Summary of scientific achievements

in relation to the habilitation procedure

Application of carbon nanomaterials in the construction of enzymatic biosensors and biofuel cells

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1. Personal details

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2. Scientific diplomas and degrees

2000 - MSc degree, University of Warsaw, Faculty of Chemistry, MSc Thesis: "Thiol Protected Gold Clusters", supervisor Prof. Renata Bilewicz, PhD

2006 - PhD degree, University of Warsaw, Faculty of Chemistry, PhD Thesis: "Properties of the electrodes modified with organothiol compounds and gold clusters", supervisor Prof. Renata Bilewicz, PhD

3. Employment history

2005 - 2006 - scientific and technical specialist, University of Warsaw, Faculty of Chemistry

2006 - 2010 - lecturer, University of Warsaw, Faculty of Chemistry

2010 - 2014 - Assistant Professor (Adjunct), University of Warsaw, Faculty of Chemistry

2014 - now - lecturer, University of Warsaw, Faculty of Chemistry

4. Scientific achievements

4.A Title of the scientific achievement:

a monothematic series of publications entitled:

Application of carbon nanomaterials in the construction of enzymatic biosensors and biofuel cells

4.B List of the publications constituting the scientific achievement

(IF according to the year of publication from the Journal Citation Reports (JCR), number of publications cited according to the Web of Science (WoS) database of 09.01.2019)

[H1] K. Stolarczyk, E. Nazaruk, J. Rogalski, R. Bilewicz*, "Mediatorless catalytic oxygen reduction at boron-doped diamond electrodes", Electrochemistry Communications 9 (2007) 115 - 118
IF (2007) = 4.186; number of citations: 35

[H2] K. Stolarczyk, E. Nazaruk, J. Rogalski, R. Bilewicz*, "Nanostructured carbon electrodes for laccase catalyzed oxygen reduction without added mediators", Electrochimica Acta 53 (2008) 3983 - 3990
IF (2008) = 3.078; number of citations: 46

[H3] K. Sadowska, **K. Stolarczyk**, J.F. Biernat*, K.P. Roberts, J. Rogalski, R. Bilewicz*, "Derivatization of single-walled carbon nanotubes with redox mediator for biocatalytic oxygen electrodes", Bioelectrochemisty 80 (2010) 73 - 80
IF (2010) = 3.520; number of citations: 28

[H4] K. Stolarczyk, M. Sepelowska, D. Łyp, K. Żelechowska, J.F. Biernat, J. Rogalski, K.D. Farmer, K.N. Roberts, R. Bilewicz*, "Hybrid biobattery based on arylated carbon nanotubes and laccase", Bioelectrochemistry 87 (2012) 154 - 163
IF(2012) = 3.947; number of citations: 46

[H5] K. Stolarczyk, D. Łyp, K. Żelechowska, J.F. Biernat, J. Rogalski, R. Bilewicz*, "Arylated carbon nanotubes for biobatteries and biofuel cells", Electrochimica Acta 79 (2012) 74 - 81
IF(2012) = 3.777; number of citations: 41

[H6] K. Stolarczyk, M. Kizling, D. Majdecka, K. Żelechowska, J.F. Biernat, J. Rogalski, R. Bilewicz*, "Biobatteries and biofuel cells with biphenylated carbon nanotubes", Journal of Power Sources 249 (2014) 263 - 269
IF(2014) = 6.217; number of citations: 26

[H7] M. Kizling, **K. Stolarczyk**, J. Sim Sin Kiat, P. Tammela, Z. Wang, L. Nyholm, R. Bilewicz*, "Pseudocapacitive polypyrrole–nanocellulose composite for sugar-air enzymatic fuel cells", Electrochemistry Communications 50 (2015) 55 - 59
IF(2015) = 4.569; number of citations: 20

[H8] M. Kizling, S. Dramińska, **K. Stolarczyk**, P. Tammela, Z. Wang, L. Nyholm, R. Bilewicz*, "Biosupercapacitors for powering oxygen sensing devices", Bioelectrochemistry 106 (2015) 34–40
IF(2015) =3.556; number of citations: 22

[H9] M. Kizling, **K. Stolarczyk**, P. Tammela, Z. Wang, L. Nyholm, J. Golimowski, R. Bilewicz*, "Bioelectrodes based on pseudocapacitive cellulose/polypyrrole composite improve performance of biofuel cell", Bioelectrochemistry 112 (2016) 184 - 190
IF(2016) = 3.346; number of citations: 7

[H10] B. Olszewski, **K. Stolarczyk*^**, "Laccase catalyzed reduction of oxygen at electrodes modified by carbon nanotubes with adsorbed promazine or acetosyringone", Catalysts (2018) 8 (2018) 414
IF(2017) = 3.465; number of citations: 0

* corresponding author
^ publications completed without the participation of professorial or doctoral degree holders

Total Impact Factor: **39.661**

Mean Impact Factor per publication: **3.966**

4.C Discussion of the scientific aims and objectives of the publications constituting the scientific achievement, their results and possible applications

I. Scientific aims and objectives

The aim of the research was to obtain new, multifunctional, environmentally safe electrode materials to immobilize enzymes and apply them in the construction of biofuel cells to power small measuring devices as well as systems that could be used as environmental sensors. The aim of this work was also to develop a new fuel cell that would work for a long period of time and generate adequately high power densities, where both biocatalysts on the anode and the cathode would be in a direct electrical contact with the surface of the electrode. In my research, I used different types of nanomaterials and the studies involved examining the interactions between these materials and the enzymes. I chose the materials so as to ensure a proper orientation of the adsorbed protein molecules and reduce the distance between the active center of the enzyme and the electrode surface. When modifying the electrodes, I searched for materials that are cheap, chemically passive, widely available, that enhance the active surface of the electrode by providing appropriate porosity, have high mechanical strength, good conductivity and energy storage properties. The objective was to develop the electrode material that could be useful in the construction of biobatteries and biofuel cells, so as to achieve such an increase in power and current density that would provide a long-time power supply for small devices, e.g. sensors, watches or potentiostats. In the course of my work I have designed biobatteries and biofuel cells characterized by the simplest design and lowest cost of materials as well as the longest possible biofeedback activity. The systems were designed so that they could be used for environmental research, for example for the construction of glucose, oxygen or phenol biosensors and for the removal of various

molecules through the use of three-dimensional materials that could adsorb various compounds in the large internal surface of the material.

II. Introduction

In recent years, as a result of a dramatic increase in energy consumption due to the development of the automotive and electronics industries, there has been much interest in electrochemical energy sources (including batteries and accumulators). Particularly intensive research is conducted in the field of fuel cells, and the so-called biofuel cells, in which chemical reaction energy can be converted into electrical energy, i.e. electrons released in the redox reaction can be used in the form of an electric current[1-3]. Although the first fuel cell was constructed as early as in 1839[4,5], major progress in this field has been made only in recent years. It is connected with the increasing awareness of the gradual depletion of our natural energy resources (such as oil, gas or coal), and, consequently, increased financing of related research in various countries.

Enzymatic fuel cells (EFCs) are currently considered to be one of the future alternative energy sources. However, the obtained bioenergy powers are too small to be commonly applied; also, their stability over time is too small[3,6]. Therefore, further progress in the construction of bioelectrodes needs to be made. The most important issues include:

1. improvement of bioelectrode working parameters,
2. elimination of the dangers associated with the mediator's diffusion to the solution,
3. increase in the number of enzyme molecules electrically connected to the electrode substrate (increasing the electrode volume),
4. selection of the enzyme so as to facilitate the anode process with the most negative potential, thus enabling the biggest open circuit potential (OCP) of the biofuel cell.

Fuel cells consist of two electrodes capable of converting chemical energy into electricity. Their special subtype are biofuel cells, in which chemical energy comes from the reduction and oxidation processes of renewable compounds naturally present in the environment, as well as in the living organisms[7,8]. Attempts at constructing an efficient enzyme-based biofuel cell are currently being made by many research teams, as it has numerous advantages over a classic fuel cell. The costs of obtaining enzymes have significantly diminished in recent years; therefore, when used as catalysts in the electrode reactions, enzymes can compete with conventional catalysts, e.g. noble metals such as platinum or gold which due to the depletion of natural resources are becoming more and more

expensive. Enzymes, being biologically active molecules, show activity in mild pH conditions close to the physiological ones and are characterized by high biocompatibility and specificity[7,9]. Moreover, enzymes have high selectivity for the substrates, which limits the necessity of introducing membranes that divide the cell into separate reaction zones[10,11]. All these factors make enzymatic cells potential sources of real power for such in-vivo devices as pacemakers, insulin pumps, drug dispensers and biosensors. The source of fuel for these biofuel cells can be found in metabolites (glucose, lactate) and oxygen present in bodily fluids. The development of nanotechnology also entails the need for miniaturization of the electronic devices, including micro- and nanoscale, so that they can be used in industry, medicine or natural sciences; this also applies to biofuel cells. The selection of an appropriate enzyme catalyzing the cathodic and anodic reaction constitutes an essential part of biofuel system design and is aimed at reducing the overpotential of the fuel reduction and oxidation, which is the condition of obtaining high voltage. The enzymes with the highest redox potential on the cathode and the most negative potential on the anode, which allows to obtain a high open circuit potential, are the best candidates for the biofuel cell construction. The most commonly used enzymes are: on the anode - glucose oxidase, glucose dehydrogenase, fructose dehydrogenase, cellobiose dehydrogenase; on the cathode - laccase and bilirubin oxidase. In the anodic part, glucose (fuel) is most often oxidized, and the reduction of oxygen to water is most often observed on the cathode.

III. Discussion of the publications

In my research on the construction of bioelectrodes I used enzymes that allow to obtain an effective electrical contact between the protein active center and the electrode surface, that are characterized by high activity in near physiological pH and are easily available. I used fructose dehydrogenase (FDH) and glucose oxidase (GOx) for the anode preparation, which oxidize fructose and glucose, respectively. In my work I chose glucose oxidase, because of its low glucose oxidation potential and high specific activity among available enzymes. GOx could also be potentially used in the construction of implanted biofuel cells and sensors determining the concentration of glucose (glucometers), due to the presence of glucose in physiological fluids. The second enzyme that I used on the anode was FDH. FDH has an affinity for only one substrate - fructose. In the last few years it has been intensively used in the preparation of biofuel cells, because it allows to obtain high catalytic currents without the presence of a mediator, at a relatively low overpotential (oxidation of

fructose at -0.34V vs. Ag/AgCl). I used laccase to prepare the biocathode which catalyzes the reaction of the oxygen reduction directly to water in the environment of pH 4 - 7 (depending on the origin of the enzyme). The reduction of oxygen with the use of laccase runs with a much lower overpotential, which results in the high efficiency of the reduction process.

One significant problem of using an enzyme as a catalyst is obtaining a good contact between the active center of the enzyme and the electrode surface. A direct electron transfer (DET) between the enzyme and the electrode surface is difficult, as the active center of the enzyme is located within the protein structure[12]. Such transfer is possible when the active center of the enzyme is located close to the surface of the electrode. Strong binding of the enzyme to the electrode can lead to a greater stability of the enzyme-modified electrode. It may also favor immobilization of a large amount of the enzymes in the orientation that is most favorable in relation to the electrode surface. The advantage of the enzyme modified electrodes is their working potential (close to the formal potential of the active enzyme center) and the ease of their miniaturization. One of the methods of obtaining a direct electrical contact between the enzyme and the electrode surface is using various nanostructures: such as carbon nanotubes, graphene or nanoparticles[13-15].

In my research I have applied various electrode-supporting materials for which I obtained a direct or mediated electron transfer between the enzyme and the electrode surface, e.g. boron doped diamond, carbon microparticles, carbon nanotubes, gold nanoparticles, a nanocellulose/polypyrrole composite **[H1-H10]**. Nanomaterials act as very efficient auxiliary materials to immobilize the enzyme; this is due to their features: a small size, very good charge transport or the possibility of attaching various functional groups enabling the covalent immobilization of the enzymes or mediators. Nanostructures also enable good contact of the enzyme active center with the substrate and the enzyme activity can be maintained for a long time. Nanomaterials significantly increase the surface area and capacitance of the electrode, which is why biofuel cells using these structures achieve much higher powers. In order to immobilize enzymes on solid substrates, physical or chemical methods are used: physical and chemical adsorption, crosslinking with conductive polymers, encapsulation or micelles closure, entrapment in a matrix, immobilization in a semi-permeable membrane[11,16]. By immobilizing the enzyme on the electrode, the concentration of the biocatalyst is increased on its surface and the electrode processes take place with a higher efficiency, much higher current densities are also obtained.

In my research **[H1, H2]**, I was the first one to obtain the unmediated catalytic reduction of oxygen in the presence of laccase on a boron doped diamond electrode (BDD), both for laccase dissolved in a solution and immobilized on the electrode. BDD is a relatively new electrode material that is finding its new applications[17-19]. A boron doped diamond can possibly work in a very large potential range of about 3.5V, therefore it allows to track redox processes in extreme potential ranges. The background currents are low, allowing the measurement of Faradaic processes with high sensitivity. BDD electrodes are chemically inert and therefore resistant to poisoning by reaction products that can accumulate on more reactive electrode materials. Moreover, BDD has excellent optical properties, thus it is possible to combine electrochemical measurements with UV-Vis and IR spectroscopy measurements. The use of an appropriate activation of the boron-doped diamond surface enables to control its hydrophobicity or hydrophilicity by creating appropriate functional groups that can interact with the enzymes. In my work, I used the procedure proposed by Marken[20] to activate the surface of the BDD electrode. I performed the activation of the BDD electrode through the cyclic potentials change in the positive potential range from 0V to 5V in the 1mol/dm^3 solution of nitric acid (V). The activation of the electrode leads to the formation of functional groups on the electrode surface, e.g. hydroxyl or carboxyl groups. In my research, I used a Cerrena unicolor laccase enzyme which has the ability to catalyze the four-electron reduction of oxygen directly into water. Unfortunately, on the classic electrodes, e.g. carbon ones, there is no direct electron transfer, because the T1 enzyme center is too far from the electrode surface (the oxygen reduction occurs at the potential of -0.6V against the Ag/AgCl reference electrode). However, by using properly activated boron doped diamond electrodes, I managed to obtain an unmediated catalytic reduction of oxygen in the presence of laccase dissolved in McIlvaine buffer (pH 5.2). When this type of an electrode was used, there was a significant reduction in the overpotential of the oxygen reduction by approximately 1.2V. The catalytic wave was observed at the potential of approximately +0.6V vs. Ag/AgCl (Fig. 1A). The immobilization of the enzyme on the electrode is important from the point of view of its application. The immobilization of the enzyme in the matrix layer on the electrode is common practice, as it allows to increase the concentration of the biocatalyst on its surface. As a result, electrode processes are more efficient and a much higher density of the catalytic current is obtained. In the course of my studies, in order to immobilize laccase, I used the cubic phase, a liquid crystal lipid system with a lipid bilayer structure intersected by a system of water channels. The water channels enable the incorporation of both hydrophobic and hydrophilic

catalysts. For the preparation of the cubic phase, monoolein was used, which in combination with water in a suitable ratio forms the liquid crystalline phase[21]. The cubic phases formed by monoolein are isotropic, have high viscosity and are stable in the presence of excess water. High viscosity and durability in aqueous solutions make it possible for the cubic phase to be easily applied as the matrix to immobilize enzymes on the electrodes surface. On a BDD electrode coated with the cubic phase containing laccase I observed a catalytic wave beginning at the same potential as when no cubic phase was applied, but the catalytic oxygen reduction densities were twice as large and amounted to about -0.8μA/cm^2. I did not observe the catalytic wave on the glassy carbon electrode covered with the cubic phase with laccase which I used as the comparative system (Fig. 1B). I ran the measurements on the tested BDD electrode in solutions with different oxygen concentrations, i.e. in the deoxygenated solution, in the solution in equilibrium with air and in the oxygen-saturated solution. I observed an almost linear dependence of the density of the catalytic oxygen reduction currents on the oxygen concentration in the solution, so this system can be successfully used as the oxygen sensor in a solution.

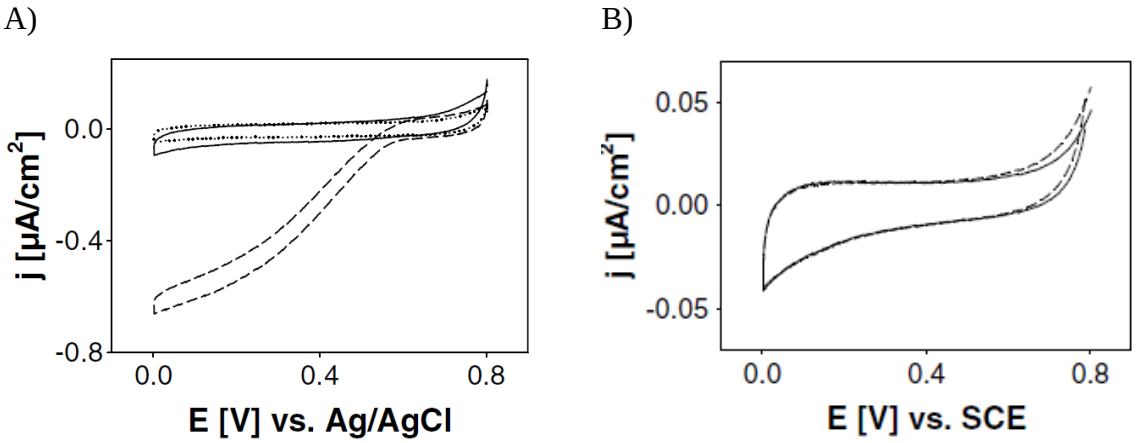


Fig. 1. Cyclic voltammetry curves recorded in (—) deoxygenated and (----) oxygenated McIlvaine buffer (pH 5.2) using electrodes A) BDD and B) GCE modified with the cubic phase containing laccase, scan rate potential: 1mV/s.

While researching for **[H2],** I developed a method of immobilizing nanomaterials: carbon nanotubes and carbon microparticles on the carbon electrode surface. I then used thus modified electrodes as surfaces for the laccase enzyme adsorption in the unmediated catalytic reduction of oxygen. Both nanomaterials and laccase were immobilized permanently on the electrode surface by means of various matrices: Nafion, chitosan, lecithin and hydrophobin. In

my research I used a glassy carbon electrode covered with carbon microparticles or carbon nanotubes in McIlvaine buffer (pH 5.2) containing dissolved laccase. The reduction of oxygen on the unmodified glassy carbon electrode proceeded with a significant overpotential at the potential of about -0.6V relative to the silver/silver chloride reference electrode. When unmodified carbon nanotubes or carbon microparticles were used to modify the glassy carbon electrode, the catalytic process of the oxygen reduction in the presence of laccase began at positive potentials of about +0.6V relative to the silver/silver chloride reference electrode (and thus at about +0.8V relative to the normal hydrogen electrode) and the oxygen reduction current densities were 10 to 40 times higher in comparison to the boron doped diamond electrode. In order to permanently immobilize carbon nanotubes and the enzyme on glassy carbon electrodes, I tested the studied matrices with the immobilized laccase enzyme (Nafion, chitosan, lecithin and hydrophobin) in hope of using them to construct systems able to catalyze the reduction of oxygen in a biofuel cell or oxygen biosensor. Having immobilized the enzyme in the matrix layer, I obtained higher densities of catalytic oxygen reduction currents, and the potentials at which the catalytic wave was initiated were similar to the results of the experiments with laccase dissolved in the solution. The largest densities of catalytic oxygen reduction currents of about -110μA/cm^2 measured at the 0.2V potential were obtained for the electrode coated with unmodified carbon nanotubes and laccase in the Nafion layer. The choice of the matrix with an immobilized enzyme meant that a smaller amount of the enzyme was needed. Also, a permanent immobilization of both the enzyme and the nanomaterial on the electrode surface was possible. The use of unmodified carbon nanotubes and microparticles ensured the optimal orientation of the enzyme molecules, which resulted in the unmediated bioelectrocatalysis.

For the majority of the enzymes from the oxidoreductase class, commonly used in the construction of biofuel cells, a direct electron transfer between the enzyme and the electrode surface is not possible. Therefore, additional substances called mediators that facilitate the electron transport between the enzyme and the electrode surface are added. This type of the electron transport with the participation of mediators is called the mediated electron transfer (MET)[11,22]. Small molecules, usually metals, metal complexes or organic molecules are used as mediators. They have the potential similar to the formal potential of the used enzyme and they undergo oxidation and reduction in the course of a reversible electrochemical process. Using mediators in the form of solutions in the tested system is sometimes disadvantageous from the point of view of the construction of biofuel cells, because the introduction of an

additional substance complicates the functioning of the biofuel cell. Oftentimes this substance can react both on the cathode and the anode, and a membrane separating the cathode and anode space must be introduced into the construction. These problems can be avoided by using mediators directly immobilized on the modified electrodes. It has also been shown that the mediators immobilized on the electrode increase the speed of the charge transfer process, help obtain higher catalytic current densities and do not require using large amounts of the catalyst. The immobilization of the mediators as well as the enzymes on the material or the electrode surface prevents them from leaking into the solution, which significantly reduces the costs of materials exploitation, but also blocks their diffusion into adjacent electrode spaces. Additionally, the immobilized enzymes show greater catalytic efficiency in comparison to the enzymes dissolved in the solution or physically adsorbed on the electrode.

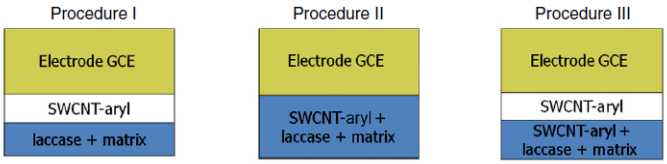
In my research, I have collaborated with Ph.D., D.Sc. Kamila Żelechowska and Prof. Jan Biernat from Gdańsk University of Technology to develop and conduct syntheses of carbon nanotubes with covalently attached aryl groups and redox compounds which I then used to modify electrodes. In my study **[H3]** I immobilized carbon nanotubes covalently modified with 2,2'-azobis- (3-ethylbenzotriazolesulfonic acid) (ABTS) on the electrode and examined the effect of various modifications of the carbon nanotubes with ABTS on the catalytic reduction of oxygen in the presence of laccase. I also compared the catalytic effects of the nanotubes modified covalently in two different ways: in the first case, the carbon nanotubes were modified with ABTS at the end of the walls, while in the second case the nanotubes were modified with the same redox compound on the side walls of the nanotubes. In addition, the electrodes coated with the ABTS modified carbon nanotubes were covered with laccase in the Nafion layer, which prevented laccase from leaking into the solution as well as blocked the detachment of the modified nanotubes from the surface of the carbon electrode. ABTS is one of the most commonly used mediators for laccase, because it is characterized by a reversible electrode process, and its potential is close to the formal potential of the T1 laccase center[23-25]. I tested the modified nanotubes in the presence of laccase in the catalytic oxygen reduction process. I showed that immobilizing ABTS on the nanotubes surface maintains its functionality, which is beneficial for the proper operation of the biocathode. One advantage of the covalent immobilization of ABTS on the nanotubes surface is that it does not diffuse into the solution and so the biocatalytic system is stable. Both types of the nanotubes modification with ABTS resulted in a significant increase in the current density value when compared to the unmodified nanotubes, as well as in decreasing

the overpotential of the oxygen reduction to 0.6V, which is beneficial from the point of view of their application in the biofuel cell construction. I showed that the mediating efficiency of ABTS modified at the ends of the nanotubes is higher, while the number of its molecules related to the surface of the nanotubes is smaller, which resulted in the lower current density of the oxygen reduction as compared to the carbon nanotubes modified on the sides. Moreover, cyclic voltammograms recorded in the presence of ABTS bound with the nanotubes showed a lower slope of the current-potential curve when compared to the unmodified nanotubes, which indicates a reduction in the limitations related to the layer resistance and the catalytic process. The largest densities of catalytic oxygen reduction currents in the presence of laccase of approximately -420μA/cm^2 were observed for single-walled carbon nanotubes modified on the sides with ABTS. The covalent immobilization of ABTS on the carbon nanotubes caused the adsorption of a bigger number of laccase molecules and their proper orientation in relation to the electrode surface, resulting in higher densities of the catalytic currents recorded in comparison to the unmodified carbon nanotubes.

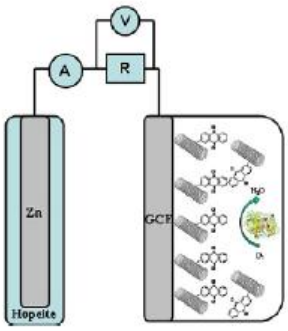
One can distinguish an active site of the enzyme in the enzyme structure where redox transformations occur. Using electrode materials properly modified with chemical compounds allows the enzyme to be properly oriented on the electrode surface. This contributes to the improvement of the electron transport between the enzyme and the electrode, resulting in higher current densities and better bioelectrode durability. The active center of laccase is a hydrophobic pocket that has a high affinity for various types of compounds. The shape of the enzyme active center is best fitted to the aromatic compounds, because it is the lignolytic enzyme. Such method of modifying graphite electrodes with anthracenyl or phenyl groups characterized by the system of conjugated double bonds was applied by the Armstrong group and a high degree of laccase molecule ordering on the electrode was achieved. As a result, a much more efficient electron transport was observed[7]. In my research done for **[H4]** I further developed Armstrong's idea by using a previously non-described carbon nanotubes covalently modified with anthracenyl or anthraquinone groups for the catalytic reduction of oxygen by laccase. I immobilized both carbon nanotubes and laccase on the glassy carbone electrode (GCE) surface and examined the influence of the carbon nanotubes, covalently modified at the ends as well as on the sides of the walls with anthracenyl or anthraquinone groups on the catalytic process of the oxygen reduction in the presence of laccase. Each type of the nanotubes modification made it possible to obtain a higher value of the catalytic oxygen reduction current density in the presence of laccase as compared to the unmodified nanotubes.

I showed that the covalent modification of the carbon nanotubes with various anthracenyl or anthraquinone groups at the end as well as on the sides of the nanotubes has a positive effect on the orientation of the enzyme molecules in relation to the electrode surface and shortens the distance of electron tunneling. Electrodes coated with the nanotubes modified on the sides with anthracenyl or anthraquinone groups and laccase showed higher catalytic currents densities of the oxygen reduction in comparison to the carbon nanotubes modified at the ends of the nanotubes. This was probably related to the increased population of aryl groups when the carbon nanotubes were modified on the sides as compared to the nanotubes modified at the ends. The work also compared three ways of modifying electrodes (Fig. 2A). The first one consisted of placing nanotubes on the electrode first and then coating them with an appropriate matrix with laccase. The second procedure consisted in placing the mixture of the nanotubes, laccase and matrix directly on the electrode. The third method consisted in applying the nanotubes directly onto the electrode as the first layer, and then coating them with the mixture of the nanotubes, laccase and matrix. Different kinds of matrices – lecithin, chitosan, unmodified Nafion, Nafion modified according to the Minteer and Lo Gorton procedure – were used in this study.

A)



B)



C)

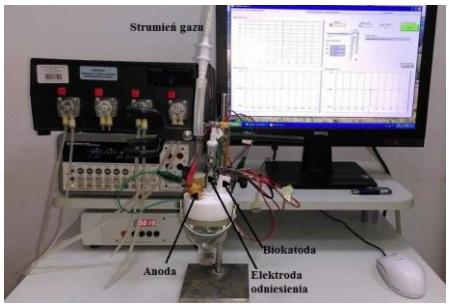


Fig. 2 A) Three ways of preparing GCE electrodes modified with the carbon nanotubes, matrix and laccase, B) Two-electrode biobattery scheme, C) Measurement set consisting of a rheostat, multimeter and computer control program.

The highest value of the current density of the oxygen reduction of about -250μA/cm^2 was obtained for the biocathodes prepared by placing carbon nanotubes modified at the ends with anthracenyl groups first, and then the mixture of the same nanotubes, laccase and Nafion prepared according to the Minteer procedure. The smallest catalytic currents were observed for the system prepared by the second electrode modification procedure. The highest currents were obtained on the electrodes modified according to the third procedure, but they were only slightly higher in comparison to other electrode modification methods; what is more, the preparation of the electrodes was very time-consuming. Therefore, from the point of view of the most economical application and taking into account preparation time and the amount of the reagents used, as well as the values of the obtained catalytic currents, the electrode prepared according to the first procedure, where the carbon nanotubes were applied directly onto the electrode, and then covered with Nafion and laccase, was chosen as the best one. Stabilities of the selected modified electrodes over time were compared. At the beginning, the catalytic current density decreased quickly, then stabilized and lasted for several days, e.g. after 12 days 40% of the initial catalytic current value was maintained. The tested electrodes were used to construct a hybrid biofuel cell called a biobattery (Fig. 2B). A biobattery is a system composed of one bioelectrode and another electrode which does not contain any biological compounds and is most often a metallic one (e.g. platinum, zinc). In my biobattery research, I used the McIlvaine buffer of pH 5.2 as the electrolyte because it has the optimal pH for laccase. In order to determine specific operational cell parameters, such as the open cicuit potential (OCP), maximum power, current and electrode resistance to discharging, resistance varying from 10MΩ to 1kΩ was applied to the biobattery, starting from high values - equivalent to the open circuit, to the very low - corresponding to real cell working conditions. For these measurements, I designed a measurement set consisting of a rheostat and a multimeter which were controlled by a special computer program (Fig. 2C). The system measured the potential difference between the anode and the cathode. Next, by applying Ohm's law, substituting resistance values and the obtained voltage, the current was calculated, and from the value of the current and voltage the power of the system was also calculated. The obtained curves of the dependence of the power on the voltage and the current on the voltage give an insight into the redox process taking place in the system. This method allows a more precise determination of the current efficiency of the system than the galvanostatic measurement performed by some researchers, because it offers the possibility of simulating the operation of the cell under specific conditions imitating the current consumption by a

device with specific parameters. A zinc wire was used as the anode because of its low standard oxidation potential of -0.76V vs. NHE. Despite the initial dissolution of zinc in aqueous solutions, after the electrode's exposure to the buffer containing phosphate (V) ions, a layer of insoluble zinc phosphate (V) - the so called hopeite, ($Zn_3(PO_4)_2 \bullet 4H_2O$) - is formed on the surface after some time. This phenomenon prevents further corrosion of zinc. Although the resulting outer layer of insoluble zinc phosphate (V) does not conduct electricity, this structure is permeable to Zn^{2+} ions. Therefore, it is possible to exchange the charge between the solution and the surface of the electrode. As it turned out, the best procedure of preparing biocathodes in the biofuel cell was the one that consisted in coating the electrode with the layer of carbon nanotubes first, and then with the mixture of the same nanotubes, Nafion and laccase enzyme. The highest currents and power densities were obtained for the carbon nanotubes modified on the sides with anthraquinone groups. The power density was 3.5mW/cm^2 at the resistance of 1kΩ and the voltage of 0.5V. All tested biobatteries showed the open circuit potential of around 1.5V. Similarly to the electrochemical studies, biobatteries using carbon nanotubes modified on the sides showed higher current and power densities than their counterparts modified at the ends of the nanotubes. It is due to a larger population of the enzyme molecules immobilized on the sides of the carbon nanotubes in comparison to those immobilized at the ends of the nanotubes. The dependence of the power of the best biobatteries on time under load with the 10kΩ resistance was also studied. At the beginning, a big decrease in power was observed, and then the power stabilized after 64 minutes at about 20% of the initial value.

In my research for **[H5]** I was the first one to use carbon nanotubes modified with non-electroactive phenyl groups (single phenyl groups, triple phenyl groups (terphenyl groups)) and naphthyl groups for the catalytic reduction of oxygen in the presence of laccase. In this study I also used covalently modified carbon nanotubes modified with ferrocene as well as carbon nanotubes modified simultaneously with GOx and catalase for glucose oxidation. I used the prepared and characterized bioelectrodes for the construction of biobatteries and full biofuel cells. Laccase was immobilized in the Nafion layer on the electrodes coated with appropriately modified carbon nanotubes. On the voltammetric curves, in the presence of covalently arylated carbon nanotubes placed on the glassy carbon electrode, the oxygen reduction wave started at the same potential as when using unmodified carbon nanotubes, but the current density was much higher. This was related to the modification of the surface of the carbon nanotubes with aryl groups, and thus the presence of more laccase molecules properly

oriented on the electrode surface, so that the unmediated electron transfer between the enzyme and the electrode surface could take place. The highest oxygen reduction current density of -400μA/cm^2 at 0.2V potential was obtained for the electrode covered with single-walled carbon nanotubes modified with terphenyl groups and laccase in the Nafion layer. The covalent binding of glucose oxidase and catalase enzymes to the surface of single-walled carbon nanotubes allowed to obtain a good electrical contact between the enzymes and the electrode surface, and, consequently, a more efficient electron transfer from and to the enzyme active center. GOx is often used in the construction of bioelectrodes in biofuel cells, due to its low glucose oxidation potential and high specific activity among available enzyme preparations. In addition, multiwall carbon nanotubes modified with ferrocene (MWCNT-Fc) were also used to modify the electrode. Mediators with a very low molecular weight, such as ferrocene, quinones or osmium complexes[26,27] are often used to prepare bioelectrodes with GOx, to allow the electron flow between the electrode and the enzyme. The tested bioanodes were stable for several days and a linear dependence of the ferrocene oxidation on the glucose concentration up to 160mM was observed. When working with GOx, hydrogen peroxide is formed on the anode as a result of the oxygen reduction reactions. H_2O_2 deactivates GOx and also reduces the currents flowing through the system by consuming the electrons generated in the anode part of the cell. The H_2O_2 formed in my system did not interfere with the process because it was disproportioning to H_2O and O_2, catalyzed by the catalase covalently bound to the same nanotubes. The tested system can also be successfully used as a glucose sensor. Electrochemically tested biocathodes coated with the carbon nanotubes modified with phenyl, naphthyl, as well as terphenyl and laccase in the Nafion layer were used to construct biofuel cells. Moreover, I was the first one to intentionally apply a silver/silver chloride reference electrode (Fig. 3A) in biobatteries and biofuel cells' design, which allowed to control the potential of each electrode during the operation of the cell. By using a reference electrode, it is possible to define which of the electrodes is the element that limits the power of the entire cell, define process limits or determine at what voltage maximum power is generated. In my research I showed that the potential of the zinc anode was constant while loading the biobattery with further resistance, and only the potential of the biocathode was changing. Also, in the biobattery system, I showed that the zinc anode may act as a kind of a reference electrode for the biobattery, because the power changes in the biobattery are controlled by potential changes occurring on the biocathode. The open circuit potential of the tested systems was 1.5V. Among the studied systems, the highest power densities were obtained for the

biobattery made of the cathode covered with phenyl-modified carbon nanotubes and laccase in the Nafion layer. The maximum power density was about 1.2mW/cm^2 with the resistance of 10kΩ and 0.9V potential. At current densities smaller than 1500μA/cm^2, the system worked like a biobattery. At higher currents, the enzyme was not sufficiently efficient and the cathodic process took place at the potentials close to 0V relative to the Ag/AgCl reference electrode, as in the case of the electrodes coated only with the nanotubes (Fig. 3B). This observation and subsequent conclusions were possible only thanks to the use of the reference electrode. I also proved that carbon nanotubes with aromatic residues play a role of insoluble molecular wires for laccase, which results in higher catalytic oxygen reduction currents, higher power and current densities in the biobattery. This is related to the better fitting of the aromatic substituents attached to the nanotubes and the hydrophobic pockets of the active enzyme center. In my research, instead of the zinc anode, I also used the bioanode made of a glassy carbon electrode coated with ferrocene modified nanotubes, as well as carbon nanotubes modified simultaneously with glucose oxidase and catalase. To fuel the tested system, I used glucose for the anode and oxygen for the cathode. This was the first construction of a full biofuel cell in my scientific career. In the studied system, I achieved 30 times lower powers in comparison to a biobattery. The maximum power was 41μW/cm^2 with 0.24V voltage and the 20kΩ resistance, and the open circuit potential was 0.41V, much lower than in the biobattery.

A) B)

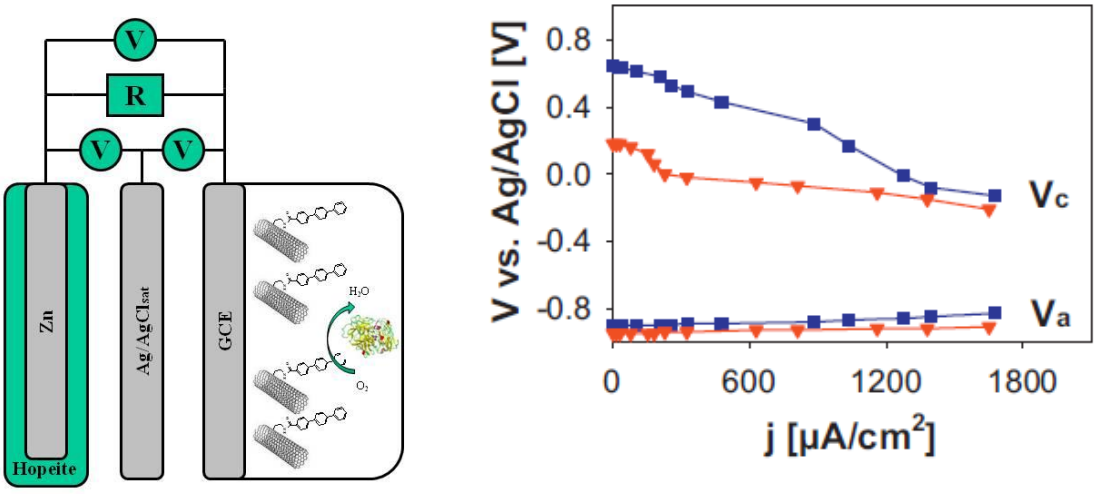


Fig. 3 A) Scheme of a biobattery with the reference electrode, B) Dependence of the cathode, Vc and the anode, Va potential vs. Ag/AgCl on the current density in the biobattery: cathode GCE electrode coated with single-wall carbon nanotubes modified with phenyl groups and coated with Nafion (■) with laccase and (▲) without laccase. Electrolyte: oxygenated McIlvaine 0.2M $NaNO_3$, pH 5.2.

As regards **[H6],** I was the first one to study the effect of various types of covalently bound biphenyl groups with carbon nanotubes on the catalytic reduction of oxygen in the presence of laccase. The nanotubes were combined with biphenyl groups using the reactions of the amine groups with a carboxyl or sulfone groups. One or two such bonds were introduced between the nanotubes and the biphenyl groups, and their effect on the catalytic oxygen reduction in the presence of laccase was studied, as were the power and current density in the biobattery. The biggest densities of the oxygen catalytic currents in the presence of laccase immobilized on the GCE electrode were observed when the nanotubes connected with biphenyl groups were used by binding the amine groups with the sulfone groups. I applied the tested biocathodes in the construction of a three-electrode biobattery, where a zinc wire formed the anode, as well as a three-electrode biofuel cell with a bioanode – a glassy carbon electrode coated with glucose oxidase and catalase-modified nanotubes, as well as ferrocene-modified nanotubes (Fig. 4). They worked both in the stationary and flow systems of the biobattery and biofuel cell. The flow systems were characterized by higher power densities in comparison to the stationary systems. I connected three biobatteries in a series to create a flow system and I used this system to power an electronic clock (Fig. 4C). Thus powered clock operated for several days. In the developed series of three connected biobatteries I obtained powers that were three times higher and a three times higher open circuit potential in comparison to a single biobattery.

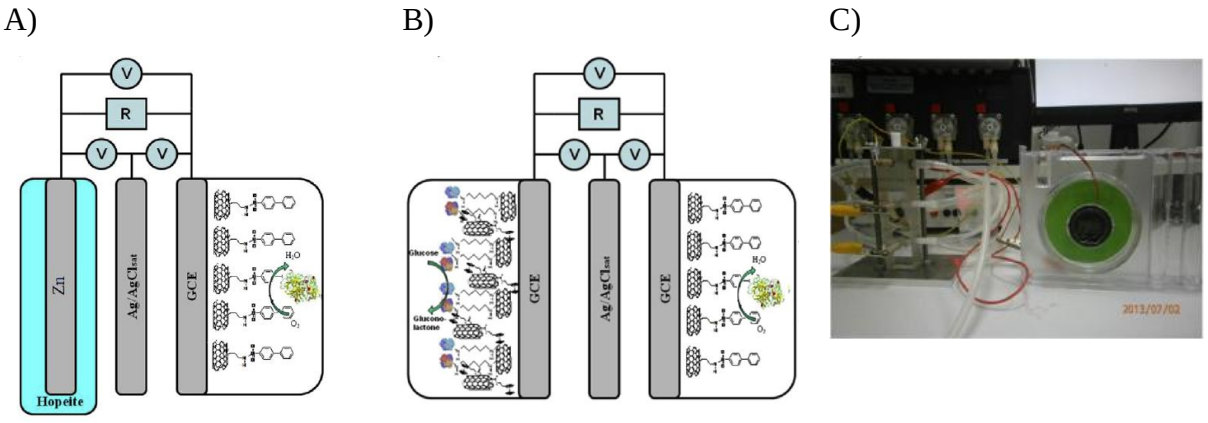


Fig. 4 Scheme of a three-electrode system A) biobattery, B) biofuel cell, C) a series of three connected flow biobatteries that supply the clock.

Recently, researchers have been looking for an effective bioanode whose potential would be as negative as possible, and thus enable the greatest open circuit potential of the

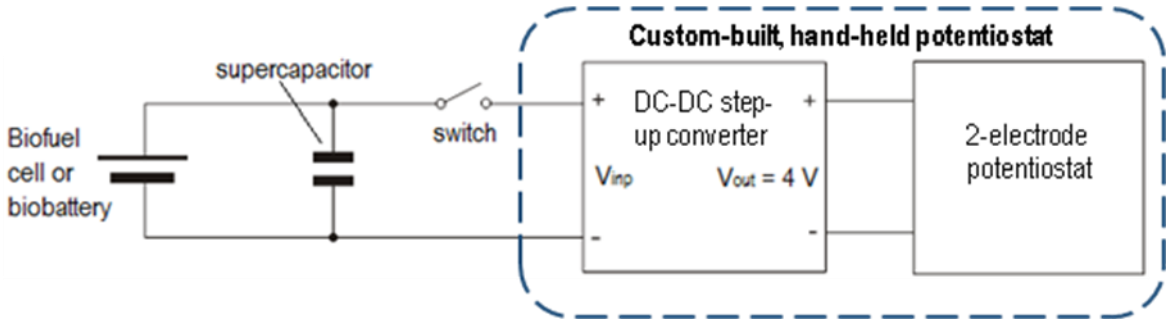
biofuel cell[28-31]. One of the enzymes used in the construction of bioanodes which has the best parameters is fructose dehydrogenase (FDH)[12,32,33]. FDH is a very promising enzyme, as far as its application in biofuel cells is concerned. It catalyzes the oxidation of fructose to 5-keto-D-fructose. In my research for **[H7]** I was the first one to apply a very interesting new material – nanocellulose/polypyrrole composite – in the catalytic oxidation of fructose in the presence of FDH. Cellulose used to prepare this composite was obtained from Cladophor algae that were collected from the Baltic Sea. This is one of the opportunities related to environmental protection – using algae, uncontrollably growing in the sea, as an electrode material in the cell construction. The composite was used to prepare an electrode – an anode containing fructose dehydrogenase. The use of FDH in the biofuel cell is particularly beneficial, because FDH is not sensitive to the presence of oxygen which is often used as the biocathode fuel for biofuel cells. As FDH does not react with oxygen, it is possible to construct a biofuel cell without a membrane. To prepare the biocathode, glassy carbon electrodes coated with multiwall carbon nanotubes modified on the sides with naphthyl groups and subsequently with laccase in the Nafion layer were used. In the course of electrochemical studies I showed that both enzymes retained their catalytic activity following the adsorption on the electrodes modified with the composite or carbon nanotubes, and that they also showed a direct electron transfer process. Fructose dehydrogenase oxidized fructose at the potential of -0.15V vs. Ag/AgCl. For this system a maximum catalytic current density of 14.1mA/cm^2 was obtained, measured at the potential of 0.5V. The electrode had very high durability over time. One of the best results presented so far in literature, the application of FDH in bioelectrocatalysis, was achieved. A glassy carbon electrode coated with an easily accessible, environmentally friendly nanocellulate/polypyrrol composite (CPPy), on which I physically immobilized FDH, was used in the biofuel cell. The construction of the bioanode combined a new idea of a bioelectrocatalytic system with the application of a good supercapacitor material as the electrode substrate. This combination of the bioelectrocatalytic system based on the composite and adsorbed FDH resulted in a very good performance of the anode in biofuel cells. As the biocathode, a glassy carbon electrode covered with carbon nanotubes and laccase in the Nafion layer was used. The prepared biofuel cell exhibited hybrid system properties, a combination of supercapacitance and bioelectrocatalytic properties. The open circuit potential of 0.76V and the maximum power density of 1.6mW/cm^2 were obtained for this biofuel cell. After the physical adsorption of FDH on the nanocellulose/polypyrrole composite: (1) FDH displays a direct electron transfer process, i.e.

without the participation of mediators; (2) CPPy acts as the matrix with a large surface area enabling proper orientation of the enzyme; (3) the biofuel cell provides very high power densities, i.e. 1.6 mW/cm^2; (4) the bioanode has a large capacity and can store significant amounts of charge; (5) the potential of the anode remains practically constant when the cell is loaded with various external resistances. Therefore, it can be concluded that the nanocellulose/polypyrrole composite is a very good material for bioelectronic applications.

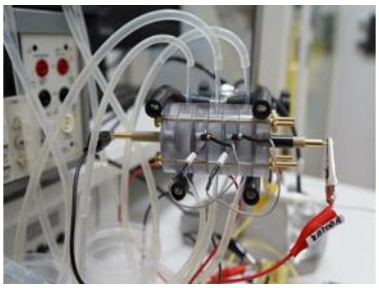
There have been attempts at using the designed biofuel cells to power real devices, for example in medicine. In medical applications, it is required that the energy source be stable, have a long life and undergo miniaturization easily. Literature describes several biofuel cells that have been implanted in various living organisms[34-36]. However, these devices do not provide too much power and current density over a longer period of time. Enzymatic fuel cells offer the open circuit potential of approx. 0.6V to 1V, and biobatteries - of approximately 1.6V. Supplying devices with even low power consumption poses a big problem. This problem can be solved by the serial linking of cells or using supercapacitors to collect the charge. Combining supercapacitors with an enzyme cell can improve the efficiency of the energy source[37-40]. In my research for **[H8]** I used the system of three connected biofuel cells combined with a supercapacitor to power a device with more power consumption: a potentiostat which I integrated with the sensor. As a test device a homemade minipotentiostat was used, specially designed and constructed by Prof. Sławomir Kalinowski, from the Faculty of Chemistry at the University of Warmia and Mazury in Olsztyn (Fig. 5A). In the study a specially designed and constructed cylindrical flow biofuel cell was used in which the bioelectrode surface was increased to 3.14cm^2, and carbon paper was used as the electrode substrate (Fig. 5B). The bioanode was prepared by applying a nanocellulose/polypyrrole composite (CPPy) and acetylene black (in the ratio of 95:5) to the carbon paper under reduced pressure, and then FDH was physically adsorbed. In the course of electrochemical studies a catalytic wave of the fructose oxidation starting at about -0.15V was observed in the presence of 100mM fructose in the McIlvaine buffer of pH 5.3, giving the maximum current of 17.5mA, and the electrode capacity was 0.6F. It was the result of the unmediated oxidation of fructose by FDH. The CPPy composite serves not only as a conductive matrix with a large surface area, but also as the material that facilitated proper orientation of the enzyme on the electrode surface and enabled a very fast mass transfer to the substrate. The biocathode was prepared by applying carbon nanotubes modified with naphthyl groups to the carbon paper (under reduced pressure) and then laccase was physically absorbed. When the buffer solution

was saturated with oxygen, the catalytic wave of the oxygen reduction starting at 0.6V was observed on the voltammetric curve recorded by the biocathode with laccase. In the case of the biocathode coated with laccase and carbon nanotubes modified with naphthyl groups, the maximum catalytic oxygen reduction current of -938μA and the capacity of the electrodes of 0.05F were achieved.

A)



B)



C)

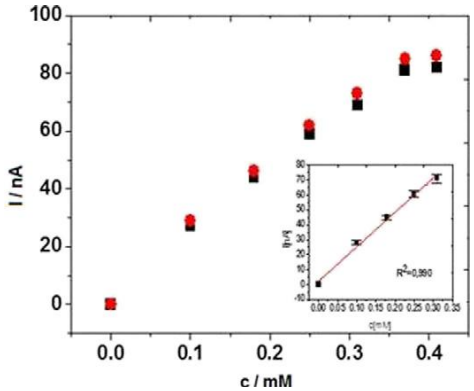


Fig. 5 A) Scheme of a minipotentiostat powered by a supercapacitor and a cell, B) System of three connected biofuel cells, C) Calibration curves for oxygen recorded in McIlvaine buffer pH 5.3 using (■) a CHI 400B potentiostat and (●) a homemade minipotentiostat.

A flow cylindrical system was used to construct the biofuel cell with the tested bioelectrodes. In addition, three biofuel cells were assembled in a series and the obtained open circuit potential for the whole biofuel system was around 2V. Such set was used to supply a homemade minipotentiostat. In this system the supercapacitor was charged by the battery built from three connected biofuel cells. It accumulated the charge and supplied the minipotentiostat in the pulse manner. The system worked in the pulse mode and was used with a two-electrode oxygen sensor. In the oxygen sensor a glassy carbon electrode was used as the working electrode, coated with multiwall carbon nanotubes modified with naphthyl groups and adsorbed laccase. The second electrode was the Ag/AgCl – a reference electrode.

Chronoamperometric experiments were carried out using both the CHI 400B potentiostat and the specially designed homemade minipotentiostat, powered by the system of three interconnected biofuel cells. The calibration curves recorded using the CHI 400 B potentiostat and the minipotentiostat were almost identical. The current was measured as the function of the oxygen concentration from 0 to 0.4mM and a linear range was observed up to 0.3mM of oxygen in the solution (Fig. 5C). The proposed integrated system can be successfully used as a specially tailored system for oxygen measurements. Hopefully, as a result of these studies, enzymatic fuel cells can indeed be used to power small devices.

In my research for **[H9]** I used the above-described nanocellulose/polypyrrole composite with capacitive properties to improve biofuel cell parameters, in order to achieve higher power densities and potential differences. Bioelectrodes were prepared using the nanocellulose/polypyrrole composite. The device had not only energy generating properties in the catalytic reaction, but also ability to accumulate charge. Such devices combining both the ability to accumulate charge and generate it in a catalytic reaction are called biosupercapacitors[3,41]. I was the first one to prove that the laccase enzyme, following physical adsorption on the nanocellulose/polypyrrole composite, retains a catalytic activity and shows a direct electron transfer in the oxygen reduction reaction. On the electrodes modified with fructose dehydrogenase, the catalytic process occurred at low overpotential, -0.15V vs. Ag/AgCl and a very big maximum catalytic current of about 8.6mA/cm^2 was achieved. The electrode also had high durability over time. On the modified biocathode, oxygen was reduced at the potential of 0.41V vs. Ag/AgCl, and the catalytic current reached the value of 1.2mA/cm^2. In order to improve the properties of the bioanode containing the composite, α-pyrrole acid (CPPy-COOH-FDH) was used for its modification, introducing a large population of carboxyl groups to the surface of the polymer. Their presence improved the stability of the electrode by a better oriented adsorption of the FDH molecules. The result was an increase in the maximum catalytic current density to 13.1mA/cm^2. In order to improve the catalytic potential and stability of the enzyme on the cathode, electrodeposition of ABTS and pyrrole was carried out on the electrode surface (CPPy-Lac-ABTS). In this process, the enzyme reaching the surface of the composite was trapped in the network of the resulting polymer. As a result of this operation, an increase of 0.09V in the catalytic potential, an increase in the maximum catalytic current and clear improvement in the electrode stability over a longer period of time were observed. For thus prepared bioelectrode, the catalytic current densities of about 2.1mA/cm^2 were obtained at the potential of +0.45V vs. Ag/AgCl,

and the electrode showed a catalytic activity for one week. Unfortunately, the durability of this electrode in time was lower in comparison to the bioanode. The electrodes were tested in a special cell designed and constructed in cooperation with Prof. Jerzy Golimowski (Fig. 6A). The device was made of transparent plexiglass, which made it possible to precisely determine the distance between the electrodes.

A) B)

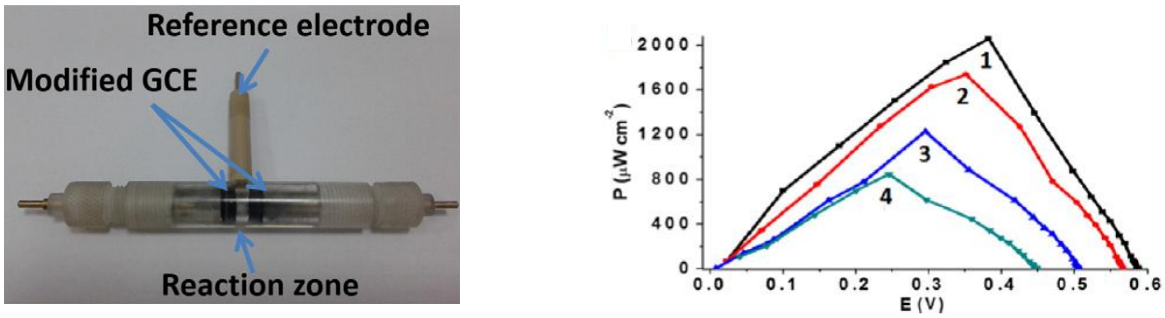


Fig. 6 A) A device used for electrochemical and biofuel cell measurements, B) Power as the function of biofuel cell voltage for different conditioning periods of the biofuel cell before the measurement; 1-24 h, 2-8 h, 3-6 h, 4-0 h.

At the beginning, biofuel cells consisting of the electrodes described above showed the OCP of 0.45V and the maximum power was about 0.8mW/cm^2. In the CPPy cell the decrease of potential of both electrodes - the cathode and the anode - was almost symmetrical. It was also shown that the power generated by the cell depends on the time of charging the cell before use (Fig. 6B). The cell reaches its maximum power after 24 hours, which can be explained by the bioelectrode charging process. During the measurements, the power of the biofuel cell did not decrease; on the contrary, it increased and after 24 hours the OCP reached 0.6V, the maximum current density was 7.7mA/cm^2 and the maximum power density equaled 2.1mW/cm^2 at the load of 1kΩ. This suggests that the capacitive current constitutes a significant part of the total electrical power and the system collects the charge over time. In fact it behaves like a biosupercapacitor. The designed device also allowed to control the distance between bioelectrodes very precisely. In the case of the biofuel cell with CPPy-COOH-FDH/CPPy-ABTS-Lac, I obtained the highest power density and OCP after 24h using the smallest distance between the electrodes, i.e. 1mm. At the end of this charging period, the fuel cell could run for the full week with a 60 percent loss of the power density initially generated. A biofuel cell using the nanocellulose/polypyrrole composite is more stable, has a

longer life span, and achieves much higher power densities than those obtained on unmodified or modified nanotubes.

While conducting research for **[H10],** I refined the biocathodes described by Cavaco-Paulo and co-workers[42]. I used carbon nanotubes to immobilize promazine or acetosyringone and performed a catalytic reduction of oxygen in the presence of laccase. Promazine is widely used in psychiatry as an antipsychotic drug with strong sedative and anti-emetic properties[43]. Acetosyringon is secreted mainly by dicotyledonous plants near the wound[44]. It acts as an indicator of tissue damage, informing the plant about the need to start the healing process. As a result, I obtained 1000 times higher current densities of the catalytic oxygen reduction in the presence of laccase. I applied the tested systems not only to the construction of a biobattery, but also to the construction of an oxygen sensor. GCE bioelectrodes were prepared by embedding multiwalled carbon nanotubes. Then acetosyringone (ASYR) or promazine (PRZ) mediators were electrochemically deposited and in the next step laccase was adsorbed on top. It was shown that the use of carbon nanotubes and mediators in the construction of bioelectrodes contributes to the improvement of the efficiency of the biocathode performance, as opposed to the GCE electrode modified only with the enzyme. In the presence of mediators and laccase on the modified electrode surface, well formed catalytic waves and an increase in the oxygen reduction current were observed. The attachment of promazine increased the level of the registered catalytic currents by 40% and the immobilization of acetosyringone resulted in an 82% current increase, as compared to the GCE electrodes modified only with carbon nanotubes and laccase. The tested biocathodes were used to construct a biobattery. The most preferred mediator for the preparation of the biobattery was acetosyringone for which the highest power density was obtained equal to 1853.9 $\mu W/cm^2$ at the 5 $k\Omega$ resistance. The prepared biobatteries were characterized by a low decrease in the generated voltage during 14 days while the system was being loaded with various resistances: 10MΩ, 100 and 10kΩ. I have also successfully applied the tested systems to the construction of oxygen biosensors in the concentration range from 0.0 to 0.3mM.

IV. Summary of the publication cycle

The most important aspects of the scientific innovation:

- I was the first one to show that properly activated boron-doped diamond electrodes enable unmediated catalytic oxygen reduction at the potential of approximately +0.6V vs. Ag/AgCl, i.e. close to the formal potential of the oxygen's reduction to water. The catalyst was laccase dissolved in a solution or immobilized in the liquid crystal layer of the lipid mesophase placed on the electrode.

- I also obtained the catalytic oxygen reduction process at approximately +0.6V potentials relative to the Ag/AgCl reference electrode (approx. +0.8V relative to a normal hydrogen electrode) by using unmodified carbon nanotubes and microparticles placed on a glassy carbon electrode in the presence of laccase. The oxygen reduction current densities were from 10 to 40 times greater than the densities achieved while using a boron-doped diamond electrode.

- I was the first one to immobilize carbon nanotubes covalently modified with the mediator - 2,2'-azobis- (3-ethylbenzotriasulfonic acid) (ABTS) on the electrode and to examine the effect of various ABTS-modifications of the carbon nanotubes on the catalytic reduction of oxygen in the presence of laccase. As a result of modifying carbon nanotubes with ABTS, the oxygen reduction currents are significantly increased.

- Further progress in the field of the catalytic oxygen reduction by introducing, for the first time, carbon nanotubes modified on the sides and at the ends with non-electroactive phenyl groups, naphthyl groups, terphenyl groups, anthracenyl groups or electroactive anthraquinone groups. The products of these modifications maintain the nonmediator oxygen reduction mechanism, but they allow to obtain significantly increased catalytic current densities. Chemically modified carbon nanomaterials served as "antennas" for the electron transport between the enzymes and the electrode surface, which significantly improved the cathode performance.

- I was the first one to use carbon nanotubes covalently modified with ferrocene and carbon nanotubes covalently modified with glucose oxidase and catalase for the catalytic oxidation of glucose and I used them as anodes in the construction of a full biofuel cell.

- I have developed electrodes in the integrated system of biofuel cells or biobatteries (this is what I simply call a hybrid cell with the zinc anode) in which the cell was exposed to resistance changes in a wide range during the measurement. In addition, I used a silver-silver chloride reference electrode, which allowed me to control the potential of each electrode during the cell work and evaluate which of the electrodes is the limiting factor of the cell's power. The best biobattery gave the density of 3.5mW/cm^2 at the 1kΩ resistance and 0.5V potential difference, which is one of the best enzyme parameters of hybrid cells described in literature.

- I used flow biofuel cells integrated with a supercapacitor to feed a specially constructed homemade minipotentiostat. The biofuel cells charged the supercapacitor which in turn accumulated the charge and powered the minipotentiostat in the pulse mode. The entire integrated system was combined with a sensor for measuring the oxygen concentration.

- In cooperation with Prof. Leif Nyholm's group, we were the first ones to apply a new composite material built of nanocellulose with polypyrrole and adsorbed FDH in the fructose oxidation. The composite enables the proper orientation of the enzyme and unmediated electron transfer. A biofuel cell composed of the anode containing a pseudocapacitive nanocellulose/polypyrrole composite with FDH, and the cathode modified with laccase, collects the charge, so it behaves like a biosupercapacitor. Very good parameters of biofuel cell work were obtained: the open circuit potential was 0.6V, the maximum current density was 7.7mA/cm^2, and the maximum power density was 2.1mW/cm^2 (with the cell load resistance of 1kΩ).

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[40] F. Conzuelo, A. Ruff, W. Schuhmann, Review Article Self-powered bioelectrochemical devices, Curr. Opin. Electrochem. (2018) 1 - 8,
[41] D. Pankratov, Z. Blum, D.B. Suyatin, V.O. Popov, S. Shleev, Self-charging electrochemical biocapacitor, ChemElectroChem 1 (2014) 343 - 346,
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5. Other scientific publications and achievements

5.A Bibliographic summary of the scientific achievements

Number of publications: **33**

Number of publications after obtaining Ph.D. degree: **28**

Total Impact Factor: **83.824**

Citations report (Web of Science, data from 09.01.2019)

Number of citations = **400** (12.1 citation per paper)

Number of citations without self-citations = **337** (10.2 citation per paper)

Hirsch index H = **11**

5.B List of the publications published before obtaining the doctoral degree in the journals listed in the Journal Citation Reports (excluding those listed in Chapter 4)

P1. **K. Stolarczyk**, R. Bilewicz*, L. Siegfried, T. Kaden, "The dual role of self assembled monolayers of tetraazamacrocyclic copper (II) complex in ascorbate oxidation catalysis", Inorganica Chimica Acta 348 (2003) 129 - 134
IF(2003) = 1.578; number of citations: 10

P2. **K. Stolarczyk**, R. Bilewicz*, A. Skwierawska, J.F. Biernat "Functionalization of electrode surfaces with monolayers of azocompounds and gold clusters", Journal of Inclusion Phenomena 49 (2004) 173 - 179
IF(2004) = 0.825; number of citations: 6

P3. **K. Stolarczyk**, B. Pałys, R. Bilewicz*, "Catalytic properties of 4-hydroxythiophenol protected gold nanoclusters supported on gold electrodes", Journal Electroanalytical Chemistry 564 (2004) 93 - 98
IF(2004) = 2.228; number of citations: 15

P4. **K. Stolarczyk**, R. Bilewicz*, "Catalytic oxidation of ascorbic acid on 2D and 3D monolayers of 4-hydroxythiophenol", Electroanalysis 16 (2004) 1609 - 1615
IF(2004) = 2.038; number of citations: 7

P5. **K. Stolarczyk**, R. Bilewicz*, "Electron transport through alkanethiolate films decorated with monolayer protected gold clusters", Electrochimica Acta 51 (2006) 2358 - 2365
IF(2006) = 2.955; number of citations: 17

In my research for **[P1-P5]** I developed electrodes modified with the monolayers of the selected organic compounds or layers of gold nanoparticles using a self-assembly method, thus providing the electrodes with catalytic properties or molecular recognition functions towards dissolved compounds. While researching for **[P1],** I demonstrated the catalytic properties of the tetraazamacrocyclic copper complex chemically immobilized on a gold electrode. This system catalyzes the oxidation of ascorbates. In the presence of oxygen and ascorbates, the complex immobilized on the electrode plays a dual role, as it catalyzes both the electrooxidation and the autoxidation of the ascorbates. This is advantageous from the point of view of practical application, since ascorbates can be determined on the basis of the oxidation signal of ascorbic acid or by using the hydrogen peroxide reduction signal.

The azocrown compound studied in **[P2]**, immobilized on the electrode with the use of a thiol group, can be used as a specific chemical "generator" of gold nanoparticles and their moieties. Such systems can be used to deposit metallic contacts and create conductive surfaces with a planned structure.

An important and difficult experimental issue was the preparation of the ordered gold nanoparticle systems immobilized on an electrode by a covalent bonding or non-covalent interactions with the use of organothiol compounds **[P3-P5]**. The aim of the research carried out in this work was to demonstrate the ability of gold nanoparticles to accumulate the charge and effectively transport it between the electrode and the compound in a solution. I proved that the concentration of the nanoparticles and the adsorption time have an effect on the efficiency of the electron transport between the electrode and the electroactive compound in the solution. A single nanoparticle can be considered as a spherical capacitor, and the gold nanoparticle system can be treated as the system of connected capacitors. I used these specific properties of the investigated system in the oxidation of ascorbic acid and 3,4-dihydroxyphenylalanine. In both cases I observed an increase in the current and a reduction of the compounds' oxidation overpotential, and so they may be used in neurotransmitter studies and in electrochemical sensors for these substances.

5.C List of the publications published after obtaining the doctoral degree in the journals listed in the Journal Citation Reports (excluding those listed in Chapter 4)

- the subject of research is related to bioelectrodes and cells

P6. K. Sadowska, E Jabłonowska, **K. Stolarczyk**, R. Wiser, R. Bilewicz, K.P. Roberts, J.F. Biernat*, "Chemically modified carbon nanotubes: synthesis and implementation", Polish Journal of Chemistry 82 (2008) 1309 - 1313
IF(2008) = 0.518

P7. R. Bilewicz*, **K. Stolarczyk**, K. Sadowska, J. Rogalski, J.F. Biernat, "Carbon nanotubes derivatized with mediators for laccase catalyzed oxygen reduction", ECS Transactions 19 (2009) 27 - 36
number of citations: 6

P8. R. Bilewicz, E. Nazaruk, K. Żelechowska, J.F. Biernat*, **K. Stolarczyk**, K.P. Roberts, G. Ginalska, J. Rogalski, "Carbon nanotubes chemically derivatized with redox systems as mediators for biofuel cell applications", Biocybernetics and Biomedical Engineering 31 (2011) 17 - 30
IF(2011) = 0.234; number of citations: 6

P9. K. Żelechowska*, **K. Stolarczyk**, D. Łyp, J. Rogalski, K.P. Roberts, R. Bilewicz, J.F. Biernat, "Aryl and N-arylamide carbon nanotubes for electrical coupling of laccase to electrodes in biofuel cells and biobatteries", Biocybernetics and Biomedical Engineering. 33 (2013) 235 - 245
IF(2013) = 0.157, number of citations: 9

P10. K. Żelechowska, **K. Stolarczyk**, D. Łyp, J.F. Biernat, J. Rogalski, R. Bilewicz*, "Materials for mediator-free electron transfer in the enzymatic electrodes of biobatteries and biofuel cells", ECS Transactions 45 (11) (2013) 1 - 8
number of citations: 2

P11. D. Majdecka, S. Dramińska, **K. Stolarczyk**, M. Kizling, P. Krysiński, J. Golimowski, R. Bilewicz*, "Sandwich biobattery with enzymatic cathode and zinc anode for powering sensors", ECS Transaction 61 (2014) 1 - 7
number of citations = 0

P12. M. Skunik-Nuckowska, K. Grzejszczyk, **K. Stolarczyk**, R. Bilewicz, P.J. Kulesza*, "Integration of supercapacitors with enzymatic biobatteries towards more effective pulse-powered use in small-scale energy harvesting devices", Journal of Applied Electrochemistry 44 (2014) 497 - 507
IF(2014) = 2.409; number of citations: 18

P13. D. Majdecka, S. Draminska, **K. Stolarczyk**, M. Kizling, P. Krysinski, J. Golimowski, J.F. Biernat, R. Bilewicz*, "Sandwich biobattery with enzymatic cathode and zinc anode integrated with sensor", Journal of The Electrochemical Society162 (6) (2015) F555 - F559
IF (2015) = 3.014; number of citations: 8

P14. I. Mazur, B. Rola, **K. Stolarczyk**, E. Nazaruk, R. Bilewicz, J. Rogalski*, S. Ohga*, "The large scale production of Cerrena unicolor laccase on waste", Agricultural Based Media Journal - Faculty of Agriculture Kyushu University 60 (2) (2015) 297 - 302
IF(2015) = 0.216; number of citations: 2

P15. M. Kizling, P. Biedul, D. Zabost, **K. Stolarczyk**, R. Bilewicz*, "Application of hydroxyethyl methacrylate and ethylene glycol methacrylate phosphate copolymer as hydrogel electrolyte in enzymatic fuel cell", Electroanalysis 28 (2016) 2444 - 2451.
IF(2016) = 2.851; number of citations: 5

P16. M Kizling, M. Dzwonek, B. Olszewski, P. Bącal, Ł. Tymecki, A. Więckowska, **K. Stolarczyk**, R. Bilewicz*, "Reticulated vitreous carbon as a scaffold for enzymatic fuel cell designing", Biosensors and Bioelectronics 95 (2017) 1 - 7
IF(2017) = 8.173; number of citations: 10

In my research for **[P6-P11]** I have shown that modifying carbon nanotubes with various aryl groups or mediators has a positive effect on the orientation of the enzyme molecules in relation to the electrode surface. It also shortens the distance of electron tunneling. These systems serve as "antennas" of the electron transport between the enzymes and the electrode surface, while significantly improving the catalytic process. In addition, the mediators do not leak into the solution. The tested systems were applied in the construction of biobatteries and biofuel cells.

An important subject of the study **[P12]** was combining catalytic and capacitive properties in one device. Together with Ph.D. Skunik-Nuckowska, I presented the combination of a carbon-nanotubes-supercapacitor with a hybrid biofuel cell (called a biobattery) that I used to power a watch. In the biobattery, the open cell potential of 1.65V, and the maximum power of 1.8mW/cm^2 were achieved under the load of 1 kΩ and the voltage of 0.7V. It was demonstrated that the use of a high-capacity system can efficiently support the enzymatic cell, significantly improving its current parameters by the pulse mode of the operation, enabling the charging of the capacitor and supplying external devices characterized by high power consumption, such as a watch with an alarm or light.

In my research for **[P13]** I used a biobattery combined with a supercapacitor to power a homemade minipotentiostat which I integrated with a sensor to measure the concentration of catechol. In the course of amperometric studies on a two-electrode system with the Ag/AgCl reference electrode it was shown that with the addition of catechol the linear dependence of the current on the catechol concentration in the range from 0 to 2mM is obtained. The tests performed with the homemade potentiostat were compared with those done using the same sensor combined with a classic Autolab potentiostat. The curves of the current dependence on

the catechol concentration plotted for these two different devices were the same. Our minipotentiostat powered by a biobattery can be successfully used for the measurements using various sensors.

From the point of view of applying the studied systems in the biobattery or biofuel cell construction, it is important to use semisolid electrolyte systems. This is related to the safety of these devices. In my research I made effective attempts at using a gel-based electrolyte in the construction of the biobattery and the biofuel cell **[P15]**. Poly (2-hydroxyethylene)methacrylate (HEMA) and ethylene glycol methacrylate (EGMP) copolymer were successfully synthesized and used as quasi-solid-state electrolytes in enzymatic fuel cells. The prepared hydrogels were used as electrolytes in the electrochemical measurements of biofuel cells with the electrodes composed of modified carbon nanotubes and enzymes. The maximum current density generated by the anode coated with single-walled carbon nanotubes (SWCNT) modified with an amino groups (SWCNT-NH_2) and fructose dehydrogenase was 5.7mA/cm^2 in 100mM fructose, and the onset potential of the catalysis was -0.12V against the Ag/AgCl reference electrode. The cathode made of the carbon nanotubes modified with naphthyl groups and laccase gave the maximum current density of 0.9mA/cm^2 and the onset catalysis potential of 0.6V vs. Ag/AgCl. In the hydrogel electrolyte no additional overpotential of the catalytic reaction was observed, and the decrease in the electrocatalytic current reached only 5% in comparison to the aqueous electrolyte. The full enzymatic fuel cell with the hydrogel electrolyte had the maximum power density of 0.198mW/cm^2.

Another important achievement was using porous carbon (Reticulated Vitreous Carbon (RVC)) for the construction of volumetric bioelectrodes **[P16]**. Porous electrodes were used as scaffolds to modify their surfaces with gold nanoparticles and enzymes. The use of a three-dimensional RVC material resulted in an effective covalent immobilization of the enzymes – FDH and laccase, thus ensuring the best contact of protein molecules with the electrode surface. The enzymes covalently bound to the gold nanoparticles retained their catalytic activity. For the electrodes modified with gold nanoparticles and fructose dehydrogenase the onset potential of the catalytic wave was equal to -0.14V vs. Ag/AgCl (the potential reached the value close to the thermodynamic potential of FDH) and the limit of the current density increased to 15mA/cm^3. This result indicates that very small gold nanoparticles play a key role in the electron transport efficiency, probably because of their redox properties. Similar behavior was observed for the electrodes with adsorbed laccase.

After the immobilization of gold nanoparticles with the diameter of 1.8nm and laccase on the RVC electrode, the catalytic process of the oxygen reduction began at the potential of 0.6V vs. Ag/AgCl, and the current density was about 7mA/cm^3. The use of volumetric RVC electrodes (3D electrodes) in the biofuel cell allowed to increase the surface area of each electrode significantly, thus increasing the number of the enzyme molecules adsorbed on its surface. Moreover, wide pores of this material allowed easy penetration of the substrates into the 3D structure and efficient removal of the catalysis products. Consequently, catalytic currents on the cathode and 3D anode and the power of the biofuel cell increased significantly.

- list of the publications in cooperation with Pharmaceutical Institute in Warsaw and Warsaw Medical University

P17. E.U. Stolarczyk*, K. Stolarczyk^, „Perspectives of nanotechnology in medicine and pharmacy and its influence on Pharmaceutical Industry", Przemysł Chemiczny 86/7 (2007) 797 - 799 (a review)
IF(2007) = 0.196; number of citations: 0

P18. E.U. Stolarczyk*, **K. Stolarczyk*^**, M. Łaszcz, M. Kubiszewski, W. Maruszak, W. Olejarz, D. Bryk, "Synthesis and characterization of genistein conjugated with gold nanoparticles and the study of their cytotoxic properties", European Journal of Pharmaceutical Sciences 96 (2017) 176 - 185
IF(2017) = 3.466; number of citations: 6

P19. W. Maruszak*, E.U. Stolarczyka, **K. Stolarczyk^**, "CE method for the in-process control of the synthesis of activesubstances conjugated with gold nanoparticles", Journal of Pharmaceutical and Biomedical Analysis 141 (2017) 52 - 58
IF(2017) = 2.831; number of citations: 1

P20. E.U. Stolarczyk*, **K. Stolarczyk***, M. Łaszcz, M. Kubiszewski, A. Leś, O. Michalak, "Pemetrexed conjugated with gold nanoparticles – Synthesis, characterization and a study of noncovalent interactions", European Journal of Pharmaceutical Sciences 109 (2017) 13 - 20.
IF(2017) = 3.466; number of citations: 1

P21. E.U. Stolarczyk*, M. Łaszcz, A. Leś, M. Kubiszewski, K. Kuziak, K. Sidoryk, **K. Stolarczyk^**, "Design and molecular modeling of abiraterone-functionalized gold nanoparticles", Nanomaterials 8 (2018) 641
IF(2017) = 3.504; number of citations: 0

P22. E.U. Stolarczyk, K. Sidoryk, M. Cybulski, M. Kubiszewski, **K. Stolarczyk*^**, "Design of therapeutic self-assembled monolayers of thiolated abiraterone", Nanomaterials 8 (2018) 1018
IF(2017) = 3.504; liczba cytowań: 0

In **[P18-P22]** the synthesis and physicochemical studies of new gold nanoparticle (AuNPs) conjugates with oncologically active substances (APIs): genistein **[P18]**, pemetrexed **[P19,P20]**, and abiraterone **[P21]** were described. Tested gold nanoparticles combined with the active substances are potential drug carriers in anti-cancer therapies. The physicochemical methods developed in **[P18-P21]** allowed a full characterization of the unique conjugates. The developed methods introduce a new approach to the full characterization of new products with predicted anti-cancer properties, in a wide qualitative and quantitative range, which was confirmed by initial cytotoxicity tests performed in **[P18]**. Analytical methodologies including electrochemical measurements, transmission electron microscopy, dynamic light scattering, zeta potential, Raman, IR, UV-Vis and NMR techniques were utilized in this work. In addition, the mechanisms of the AuNPs-API interactions were determined based on newly developed analytical methodology and theoretical calculations.

5.D List of the monographies and scientific publications published in international or domestic journals not listed in the Journal Citation Reports

P23. E.U. Stolarczyk*, **K. Stolarczyk**^, M. Kubiszewski, ”Properties and application of gold nanoparticles as selective drug nanocarriers in the transport of anti-cancer active substances”, Farmacja Polska 71(2) (2015) 111 - 118 (a review)