ANNOTATIONS

on the materials under Art. 76 of the Regulations for the Development of the Academic Staff of Plovdiv University (in English language);

of Assoc. Prof. Dr. Nina Dimitrova Dimcheva,

participating in a competition for the academic position of "Professor" in the area of higher education: 4. Natural Sciences, Mathematics and Informatics, Professional Field 4.2 Chemical Sciences (Physical Chemistry) at the Department of Physical Chemistry of the Faculty of Chemistry at Plovdiv University "Paisii Hilendarski", announced in the State Gazette, issue 96 of 11.11.2025.

The materials submitted for participation in the competition for the academic position "Professor" were developed after acquiring the academic position "Associate professor" and include 40 articles, 31 of which have an impact factor, 2 book chapters, 1 patent, and 2 instructional students' textbooks. Habilitation thesis is based on 6 of these publications, all of which are indexed in the Web of Science database.

- ✓ All papers for the competition were published in journals indexed in databases with scientific information (Web of Science and/or Scopus).
- ✓ 31 of the publications participating in the competition are in journals with an impact factor, and the sum IF is 83.653.
- ✓ h-index 17/15 (according to Web of Science/Scopus)

Link to SCOPUS profile:
https://www.scopus.com/authid/detail.uri?authorId=6602924420

I. Annotation of publications equivalent to a habilitation thesis.

According to IUPAC definitions, biosensors are analytical devices in which a biological element for molecular recognition (bioreceptor) is in intimate contact with a transducer of the signal obtained during the biochemical transformation of the substance being analyzed (analyte).Signal transducers can be thermal, optical, or electrochemical, with the latter being the most common.The review article examines the functions of micro- or nano-sized metal structures in the mechanisms of action of electrochemical biosensors.Among them, the most common are amperometric enzyme-based sensor platforms, where the measurable signal is an electric current proportional to the analyte concentration. In amperometric biosensors, the signal is generated as a result of one of the following three events:

a) oxidation/reduction of a product from an enzyme-catalysed reaction with the analyte (first-generation biosensors);

b) oxidation/reduction of small molecules called mediators, which transfer electrons between the active centre of the enzyme and the electrode surface and are regenerated during the process (second-generation biosensors); or

c) oxidation or reduction at the enzyme's active site itself as a result of the addition of the analyte (third-generation biosensors).

The articles presented as equivalent to a habilitation thesis demonstrate that the deposition of metal nano- or microstructures on electrically conductive materials (graphite, glassy carbon) performs extremely important functions in the creation of electrochemical sensors and biosensors, as they can function as an "anchor" facilitating the attachment of various bioreceptors to the electrode surface, as well as electrocatalysts for the conversion of products from the enzyme-catalyzed reaction, but they can also replace the biocatalyst itself with sufficient reliability and selectivity.

1. Dimcheva, N., Horozova, E., Electrochemical enzymatic biosensors based on metal micro-/nanoparticles-modified electrodes: a review. *Chemical Papers*, **69** (1) (2015) 17-26. DOI: https://doi.org/10.1515/chempap-2015-0011 (IF_{2015} = 1.326), **Q2**

Abstract: In this review, the applications of materials modified with metallic mono- or bimetallic nanoparticles as catalytic electrodes in the production of first-generation electrochemical biosensors, as well as the role that metallic nanoparticles play in promoting or enhancing the rate of electron transfer in third-generation electrochemical biosensors. Some examples of gold nanoparticles functionalized with enzymes via gold-thiol chemistry as a strategy for enzyme immobilisation and spatial orientation in the development of amperometric biosensors are also discussed.

Personal contribution: conceptualization, writing, corresponding author

2. Dimcheva, N., Horozova, E., Ivanov, Y., Godjevargova, T., Self-assembly of acetylcholinesterase on gold nanoparticles electrodeposited on graphite. *Central European Journal of Chemistry* **11**(11): (2013) 1740-1748 DOI: https://doi.org/10.2478/s11532-013-0307-3 (IF_{2013} = 0.829) **Q2**

Abstract: The immobilisation of AChE enzyme through chemisorption on Au-modified graphite was examined with view of its prospective application in the design of membraneless electrochemical biosensors for the assay of enzyme inhibitors. The developed immobilisation protocol has been based on a two-stage procedure, comprising i) electrodeposition of gold nanostructures on spectroscopic graphite; followed by ii) chemisorption of the enzyme onto gold nanoparticles. Both the coverage of the electrode surface with Au nanostructures and the conditions for enzyme immobilisation were optimised. The proposed electrode architecture, together with the specific enzyme immobilisation method, enables long-term retention of enzyme catalytic activity. The extent of inhibition of the immobilised acetylcholinesterase enzyme by the organophosphorous compound monocrotophos has been found to depend linearly on its concentration over the range from 50 to 400 nmol mL^{-1}, with sensitivity 77.2% inhibition per 1 µmol mL^{-1} of monocrotophos.

Personal contribution: conceptualization, biosensor acquisition, characterization, writing, corresponding author.

3. Dodevska, T., Horozova, E., Dimcheva, N., Electrochemical behavior of ascorbate oxidase immobilized on graphite electrode modified with Au – nanostructures. *Material Science and Engineering B* **178** (20) (2013) 1497-1502 DOI: https://doi.org/10.1016/j.mseb.2013.08.012 (IF_{2013} = 2.122) **Q2**

Abstract: Direct electrochemistry of ascorbate oxidase was observed when immobilized on graphite modified with nano-sized gold structures. Au-structures were electrodeposited onto the graphite surface by means of cyclic voltammetry, then the enzyme was chemisorbed onto their surface. The electron transfer between the enzyme active center and the modified electrode surface was probed by square wave voltammetry (SWV) and cyclic voltammetry (CV). The dependence of the current maxima on the scan rate was found linear, suggesting that the redox process is controlled by surface chemistry. Bioelectrocatalytic oxidation of the enzyme substrate l-ascorbic acid was explored by constant-potential amperometry over the potential range of 200-350 mV (vs. Ag/AgCl, 3 M KCl) at pH 5.6 and 7.0. At a potential as low as 200 mV, pH 7.0 and temperature 25 ^{0}C, the following operational parameters were determined for the enzyme electrode: a sensitivity of 1.54 μA mM^{-1}mm^{-2} ($R^2 = 0.995$), a linear dynamic range up to 3.3 mM, a detection limit of 1.5 μM, and a response time up to 20 s.

Personal contribution: conceptualization, biosensor acquisition, characterization, data analysis.

4. Dodevska, T., Horozova, E., <u>Dimcheva, N.</u>, Design of an amperometric xanthine biosensor based on a graphite transducer patterned with noble metal microparticles. *Central European Journal of Chemistry* **8** (2010) 19-27. https://doi.org/10.2478/s11532-009-0102-3 (IF$_{2010}$= 0.991) **Q3**

Abstract: A mesoporous graphite material micro-structured with palladium-platinum deposits (mixed in the ratio of 70:30% Pd:Pt) has been used as a cost-effective electrode material for designing an amperometric biosensor for xanthine. The here reported biosensor shows significantly improved operational parameters as compared to previously published results. At a constant applied potential of -0.05 V (*vs.* Ag/AgCl) it is distinguished with enhanced selectivity of the determination: at the working potential the current from the electrochemical transformation of various electrochemically active substances usually attending biological fluids (incl. uric acid, L-ascorbic acid, glutathione and paracetamol) has been eliminated. The effect of both the temperature and buffer composition on the analytical performance of the sensor has been investigated. Under optimal operational conditions (25°C, -0.05 V *vs.* Ag/AgCl, phosphate buffer, pH 8.4), the following have been defined for the biosensor: sensitivity 0.39 μA μM^{-1}, strict linearity of the response up to xanthine concentration 70 μM, detection limit of 1.5 μM (S/N=3) and a response time of at most 60 s.

Personal contribution: conceptualization, biosensor acquisition, characterization, data analysis.

5. Horozova, E., Dodevska, T., <u>Dimcheva, N.</u>, Mussarlieva, R., Electrocatalytic reduction of hydrogen peroxide on palladium-gold codeposits on glassy carbon: applications to the design of interference-free glucose biosensor. *International Journal of Electrochemistry* – Volume 2011, Article ID 697698, 8 pages; (SI Electrocatalysis: Fundamentals and Applications (2011)) http://www.hindawi.com/journals/ijelc/2011/697698/. DOI: 10.4061/2011/697698 **(WoS)**

Abstract: Following our previous studies on the catalytic activity electrochemically codeposited on graphite Pd-Pt electrocatalysts for hydrogen peroxide electroreduction, a series of glassy carbon electrodes were modified with Pd or (Pd+Au) deposits aiming at the development of even more efficient electrocatalysts for the same process. The resulting electrodes were found to be very effective at low applied potentials (−100 and −50 mV versus Ag/AgCl, 1M KCl). The surface topography of the electrode modified with Pd+Au mixed in proportions 90% : 10%, exhibiting optimal combination of sensitivity and linear dynamic range towards hydrogen peroxide electrochemical reduction, was studied with SEM and AFM. The applicability of the same electrode as transducer in an amperometric biosensor for glucose assay was demonstrated. At an applied potential of −50mV, the following were determined: detection limit (*S/N* = 3) of

6×10^{-6} M glucose, electrode sensitivity of 0.15 μA μM^{-1}, and strict linearity up to concentration of 3×10^{-4} M.

Personal contribution: conceptualization, data analysis.

6. Demkiv, O.; Nogala, W.; Stasyuk, N.; Holdynski, M.; Dimcheva, N.; Danysh, T.; Asztemborska, M.; Gonchar, M., Laccase mimetics as sensing elements for amperometric assay of 5-hydroxyindoleacetic acid in urine. *Bioelectrochemistry* **2025**, *161*, DOI: https://doi.org/10.1016/j.bioelechem.2024.108839 ($IF_{2024} = 4.5$) **Q1**

Abstract: Monitoring of the levels of 5-hydroxyindole-3-acetic acid (5-HIAA) is of significant importance for diagnostics of carcinoid tumors. We propose simple catalytic electrochemical sensors for the determination of 5-HIAA in urine using laccase and its mimetics. Laccase-like nanozymes (LacNZs) were synthesized *via* a chemical reduction, and resulting PtMn and MnO2 nanoflowers (NFs) demonstrated laccase-like activity similar to the laccase from the *Trametes zonata*. In addition, these LacNZs showed enhanced stability under a wide range of pH (3.0–7.5), temperatures (4–70 °C), and ionic strengths (up to 500 mM NaCl). The developed PtMn NF/graphite electrode, similar to a laccase/graphite electrode, can detect 5-HIAA with a high sensitivity ($25\,000 \pm 12$ A·M^{-1}·m^{-2} and 1900 ± 9 A·M^{-1}·m^{-2}, respectively) and have linear ranges of 0.3 – 15 μM and 2 – 50 μM. The sensors work at low working potentials with a detection limit of 0.16 and 1.4 μM, covering the normal and pathological ranges of 5-HIAA (1–50 μM) content in urine. They have been successfully applied to 5- HIAA assay in urine samples of people with various diseases and revealed good recovery values and reproducibility. Additionally, the LacNZ-sensor has the best stability and can be used up to 20 days.

Personal contribution: conceptualization, protocol for obtaining metal deposits.

II. Annotation of publications indexed in Scopus and Web of Science databases

7. Dodevska, T., Horozova, E., Dimcheva, N., Electrocatalytic reduction of hydrogen peroxide on modified graphite electrodes: application to the development of glucose biosensors. *Anal. Bioanal. Chem.* **386** (5) (2006) 1413-1418. https://doi.org/10.1007/s00216-006-0682-0; (IF =2.591), **Q1**

Abstract: The electrocatalytic activities of a series of compact graphites modified with microquantities of platinum metals (Pd or Pt+Pd) towards the electrochemical reduction of hydrogen peroxide were characterised. Operational parameters such as the optimal working potential, the influence of temperature and the resulting electrode characteristics were examined. The benefits of using graphite modified with Pt+Pd (mixture ratio 30%:70%) as the basic transducer in a glucose biosensor with improved sensitivity were demonstrated. It was proven that, under the working conditions chosen, the selected electrode (whether bare or covered with an enzyme layer) did not respond to any glutathione, uric acid or ascorbic acid (which all normally occur in biological fluids) present.

Personal contribution: conceptualization, characterization, data analysis.

8. Guschin, DA, Castillo, J., Dimcheva, N., Schuhmann, W., Redox electrodeposition polymers: Adaptation of the redox potential of polymer-bound Os complexes for bioanalytical applications. ABC **389** (4) (2010) 1661-1673 https://doi.org/10.1007/s00216-010-3982-3 (IF_{2010} =3.841). **Q1**

Abstract: The design of polymers carrying suitable ligands for coordinating Os complexes in ligand exchange reactions against labile chloro ligands is a strategy for the synthesis of redox polymers with bound Os centers which exhibit a wide variation in their redox potential. This strategy is applied to polymers with an additional variation of the properties of the polymer backbone with respect to pHdependent solubility, monomer composition, hydrophilicity etc. A library of Os-complex-modified electrodeposition polymers was synthesized and initially tested with respect to their electron-transfer ability in combination with enzymes such as glucose oxidase, cellobiose dehydrogenase, and PQQ-dependent glucose dehydrogenase entrapped during the pH-induced deposition process. The different polymer-bound Os complexes in a library containing 50 different redox polymers allowed the statistical evaluation of the impact of an individual ligand to the overall redox potential of an Os complex. Using a simple linear regression algorithm prediction of the redox potential of Os complexes becomes feasible. Thus, a redox polymer can now be designed to optimally interact in electron-transfer reactions with a selected enzyme.

Personal contribution: experimental characterization of a biosensor with glucose oxidase-Os-polymer, data analysis.

9. Dimcheva, N., Horozova, E., Direct electrochemistry of Penicillium chrysogenum catalase adsorbed on spectroscopic graphite. *Bioelectrochemistry* **90** (2013) 1-7 DOI: https://doi.org/10.1016/j.bioelechem.2012.09.003 . (IF_{2013} = 3.870), **Q1**

Abstract: The voltammetric studies of Penicillium chrysogenum catalase (PcCAT) adsorbed on spectroscopic graphite, showed direct electron transfer (DET) between its active site and the electrode surface. Analogous tests performed with the commercially available bovine catalase revealed that mammalian enzyme is much less efficient in the DET process. Both catalases were found capable to catalyse the electrooxidation of phenol, but differed in the specifics of catalytic action. At an applied potential of 0.45 V the non-linear regression showed the kinetics of the bioelectrochemical oxidation catalysed by the PcCAT obeyed the Hill equation with a binding constant $K=0.034\pm0.002\ M^2$ (Hill's coefficient $n=2.097\pm0.083$, $R^2=0.997$), whilst the catalytic action of the bovine catalase was described by the Michaelis–Menten kinetic model with the following parameters: $V_{max,}{}^{app}=7.780\pm0.509\ \mu A$, and $K_{M,}{}^{app}=0.068\pm0.070\ mol\ L^{-1}$. The performance of the electrode reaction was affected by the electrode potential, the pH, and temperature. Based on the effect of pH and temperature on the electrode response in presence of phenol a tentative reaction pathway of its bioelectrocatalytic oxidation has been hypothesised. The possible application of these findings in biosensing phenol up to concentration 30 mM at pHs below 7 and in absence of oxidising agents (oxygen or H_2O_2) was considered.

Personal contribution: conceptualization, experimental characterization of the biosensor, data analysis.

10. Dimcheva, N., Dodevska, T., Horozova, E., Direct electrochemistry of ascorbate oxidase self-assembled on Au – modified glassy carbon: *Journal of the Electrochemical Society,* **160** (8) H414-H419, 2013 DOI: https://doi.org/10.1149/2.025308jes . (IF_{2013} = 2.859), **Q1**

Abstract: Ascorbate oxidase (AOx) was chemisorbed on glassy carbon modified with Au nanoparticles. Direct electron transfer (DET) between the copper sites of the immobilized enzyme and the electrode surface was examined with voltammetry. The dependence of the T1 site current maxima on the scan rate was found linear, suggesting a redox process controlled by surface chemistry with the anodic peak potential shifting negatively with the pH over the range from 5.6 to 7.0. The bioelectrocatalytic oxidation of the enzyme substrate L-ascorbate was observed under both aerobic and anaerobic conditions with a current response being more than 3.5 times higher in absence of oxygen than in its presence. Inhibition studies confirmed that the L-ascorbate oxidation results from the bioelectrocatalytic activity of the immobilized AOx. Amperometry carried out at pH = 7.0 and 298 K at a constant potential as low as 150mVgave the following operational parameters: a detection limit of 1.5 μM; linear dynamic range up to 4.8mMand a sensitivity of 2.56 $\mu AmM^{-1}mm^{-2}$ (4.03 $\mu AmM^{-1}mm^{-2}$ at 200 mV). Under these conditions a real sample was assayed with a recovery between 95 and 102%.The enzyme electrode preserved up to 63% of its initial activity after 8 months storage.

Personal contribution: experimental characterization of the biosensor with ascorbate oxidase, data analysis, writing the article

11. Nagaiah, T.C., Schäfer, D., Schuhmann, W., Dimcheva, N., Electrochemically deposited Pd-Pt and Pd-Au co-deposits on graphite electrodes for electrocatalytic H_2O_2 reduction. *Analytical Chemistry* **85** (2013) 7897–7903 DOI: https://doi.org/10.1021/ac401317y (IF_{2013} = 5.578), **Q1**

Abstract: Improved electrocatalytic activity and selectivity for the reduction of H_2O_2 were obtained by electrodepositing Pd−Pt and Pd−Au on spectrographic graphite from solutions containing salts of the two metals at varying ratio. The electrocatalytic activity of the resulting binary codeposits for H_2O_2 reduction was evaluated by means of the redox-competition mode of scanning electrochemical microscopy (SECM) and voltammetric methods. In a potential range from 0 to −600 mV (vs. Ag/AgCl/3 M KCl) at pH 7.0 in 0.1 M phosphate citrate buffer, the electrocatalytic activity of both Pd−Pt and Pd−Au codeposits was substantially improved as compared with the identically deposited single metals suggesting an electrocatalytic synergy of the codeposits.

Pd−Pt and Pd−Au codeposits were characterized by X-ray diffraction (XRD) analysis and scanning electron microscopy (SEM). Codepositing with Au caused a change of hedgehog-like shaped Pd nanoparticles into cauliflower-like nanoparticles with the particle size decreasing with increasing Au concentration. Codepositing Pd with Pt caused the formation of oblong structures with the size initially increasing with increasing Pt content. However, the particle size decreases with further increase in Pt concentration. The improved electrocatalytic capability for H_2O_2 reduction of the Pd−Pt electrodeposits on graphite was further demonstrated by immobilizing glucose oxidase as a basis for the development of an interference-free amperometric glucose biosensor.

Personal contribution: conceptualization, experimental characterization of electrocatalysts using a rotating disk electrode, data analysis, article writing.

12. Teneva, O., Dimcheva, N., Electrochemical assay of the antioxidant ascorbyl palmitate in mixed medium. (2016) *Food Chemistry*, **203**, 35-40, doi: https://doi.org/0.1016/j.foodchem.2016.02.008 (IF_{2016} = 4.83) **Q1**

Abstract: Electrooxidation of ascorbyl palmitate (AP) over gold screen-printed electrode (AuSPE) and gold nanoparticles modified graphite (AuNPs/gr) was examined in mixed water-alcohol medium. Voltammetric and amperometric studies showed that: (i) AP oxidation on the AuSPE proceeds at higher potential than on AuNPs/gr; (ii) the current density on AuNPs/gr was 2.4 times higher than on AuSPE; (iii) the linear dynamic range for AuNPs/gr doubled that for AuSPE. At the optimal for AuNPs/gr operating potential (250 mV) the following operational parameters were determined: sensitivity 1.627 ± 0.138 1,627 ± 0,138 μAmM^{-1} mm^{-2}; linearity up to 500 μM; LOD = μ5.8 M. Quantification of the AP content in a real sample – stabilised flaxseed oil, was performed.

Personal contribution: conceptualization, optimization of the gold electrodeposition procedure, supervision, data analysis, article editing.

13. Dimcheva, N., Nanostructures of noble metals as functional materials in (protein -based) biosensors. *Current Opinion in Electrochemistry*, 19 (SI Bioelectrochemistry) (pp. 35–41) 2020. DOI: https://doi.org/10.1016/j.coelec.2019.09.008 (IF_{2020} = 6.68) **Q1**

Abstract: Recent advancement in nanoscience and nanotechnologies inspired a wide spectrum of uses of nanodimensional materials ranging from industrial sector to biomedical applications. Inorganic nanomaterials made of noble metals, which are corrosion-resistant, are often included as electrode modifiers in designing electrochemical chemosensors and biosensors because of their unique catalytic, electric, and surface-related properties. This review summarizes the developments in electrochemical biosensors with integrated in their architecture metal nanostructures reported mainly during the last two years with a summary on some of the commonly used methods for the synthesis of metallic nanostructures. Nanodimensional noble metal structures might be considered as multipurpose electrode modifiers because of their abilities to act at the same time as electrocatalysts, signal amplifiers, and tools for immobilization and spatial orientation of redox proteins/enzymes or other type of bioreceptors.

Personal contribution: concept, literature analysis, writing, and editing. The review is by invitation.

14. Pimpilova, M., Kamarska, K., Dimcheva, N., Biosensing dopamine and L-epinephrine with laccase (*Trametes pubescens*) immobilized on a gold modified electrode. *Biosensors-Basel* 2022, 12,719. DOI: https://doi.org/10.3390/bios12090719 (IF_{2022} = 5.7), **Q1**

Abstract: Engineering electrode surfaces through the electrodeposition of gold may provide a range of advantages in the context of biosensor development, such as greatly enhanced surface area, improved conductivity and versatile functionalization. In this work we report on the development of an electrochemical biosensor for the laccase-catalyzed assay of two catecholamines—dopamine and L-epinephrine. Variety of electrochemical techniques—cyclic voltammetry, differential pulse voltammetry, electrochemical impedance spectroscopy and constant potential amperometry have been used in its characterization. It has been demonstrated that the laccase electrode is capable of sensing dopamine using two distinct techniques—differential pulse voltammetry and constant potential amperometry, the latter being suitable for the assay of L-epinephrine as well. The biosensor response to both catecholamines, examined by constant potential chronoamperometry over the potential range from 0.2 to – 0.1 V (vs. Ag|AgCl, sat KCl) showed the highest electrode sensitivity at 0 and – 0.1 V. The dependencies of the current density on either catecholamine's concentration was found to follow the

Michaelis—Menten kinetics with apparent constants $K_M^{app} = 0.116 \pm 0.015$ mM for dopamine and $K_M^{app} = 0.245 \pm 0.031$ mM for L-epinephrine and linear dynamic ranges spanning up to 0.10 mM and 0.20 mM, respectively. Calculated limits of detection for both analytes were found to be within the sub-micromolar concentration range. The biosensor applicability to the assay of dopamine concentration in a pharmaceutical product was demonstrated (with recovery rates between 99% and 106%, n = 3).

Personal contribution: conceptualization, optimization of the gold electrodeposition procedure, supervision, data analysis, article editing.

15. Shleev S, Cristea C, Dimcheva N., Electrochemical (Bio-) Sensors in Biological Applications—2nd Edition. *Biosensors.* 2025; 15(11):746. https://doi.org/10.3390/bios15110746 (IF $_{2024}$ = 5.6), **Q1**

Abstract: The Special Issue "Electrochemical (Bio-)Sensors in Biological Applications—2nd Edition" brings together nine diverse contributions that showcase both cutting-edge research and comprehensive reviews in the field of (bio-)sensor technology. The Special Issue covers advancements in both electrochemical sensors and electrochemical biosensors, highlighting their applications in various biological contexts.

Role – guest editor of the special issue.

16. Stoica, L., Dimcheva, N., Ackermann, Y., Karnicka, K., Guschin, D.A., Kulesza, P.J., Rogalski, J., Haltrich, D., Ludwig, R., Gorton, L., Schuhmann, W., Membrane-less biofuel cell based on cellobiose dehydrogenase (anode)/laccase (cathode) wired via specific Os-redox polymers *Fuel Cells* **9** (2009) 53-62. https://doi.org/10.1002/fuce.200800033 (IF$_{2009}$ 2.557), **Q2**

Abstract: A membrane-free biofuel cell (BFC) is reported based on enzymes wired to graphite electrodes by means of Os-complex modified redox polymers. For the anode cellobiose dehydrogenase (CDH) is used as a biocatalyst whereas for the cathode a laccase was applied. This laccase is a highpotential laccase and hence able to reduce O_2 to H_2O at a formal potential higher than +500 mV *versus* Ag/AgCl. In order to establish efficient electrochemical contact between the enzymes and graphite electrodes electrodeposition polymers containing Os-complex with specifically designed monomer compositions and formal potentials of the coordinatively bound Os-complex were synthesised and used to wire the enzymes to the electrodes. The newly designed CDH/Os-redox polymer anode was characterised at different pH values and optimised with respect to the nature of the polymer and the enzyme-to-polymer ratio. The resulting BFC was evaluated running on *b*-lactose as a fuel and air/O_2 as an oxidising agent. The power output, the maximum current density and the electromotor force (*E*emf) were found to be affected by the pH value, resulting in a maximum power output of 1.9 $\mu W\ cm^{-2}$ reached at pH 4.3, a maximum current density of about 13 $\mu A\ cm^{-2}$ at pH 3.5, and the highest E_{emf} approaching 600 mV at pH 4.0.

Personal contribution: development and characterization of the anode, characterization of the biofuel cell during prolonged operation, data analysis and visualization.

17. Dodevska, T., Hadzhiev, D., Dimcheva, N., Highly Selective and Stable Electrochemical Sensor for Hydrogen Peroxide—Application in Cosmetics Quality Control. *Chemosensors* **13**, (2025), no. 11: 376. https://doi.org/10.3390/chemosensors13110376 (IF $_{2024}$ = 3.7), **Q2**

Abstract: Nowadays, electrochemical sensors have become a popular topic in cosmetics quality control. A simple and stable electrochemical sensor for hydrogen peroxide (H_2O_2) was developed on the basis of a rhodium-modified glassy carbon electrode (Rh/GCE). A quick, one-step, reproducible, and cost-effective electrodeposition procedure was applied to modify GCE with Rh nanoparticles. The sensor shows a high selectivity for H_2O_2 at a low applied potential of −0.1 V (vs. Ag/AgCl, 3 M KCl), with an excellent stability and good repeatability (RSD = 3.2%; n = 5). The modified electrode Rh/GCE demonstrates a high sensitivity of 172.24 ± 1.95 $\mu A\ mM^{-1}\ cm^{-2}$ (n = 3), a linear response to H_2O_2 between 5 and 1000 μM, and a detection limit estimated to be 1.2 μM. Furthermore, Rh/GCE has been successfully used to measure H_2O_2 concentrations in hair dye and antiseptic solution, yielding satisfactory recovery rates. These findings highlight the potential of the Rh/GCE for the reliable quantitative detection of H_2O_2 in complex cosmetics matrices.

Personal contribution: analysis of SEM studies, determination of the electrochemically active surface area.

18. Horozova, E., Dodevska, T., Dimcheva, N., Modified graphites: Application to the development of enzyme-based amperometric biosensors. *Bioelectrochemistry* **74** (2009) 260 - 264. https://doi.org/10.1016/j.bioelechem.2008.09.003; (IF_{2009} =2.652), **Q2**

Abstract: For a series of graphite electrodes, modified with microquantities of Pd+Pt mixture in varied proportions, surface morphology of the catalytically active phase was studied with scanning electron microscopy (SEM), while the catalytic activity was examined at electrochemical reduction of hydrogen peroxide by means of steady-state polarization curves and constant potential amperometry. It was proven that the graphite, exhibiting the highest electrocatalytic activity (modified with Pd+Pt mixed in the ratio 70%:30% at $t_{deposit}$=10 s) is distinguished with the smallest average size of the microformations. The operational characteristics of the same electrode and graphite modified with microquantities of Pd+Au mixed in the same ratio (70%:30%; $t_{deposit}$ =10 s) were compared. The application of these electrodes as basic transducers in highly selective biosensors for glucose and xanthine was demonstrated.

Personal contribution: obtaining electrodeposits on graphite, development and characterization of biosensors.

19. Pimpilova, M., Ivanova-Kolcheva, V., Stoyanova, M., Dimcheva, N., 2D Nanomaterial-Based Electrocatalyst for Water Soluble Hydroperoxide Reduction. *Catalysts* **12** (8) (2022), 807, DOI: https://doi.org/10.3390/catal12080807 (IF_{2022} = 3.9), **Q2**

Abstract: Hydroperoxides generated on lipid peroxidation are highly reactive compounds, tend to form free radicals, and their elevated levels indicate the deterioration of lipid samples. A good alternative to the classical methods for hydroperoxide monitoring are the electroanalytical methods (e.g., a catalytic electrode for their redox-transformation). For this purpose, a series of metal oxides—doped graphitic carbon nitride 2D nanomaterials—have been examined under mild conditions (pH = 7, room temperature) as catalysts for the electrochemical reduction of two water-soluble hydroperoxides: hydrogen peroxide and tert-butyl hydroperoxide. Composition of the electrode modifying phase has been optimized with respect to the catalyst load and binding polymer concentration. The resulting catalytic electrode has been characterized by impedance studies, cyclic voltammetry and chronoamperometry.

Electrocatalytic effect of the Co-g-C_3N_4/Nafion modified electrode on the electrochemical reduction of both hydroperoxides has been proved by comparative studies. An optimal range of operating potentials from -0.215 V to -0.415 V (vs. RHE) was selected with the highest sensitivity achieved at -0.415 V (vs. RHE). At this operating potential, a linear dynamic range from 0.4 to 14 mM has been established by means of constant-potential chronoamperometry with a sensitivity, which is two orders of magnitude higher than that obtained with polymer-covered electrode.

Personal contribution: conceptualization, optimization of the electrode modification procedure, scientific guidance, data analysis, article editing.

20. Rabadzhiyska, S.; Dechev, D.; Ivanov, N.; Shipochka, M.; Atanasova, G.; Strijkova, V.; Katrova, V.; Dimcheva, N., Mechanical, Tribological, and Corrosion Behavior of Magnetron-Sputtered VN Coatings Deposited at Different Substrate Temperatures. *Metals*, ***15*** (2025), 955. DOI: https://doi.org/10.3390/met15090955 (IF$_{2024}$ = 2.5) **Q2**

Abstract: Vanadium nitride (VN) ceramic layers were deposited on 304L stainless steel specimens by direct current (DC) magnetron sputtering in an Ar/N_2 gas mixture at substrate temperatures of 250 °C, 300 °C, and 350 °C. The obtained films were evaluated by X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM). The results showed the existence of VN and V2N phases in the as-deposited coatings. It was found that the surface roughness parameter (Ra = 10 nm) decreased with increasing substrate temperatures up to 350 °C. The highest hardness (10.6 GPa) was achieved in the layer produced at 300 °C. The low values of plastic and elastic deformation, as well as a low friction coefficient (0.38), led to an enhancement in the coatings' tribological properties. The film's thickness increased with increasing temperature due to the presence of nucleation centers in the films. The highest thickness (557 nm) was achieved in the layer deposited at 350 °C. The electrochemical tests exhibited reliable protection against corrosion in strongly aggressive electrolytes. It has been proven that the temperature significantly affects the ceramic coatings' structural, morphological, tribological, and corrosion properties.

Personal contribution: corrosion research, data analysis, and description.

21. Rabadzhiyska, S., Kotlarski, G., Shipochka, M., Rafailov, P., Ormanova, M., Strijkova, V., Dimcheva, N., Valkov, S., Duplex Surface Modification of 304-L SS Substrates by an Electron-Beam Treatment and Subsequent Deposition of Diamond-like Carbon Coatings. *Coatings* **12**(3) (2022), 401, DOI: https://doi.org/10.3390/coatings12030401 (IF$_{2022}$ = 3.4) **Q2**

Abstract: In this study, we present the results of the effect of duplex surface modification of 304-L stainless steel substrates by an electron-beam treatment (EBT) and subsequent deposition of diamond-like carbon coatings on the surface roughness and corrosion behavior. During the EBT process, the beam power was varied from 1000 to 1500 W. The successful deposition of the DLC coatings was confirmed by FTIR and Raman spectroscopy experiments. The results showed a presence of C–O, C=N, graphite-like sp^2, and mixed sp^2-sp^3 C–C bond vibrations. The surface topography was studied by atomic force microscopy. The rise in the beam power leads to a decrease in the surface roughness of the deposited DLC coatings. The studies on the corrosion resistance of the samples have been performed using three electrochemical techniques: open circuit potential (OCP), cyclic voltammetry (polarization measurements), and non-destructive electrochemical impedance spectroscopy (EIS). The measured corrosion potentials suggest that these samples are corrosion-resistant even in a medium, containing corrosive agents such as chloride ions. It can be concluded that the most corrosion-resistant specimen is DLC coating deposited on electron-beam-treated 304-L SS substrate by a beam power of 1500 W.

Personal contribution: corrosion research, data analysis, and description.

22. Rabadzhiyska, S., Dechev, D., Ivanov, N., Ivanova, T., Strijkova, V., Katrova, V., Rupetsov, V., Dimcheva, N.; Valkov, S., Wear and Corrosion Resistance of ZrN Coatings Deposited on Ti_6Al_4V Alloy for Biomedical Applications. *Coatings,* **14** (11) (2024) 1434; DOI: https://doi.org/10.3390/coatings14111434 (IF $_{2024}$ = 3.0) **Q2**

Abstract: Zirconium nitrides films were synthesized on Ti6Al4V substrates at a bias voltage of −50 V, −80 V, −110 V and −150 V by the direct current (DC) reactive magnetron sputtering technique. The asdeposited coatings were characterized by X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and atomic force microscopy (AFM). The wear and corrosion resistance of the obtained ZrN coatings were evaluated to determine the possibility for their implementation in modern biomedical applications. It was found that the intensity of the diffraction peak of the Zr-N phase corresponding to the (1 1 1) crystallographic plane rose as the bias voltage increased, while the ZrN coatings' thickness reduced from 1.21 μm to 250 nm. The ZrN films' surface roughness rose up to 75 nm at −150 V. Wear tests showed an increase in the wear rate and wear intensity as the bias voltage increased. Corrosion studies of the ZrN coatings were carried out by three electrochemical methods: open circuit potential (OCP), cyclic voltammetry (polarization measurements) and electrochemical impedance spectroscopy (EIS). All electrochemical measurements confirmed that the highest protection to corrosion is the ZrN coating, which was deposited on the Ti_6Al_4V substrate at a bias voltage of −150 V.

Personal contribution: corrosion research, data analysis, and description.

23. Ivanova, V., Pimpilova, M., Stoyanova, M., Dimcheva, N., Electrochemical Method for the Assayof Organic Peroxides Directly inAcetonitrile. *Molecules* **30**, (2025), 374. https://doi.org/10.3390/molecules30020374; (IF $_{2024}$ = 4.6) **Q2**

Abstract: Lipid peroxidation is a major process that determines the quality of various oil samples during their use and storage, in which the primary products are hydroperoxides (HP'S). HP'S are very stable compounds at ambient conditions and are harmful to human health. Therefore, the evaluation of the degree of oil oxidation is an excellent tool for ensuring food safety. The peroxide value (PV) is the main parameter used for quality control in oils. Herein, we propose an alternative electrochemical method to the classical iodometric titration method most widely used for determining the PV. Our approach is based on the electrochemical quantification of hydroperoxides/peroxides in an organic solvent medium (acetonitrile and organic ammonium salt) using a composite electrocatalyst–glassy carbon electrode modified with 2D-nanomaterial graphitic carbon nitride doped with Co_3O_4. Calibration was made by the method of standard addition using benzoyl peroxide (BPO) as a model peroxide compound, dissolved in chloroform and added to fresh Rivana-branded anti-cellulite oil, used as a model oil sample. Calibration plots showed a linear response and the very good reproducibility of the analytical result ($R^2 > 0.99$). Further, in terms of accuracy, the method showed good results, since the BPO quantitative analysis was close to the theoretical response. In addition, the accuracy of the electrochemical method was compared with that of the standard iodometric titration method for determining the PV of vegetable fats (according to a standard method). Finally, using the electrochemical method, the concentration of peroxides was determined in a real sample—an anti-cellulite oil of the trademark Rivana with an expired shelf life.

Personal contribution: conceptualization, supervision, data analysis, article editing

24. Ivanov, Y., Marinov, I., Gabrovska, K., Dimcheva, N., Godjevargova. T., Amperometric biosensor based on a site-specific immobilization of acetylcholinesterase via affinity bonds on a nanostructured polymer membrane with integrated multiwall carbon nanotubes. *Journal of Molecular Catalysis B- Enzymatic* **63** (2010) 141-148. https://doi.org/10.1016/j.molcatb.2010.01.005 ($IF_{2010} = 2.330$) **Q2**

Abstract: Acetylcholinesterase (AChE) was immobilized on chemically modified poly-(acrylonitrile-methylmethacrylate- sodium vinylsulfonate) membranes in accordance with three different methods, the first of which involved random enzyme immobilization via glutaraldehyde, the second one—site-specific enzyme immobilization via glutaraldehyde and Concanavalin A (Con A) and the third method—modified site-specific enzyme immobilization via glutaraldehyde in the presence of a mixture of multiwall carbon nanotubes and albumin (MWCNs+ BSA), glutaraldehyde and Con A. Preliminary tests for the activity of immobilized AChE were carried out using these three methods. The third method was selected as the most efficient one for the immobilization of AChE and the prepared enzyme carriers were used for the construction of amperometric biosensors for the detection of acetylthiocholine (ATCh). A five level three factorial central composite design was chosen to determine the optimal conditions for the enzyme immobilization with three critical variables: concentration of enzyme, Concanavalin A and MWCNs. The design illustrated that the optimum values of the factors influencing the amperometric current were : C_E =70 UmL^{-1}; $C_{Con\ A}$: 1.5 $mgmL^{-1}$ and C_{MWCN}: 11$mgmL^{-1}$, with an amperometric current 0.418 μA. The basic amperometric characteristics of the constructed biosensor were investigated. A calibration plot was obtained for a series of ATCh concentrations ranging from 5 to 400 μM. A linear interval was detected along the calibration curve from 5 to 200 μM. The correlation coefficient for this concentration range was 0.995. The biosensor sensitivity was calculated to be $0.065 \mu A \mu M^{-1}\ cm^{-2}$. The detection limit with regard to ATCh was calculated to be 0.34 μM. The potential application of the biosensor for detection and quantification of organophosphate pesticides was investigated as well. It was tested against sample solutions of Paraoxon. The biosensor detection limit was determined to be $1.39 \times 10^{-12}\ gL^{-1}$ of Paraoxon, as well as the interval (10^{-11} to $10^{-8}\ gL^{-1}$) within which the biosensor response was linearly dependant on the Paraoxon concentration. Finally, the storage stability of the enzyme carrier was traced for a period of 120 days. After 30-day storage the sensor retained 76% of its initial current response, after 60 days—68% and after 120 days—61%.

Personal contribution: experimental characterization of the sensor in the presence of an enzyme substrate – acetylthiocholine chloride.

25. Shukri, M., Cherneva, T., Peshkov, A., Nikolova, M., Iliev, I., Dimcheva, N., Comparison of Two Laccase Enzymes from *Trametes versicolor* and *Trametes pubescens* for the Assessment of Phenolic Acids Content Using Laccase-Based Biosensor. *Applied Food Biotechnology*, **12**(1), (2025) 1-11. https://doi.org/10.22037/afb.v12i1.46955 ; ($IF_{2024} = 1.3$) **Q3**

Abstract: **Background and Objective:** Although biochemically similar, two laccase enzymes isolated from basidiomycetes (*Trametes* sp.) showed differences in their affinity to two types of phenolic compounds, interacting stronger with diphenols (catechol and caffeic acid), compared to interactions with benzenetriols (pyrogallol and gallic acid). Catalytic efficiency of *Trametes pubescens* laccase was detected 4-5 times higher than determined for commercial laccase (*Trametes versicolor*). In this study, the interactions of the two immobilized enzymes with di and triphenols were examined by various electrochemical techniques.
Material and Methods: Following electrochemical techniques: cyclic voltammetry, chronoamperometry and differential pulse voltammetry were used in this study. Experiments were carried out in varying substrate concentrations. Activity and sensitivity of the two alternative laccase – based biosensors were compared using DPV and chronoamperometry.
Results and Conclusion: Constant potential amperometric measurements indicated that the biosensor produced with *Trametes pubescens* laccase was much more active than biosensor based on laccase from *Trametes versicolor* when interacting with caffeic and gallic acids. The phenolic content of three different herbal extracts was evaluated with the developed laccase biosensors and results were found to be similar to those from chromatographic analysis used as a reference method. Therefore, biosensors can be used for rapid testing of phenolic content in real samples.

Personal contribution: concept, optimization of the electrode modification procedure, scientific guidance, data analysis, writing and editing of the article.

26. Velichkova, Y., Ivanov, Y., Marinov, I., Ramesh, R., Kamini, N.R., Dimcheva, N., Horozova, E., Godjevargova, T., Amperometric electrode for determination of urea using electrodeposited rhodium and immobilized urease. *Journal of Molecular Catalysis B: Enzymatic* **69** (2011) 168–175 https://doi.org/10.1016/j.molcatb.2011.01.015 ; (IF$_{2011}$ = 1.8) **Q3**

Abstract: An amperometric biosensor was developed for determination of urea using electrodeposited rhodium on a polymer membrane and immobilized urease. The urease catalyzes the hydrolysis of urea to NH^{4+} and HCO^{3-} ions and the liberated ammonia is catalytically and electrochemically oxidized by rhodium present in the rhodinized membrane on the Pt working electrode. Three types of rhodinized polymer membranes were prepared by varying the number of electrodeposition cycles: membrane 1 with 10 deposition cycles, membrane 2 with 40 cycles and membrane 3 with 60 cycles. The morphologies of the rhodinized membranes were investigated by scanning electron microscopy and the results showed that the deposition of rhodium was like flowers with cornices-like centers. The influence of the amount of electrodeposited rhodium over the electrode sensitivity to different concentrations of ammonia was examined initially based on the cyclic voltammetric curves using the three rhodium modified electrodes. The obtained results convincingly show that electrode with rhodinized membrane 1, which contain the lowest amount of electrodeposited rhodium is the most active and sensitive regarding ammonia. It was found that the anodic oxidation peak of ammonia to nitrogen occurs at 0.60 V. In order to study the performance of urease amperometric sensor for the determination of urea, experiments at constant potential (0.60 V) were performed. The current–time experiments were carried out with urease rhodinized membrane 1 (10 cycles). The amperometric response increased linearly up to 1.75 mM urea. The detection limit was 0.05 mM. The urea biosensor exhibited a high sensitivity of 1.85 μAmM^{-1} cm^{-2} with a response time 15 s. The Michaelis–Menten constant K_M for the urea biosensor was calculated to be 6.5 mM, indicating that the immobilized enzyme featured a high affinity to urea. The urea sensor showed a good reproducibility and stability. Both components rhodium and urease contribute to the decreasing of the production cost of biosensor by avoiding the use of a second enzyme.

Personal contribution: optimization of the procedure for electrochemical deposition of rhodium.

27. Peshkov, A., Shukri, M., Pimpilova, M., Iliev, I., Dimcheva, N., Electrochemical approach for monitoring the catalytic action of immobilized catalase. *Open Chemistry*, vol. **23** (1), (2025), 20250143. https://doi.org/10.1515/chem-2025-0143 (IF $_{2023}$ = 2.1) **Q3**

Abstract: Heterogeneous enzymatic activity of fungal catalase from Penicillium chrysogenum in the hydrogen peroxide (H_2O_2) disproportionation reaction in a neutral aqueous buffer solution has been examined by means of constant-potential chronoamperometry. A recently developed catalytic peroxide electrode has been used as both an electrochemical detector and a high-speed low-noise stirrer. The determination of immobilized enzyme activity has been performed in an electrochemical system containing a sample of immobilized fungal catalase (from P. chrysogenum) and a rotating catalytic electrode sensitive to H_2O_2 concentration placed at a close distance to the sample. Heterogeneous catalytic activities of fungal catalase for H_2O_2 decomposition have also been assayed in the presence of 0.5 and 1.0% ethanol andmethanol. Alterations of catalase catalytic action have been found in the presence of alcohols: while in the absence of alcohols its kinetics obeys the Michaelis–Menten mechanism, in the presence of alcohols it is described by Hill's model. The apparent kinetic constants of the enzyme-catalyzed process have been proven to differ depending on the type of alcohol and its amount in the medium. The obtained results imply that the use of the electrocatalytic approach can be considered a promising alternative for kinetic characterization of immobilized enzymes, provided that the enzyme substrate is electrochemically active.

Personal contribution: concept, optimization of the procedure for determining enzyme activity, scientific guidance, data analysis, article editing.

28. Horozova, E., Dimcheva, N., Miteva, M., Jordanova, Z., Rhodium deposits on graphite: The impact of the graphite pad on the electrocatalysic activity in the electrooxidation of formic acid. *Bulgarian Chemical Communications* **40** (2), (2008) 129 – 136. (IF_{2008} = 0.152) **Q4**

Abstract: The impact of the graphite pad on the electrocatalytic activity of rhodium microdeposits has been studied using the electrooxidation of formic acid as a model reaction. It has been established that rhodium-modified electrodes show different electrocatalytic activity depending on the type of carbonaceous support. Particular attention has been paid to the electrocatalytic activity of both rhodium underpotential (*upd*) and overpotential deposits, obtained at $E_r^{equil} < E_r^{ads}$ and $E_r^{equil} > E_r^{ads}$, respectively. Several times higher electrocatalytic activity during the oxidation of formic acid, compared to the same process on graphite, modified with thick rhodium electrodeposits (ED), was observed when Rh was deposited as adatoms. The electron microscopy of rhodanized graphite electrodes indicates that the graphite pad role is confined to the formation of rhodium depositions of different structure.

Personal contribution: data analysis and description.

29. Horozova, E., Dodevska, T., Dimcheva, N., Modified graphites as catalysts for electroreduction of hydrogen peroxide. *Bulgarian Chemical Communications* **40** (3), (2008) 233-239. (IF_{2008} = 0.152) **Q4**

Abstract: The adsorption behaviour and electrocatalytic activity of compact graphite electrodes, modified only with microquantities of palladium or with a mixture of (Pd+Au), have been studied in the reaction of electroreduction of hydrogen peroxide. The influence of the following factors has been investigated: composition of the promoter, pH and temperature of the working ambience on the electrocatalytic activity of the series of modified graphites at potentials of 0 and –50 mV (vs. Ag/AgCl). The electrode modified with microquantities of (Pd+Au) mixed in a proportion of 70:30% exhibits optimal operational characteristics (electrode sensitivity, linear concentration area and low background currents).

Personal contribution: obtaining the modified electrodes, characterizing the electrocatalysts during electroreduction of H_2O_2, data analysis, and description.

30. Dimcheva, N., Horozova, E., Dodevska, T., Direct electrochemistry of myoglobin immobilized on non-modified and modified graphite. *Bulgarian Chemical Communications* **43** (1), (2011), 17-22 ($IF_{2011} = 0.283$), **Q4**

Abstract: Immobilization of redox protein myoglobin (Mb) through adsorption was optimized so that a direct redox transformation of the protein active site onto the surface of spectroscopic graphite was observed. The phenomenon of the direct electron transfer (DET) between the myoglobin redox centre and the electrode surface, detected both on nonmodified and on graphite, patterned with gold nano-deposits, was studied using a wide range of electrochemical methods such as linear and cyclic voltammetry, square-wave voltammetry, and differential pulse voltammetry. It was shown that the immobilized Mb exhibits a high catalytic activity at the electroreduction of H_2O_2.

Personal contribution: obtaining the modified with myoglobin electrodes, characterizing the electrocatalysts during electroreduction of H_2O_2, data analysis, and description.

31. Dodevska, T., Horozova, E., Dimcheva, N., Electrochemical and structural characteristics of carbonaceous electrodes modified with micro- and nanodeposits of platinum metals. *Bulgarian Chemical Communications* ***45 A*** (2013), 171-178. ($IF_{2013} = 0.320$) **Q4**

Abstract: For a series of graphite and glassy carbon electrodes, modified with micro- and nanodeposits of (Pd+Pt) and (Pd+Au) mixtures in varied proportions of the catalytically active components, surface topography of the modified electrodes has been studied with atomic force microscopy (AFM). It has been established that, catalytic activity of the modified electrodes in the reduction of H_2O_2 depends on the specific nature and structure of the metal phase, electrochemicaly deposited on the carbonaceous carrier. Moreover, the specifics of the structure, morphology of the metal deposits as well as their surface coverage has been shown to dramatically depend on the type of catalytically active components (nature and ratio of the two metals in the modifying mixture) and the nature of the carbonaceous matrix (type, porosity and surface condition).

Personal contribution: obtaining the modified electrodes, characterizing the electrocatalysts during electroreduction of H_2O_2, data analysis, and description.

32. Dodevska, T., Dimcheva, N., Horozova, E., Lazarova, Y., Electrochemically modified with osmium graphite: catalytic activity and application to the amperometric detection of hydrogen peroxide. *Bulgarian Chemical Communications* ***49*** *G* (2017) 177-182. ($IF_{2017} = 0.242$), **Q4**

Abstract: A new electrochemical method for deposition of Os on carbon carrier is reported. The metal phase was electrodeposited onto graphite under potentiodynamic conditions, using cyclic voltammetry over the potential range from -0.8 to 0 V (vs. Ag/AgCl, 3 M KCl). In order to obtain modified electrode with high catalytic activity in the reduction of hydrogen peroxide, the number of cycles and the scan rate of the electrodeposition process were optimized. It was found that the graphite modified with Os for 10 cycles with a scan rate of 0.01 V s^{-1} possesses the highest catalytic activity with respect to the target reaction. The electrochemical behavior of the so produced electrode-catalyst was examined at different pHs at potentials of 0 V and -0.05 V. The modified electrode shows good analytical performance at hydrogen peroxide determination in neutral medium: a wide dynamic range (up to 5 mM) and a sensitivity of 426 $\mu A\ mM^{-1}\ cm^{-2}$ at an applied potential of -0.05 V (sensitivity of 364 $\mu A\ mM^{-1}\ cm^{-2}$ at potential of 0 V, respectively), which makes it a promising transducing material for the development of electrochemical peroxide sensors and, eventually, biosensors.

Personal contribution: testing different procedures for electrochemical deposition of Os.

33. Chorbadzhiyska, E., Mitov, M., Hristov, G., Dimcheva, N., Nalbandian, L., Evdou, A., Hubenova, Y., Pd-Au Electrocatalysts for Hydrogen Evolution Reaction at Neutral pH. *International Journal of Electrochemistry* 04/2014 (2014)239270; DOI: http://dx.doi.org/10.1155/2014/239270 (WoS)

Abstract: Pd-Au codeposits with different ratio of both metals were electrodeposited on carbon felt, characterized by scanning electron microscopy, and investigated as electrocatalysts towards hydrogen evolution reaction in neutral phosphate buffer solution. The quantities of the produced hydrogen gas with different electrocatalysts, estimated from data obtained by chronoamperometry, were confirmed by mass spectrometry analysis. The highest hydrogen evolution rate was achieved with the electrocatalysts, produced from electrolyte with equal Pd and Au content.
Personal contribution: obtaining the modified electrodes, characterizing the electrocatalysts , data analysis, and description.

34. Rabadzhiyska, S., Kolaklieva, L., Chitanov, V., Cholakova, T., Kakanakov, R., Dimcheva, N., Balashev, K., Mechanical, wear and corrosion behavior of CrN/TiN multilayer coatings deposited by low temperature unbalanced magnetron sputtering for biomedical applications. *Materials Today: Proceedings* **5**(8) (2018):16012-16021; https://doi.org/10.1016/j.matpr.2018.05.046; SJR_{2018} = 0.299

Abstract: A multilayer CrN/TiN coating was deposited onto a high-speed steel substrate by unbalanced magnetron sputtering at different nitrogen flow, temperatures of 130-200 ^oC and a bias voltage of -60 V, -70 V and -80 V. The nanohardness and elastic modulus were in the range of 23-32 GPa and 245-378 GPa, respectively. The results of the scratch tests showed good adhesion of the coating to the substrate. The coefficient of friction was ranged of 0.09- 0.25. The multilayer CrN/TiN coatings demonstrated low average surface roughness of 16.2 nm. The electrochemical tests indicated high corrosion resistance of the samples. The wear rates of the multilayer CrN/TiN coatings were evaluated to be 7.04 $x10^{-5}mm^3N^{-1}m^{-1}$ and $5.44x10^{-5}mm^3N^{-1}m^{-1}$ by a scratch test and a ball on flat test, respectively.
Personal contribution: corrosion research, data analysis, and description.

35. Dimcheva, N., Horozova, E., Improved operational stability of a laccase-based electrode applicable in biofuel cells. *Bulgarian Chemical Communications*, **50**, Special Issue B, (2018), 130 – 135; SJR = 0.148

Abstract: To ensure high electrocatalytic activity and operational stability of immobilized laccase enzyme (isolated from the basidiomycetes *Trametes pubescens*), different immobilization protocols on various electrode materials (graphite, gold and glassy carbon) were studied. The physical methods of immobilization – physisorption or entrapment of the enzyme in a composite layer, did not yield bioelectrodes with long-lasting activity, whilst the immobilization approach based on covalent attachment of the enzyme to the electrode surface was found to produce a bioelectrode with extended operational stability. The basic electrode material was found to play an important role in bioelectrode's performance. The comparative studies carried out with two different electrode materials – polycrystalline gold and glassy carbon, modified with electrodeposited gold nanoparticles, with identically immobilized laccase on them, showed much higher current density for the second type of enzyme electrode. An extended operational stability of ca. 3 weeks was achieved for the laccase immobilized on gold-nanoparticles modified glassy carbon electrode. The value of the open circuit voltage (over 450 mV *vs*. Ag/AgCl, 3M KCl at pH = 4.5) of the laccase based electrode suggests that it is a good candidate for cathode when engineering biofuel cells.

Personal contribution: concept, enzyme immobilization via covalent bonding to the electrode surface (solid-phase organic synthesis) and via physical methods, data analysis, writing of the article.

36. Kamarska, K., Dimcheva, N., Electrooxidation and bioelectrooxidation of L- and D-ascorbic acids. *Bulgarian Chemical Communications*, **51**, Special issue D (2019), 149 –152; SJR = 0.148

Abstract: Electrooxidation and bioelectrooxidation of L- and D-iso ascorbic acids over gold nanoparticles-modified glassy carbon electrode was examined at pH 5.6 (optimal for enzyme activity). The glassy carbon electrode was modified through electrodeposition of gold nanoparticles under potentiodynamic conditions. The enzyme ascorbate oxidase was then chemisorbed onto their surface after electrochemical pre-treatment in 0.5 M H_2SO_4. Electrochemical oxidation of the enzyme substrates L- and D-iso ascorbic acid was investigated by cyclic voltammetry under both aerobic and anaerobic conditions and by constant potential amperometry at the same pH. The experimental results showed that there is no difference in the electrooxidation rates of both enantiomers when electrooxidised on bare gold nanoparticles-modified glassy carbon electrode; whilst the enzyme electrode showed ca. 30% higher electrocatalytic activity to D-iso ascorbic acid as deduced from cyclic voltammograms and ca. 12% higher sensitivity towards D-iso ascorbic acid than to L-ascorbic acid as estimated by constant potential amperometry.

Personal contribution: conceptualization, scientific guidance, article review and editing.

37. Rabadzhiyska, S., Kotlarski, G., Valkov, S., Ormanova, M., Strijkova, V., Dimcheva, N., Shipochka, M., Petrov, P., Study of diamond–like carbon coatings for biomedical applications produced by electron-beam physical vapor deposition. *AIP Conference Proceedings*, **2803**(1), (2023), 040014; DOI: https://doi.org/10.1063/5.0143804 ; SJR_{2022} = 0.164

Abstract: Coatings on the base of diamond-like carbon (DLC) were grown on 304 L stainless steel substrates at temperatures in range of 300°C to 500°C by electron-beam physical vapor deposition. X-ray photo electron spectroscopy (XPS) analysis was used to determine the binding energy and the phase composition of the obtained films. The surface morphology and roughness of the as-deposited samples were investigated via Atomic Force Microscopy (AFM).

The XPS measurements indicated that the films contain high quantity of C-C bonds in sp^3 state in the deposited DLC films. It was found that by increasing of the temperature from 300 to 500°C, the surface roughness of the produced films decreased from 27 nm to 15.6 nm. The friction coefficient and corrosion behavior of the DLC films deposited at different temperatures were also investigated. The results exhibited that the friction coefficient diminished with increasing of the temperatures and the lowest values of the friction coefficient were achieved in the films obtained at 400 °C and 500 °C. The studies on the corrosion resistance of three samples have been performed using 3 electrochemical techniques: open circuit potential (OCP), cyclic voltammetry (polarization measurements), and non-destructive electrochemical impedance spectroscopy (EIS). It can be concluded from the EIS studies that the most-corrosion resistant sample is DLC film produced at 500° C which was confirmed by OCP measurements. *Personal contribution: corrosion research, data analysis, and description.*

38. Peshkov, A., Angelova, S., Avesque, C., Iliev, I., Dimcheva, N., Determination of kinetic parameters of catalase of different origin immobilized on water-insoluble glucan synthesized by recombinant glycosyltransferase URE13-300. *Ecologia Balkanica*, 15 (2), (2023), 190-198, http://eb.bio.uni-plovdiv.bg ; SJR_{2023} = 0.192

Abstract: The kinetic constants determined for three catalase enzymes of different origins – fungal, bacterial, and mammal, have been compared in the process of hydrogen peroxide dispropor-tionation as a model reaction. In order to stabilize the enzyme, it has been immobilized on water-insoluble glucan whilst the studied process has been carried out in both the absence and the presence of low molecular weight aliphatic alcohols over a wide range of concentrations. In this study, purified catalases originating from the fungus *Penicilium chrysogenum*, bacteria *Micrococcus lysodeikticus*, and mammalian from the bovine liver, have been used. Their activities were determined by means of a spectrophotometric method, following the decay of the absorbance at 240 nm at a constant temperature, over the temperature range from 0 to 25°C. The catalytic processes were performed in the presence of methyl and ethyl alcohols in concentrations from 1% to 10%. For catalase immobilization, the water-insoluble glucan URE13-300 was used as a carrier when determining heterogeneous catalytic enzyme activity. The best and most stable was found to be the enzyme from *P. chrysogenum*, both immobilized and in the native state. It was found that the immobilized enzyme retains its activity over the temperature range – from 0 to 25°C. Its apparent kinetic constants were calculated to be: K_M =109.4 mM and V_{max} =14.42 μmol l^{-1} s^{-1}. Preservation of the catalytic activity above 90% of the initial one was found in the presence of ethanol and methanol at concentrations not exceeding 3%.

Personal contribution: conceptualization, scientific guidance, data analysis, article review and editing.

39. Shukri, M., Dimcheva, N., Biosensing L-DOPA with laccase-based enzyme electrodes: a comparative study. *Bulgarian Chemical Communications*, 56, (2024) 95-99, DOI: 10.34049/bcc.56.D.S1P36 ; SJR_{2024} = 0.148

Abstract: The focus of the present work is to develop and optimize an electrochemical laccase-based biosensor for the determination of 3,4-dihydroxyphenyl-alanine, also known as L-DOPA. The biosensor was assembled on a conventional glassy carbon electrode, the surface of which was covered with laccase enzyme retained under a thin Nafion™ membrane. The enzymes used for this purpose were isolated and purified from the white-rot basidiomycetes (*Trametes sp.*) *Trametes pubescens* and *Trametes versicolor*. Although biochemically similar, the two

enzymes demonstrated some differences in their affinity not only using 3,4-dihydroxyphenyl-L-alanine as enzyme substrate, but also when catecholamines such as dopamine and L-epinephrine were used. A range of electrochemical techniques were used for the study, such as cyclic voltammetry, chronoamperometry, and electrochemical impedance spectroscopy. Experiments were performed using buffers with different pH and applying various substrate concentrations. Activity and sensitivity of the two alternative laccase – based biosensors were compared by means of chronoamperometry. The biosensor produced on the basis of *Trametes pubescens* laccase, operating in citrate buffer with pH 4 proved to be more suitable than the one based on laccase purified from *Trametes versicolor* for biosensing L-DOPA.
Personal contribution: conceptualization, scientific guidance, article review and editing.

40. Peshkov, A., Iliev, I., Dimcheva, N., Laccase-Based Biosensors: Advancements and Applications in Environmental, Biochemical and Biomedical Sensing. *Ecologia Balkanica* 17 (1), (2025) 104 - 111; DOI: 10.69085/eb20251104; http://eb.bio.uni-plovdiv.bg; SJR_{2024} = 0.192

Abstract: Laccase-based biosensors represent a promising and innovative area of biotechnology that harnesses the natural enzymatic properties of laccase, a copper-containing oxidative enzyme, for detecting various aromatic compounds. These biosensors have garnered attention due to their high specificity, sensitivity, and eco-friendly nature, making them suitable for multiple applications, including environmental monitoring, biomedical diagnostics, and industrial process control. This overview provides insights into the fundamental principles, advantages, and key applications of laccase-based biosensors, along with emerging trends and challenges in this rapidly advancing field.
Personal contribution: conceptualization, supervision, editing of the article.

Book chapter:

1. V. Rangelova, D. Tsankova, N. Dimcheva: **Soft Computing Techniques in Modelling the Influence of pH and Temperature on Dopamine Biosensor** in (Vernon S. Somerset – Ed) «Intelligent and Biosensors», pp. 99-122 , Publ. INTECH, Croatia, (2010) ISBN 978 -953 – 7619 – 58 -9, 386 pages. (WoS)

Abstract: The work aims to model the influence of pH and temperature separately and simultaneously, on the dopamine biosensor response by means of soft computing. The problem to solve is to find a way of increasing the accuracy (and the rapidity) of the modelling process, under a condition of insufficient experimental data. To this end, the following soft computing techniques were compared in MATLAB environment: (1) *Cerebellar Model Articulation Controller* (CMAC) neural network, (2) *neural network with backpropagation learning algorithm* (NNBP), (3) *fuzzy logic* (FL), and (4) *adaptive network- based fuzzy inference system* (ANFIS). The relative errors over a few new experimental samples were calculated for validation of the proposed models.

Personal contribution: conceptualization of the experiment, writing theoretical part and analysis of enzyme kinetics experiments.

2. N. Dimcheva, "Nanoparticles of Noble Metals: Electrochemical Synthesis, Properties and Application" in "Nanosciences and Nanotechnologies" (Y. Dimova - compiler), Paisii Hilendarski University Press, 2017, pp. 54-64, ISBN 978-619-202-282-2.

Abstract: This article will teach you how noble metal nanoparticles can be synthesized and attached to the surfaces of various solid conductive materials using electrochemical methods, what they look like, what methods can be used to characterize them, and what they can be used for.

Patent for invention

1. Patent No. 66837 B1: "Bio-electrocatalytic method for quantitative analysis of L-ascorbic acid" with authors: Nina Dimcheva, Elena Horozova, Totka Dodevska; published in issue 02.2/28.02.2019.Official Bulletin of the Patent Office of the Republic of Bulgaria; Intellectual Property: Plovdiv University "P. Hilendarski"; N. Dimcheva and E. Horozova;

Abstract: A bio-electrocatalytic method for the quantitative analysis of L-ascorbic acid in an analyte by amperometric detection, using an enzyme electrode positioned in a standard three-electrode electrochemical cell, immersed in a background electrolyte medium and connected to an electrochemical workstation, characterized in that the enzyme electrode is obtained by modifying a standard glassy carbon working electrode by cyclic voltammetry in a 2% solution of chloroauric acid in 0.1 M hydrochloric acid, at a potential varying linearly with time at a rate of 0.1 V/s in the range from -600 mV to 0 mV and back to -600 mV versus Ag/AgCl, 3 M potassium chloride, electrochemically cleaned in 0.5 M sulfuric acid by cyclic voltammetry in a potential range from 0 to 1.7 V versus Ag/AgCl, 3 M potassium chloride, rinsed with double-distilled water, after which the modified electrode with a layer of gold nanoparticles with a thickness of up to 50 nm deposited on its surface is immobilized with the biocatalyst ascorbate oxidase by chemisorption, by immersing the electrode for 12 h at a temperature of 4°C in a solution containing 2 or 5 mg/ml ascorbate oxidase dissolved in 0.1 M phosphate buffer with pH 5.6, the unbound enzyme is removed from the surface by soaking the electrode in buffer with pH 5.6 at room temperature, and the resulting enzyme electrode, together with a reference electrode Ag/AgCl, 3M potassium chloride and an auxiliary electrode - platinum wire, are positioned in a standard three-electrode electrochemical cell without separators, with 0.1 M phosphate buffer with pH 7.0 added as a background electrolyte, and a constant working potential of 150 or 200 mV is set on the electrochemical workstation to which the electrodes are connected, a stationary background current of 0.2-0.3 μA is waited for, and the analyte is added to the electrolyte in the cell with continuous stirring in portions with an initial volume of 20 to 100 μl and an increase in each subsequent added portion with a volume equal to the initially introduced one, and after each added portion of analyte, the current value is measured by the electrochemical workstation, and the quantitative content of ascorbic acid in the studied analyte is determined using a calibration graph prepared under the same working conditions.

Instructional textbooks:

1. E. Horozova, St. Hristoskova, K. Kanchev, R. Mancheva, M. Stoyanova, N. Dimcheva and V. Delchev *"Exercises and Problems in Applied Physical Chemistry"* (Master's Course)Biocatalysis and Electrocatalysis - 9 Laboratory Exercises). Paisii Hilendarski University Press, Plovdiv, 2007, ISBN 978-954-423-385-3

Abstract: The laboratory manual for applied physical chemistry is prepared in accordance with the curricula of the core courses of the master's program "Applied Physical Chemistry" at the Faculty of Chemistry of Plovdiv University "Paisii Hilendarski". The structure of each lab exercise is as follows: Theoretical background, principle of the method, aim and objectives, procedure, processing and presentation of experimental data.

The candidate's personal contribution consists of developing 9 laboratory exercises from the sections "Biocatalysis" and "Electrocatalysis".

2. Horozova, E., Hristoskova, St., Semkova, R., Stoyanova, M., Dimcheva, N., Delchev, V., Petrov, D., Manual for Laboratory Exercises in Physical Chemistry and Colloid Chemistry (II Revised and Expanded Edition). University Publishing House "P. Hilendarski", Plovdiv, 2017, ISBN 978-619-202-242-6

Abstract: The laboratory manual for physical chemistry and colloid chemistry is the second revised edition of the same manual from 1999. It is prepared in accordance with the curricula of the basic courses in physical chemistry and colloid chemistry for students of all bachelor's specialties of the Faculty of Chemistry and for the specialty "Biology and Chemistry" of the Faculty of Biology at Plovdiv University "Paisii Hilendarski". The manual includes 48 laboratory exercises, 18 of which are new to this edition, and a large portion of the rest have been revised in both the theoretical sections and the experimental setups. The structure of each lab exercise is as follows: Theoretical background, principle of the method, aim and objectives, procedure, processing and presentation of experimental data.

The candidate's personal contribution consists of developing 4 laboratory exercises: 1) determination of the universal gas constant; 2) determination of the dissociation constant of a weak acid and buffer capacity, 3) primary salt effect and 4) determination of the Michaelis constant and maximum velocity of enzymatic hydrolysis of urea with urease.