPARTICLES CONTAINING A THERAPEUTIC SUBSTANCE AS POTENTIAL DRUG CARRIERS

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Polymeric nanoparticles are widely used as drug carriers. An example of a polymer used to synthesize nanoparticles is albumin. To cross-link albumin to form nanoparticles, glutaraldehyde is most commonly used to bind the free lysyl groups of albumin. Nanoparticles can be studied using many techniques, i.e. UV-VIS spectroscopic technique, which measures the amount of ultraviolet (UV) and visible light (VIS) that is absorbed by a sample. An example of the drug encapsulated into nanoparticles can be ascorbic acid, commonly known as vitamin C. Deficiency of ascorbic acid in human body can affect the development of scurvy. The aim of the study was to encapsulate ascorbic acid into albumin nanoparticles (from human, sheep and bovine serum) with different amount of cross-linking factor.
Albumin nanoparticles were prepared by desolvation method. Albumin from human (HSA) and two species of the other mammals, sheep (SSA) and bovine (BSA), were purchased commercially. Albumin was dissolved in phosphate buffer. Then ascorbic acid and ethyl alcohol were added. As a cross-linking factor, 8% glutaraldehyde was used in amounts of: 0 µl, 12 µl, 50 µl, respectively. Measurements of unbound ascorbic acid were conducted using JASCO V-730 UV–VIS spectrophotometer (JASCO International Co., Ltd., Hachioji, Tokyo, Japan) at 266 nm.
In each sample, ascorbic acid was encapsulated with an efficiency of more than 90%. The highest encapsulation efficiency was for HSA nanoparticles, and the lowest for SSA nanoparticles. The amount of glutaraldehyde added affected the encapsulation efficiency for samples made with BSA and SSA - the more crosslinking agent added, the lower encapsulation efficiency. For HSA nanoparticles, encapsulation efficiency increased respectively for samples: 12 µl, 0 µl, 50 µl.
Ascorbic acid can be successfully encapsulated into albumin nanoparticles. The slight differences in the amino acids sequence allow albumin from any species to be used as a polymer. Further research is needed to understand the resulting variations of encapsulation efficiency for HSA nanoparticles.

Keywords: albumin, nanoparticles, ascorbic acid

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P_39 THE NOVEL USE OF TEREPHTHALIC ACID (TPA) IN THE STUDY OF HYDROXYL RADICAL DETECTION A SHORT LITERATURE REVIEW

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Understanding of free radical reactions mechanisms is important in the analysis of antioxidant activity. This term refers to the ability of specific substances to neutralize or inhibit the harmful effects of free radicals in the body. Free radicals are unstable molecules that can cause oxidative stress which may lead to cell damage and various health problems, including inflammation, aging and chronic diseases such as cancer, cardiovascular diseases and neurodegenerative disorders.

The aim of this work was to analyse existing data on terephthalic acid (TPA) used as hydroxyl radicals (•OH) scavenger in the studies of free radical reactions. Terephthalic acid (benzene-1,4-dicarboxylic acid, TPA) is a sensitive and specific probe for •OH [3]. TPA assay can be used in many *in vitro* and *in vivo* studies to detect •OH [1,2]. Unfortunately, according to literature data, there are not many studies on the analysis of drugs using TPA assay as an •OH trap.

Terephthalic acid is an organic chemical substance ($C_8H_6O_4$) that is synthesized by the oxidation of p-xylene [4]. The UV-Vis absorption spectrum of TPA has a maximum absorbance at λ_{abs} = 243 nm and its molar absorptivity (ε) value equals to 11 900 $M^{-1}cm^{-1}$ [5]. The free radical scavenging assay can be performed for various concentration of TPA [1,3] in NaOH solutions [1] or sodium phosphate buffer [3] exposed to an •OH source (radiolysis, light irradiation, Fenton reaction) [1,4,5]. TPA, a non-fluorescent substrate, reacts with •OH to form highly fluorescent, non-toxic and stable 2-hydroxyterephthalic acid (TPA-OH), which can be detected by spectrofluorimetric measurements. When excited by light with a wavelength at λ_{ex} = 312 nm or 315 nm, TPA-OH emits fluorescence at λ_{em} = 426 nm [1-3,5]. An increase of TPA-OH concentration correlates with an increase in fluorescence intensity [2].

The literature data confirm the using of TPA assay in the studies of free radicals and the analysis of the drugs based on this is a challenge faced by researchers.

Keywords: terephthalic acid, free radicals, spectroscopy

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P_40

THE ANALYSIS OF STREPTOMYCIN BINDING TO HUMAN SERUM ALBUMIN WITH THE USE OF SPECTROSCOPIC AND CALORIMETRIC METHODS

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Streptomycin (STR) belongs to the aminoglycoside antibiotics. It was isolated from *Streptomyces griseus*. STR has a broad spectrum of activity against Gram-negative bacteria. Its use is limited due to its strong ototoxic and nephrotoxic effects [1]. There is lack of consistent information in the literature to confirm the complex formation between the human serum albumin (HSA), transporting protein, and STR [2,3]. The aim of this work was the physicochemical characterization of the binding of STR to HSA. In order to achieve this goal, CD spectroscopic (JASCO J-1500 Spectropolarimeter), UV-Vis spectrophotometric (JASCO V-730 UV–Vis Spectrophotometer) and nanoITC (TA Instruments) techniques have been used.

Based on the near-UV and far-UV CD (from λ = 245 nm to 400 nm and from λ = 200 nm to 250 nm, respectively) measurements and the second derivative of differential absorption spectra analysis, the spatial structure of protein, especially in the surroundings of HSA aromatic amino acid residues, were characterized. Compared to HSA, the percentage of α-helix and β-sheet in the $(HSA\text{-}STR)_{complex}$ slightly decreased and an impact of STR on HSA secondary structure was probably possible. Due to the influence of STR, the HSA tertiary structure changes (in the regions of tryptophanyl and tyrosyl amino acid residues) were also probable. In order to precisely characterize the binding reaction between HSA and STR, calorimetric measurements were performed. Association constant (K_a), stoichiometry (n), the enthalpy (ΔH), entropy (ΔS) and Gibbs free energy (ΔG) changes have been determined. The binding reaction of STR to HSA was endoenergetic ($\Delta H>0$) and spontaneous ($\Delta G<0$). Moreover, probably four STR molecules interact with a single HSA molecule. Based on the K_a value ($(5.95\pm0.73)\times10^5$ M^{-1}) and taking into account the stoichiometry of the reaction (4.34±0.43) it can be concluded that the affinity of HSA towards STR is low. Based on the values of enthalpy and entropy changes ($\Delta H>0$, $\Delta S>0$) it can be noted that both hydrophobic and ionic bonds play the main role in the $(KAN\text{-}HSA)_{complex}$ stabilization.

In accordance with the performed preliminary studies it has been concluded that the analysis of STR-HSA complex using spectroscopic and calorimetric methods is possible and it could be the subject of many researches on this system in the future.

Keywords: streptomycin, albumin, spectroscopy, calorimetry

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P_41 CALORIMETRIC STUDY OF PREDNISONE AND PREDNISOLONE INTERACTION WITH HUMAN SERUM ALBUMIN

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Prednisone (PRN) and it's active form, prednisolone (PRL), are classified as synthetic glucocorticoids. They are used i.a. in acute and chronic inflammatory, autoimmune diseases and cancer therapy [1]. Glucocorticoids can cause wide range of side effects. In order to reduce them, it is necessary to better understand their pharmacokinetics. There are available studies which describe the interactions between PRN, PRL and plasma proteins, especially human serum albumin (HSA) [2,3]. Nevertheless, the historical data should be refined and updated with the help of novel research techniques.

The aim of this study was to characterize the binding reaction of PRN and PRL with HSA using Nano Isothermal Titration Calorimetry (nanoITC, TA Instruments). Based on the validated methodology, both binding and thermodynamic parameters have been determined. The obtained association constant (K_a) values for the reactions between HSA and studied ligands were not very high: 4.93 (± 2.33) × 10^5 [M^{-1}] for $(HSA\text{-}PRN)_{complex}$ and 2.79 (± 1.30) × 10^5 [M^{-1}] for $(HSA\text{-}PRL)_{complex}$. Simultaneously, the stoichiometry of $(HSA\text{-}PRN)_{complex}$ and $(HSA\text{-}PRN)_{complex}$ formation was 2.00 ± 0.17 and 2.14 ± 0.21, respectively. This phenomenon probably means that two molecules of PRN as well as PRL can interact with one molecule of HSA. The binding reaction of both PRN and PRL to HSA molecule has spontaneous ($\Delta G<0$) and exothermic ($\Delta H<0$) character. Moreover, the negative value of both ΔH and ΔS in the case of $(HSA\text{-}PRN)_{complex}$ indicates that the complex is stabilized by hydrogen bonds and van der Waals forces. On the other hand, in the $(PRL\text{-}HSA)_{complex}$, ionic bonds play the important role in complex stabilization ($\Delta H<0$, $\Delta S\sim0$).

Based on the presented data it can be concluded that the studies with the use of nano Isothermal Titration Calorimetry allowed to characterize the PRN-HSA and PRL-HSA complexes and this technique can be a useful tool for the study of ligands (PRN, PRL)-HSA interactions.

Keywords: prednisone, prednisolone, albumin, calorimetry

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P_42 IN VITRO INVESTIGATION OF THE INTERACTION OF LOSARTAN WITH SERUM ALBUMIN UNDER PATHOLOGICAL CONDITIONS

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Serum albumin (SA) plays a significant role in drugs pharmacokinetics and can affect pharmacological or toxicity effect of the drug. The drug-serum albumin interaction is an important component in understanding mechanism of action and drugs distribution. One of the processes causing the loss of albumins properties is the increased glycation in a state of hyperglycemia. Heterogeneous, stable compounds formed at the end of this process—Advanced Glycation End-Products (AGEs)—play a significant role in the development of chronic micro- and macroangiopathic diabetic complications as well as degenerative processes related to age [1]. To investigate how glycation affects binding affinity, comparative analyses of losartan (LOS) interactions with non-glycated (HSA) and glycated human serum albumin ($gHSA_{GLC}$, $gHSA_{FRC}$) were performed. Losartan is one of the significant drugs used in the regulation of arterial hypertension as well as in the treatment for chronic heart failure and the prevention of cardiovascular diseases in order to reduce the risk of a stroke in patients with hypertension and left ventricular hypertrophy [2].
Study of LOS-HSA, LOS-$gHSA_{GLC}$ and LOS-$gHSA_{FRC}$ systems has been carried out on the basis of human serum albumin quenching fluorescence technique at excitation wavelength λ_{ex} = 275 nm and λ_{ex} = 295 nm. The lower values of binding parameters – association K_a and Stern-Volmer K_{SV} constants – confirmed that the glycation decreases the stability of ligand-serum albumin complex (for LOS-HSA: $K_{SV(275nm)} = (1.92 \pm 0.04)\cdot10^4$ mol^{-1}·L and $K_{SV(295nm)} = (1.64 \pm 0.05)\cdot10^4$ mol^{-1}·L; $K_{a(275nm)} = (3.76 \pm 0.05)\cdot10^4$ mol^{-1}·L and $K_{a(295nm)} = (3.61 \pm 0.07)\cdot10^4$ mol^{-1}·L; for LOS-$gHSA_{GLC}$: $K_{SV(275nm)} = (1.42 \pm 0.01)\cdot10^4$ mol^{-1}·L and $K_{SV(295nm)} = (0.99 \pm 0.02)\cdot10^4$ mol^{-1}·L; $K_{a(275nm)} = (2.31 \pm 0.19)\cdot10^4$ mol^{-1}·L and $K_{a(295nm)} = (2.40 \pm 0.26)\cdot10^4$ mol^{-1}·L; for LOS-$gHSA_{FRC}$: $K_{SV(275nm)} = (0.84 \pm 0.01)\cdot10^4$ mol^{-1}·L and $K_{SV(295nm)} = (0.40 \pm 0.01)\cdot10^4$ mol^{-1}·L; $K_{a(275nm)} = (0.93 \pm 0.05)\cdot10^4$ mol^{-1}·L and $K_{a(295nm)} = (0.89 \pm 0.07)\cdot10^4$ mol^{-1}·L). Change of HSA structure caused by *in vitro* glycation results in reduction of losartan binding affinity to Sudlow's site I and II of macromolecule. These phenomena may influence the pharmacokinetics of losartan, thus monitored pharmacotherapy is reasonable in case of polypharmacy of diabetes and hypertension.

Keywords: losartan; spectroscopic methods; glycated human serum albumin

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ANALYTICAL PHYTOCHEMICAL PROFILING AND GLUCOSE UPTAKE POTENTIAL VIA GLUT4 OF *PTEROCARPUS MARSUPIUM* AQUEOUS BARK EXTRACT

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Pterocarpus marsupium (Fabaceae), also known as the Indian Kino tree, has been traditionally used to treat many ailments, especially diabetes [1-2]. Although there are numerous studies reporting the pharmacological activities of *P. marsupium*, little is known about the glucose uptake potential the bark of this plant possesses [3]. Hence, this study aimed to identify the putative compounds present in the aqueous bark extract of *P. marsupium* followed by its potential in increasing glucose uptake in insulin-resistant rat L6 myoblasts through the translocation of GLUT4. The phytochemical profiling of *P. marsupium* was analysed using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Rat L6 myoblasts were differentiated into myotubes and induced with insulin resistance using a high glucose/high insulin media before treating the cells with 10, 50, 100, 150, and 200 µg/mL of *P. marsupium* extract. Consequently, glucose uptake was performed using the Promega Glucose Uptake-Glo™ Assay Kit while the proteins were extracted from the cells to determine the GLUT4 protein concentrations through ELISA. The findings of the study revealed the presence of 10 major compounds at different retention times. The glucose uptake activity of *P. marsupium* extract showed a significant increase in a concentration-dependent manner and this result corroborated with the increased GLUT4 protein concentrations in the *P. marsupium*-treated L6 myoblasts. Conclusively, *P. marsupium* showed strong potential as a glucose uptake inducer through GLUT4 and the revelation of phytochemicals present in this extract sheds light in the development of therapeutic interventions in diabetes treatment.

Keywords: Pterocarpus marsupium; phytochemicals; glucose uptake; GLUT4; diabetes

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P_44 AN *IN VITRO* STUDY OF ANTIHYPERLIPIDEMIC EFFECT OF ALKILOSULFONIC ACIDS WITH QUINOBENZOTHIAZINLY SUBSTITUENTS

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Hyperlipidaemia, a condition characterized by high blood fat levels, increases the risk of onset of non-communicable diseases such as type 2 diabetes mellitus, cardiovascular disorders, cancer, and many other related diseases. Clinically, many drugs such as Orlistat, Simvastatin, and Atorvastatin have been used for the treatment and management of hyperlipidaemia. However, these medications often present unwanted side effects and many complains from users. Hence there is a need to find new leads that have less side effects, at the same time high efficacy and cheap. Propano- and butanosulfonic acids with 9-substituted quinobenzothiazinly substituents obtained in the reactions of 9-substituted quinobenzothiazines with propane sulton or butane sulton, respectively. The structures of new quinobenzothiazine derivatives confirmed by ^{1}H NMR, ^{13}C NMR and HR MS analyses. Therefore, this study aimed to evaluate the toxicity and antihyperlipidemic effect of 4-quinobenzothiazini butanesulfonic acids (MY-A - MY-H) on pancreatic lipase, cholesterol esterase and HMG CoA reductase activity. 3T3 L1 fibroblast cell line was used to evaluate cytotoxicity of MY-A - MY-H. MTT assay was done using concentrations of 10 µg/ml to 100 µg/ml. An *in vitro* antihyperlipidemic study of MY-A - MY-H was performed by evaluating the inhibition of pancreatic lipase, cholesterol esterase and HMG CoA reductase by MY-A - MY-H. Orlistat, Simvastatin, and Atorvastatin respectively was used as positive control and a concentration rage of 10 – 100 ug/ml of MY-A - MY-H was evaluated. The study found that synthetic compounds of MY-A - MY-H compounds was not toxic to the cells and it effectively inhibited pancreatic lipase, cholesterol esterase and HMG CoA reductase activities. MY-B and MY-H showed the highest activity. These compounds possess significant antihyperlipidemic properties, potentially impacting cholesterol metabolism.

Keywords: Antihyperlipidemic, MTT, Pancreatic lipase, Cholesterol esterase, HMG-CoA reductase, Quinobenzothiazine, Phenothiazine.

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