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Ph.D. thesis

DESIGN, FABRICATION AND CHARACTERISTICS OF ELECTROACTIVE HYBRID MATERIALS FOR SENSOR DETECTION SYSTEMS

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"Instead of worrying about what you cannot control,
shift your energy to what you can create"
— Roy T. Bennett

I dedicate this doctoral thesis to
my **Godmother –** forever in my Heart.

List of abbreviations and symbols used at work

AA	-	ascorbic acid
ADH	-	alcohol dehydrogenase
AFM	-	atomic force microscopy
βCD	-	β-cyclodextrins
BQ	-	1,4-benzoquinone
CA	-	caffeic acid
Chit	-	chitosan
ChOx	-	cholesterol oxidase
CNTs	-	carbon nanotubes
CV	-	cyclic voltammetry
DA	-	dopamine
DET	-	direct electron transfer
DOPA	-	3,4-dihydroxy-L-phenylalanine
DLS	-	dynamic light scattering
EDS	-	energy-dispersive X-ray spectroscopy
EIS	-	electrochemical impedance spectroscopy
ELS	-	electrophoretic light scattering
FAD	-	flavin adenine dinucleotide, oxidized form
$FADH_2$	-	flavin adenine dinucleotide, reduced form
Fe_3O_4	-	magnetite
Fc	-	ferrocene
FDA	-	Food and Drug Administration
FFC	-	potassium ferrocyanide and potassium ferricyanide mixture
FTIR	-	Fourier transform infrared spectroscopy
GC	-	glassy carbon electrode
GOx	-	glucose oxidase
Glu	-	glucose
HFc	-	(hydroxymethyl)ferrocene
HQ	-	hydroquinone
HR-TEM	-	high-resolution transmission electron microscopy

IUPAC	-	International Union of Pure and Applied Chemistry
Km	-	Michaelis-Menten constant
L-DOPA	-	3,4-dihydroxy-L-phenylalanine
LOD	-	limit of detection
LOQ	-	limit of quantification
MB	-	methylene blue
Med_{ox}	-	mediator, oxidized form
Med_{red}	-	mediator, reduced form
MET	-	mediated electron transport
MNPs	-	magnetic nanoparticles
MOFs	-	metal-organic frameworks
MWCNT	-	multi-walled carbon nanotube
NAD^+	-	nicotinamide adenine dinucleotide, oxidized form
NADH	-	nicotinamide adenine dinucleotide, reduced form
NIBS	-	non-invasive light scattering
PBS	-	phosphate-buffered saline
PCA	-	poly(caffeic acid)
PDA	-	polydopamine
PdI	-	polydispersity index
POCT	-	point-of-care testing
SEM	-	scanning electron microscopy
SH-βCD	-	6-thio-β-cyclodextrins
SPE	-	screen-printed electrode
SWCNT	-	single-walled carbon nanotube
TEM	-	transmission electron microscopy
TRIS	-	tris(hydroxymethyl)aminomethane
UA	-	uric acid
UV-Vis	-	ultraviolet-visible spectroscopy
XPS	-	X-ray photoelectron spectroscopy
WHO	-	World Health Organization
ζ	-	zeta potential

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List of selected publications as the basis for the Ph.D. procedure

According to: *Ustawa o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz.U. 2003 Nr 65 poz. 595) - 2. Rozprawa doktorska może mieć formę maszynopisu książki, książki wydanej lub spójnego tematycznie zbioru rozdziałów w książkach wydanych, spójnego tematycznie zbioru artykułów opublikowanych lub przyjętych do druku w czasopismach naukowych, określonych przez ministra właściwego do spraw nauki na podstawie przepisów dotyczących finansowania nauki (...)* six selected publications were presented in Ph.D. procedure, constituting a monothematic series of scientific articles in the field of design, fabrication and charactristics of electroactive hybrid materials for sensor and biosensor applications.

No.	Publication	IF	5-Year IF	MEN points
1.	**M. Kuznowicz**, A. Jędrzak, A. Leda, T. Rębiś, T. Jesionowski; *Measurements of working parameters of external mediators for biodetectors based on the polydopamine@magnetite nanoparticles*; Measurement, 184 (2021) 109950. I contributed to the preparation of the hybrid material, the immobilization process, construction of the biosensor, performed electrochemical tests, wrote part of the manuscript, and drew up charts and tables.	5.131	4.392	200
2.	**M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; *Biomimetic magnetite/polydopamine/β-cyclodextrins nanocomposite for long-term glucose measurements*; Biochemical Engineering Journal, 174 (2021) 108127. I carried out the synthesis of the hybrid nanomaterial, enzyme immobilization, and whole electrochemical tests. I described the analysis results, wrote the manuscript, drawing up charts and tables.	4.446	4.020	100

3.	T. Rębiś, **M. Kuznowicz**, A. Jędrzak, G. Milczarek, T. Jesionowski; *Design and fabrication of low potential NADH-sensor based on poly(caffeic acid)@multi-walled carbon nanotubes*; Electrochimica Acta, 386 (2021) 138384. I designed the methodology of the synthesis, obtained the hybrid material, I described part of the manuscript and the results.	7.336	6.498	100
4.	**M. Kuznowicz**, T. Rębiś, A. Jędrzak, G. Nowaczyk, M. Szybowicz, T. Jesionowski; *Glucose determination using amperometric non-enzymatic sensor based on electroactive poly(caffeic acid)@MWCNT decorated with CuO nanoparticles*; Microchimica Acta, 189 (2022) 159. I contributed to the synthesis of the nanomaterial, construction of a non-enzymatic sensor, and electrochemical research, wrote the manuscript, drew up charts, tables, and drawings, and discussed with reviewers.	5.700	5.350	140
5.	**M. Kuznowicz**, T. Rębiś, A. Jędrzak, G. Nowaczyk, T. Jesionowski; *Preparation of poly(caffeic acid)@MWCNT- $Ni(OH)_2$ hybrid nanomaterial and its application as non-enzymatic glucose sensor*; Chemosensors, 11 (2023) 452. I contributed to synthesizing the hybrid nanomaterial, conducted electrochemical tests, described the results, wrote the entire manuscript, prepared graphs and tables, and discussed with the reviewers.	4.200	4.200	20
6.	**M. Kuznowicz**, A. Jędrzak, T. Jesionowski; *Nature-inspired biomolecular corona-based on poly(caffeic acid) as a low potential and time-stable glucose biosensor;* Molecules, manuscript ID: 2647041, under review. I received the hybrid material, conducted and assessed the effectiveness of immobilization, performed electrochemical tests, wrote part of the manuscript, prepared charts and tables.	4.600	4.900	140
	Summary	**31.413**	**29.360**	**700**

1. Abstract

Glucose measurements are one of the most important analyzes in medical diagnosis. They also play a key role in other fields of science and industry, i.e., environmental monitoring, pharmacy, and the food industry. However, commercial glucometers available on the market often present numerous errors and limitations. They result from many factors, including the design of materials, improper storage, coding errors, problems with the calibration of devices, the production process, and the significant impact of environmental conditions i.e., temperature and pH. The solution to some of these problems may include designing novel hybrid materials with improved properties (i.e., adsorption properties, stability).

The improvement of the sensor properties, in particular the sensitivity, selectivity, and time stability, was the motivation to undertake research on electroactive hybrid materials for sensor applications. Moreover, miniaturization of the system's setup was carried out to reduce the potential unit cost of analysis and increase the simplicity of quantification. An additional scientific value of the research was the extension of the time stability of the proposed systems.

As part of the presented doctoral dissertation, six scientific publications on the preparation of hybrid nanomaterials for electrochemical enzymatic biosensors and non-enzymatic sensors were performed. The publications attached as a part of the Ph.D. thesis presented the design, synthesis, characterization, and application of the resulting electroactive hybrid material in sensors. The obtained nanomaterials showed redox-active groups responsible for generating a signal on the electrode surface and using them to construct electrochemical biosensors and sensors.

To characterize the presented hybrid materials, numerous physicochemical and structural analyses were carried out, i.e., atomic force microscopy (AFM), electrophoretic light scattering (ELS), Fourier transform infrared spectroscopy (FTIR), high-resolution transmission electron microscopy (HR-TEM), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), as well as measurement of the zeta potential (ζ). The obtained sensors were thoroughly tested using electrochemical techniques, including amperometry, cyclic voltammetry, and electrochemical impedance spectroscopy.

In the **Publication 1** entitled "***Measurements of working parameters of external mediators for biodetectors based on the polydopamine@magnetite nanoparticles***" (**M. Kuznowicz**, A. Jędrzak, A. Leda, T. Rębiś, T. Jesionowski; *Measurement*, 184 (2021) 109950), the synthesis of a hybrid nanomaterial magnetite@polydopamine (Fe_3O_4@PDA) with immobilized glucose oxidase (GOx) derived from *Aspergillus niger* was presented. The Fe_3O_4@PDA-GOx was used to construct a second generation biosensor by modifying a glassy carbon electrode (GC). An important part of the research was to check the effect of different charge transfer mediators: (i) (hydroxymethyl) ferrocene (HFc); (ii) 1,4-benzoquinone (BQ); (iii) hydroquinone (HQ); (iv) methylene blue (MB); and (v) a mixture of potassium hexacyanoferrate(III) and potassium hexacyanoferrate(II) (FFC). The proposed hybrid material Fe_3O_4@PDA-GOx with (hydroxymethyl)ferrocene (HFc) was characterized by: (i) the lowest limit of detection (LOD); (ii) the widest linear range; and (iii) higher reproducibility compared to other tested mediators. Therefore, it was found beneficial in the constructed system with the enzyme glucose oxidase and served as an external mediator in subsequent glucose biosensors.

However, to increase the enzyme loading, linear range and time stability of the magnetite@polydopamine nanomaterial, the research was continued and the proposed material was enriched with β-cyclodextrins as an example of nanocontainers. The results were described in the **Publication 2** entitled "***Biomimetic magnetite/polydopamine/β-cyclodextrins nanocomposite for long-term glucose measurements***" (**M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; *Biochemical Engineering Journal*, 174 (2021) 108127). The resulting electroactive matrix was used to construct an amperometric enzymatic sensor using a glassy carbon electrode and (hydroxymethyl)ferrocene as a charge transfer mediator selected based on previous results (the **Publication 1**).

In the work mentioned above, it was observed that the addition of β-cyclodextrins to the matrix allowed: (i) an increase in the efficiency of the immobilization process; (ii) extending the linear range for glucose detection; (iii) longer time stability; and (iv) a lower limit of quantification (LOD) compared to the Fe_3O_4@PDA-GOx material. The presented biosensor system was finally used for electrochemical measurements on commercial real glucose solutions, i.e., standard glucose solutions, infusion fluids, and food products containing glucose.

The results observed for the polydopamine coating prompted the search for another catechol biopolymer that would be characterized by better electrochemical activity. Therefore, research was undertaken to optimize and design the synthesis of poly(caffeic acid).

In the **Publication 3** entitled "***Design and fabrication of low potential NADH-sensor based on poly(caffeic acid)@multi-walled carbon nanotubes***" (T. Rębiś, **M. Kuznowicz**, A. Jędrzak, G. Milczarek, T. Jesionowski; *Electrochimica Acta*, 386 (2021) 138384) research focused on a novel biopolymer - poly(caffeic acid) (PCA), to improve the electrochemical properties of the final hybrid material. The structure of poly(caffeic acid) contains both reduced and oxidized o-hydroquinone/o-quinones pairs that affect electron transfer reactions. As a result of chemical synthesis, multi-walled carbon nanotubes (MWCNT) were functionalized with poly(caffeic acid). The amperometric sensor GC/PCA@MWCNT was obtained and then used to detect nicotinamide adenine dinucleotide (NADH). The presented modification of poly(caffeic acid) resulted in: (i) increasing the amount of reactive functional groups compared to unmodified multi-walled carbon nanotubes; (ii) lower electrocatalytic potential of NADH oxidation; (iii) improvement of properties, i.e., sensitivity, linear range compared to poly(caffeic acid) obtained in the electropolymerization process.

The results obtained for the PCA@MWCNT hybrid material were continued to obtain non-enzymatic sensors in which the receptor material would be a metal-based compound. This activity was to try to improve the sensor's properties (sensitivity, linear range, limit of detection) compared to enzymatic sensors.

In the **Publication 4** entitled "***Glucose determination using amperometric non-enzymatic sensor based on electroactive poly(caffeic acid)@MWCNT decorated with CuO nanoparticles***" (**M. Kuznowicz**, T. Rębiś, A. Jędrzak, G. Nowaczyk, M. Szybowicz, T. Jesionowski; *Microchimica Acta*, 189 (2022) 159) a non-enzymatic sensor for detecting glucose was presented. Copper(II) oxide (CuO) was used to modify the surface of the PCA@MWCNT nanomaterial due to its electrocatalytic properties. To check the application possibilities, tests were carried out on real solutions, including human blood and serum. The design of the non-enzymatic sensor enabled: (i) reduction of unit measurement costs; (ii) reducing the limit of quantification; (iii) higher sensitivity; (iv) the ability to detect glucose at lower concentrations compared to enzyme sensors. The proposed method of synthesis of non-enzymatic electrode material presented an efficient and relatively cheap glucose detection

technique and the possibility of designing further nanomaterials that would use the properties of other transition metals and their oxides.

In order to improve the linear range and potentially reduce the impact of interference interference in further stages, it was decided to replace copper(II) oxide with nickel(II) hydroxide. For this purpose, the material poly(caffeic acid)@multi-wall carbon nanotubes was proposed - nickel(II) hydroxide in the **Publication 5** entitled "***Facile fabrication of selective poly(caffeic acid)@MWCNT-Ni(OH)$_2$ hybrid nanomaterial and its application as a non-enzymatic glucose sensor***" (**M. Kuznowicz**, T. Rębiś, A. Jędrzak, G. Nowaczyk, T. Jesionowski; *Chemosensors*, 11 (2023) 452). During the research, the process of electrodeposition of nickel(II) hydroxide on a hybrid nanomaterial based on poly(caffeic acid)@multi-walled carbon nanotubes (PCA@MWCNT) was carried out. The proposed sensor was characterized by a quick response to glucose additions, and the electrochemical tests, mainly amperometry and cyclic voltammetry, were used for detailed characteristics of the presented detector. Tests on real solutions, i.e., human blood and human serum, were characterized by high efficiency of glucose detection. They were compared with the results of tests carried out on a chemical analyzer adapted to measure glucose in the human blood of patients in the hospital. Moreover, the proposed non-enzymatic electrode material compared to GC/MWCNT@PCA-CuO (the **Publication 4**) was characterized by: (i) lower response time; (ii) higher time stability; (iii) lower limit of quantification.

The electrochemical properties of poly(caffeic acid) and the need to conduct measurements in pH corresponding to human blood, not in alkaline conditions as in the case of non-enzymatic sensors, were the inspiration to undertake research on an enzymatic glucose biosensor based on this biopolymer. Additionally, a key aspect of the conducted research was reducing the influence of interferents and extending the linear range in accordance with the guidelines of the World Health Organization (WHO) compared to previous proposed enzymatic biosensors and non-enzymatic sensors. These activities are presented in the **Publication 6** entitled "***Nature-inspired biomolecular corona-based on poly(caffeic acid) as a low potential and time-stable glucose biosensors***" (**M. Kuznowicz**, A. Jędrzak, T. Jesionowski*; Molecules,* manuscript ID: 2647041, under review). The novel core-shell magnetite@poly(caffeic acid) (Fe_3O_4@PCA) nanomaterial was designed and obtained to facilitate the magnetic separation of the material and increase biocompatibility. The crucial aspect of the research was the miniaturization of the system with the use of the screen-printed

electrode (SPE). This made it possible to reduce the volume of the sample necessary for the research, and the use of poly(caffeic acid) allowed the measurements to be carried out at a lower electrochemical potential than in the case of the previously obtained enzyme systems. The proposed hybrid nanomaterial was an electrocatalytic material for glucose detection characterized by the selective and sensitive detection of this monosaccharide. An additional advantage of the work was the extension of the time stability of the proposed system to 10 months of use. Compared to the previously presented papers (in the **Publications 1, 2, 4, 5**), the following were improved: (i) selectivity by using an enzyme and detection at a lower potential value; (ii) the linear range corresponding to the guidelines of the World Health Organization (WHO); (iii) miniaturization of the system was carried out, allowing measurements of a smaller sample volume; (iv) high recoveries of real samples. Moreover, it was decided to design an enzymatic sensor due to the possibility of conducting pH measurements corresponding to human blood, without the need for tests at alkaline pH as in the case of non-enzymatic sensors.

The proposed electroactive hybrid materials for biosensors and sensors in this Ph.D. dissertation, described in the publications mentioned above, may be an interesting alternative for sensitive, selective, and stable glucose measurement over time due to their unique physicochemical and electrochemical properties. The presented systems may be used mainly in medicine and also in the food and pharmaceutical industries.

2. Streszczenie

Pomiary glukozy są jedną z najważniejszych analiz w diagnostyce medycznej. Odgrywają także kluczową rolę w innych dziedzinach nauki i przemysłu, takich jak monitoring środowiska, farmacja czy przemysł spożywczy. Dostępne na rynku glukometry komercyjne często obarczone są jednak licznymi błędami i ograniczeniami. Wynikają one z wielu czynników, m.in. z zaprojektowania materiałów, niewłaściwego przechowywania, błędów w kodowaniu, problemów z kalibracją urządzeń, procesem produkcyjnym, a także znaczącym wpływem warunków środowiskowych takich jak temperatura, pH. Rozwiązaniem niektórych z tych problemów może być projektowanie nowych materiałów hybrydowych o ulepszonych właściwościach (tj. adsorpcja, stabilność).

Poprawa właściwości sensora, w szczególności czułości, selektywności i stabilności pracy w czasie, była motywacją do podjęcia badań nad elektroaktywnymi materiałami hybrydowymi do zastosowań sensorowych. Ponadto przeprowadzono miniaturyzację systemu, aby zmniejszyć potencjalny koszt jednostkowy analizy i zwiększyć prostotę oznaczania ilościowego. Dodatkową wartością naukową badań było wydłużenie stabilności czasowej proponowanych układów.

W ramach prezentowanej rozprawy doktorskiej przedstawiono sześć publikacji naukowych dotyczących przygotowania nanomateriałów hybrydowych do elektrochemicznych biosensorów enzymatycznych i nieenzymatycznych sensorów. Publikacje załączone w ramach niniejszej rozprawy doktorskiej przedstawiały projektowanie, syntezę, charakterystykę i zastosowanie powstałego elektroaktywnego materiału hybrydowego w biosesnorach i sensorach. Otrzymane nanomateriały wykazały grupy aktywne redoks odpowiedzialne za generowanie sygnału na powierzchni elektrody i wykorzystanie ich w konstrukcji biosensorów i sensorów elektrochemicznych.

W celu scharakteryzowania prezentowanych materiałów hybrydowych przeprowadzono liczne analizy fizykochemiczne i strukturalne, tj. mikroskopia sił atomowych (AFM, z ang. *atomic force microscopy*), elektroforetyczne rozpraszanie światła) (ELS, z ang. *electrophoretic light scattering*), spektroskopia w podczerwieni z transformacją Fouriera (FTIR, z ang. *Fourier transform infrared spectroscopy*), wysokorozdzielcza transmisyjna mikroskopia elektronowa (HR-TEM, z ang. *high-resolution transmission electron microscopy*), skaningowa mikroskopia elektronowa

(SEM, z ang. *scanning electron microscopy*), spektroskopia rentgenowska z dyspersją energii (EDS, z ang. *energy-dispersive X-ray spectroscopy*), transmisyjna mikroskopia elektronowa (TEM, z ang. *transmission electron microscopy*), rentgenowska spektroskopia fotoelektronów (XPS, z ang. *X-ray photoelectron spectroscopy*), a także pomiar potencjału dzeta (ζ). Otrzymane sensory zostały szczegółowo przebadane z wykorzystaniem technik elektrochemicznych tj., amperometria, woltamperometria cykliczna oraz elektrochemiczna spektroskopia impedancyjna.

W **Publikacji** 1 pt. „***Measurements of working parameters of external mediators for biodetectors based on the polydopamine@magnetite nanoparticles***" (**M. Kuznowicz**, A. Jędrzak, A. Leda, T. Rębiś, T. Jesionowski; *Measurement*, 184 (2021) 109950), synteza hybrydowego nanomateriału magnetyt@polidopamina (Fe_3O_4@PDA) z unieruchomioną oksydazą glukozową (GOx) pochodzącą z *Aspergillus niger* została przedstawiona. Nanomateriał Fe_3O_4@PDA-GOx wykorzystano do skonstruowania biosensora drugiej generacji poprzez modyfikację elektrody z węgla szklistego (GC, z ang. *glassy carbon electrode*). Ważną częścią badań było sprawdzenie wpływu różnych mediatorów przeniesienia ładunku: (i) (hydroksymetylo)ferrocenu (HFc); (ii) 1,4-benzochinon (BQ); (iii) hydrochinon (HQ); (iv) błękit metylenowy (MB); oraz (v) mieszaninę heksacyjanożelazianu(III) potasu i heksacyjanożelazianu(II) potasu (FFC). Zaproponowany materiał hybrydowy Fe_3O_4@PDA-GOx z (hydroksymetylo)ferrocenem (HFc) charakteryzował się: (i) najniższą granicą wykrywalności (LOD); (ii) najszerszym zakresem liniowości; oraz (iii) wyższą powtarzalność w porównaniu z innymi testowanymi mediatorami. W związku z czym został uznany za korzystny w skonstruowanym układzie z enzymem - oksydazą glukozową i posłużył jako zewnętrzny mediator w kolejnych biosensorach glukozy.

Jednakże, aby zwiększyć załadunek enzymu, zakres liniowości i badania stabilności w czasie nanomateriału magnetyt@polidopamina, badania kontynuowano a zaproponowany materiał wzbogacono o β-cyklodekstryny, jako przykład nanokontenerów. Wyniki opisano w **Publikacji 2** pt. „***Biomimetic magnetite/polydopamine/β-cyclodextrins nanocomposite for long-term glucose measurements***" (**M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; *Biochemical Engineering Journal*, 174 (2021) 108127). Materiał hybrydowy Fe_3O_4@PDA został wzbogacony o β-cyklodekstryny, w celu zwiększenia załadunku enzymu - oksydazy glukozowej. Powstała elektroaktywna matryca posłużyła do konstrukcji

amperometrycznego sensora enzymatycznego z wykorzystaniem elektrody z węgla szklistego oraz (hydroksymetylo)ferrocenu jako mediatora przeniesienia ładunku wybranego na podstawie wcześniejszych wyników (**Publikacja 1**).

W powyższej pracy zaobserwowano, że dodatek β-cyklodekstryn do matrycy pozwolił na: (i) zwiększenie efektywności procesu immobilizacji; (ii) rozszerzenie zakresu liniowości wykrywania glukozy; (iii) dłuższą stabilność czasu pracy; oraz (iv) niższą granicę oznaczalności (LOD) w porównaniu z materiałem Fe_3O_4@PDA-GOx. Zaprezentowany biosensor finalnie posłużył do pomiarów elektrochemicznych w komercyjnych roztworach rzeczywistych glukozy m.in. wzorcowych roztworach glukozy, płynach infuzyjnych i produktach spożywczych zawierających glukozę.

Wyniki zaobserwowane dla powłoki polidopaminy skłoniły do poszukiwań innego katecholowego biopolimeru, który charakteryzowałaby się większą aktywnością elektrochemiczną. W związku z tym podjęto badania mające na celu optymalizację i zaprojektowanie syntezy poli(kwasu kawowego).

W **Publikacji 3** zatytułowanej „***Design and fabrication of low potential NADH-sensor based on poly(caffeic acid)@multi-walled carbon nanotubes***" (T. Rębiś, **M. Kuznowicz**, A. Jędrzak, G. Milczarek, T. Jesionowski; *Electrochimica Acta*, 386 (2021) 138384) badania skupiały się na nowatorskim biopolimerze – poli(kwasie kawowym) (PCA, z ang. *poly(caffeic acid)*), w celu poprawy właściwości elektrochemicznych końcowego materiału hybrydowego. Struktura poli(kwasu kawowego) zawiera zarówno zredukowane, jak i utlenione pary o-hydrochinon/o-chinon, które wpływają na reakcje przeniesienia elektronu. W wyniku syntezy chemicznej wielościenne nanorurki węglowe (MWCNT, z ang. *multi-walled carbon nanotubes*) funkcjonalizowano poli(kwasem kawowym). Otrzymano czujnik amperometryczny GC/PCA@MWCNT, a następnie wykorzystano do wykrywania dinukleotydu nikotynoamidoadeninowego (NADH, z ang. *nicotinamide adenine dinucleotide*). Przedstawiona modyfikacja poli(kwasem kawowym) spowodowała: (i) zwiększenie ilości reaktywnych grup funkcyjnych w porównaniu z niemodyfikowanymi wielościennymi nanorurkami węglowymi; (ii) obniżenie potencjału elektrokatalitycznego utleniania NADH; (iii) poprawę właściwości, tj. czułość, zakres liniowości w porównaniu do poli(kwasu kawowego) otrzymanego w procesie elektropolimeryzacji.

Wyniki uzyskane dla materiału hybrydowego PCA@MWCNT kontynuowano w celu konstrukcji sensorów nieenzymatycznych, w których częścią receptorową byłby związek na bazie metalu. Miało to na celu poprawę właściwości czujnika (czułość, liniowość, granicę oznaczalności) w porównaniu z czujnikami enzymatycznymi.

W **Publikacji 4** pt. „***Glucose determination using amperometric non-enzymatic sensor based on electroactive poly(caffeic acid)@MWCNT decorated with CuO nanoparticles***" (**M. Kuznowicz**, T. Rębiś, A. Jędrzak, G. Nowaczyk, M. Szybowicz, T. Jesionowski; *Microchimica Acta*, 189 (2022) 159) przedstawiono nieenzymatyczny sensor do detekcji glukozy. Do modyfikacji powierzchni nanomateriału PCA@MWCNT ze względu na jego właściwości elektrokatalityczne wykorzystano tlenek miedzi(II) (CuO). Aby sprawdzić możliwości zastosowania, przeprowadzono badania na rzeczywistych roztworach, w tym na ludzkiej krwi i osoczu. Konstrukcja czujnika nieenzymatycznego umożliwiła: (i) redukcję jednostkowych kosztów pomiaru; (ii) zmniejszenie granicy oznaczalności; (iii) wyższą czułość; (iv) zdolność do wykrywania glukozy w niższych stężeniach w porównaniu z czujnikami enzymatycznymi. Zaproponowana metoda syntezy nieenzymatycznego materiału elektrodowego przedstawiła wydajną i stosunkowo tanią technikę wykrywania glukozy oraz możliwość projektowania kolejnych nanomateriałów, które wykorzystywałyby właściwości innych metali przejściowych i ich tlenków.

Aby poprawić zakres liniowości i potencjalnie zmniejszyć wpływ zakłóceń interferentów w dalszych etapach zdecydowano się zastąpić tlenek miedzi(II) wodorotlenkiem niklu(II). W tym celu zaproponowano materiał poli(kwas kawowy)@wielościenne nanorurki węglowe - wodorotlenek niklu(II) w **Publikacji 5** pt. „***Facile fabrication of selective poly(caffeic acid)@MWCNT-Ni(OH)$_2$ hybrid nanomaterial and its application as a non-enzymatic glucose sensor***" (**M. Kuznowicz**, T. Rębiś, A. Jędrzak, G. Nowaczyk, T. Jesionowski; *Chemosensors*, 11 (2023) 452). W trakcie badań przeprowadzono proces elektroosadzania wodorotlenku niklu(II) na nanomateriale hybrydowym wielościenne nanorurki węglowe@poli(kwas kawowy) (PCA@MWCNT). Zaproponowany sensor charakteryzował się szybką reakcją na dodatki glukozy, a do szczegółowej charakterystyki prezentowanego sensora wykorzystano badania elektrochemiczne, głównie amperometrię i woltamperometrię cykliczną. Badania na roztworach rzeczywistych, tj. krew ludzka i osocze, charakteryzowały się wysoką skutecznością wykrywania glukozy. Porównano je z wynikami badań przeprowadzonych na analizatorze chemicznym przystosowanym do

pomiaru glukozy we krwi ludzkiej pacjentów szpitala. Ponadto zaproponowany nieenzymatyczny materiał elektrodowy w porównaniu do GC/MWCNT@PCA-CuO (**Publikacja 4**) charakteryzował się: (i) krótszym czasem odpowiedzi; (ii) większą stabilnością w czasie; (iii) niższą granicą oznaczalności.

Właściwości elektrochemiczne poli(kwasu kawowego) oraz potrzeba prowadzenia pomiarów w pH odpowiadających krwi ludzkiej, nie w warunkach alkalicznych jak w przypadku nieenzymatycznych sensorów były inspiracją do podjęcia badań nad enzymatycznym biosensorem glukozy opartym na tym biopolimerze. Dodatkowo kluczowym aspektem prowadzonych badań było ograniczenie wpływu czynników zakłócających i poszerzenie zakresu liniowości zgodnie z wytycznymi Światowej Organizacji Zdrowia (WHO) w porównaniu do wcześniej proponowanych biosensorów enzymatycznych i czujników nieenzymatycznych. Ponadto zdecydowano się na konstrukcję sensora enzymatycznego ze względu na możliwość prowadzenia pomiarów pH odpowiadającym krwi ludzkiej, bez konieczności testów w alkalicznym pH jak w przypadku nieenzymatycznych sensorów. Działania te zostały zaprezentowane w **Publikacji 6** zatytułowanej „***Nature-inspired biomolecular corona-based on poly(caffeic acid) as a low potential and time-stable glucose biosensors***" (**M. Kuznowicz**, A. Jędrzak, T. Jesionowski, *Molecules,* manuscript ID: 2647041, w trakcie recenzji). Zaprojektowano i otrzymano nowatorski nanomateriał typu *core-shell* magnetyt@poli(kwas kawowy) (Fe_3O_4@PCA), aby ułatwić separację magnetyczną materiału i zwiększyć biokompatybilność. Kluczowym aspektem badań była miniaturyzacja układu za pomocą elektrody sitodrukowej (SPE z ang. *screen printed electrode*). Umożliwiło to zmniejszenie objętości próbki niezbędnej do badań, a zastosowanie poli(kwasu kawowego) pozwoliło na prowadzenie pomiarów przy niższym potencjale elektrochemicznym, niż w przypadku otrzymanych wcześniej układów enzymatycznych. Zaproponowane nanomateriały hybrydowe były materiałem elektrokatalitycznym do wykrywania glukozy charakteryzującym się selektywną i czułą detekcją tego monosacharydu. Dodatkowym atutem pracy było wydłużenie stabilności czasowej zaproponowanego systemu do 10 miesięcy użytkowania. W porównaniu do wcześniej przedstawionych prac (**w Publikacjach 1, 2, 4, 5**) poprawiono: (i) selektywność poprzez zastosowanie enzymu i detekcję przy niższej wartości potencjału; (ii) zakres liniowości odpowiadający wytycznym Światowej Organizacji Zdrowia (WHO z ang. *World Health Organization*); (iii) przeprowadzono

miniaturyzację układu, umożliwiając pomiary mniejszej objętości próbki; (iv) uzyskano wysokie odzyski próbek rzeczywistych.

Zaproponowane w tej rozprawie doktorskiej elektroaktywne materiały hybrydowe do biosensorów i czujników. Rozprawa doktorska opisana w powyższych publikacjach może stanowić interesującą alternatywę dla czułego, selektywnego i stabilnego w czasie pomiaru glukozy ze względu na ich unikalne właściwości fizykochemiczne i elektrochemiczne. Prezentowane systemy mogą znaleźć zastosowanie głównie w medycynie, ale także w przemyśle spożywczym i farmaceutycznym.

3. Introduction

3.1. Biomimetic coatings based on catechol polymers

Adhesive materials derived from mussels, which contain catechol groups in their structure, are valuable materials for industrial, biomedical, and pharmaceutical applications. Their usefulness results from their properties, including hydrophilicity, permeability, and stability on various surfaces [1].

Catechol moieties in 3,4-dihydroxy-L-phenylalanine (L-DOPA) can form chemical interactions, i.e., cross-linking by oxidation, which show excellent adhesion properties for various materials, like inorganic metals, metal oxides, alloys, polymers, glass, wood, and ceramics [1,2].

The growing material chemistry market demand is determined by new research efforts to create new catechol-based adhesive compounds that provide better adhesion of components and surface coatings [3]. The examples of catechol compounds used for surface modification are shown in Fig. 1.

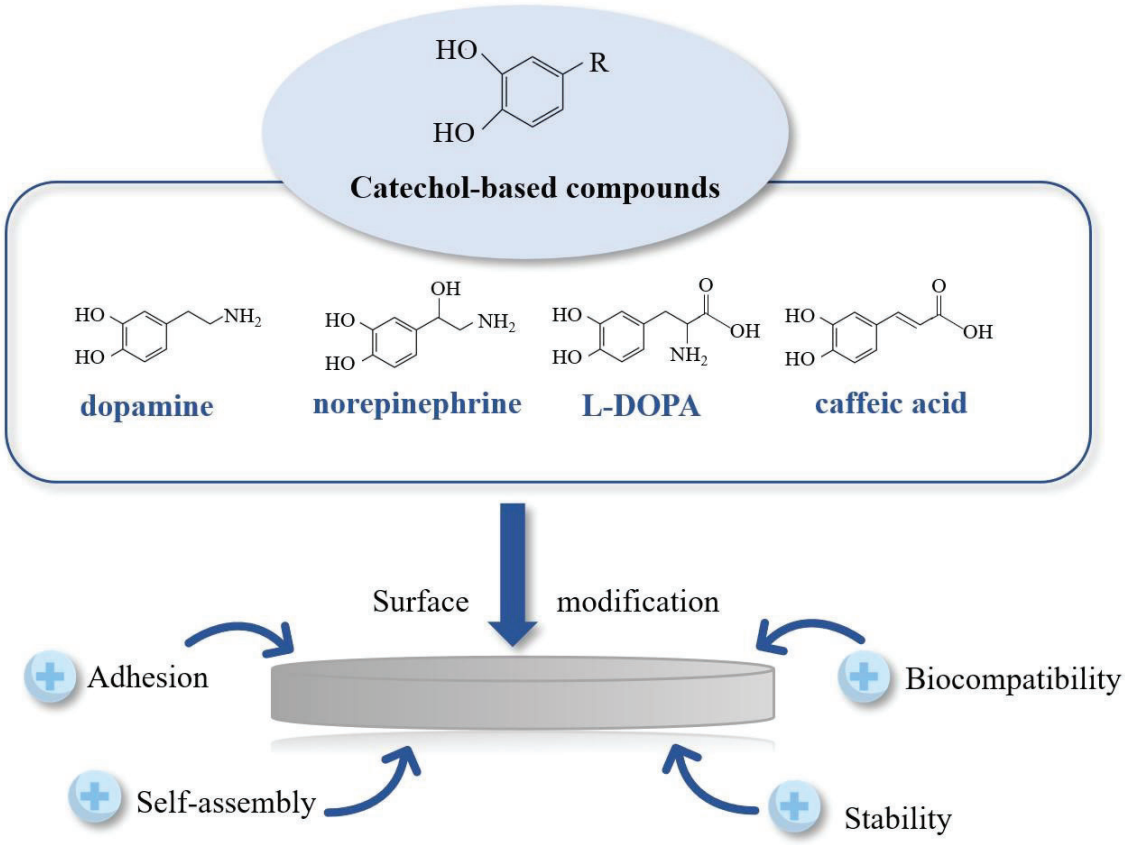


Fig. 1. *Catechol compounds and their influence on modified surfaces, based on* [4].

Recent research on catechol polymers focuses on their unique properties, shown schematically in Fig. 2.

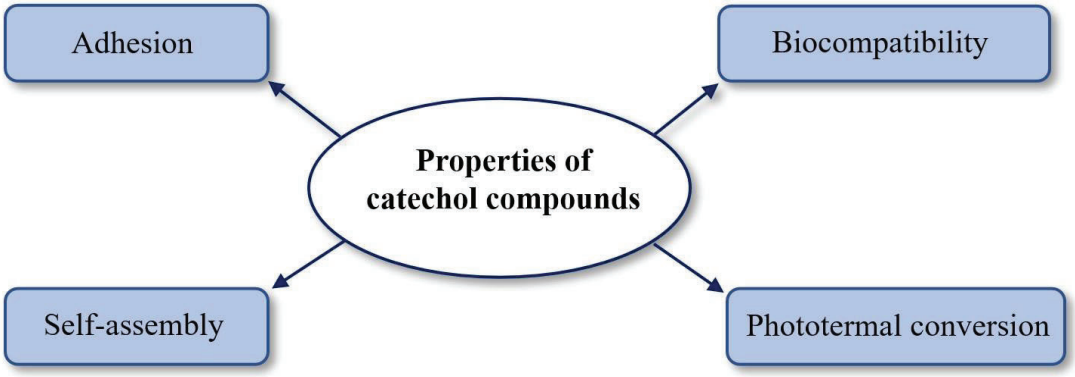


Fig. 2. *The properties of catechol compounds for their use in the construction of hybrid materials, based on* [4,5].

Catechol polymers present adhesive properties, enabling various applications, i.e., coatings and sealants. They are biocompatible and not harmful to living tissue, which is valuable in drug delivery and tissue engineering. Due to their stability, they can withstand harsh environmental conditions without degradation. Moreover, these materials can organize themselves into ordered structures without external intervention, which allows their applications in nanotechnology and drug delivery [4,5].

An adhesive material should have two key properties: (i) **interfacial adhesion**, which assists material adsorption onto surfaces, and (ii) **cohesion,** which promotes intermolecular attraction. The catechol component, which can interact with various inorganic or organic surfaces, is assumed to cause the interfacial adhesion [6].

Catechols' phenyl rings interact hydrophobically with surfaces through van der Waals forces, which are essential to the adsorption process [7]. These compounds primarily bind to metallic or metal oxide surfaces by coordination bonding. Due to the numerous uses of titanium-related technologies in biomedicine and energy, the interactions between catechols, titanium, and titanium(II) oxide were explored [8].

In addition, catechols covalently link to organic substrates that present amino or thiol groups in their structure via Michael addition or Schiff base generation. This process starts when these functionalities react with the quinone moiety generated from the catechols [9].

Figure 3 shows different mechanisms of catechol attachment depending on the type of bonds.

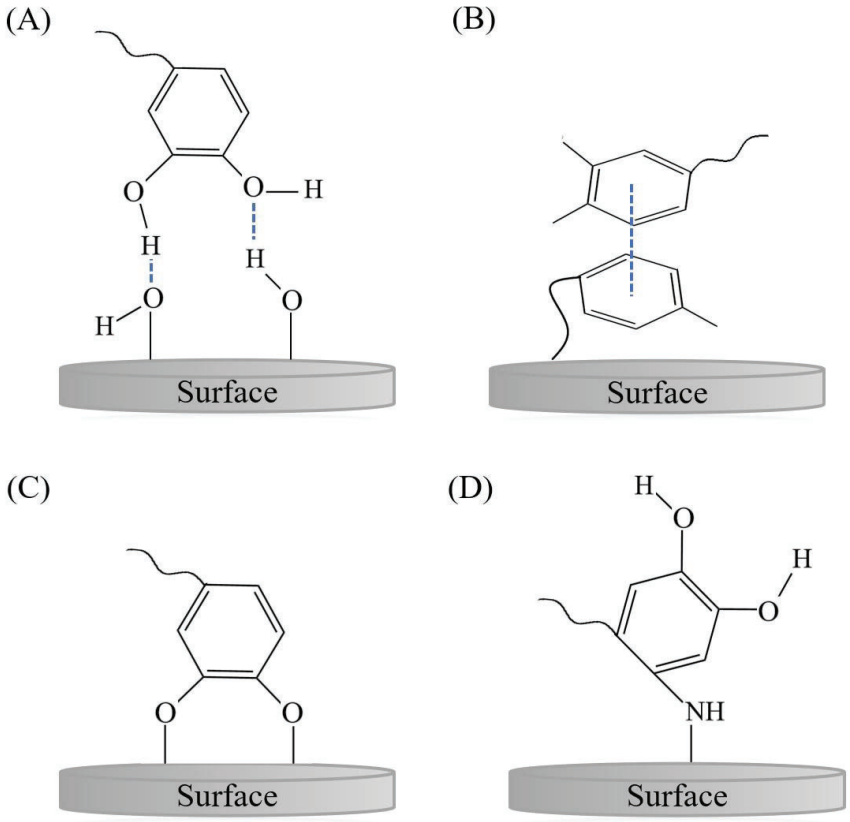


Fig. 3. *Schematic representation of catechol–surface interactions: hydrogen bonding (A); π–π stacking (B); coordination (C); covalent bonding with surface amine (D), based on* [6].

3.1.1. Characteristics of polydopamine coatings

Polydopamine (PDA) is a biomimetic polymer formed as a result of oxidation, which, due to its adhesive properties, enables the coating of various substrates [10].

The precursor for obtaining PDA is an organic compound – dopamine (DA). The oxidation of dopamine is typically initiated by an oxidizing agent, i.e., oxygen, which promotes the formation of reactive quinone intermediates. These compounds undergo a series of reactions that ultimately lead to the formation of PDA [11]. Two key intermediates of this synthesis are distinguished: dopamine quinone and 5,6-dihydroxyindole [11].

This biopolymer is a material composed of oligomeric building blocks generated spontaneously by further oxidation of 5,6-dihydroxyindole and coupling via 2-2', 2-4', or 2-7' bonds [12]. The PDA polymerization process is shown schematically in Fig. 4.

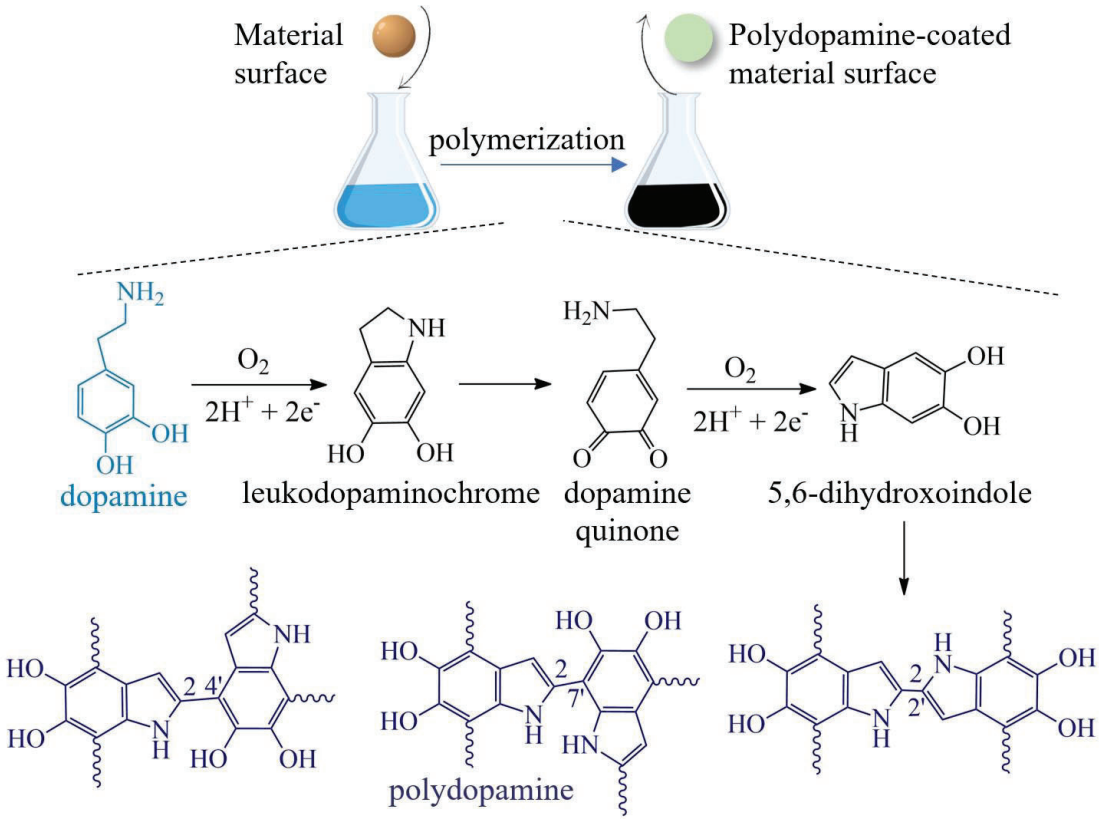


Fig. 4. *Scheme of PDA coating, with polymerization reactions, based on* [13].

The properties of polydopamine, including natural hydrophilicity or adhesiveness, result from the presence of catechol and amino functional groups. Characteristics of polydopamine are presented in Fig. 5.

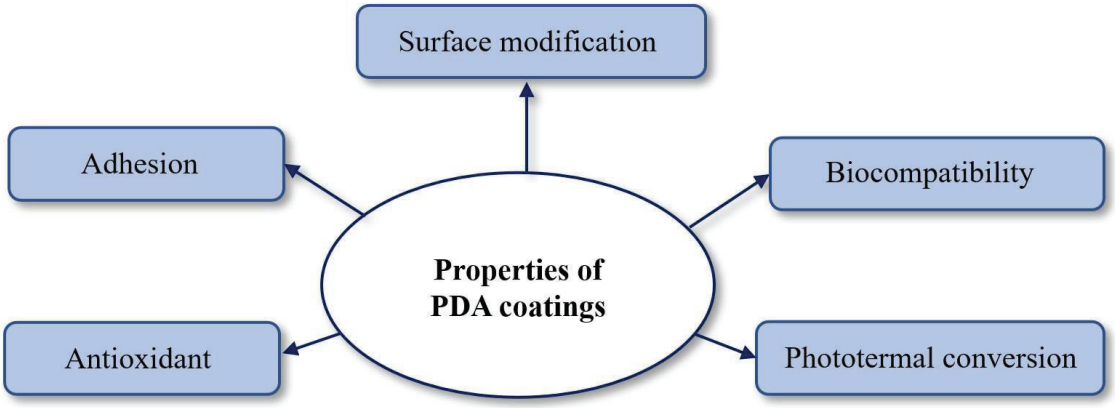


Fig. 5. *Examples of properties of the polydopamine coatings, based on* [14,15].

The PDA–coating enables to adhere to a wide range of substrates, including metals, polymers, ceramics, and biological surfaces [12]. These coatings are biocompatible and do not cause an adverse reaction during contact with biological

systems. This biopolymer can absorb light and convert it into heat. Make it a potential agent for photothermal therapy or energy conversion. This biomimetic compound presents antioxidant properties, which makes it an effective scavenger of free radicals. The PDA can modify the surface properties of a substrate, i.e., wettability, surface charge, and zeta potential [15].

The PDA structure presents a wide range of applications, and this biomimetic compound can be a surface coating for various substrates, including metals, polymers, ceramics, and biological. In this case, the coating can improve adhesion, biocompatibility, and durability of the matrix [16]. Example fields of polydopamine applications are schematically present in Fig. 6.

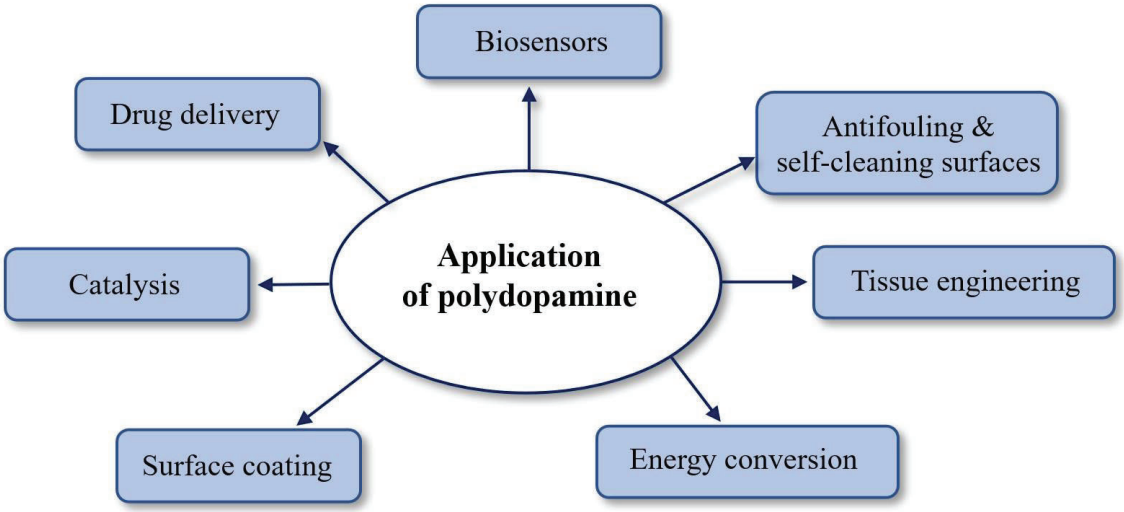


Fig. 6. *Exemplary directions of polydopamine application, based on* [17].

Polydopamine can be used as a carrier for drug delivery due to its biocompatibility and surface capabilities modification [18]. Moreover, this biomimetic polymer, due to its adhesion and surface modification properties, finds application in scaffold materials for tissue engineering [17]. PDA can modify the surface properties of a substrate to make it antifouling and self-cleaning. This property makes it useful for various applications, i.e., marine coatings and food packaging [19].

This biomimetic polymer can be applied as a catalyst for various reactions due to its ability to absorb and transfer electrons. This characteristic led to its use, for instance, in water treatment and organic synthesis [20,21]. Moreover, polydopamine can be used in solar cells and other energy conversion [22].

The nanomaterial coating with PDA finds the application for sensors to improve their sensitivity and selectivity and increase the loading of biological material [23].

Polydopamine as a surface layer in glucose biosensors was presented by Fedorenko et al. The proposed ZnO/PDA structure was applied for the photo-electrochemical determination of glucose in aqueous solutions. Fast and reliable sensor responses were recorded using this technique in the concentration range from 0.0062 to 0.120 mM [15].

Martín et al. developed the Fe_3O_4/PDA system for glucose detection. The magnetite nanoparticles (obtained via co-precipitation) were covered with *in situ* polymerization. The hybrid material obtained by this technique showed a core diameter of 17 nm and a polymer coating thickness of 2 nm. Using produced nanoparticles, an amperometric biosensor for glucose detection was built and applied to determine glucose in blood samples [24].

Wei et al. used polydopamine foils with gold nanoparticles to provide an effective approach for immobilizing enzymes for electrochemical biosensors. This enzyme loading was carried out in mild circumstances and kept for time stability with bioactivity. The manufactured amperometric glucose biosensor showed repeatable sensitivity and a broad linear range [25].

3.1.2. General information about poly(caffeic acid) coatings

Poly(caffeic acid) (PCA) is an aromatic, catechol biopolymer obtained by polymerization of its monomer caffeic acid (3,4-hydroxycinnamic acid, CA) [26].

It was demonstrated that an electroactive polymer layer is produced when caffeic acid (CA) is oxidized over various electrode materials, i.e., platinum, carbon, and gold. The obtained polymer was examined for its electrochemical properties and use as a sensing material due to the presence of functional groups that enable electron transfer [27].

Polymerization of poly(caffeic acid) by polycondensation was presented by Ishii et al. They defend the effect on the solubility and thermal properties of the obtained product. In the proposed procedure, sodium acetate and acetic anhydride were used for transesterification of caffeic acid [28].

In oxidative coupling, the polymerization occurs spontaneously in the presence of an oxidizing agent, i.e., hydrogen peroxide (H_2O_2) or sodium periodate ($NaIO_4$).

The reaction starts with the oxidation of the phenolic hydroxyl group of the caffeic acid monomer to form a phenoxy radical. This compound then reacts with another caffeic acid monomer to form a dimer, which is repeated to form longer chains.

The polymerization of PCA can be controlled by setting the concentration of the oxidizing agent and the reaction time [29]. Figure 7 shows a scheme of the synthesis of poly(caffeic acid).

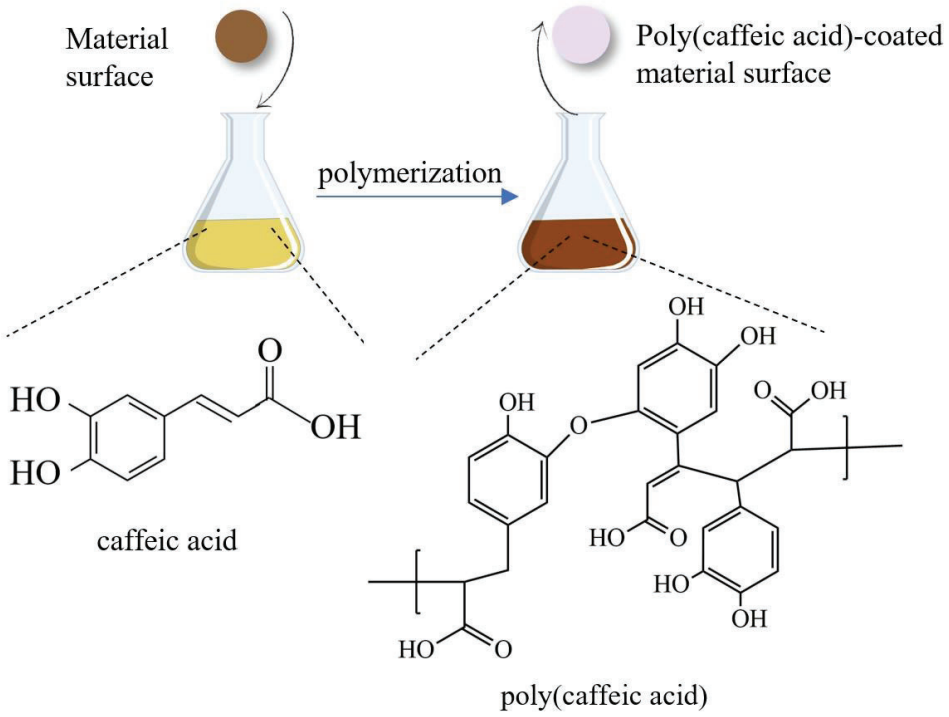


Fig. 7. *Schematic representation of the poly(caffeic acid) polymerization process, based on* [30].

The prooxidant effects of poly(caffeic acid) selectively induce oxidative stress in cancer cells, resulting in apoptosis and tumor growth inhibition while sparing healthy cells due to its antioxidant properties, thus conferring its anticancer qualities [31].

Additionally, this biopolymer participates in reversible electron transfer and mediates the target molecule's reduction [32]. Poly(caffeic acid) films are biocompatible, and do not cause an adverse reaction with biological tissues. Moreover, The polymer's structure and properties may make it suitable for controlled drug delivery systems, where drugs are gradually released from the film [30]. Examples of properties of PCA coatings are presented in Fig. 8.

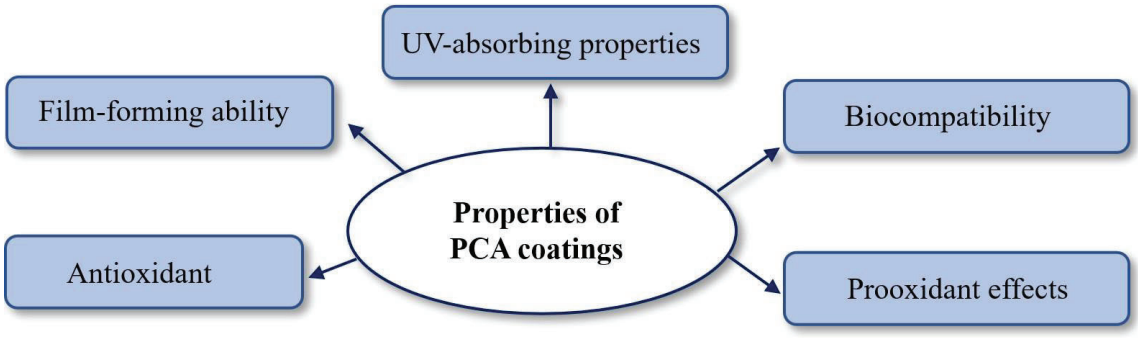


Fig. 8. *Examples of PCA coating features in hybrid materials, based on* [29].

Furthermore, PCA can absorb ultraviolet (UV) radiation, which makes it useful in the development of sunscreens and other UV-protective coatings [33]. The mechanical properties of poly(caffeic acid) films, including flexibility and strength, can potentially be customized by modifying polymer structure or processing conditions. PCA could form thin films, which can be used in various applications, i.e., coatings, adhesives, and composites [34]. Moreover, the catechol compound exhibited antibacterial activity against certain strains of bacteria [33].

Poly(caffeic acid), during its properties, has gained popularity over the past few years. Examples of the application and recommendations for using poly(caffeic acid) are presented in Fig. 9.

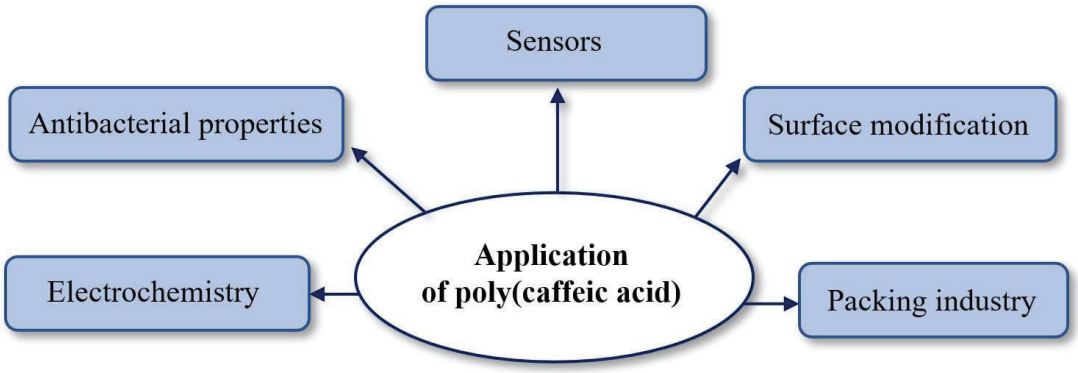


Fig. 9. *Potential applications of poly(caffeic acid), based on* [29].

This phenolic compound has drawn attention to its potential medical uses in regenerative medicine and tissue engineering as a scaffold material. Its biocompatibility and ability to promote cell adhesion and tissue growth make it valuable for creating artificial tissues and organs [35]. Furthermore, PCA was studied for its possible use in biomedical engineering applications [36].

Poly(caffeic acid) is a synthetic polymer based on natural ingredients that can be used in ecological packaging materials, reducing the impact of single-use plastics on the environment [37]. There is estimated to be further development of PCA implementation in the packaging sector [29].

Li et al. proposed a poly(caffeic acid) thin film, which was made by the potentiostatic technique in a solution containing caffeic acid on the GC electrode surface. Through the use of cyclic voltammetry, the ascorbic acid (AA), dopamine (DA), and their combination were measured on the poly(caffeic acid)-modified electrode. A well-separated oxidation peak toward AA and DA was followed by a potent and sustained electron-mediating behavior on this modified electrode [38].

Lee et al. presented the electropolymerization of caffeic acid with carbon nanotubes onto the electrode surface. The voltammetric behavior of the electrode was described by the ortho-quinone moiety on the caffeic acid unit and the optimal surface loading for the current response. A reported nanocomposite-mediated electrode allowed for the exact detection of glutathione [39].

Rohanifar et al. present an electrochemical sensor based on the PCA for the 3,4-dihydroxy-L-phenylalanine (L-DOPA). The glassy carbon electrode (GC) was used as the substrate for the electrodeposition of a thin layer of poly(caffeic acid). In the presence of typical biological interferences, such as ascorbic acid and uric acid, the modified electrode demonstrated good selectivity for L-DOPA [40].

Li et al. proposed an Au/poly(caffeic acid) composite and the electrocatalytic activity by the AuNPs/PCA-GC electrode related to the oxidation of acetaminophen. Analyzing AP in blood, urine, and drug sample has successfully implemented and validated the AuNPs/PCA-GC electrode [41].

3.2. Hybrid materials for electrochemical applications

Hybrid materials are composed of two or more components, typically combined at the nanoscale level, including organic and inorganic elements. This combination results in properties and functionalities that differ from their initial features [42].

The organic component usually creates a bond between the inorganic building blocks and often contains functional groups, such as hydroxyl (-OH) or amino ($-NH_2$). These chemical groups can participate in reactions, i.e., hydrogen bonding or covalent

bonding with the inorganic components. In contrast, the inorganic part typically provides mechanical strength and an overall structure to natural materials [43].

The division of hybrid materials involves categorizing based on their composition, properties, and the nature of interactions. According to their chemical bonding patterns, Makishima divided substances into metals, organic materials, polymers, and ceramics. In his theory, hybrid materials are combinations of two or more materials having recently established chemical connections [44]. He suggested the following categories for hybrid materials and items connected to them: (i) **composites** are materials mixture with micro-scale dispersion; (ii) **nanocomposites** are the sub-micron scale combination of similar kinds of materials; (iii) **hybrids** are a variety of materials at the sub-micron scale; (iv) **nanohybrids** are a combination of diverse materials at the atomic or molecular level with chemical linkages between them [44].

One of the most popular classifications is the division into two classes, often used in materials science to provide a framework for understanding and categorizing hybrid materials.

Hybrid class I have weak interactions between the two components, for instance, van der Waals and hydrogen bonds. Moreover, hybrids class II present strong chemical bonds (covalent, coordination, and ionic bonds) [45]. Both classes have their applications and advantages, depending on the desired properties and performance characteristics. The classification of hybrid materials is shown in Fig. 10.

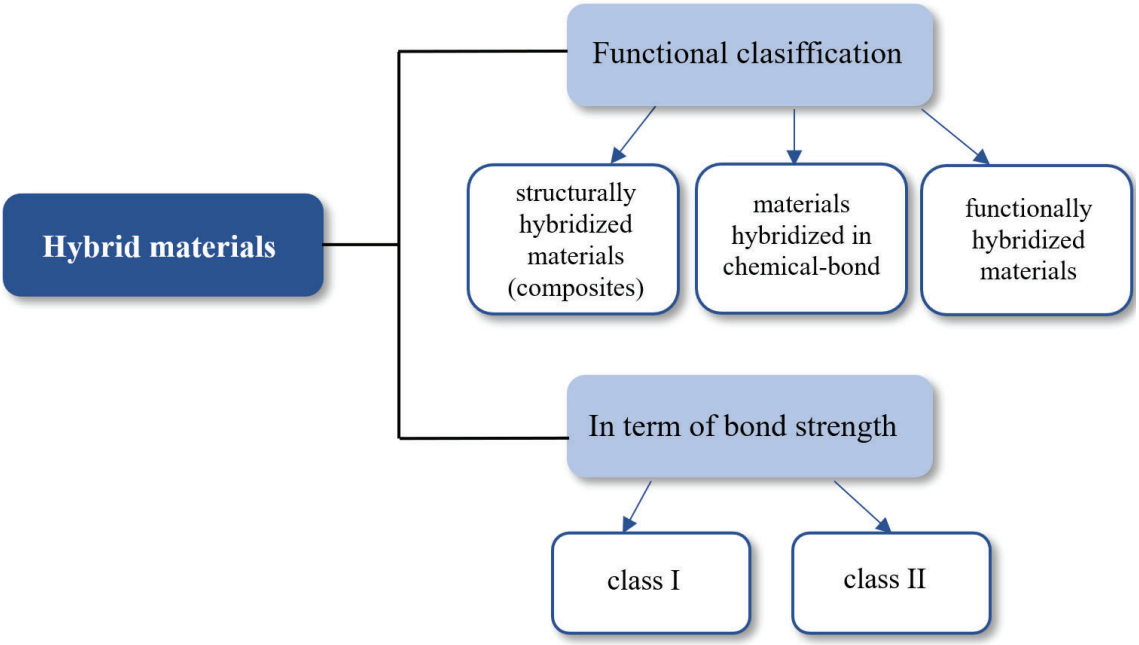


Fig. 10. *Classification of hybrid materials, based on* [46].

Hybrid materials can be divided based on functionality into three types: structurally hybridized materials (composites), inorganically hybridized materials, and functionally hybridized materials [46].

To form hybrid materials, inorganic components (metal oxides, phosphates, carbonates, chalcogenides, and their derivatives) are mixed at the molecular or nanoscopic level with organic ingredients (molecules) or networks (organic polymers) [47].

There are two ways that organic components can be included in an inorganic network: as network modifiers (molecules) or network formers (macromolecules). Organosilicon alkoxides or chlorides connect the most popular network modifiers or formers to inorganic moieties [48].

A comparison of selected properties of the organic and inorganic hybrid materials is summarized in Table 1.

Table 1. *Comparison of properties of an inorganic and organic part of hybrid materials, based on* [49].

Qualities of components	**Organic material**	**Inorganic material**
Density	lower	higher
Mechanical properties	soft	hard
Thermal stability	lower	higher
Photoelectric properties	lower	higher
Refractive index	lower	higher
Surface charges	versatile	limited
Colloidal & nanoparticles stability	higher	lower

Due to the possibility of designing properties, hybrid materials are used in many areas of materials chemistry. Currently, the synthesis of inorganic-organic materials focuses on four key areas: (i) molecular engineering; (ii) organization at the nano- and micrometer scales; (iii) the shift from functional to multifunctional hybrids; (iv) the incorporation of bioactive components [43]. The most visible advantage of these materials is the ability to combine even different characteristics of inorganic and organic components in one material [43].

Hybrid materials were applied in many fields of science and industry and have become increasingly popular for enzyme immobilization as a result of their unique properties and ability to enhance enzyme stability, activity, and selectivity. Immobilization of enzymes on hybrid materials can improve their operational stability,

reusability, and biocompatibility, making them attractive for various applications in biotechnology, the food industry, and medicine [50].

The advantage of immobilization of biological material is the increase in the efficiency of the biotechnological process [51]. The examples of the application of hybrid materials are shown in Fig. 11.

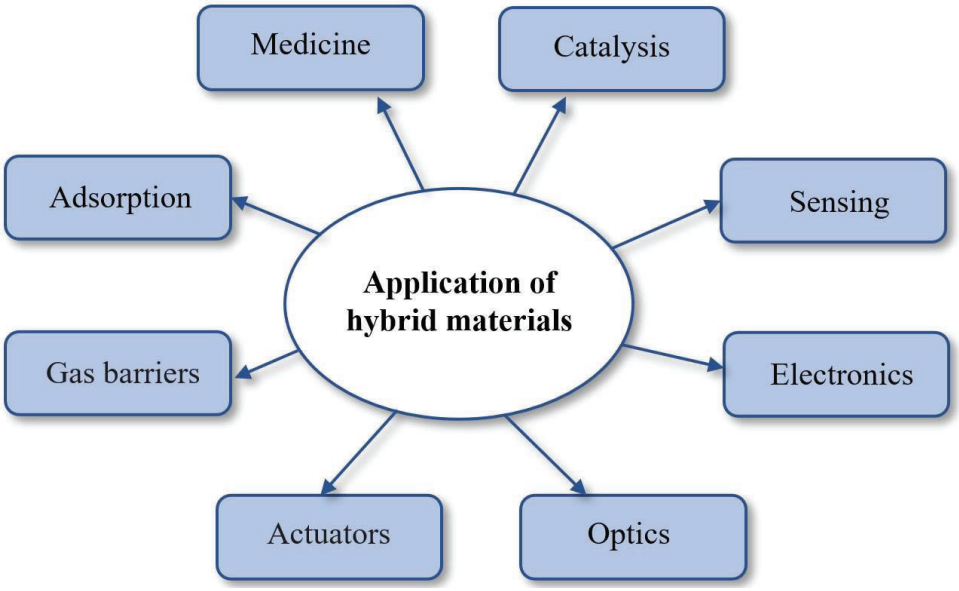


Fig. 11. *Application of hybrid materials, based on* [52].

Due to their synergistic effect obtained by combining inorganic and organic parts, hybrid materials present the potential for immobilization [53].

The use of inorganic components when designing the system allows for an increase in both mechanical and environmental stability (lower influence of factors, i.e., pH or temperature). In addition, they increase the sorption capacity and facilitate the functionalization of the system [53]. The organic part is responsible for biocompatibility, and modification of the material provides functional groups and is compatible with enzymes. Furthermore, the carrier material used for the immobilization should be insoluble in the reaction medium [54].

Due to the individual organic-inorganic components, the hybrid material allows for increased stability, improved loading of enzymes, and higher similarity to biological material. In addition, the appropriate design of hybrid materials allows for obtaining improved, specific, and desired properties [54].

Examples of the characteristics of individual component materials and their properties are schematically shown in Fig. 12.

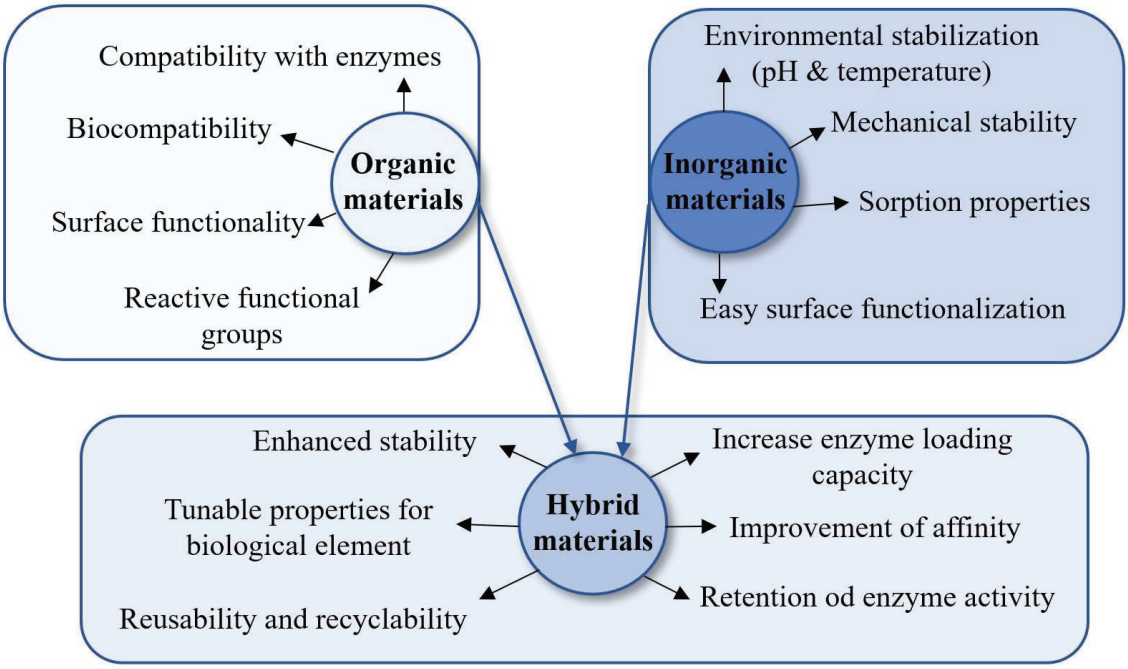


Fig. 12. *Properties of hybrid materials for immobilization of biomolecules, based on* [54,55].

In the context of sensor applications, hybrid materials have emerged as promising candidates for use in various sensing applications due to their unique properties that can lead to improved performance compared to traditional materials caused by the customization of sensor responses for different analytes or environmental conditions.

Hybrid materials in electrochemical biosensors and sensors can play different roles, depending on their properties. The most important functions of these materials include: (i) **immobilization support for biomaterial**; (ii) **mediator providing electron transport**; (iii) **signal amplifier** [57]. Appropriate features resulting from the synergistic effect of building ingredients determine its final role in this field [56].

The use of hybrid materials in the design and production of sensors brings many benefits, as presented in Fig. 13.

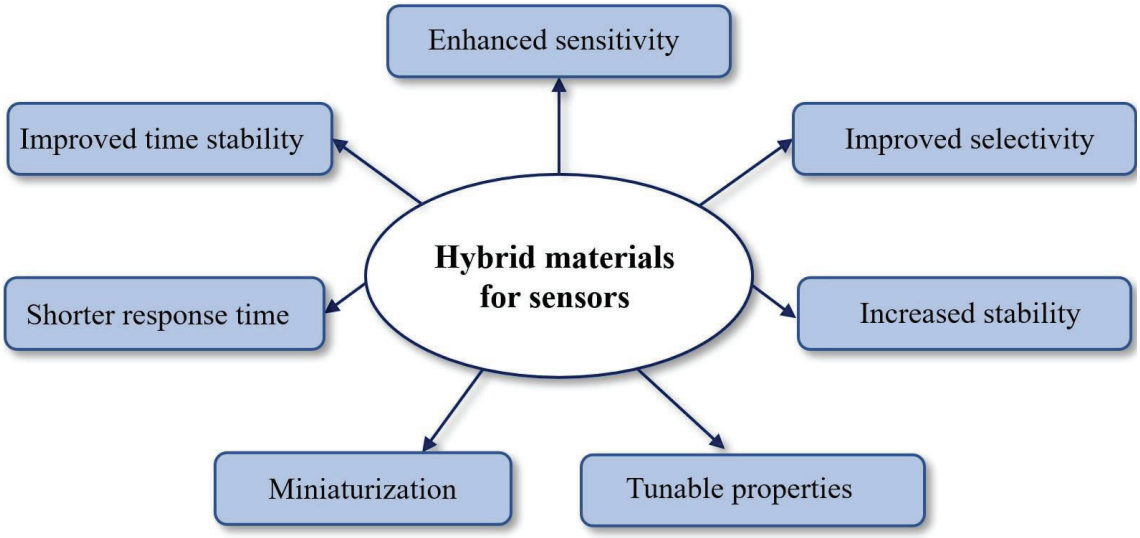


Fig. 13. *The key properties of hybrid materials for sensors application, based on* [58].

Hybrid materials can be designed to exhibit enhanced sensitivity to a particular analyte, which allows for more accurate detection and quantification. Moreover, selecting the components and their properties can demonstrate improved selectivity over interfering compounds. Hybrid materials could be engineered to exhibit improved stability under various environmental conditions and can be tuned by adjusting the composition, structure, and processing conditions, which enables tailoring sensor performance to specific applications. Hybrid materials can be integrated into small-scale and microscale sensor devices, enabling miniaturization and portability. In addition some of them exhibit rapid response and recovery times, allowing sensors to provide real-time data [58].

3.2.1. Carbon-based nanomaterials

Carbon is a material with various allotropes, which determines its unique properties. The distinctive qualities and diversity of carbon-based systems are the appearance of new opportunities in chemistry, physics, and engineering subfields [59].

The symmetry-rich hybrid atomic orbitals created by the linear combination of valence carbon orbitals can form 0D to 3D polymeric structures. Quantum dots and fullerenes are an example of 0D nanostructures [60]. Carbon nanotubes (examples of 1D structure) are materials with distinctive physicochemical characteristics. The formation of functional compounds is currently dominated by aromatic compounds -

two-dimensional carbon structures (2D). Due to the stability and inertia of 3D carbon structures, they are commonly used in many directions [60].

The division and examples of carbon materials, due to their structure, are presented in Fig. 14.

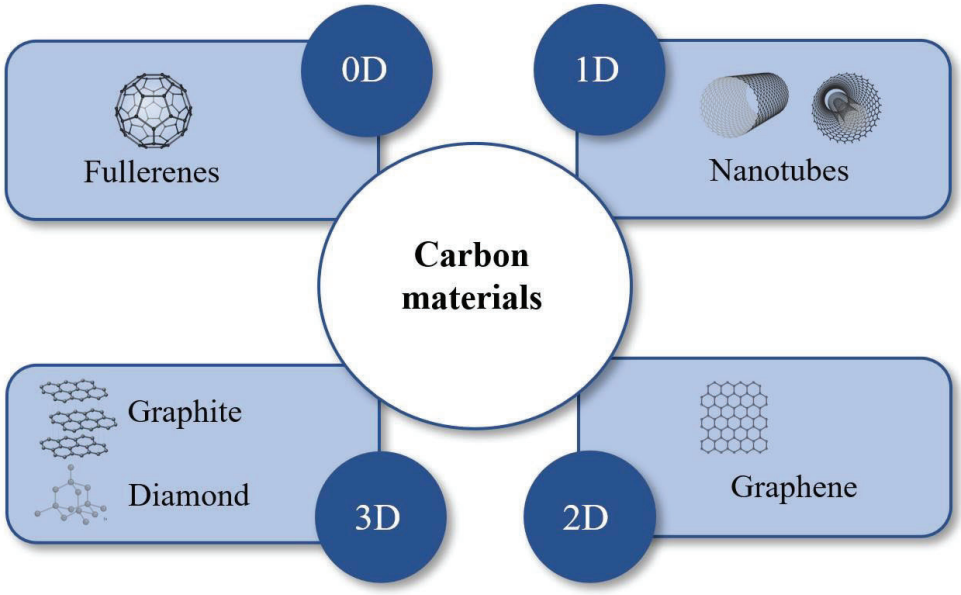


Fig. 14. *Types of carbon-based materials according to the arrangement of carbon atoms, based on* [61].

Due to its electrical conductivity, quick charge transfer, high stability, and ease of modification, carbon-based materials continue to catch attention [62].

Hybrid materials comprise two or more types of carbon structures, i.e., graphene, carbon nanotubes, carbon fibers, or diamond. These hybrid materials can exhibit unique properties, including good electrical conductivity, thermal stability, and chemical resistance (not present in the individual carbon structures), making them ideal for a wide range of applications [63].

Carbon nanotubes are suitable as individual nanoelectrodes due to their conductivity and size. Numerous investigations demonstrated the power to facilitate electron-transfer reactions using this material. These materials present strength-to-weight ratios. The combination of different carbon structures in a hybrid material can lead to increased strength compared to individual carbon structures [64].

The properties of carbon-based hybrid materials are presented in Fig. 15.

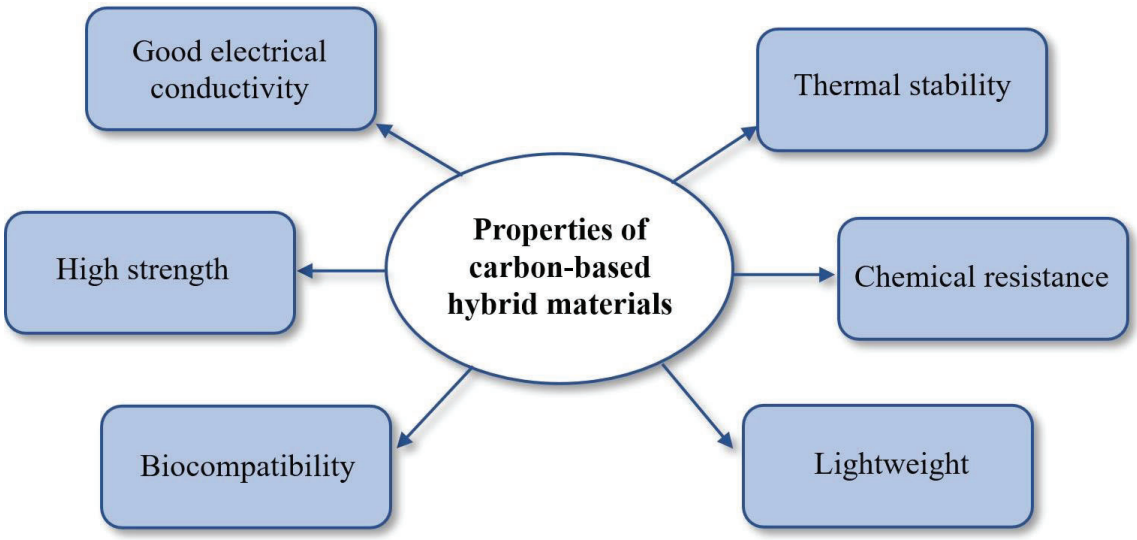


Fig. 15. *Properties of carbon-based hybrid materials, based on* [63].

The high electrical conductivity of carbon-based hybrid materials enables their applications as electronic devices, sensors, and energy storage. These hybrid materials are highly heat-resistant and can maintain structural integrity even at high temperatures. Moreover, their high resistance to chemical degradation makes them suitable for harsh environments like chemical processing and wastewater treatment [63].

Carbon nanotubes (CNTs) are one-dimensional carbon with a hollow cylindrical shape. Single-walled carbon nanotubes (SWCNT) are produced by rolling up a single graphene layer, whereas multi-walled carbon nanotubes (MWCNT) are created by rolling up multiple graphene sheets. Carbon nanotubes and graphene are comparable in their geometrical structure and electrical characteristics [65]. The walls of pure CNTs have a hydrophobic character [65].

Covalent and non-covalent carbon nanotube functionalization techniques are used to change their properties. The process of covalent functionalization entails the creation of covalent bonds between a functional group and a carbon atom. Covalent chemical bonds between CNTs and functional entities are created during the covalent functionalization process, which entails several chemical reactions [65,66].

Developing architectural designs and understanding electrochemical principles was a protracted experimental process in the history of carbon-based electrochemical sensors. The electrochemical efficiency depends on various factors, including surface

chemistry, the functional groups attached to draw specific molecules, the ability to produce them in large quantities, and exceptional sensitivity and selectivity [67].

Single- and multi-walled carbon nanotubes were used to construct various electrochemical sensors due to the properties of the carbon atoms on the surface, i.e., good mobility and high sensitivity [67].

Shi et al. created an electrochemical sensor based on functionalized MWCNT and gold nanoparticles with limits of detection of 0.44 μM and 0.025 μM, respectively, for the simultaneous detection of p-acetaminophen and p-aminophenol [68].

Moreover, a further attempt at electrochemical glucose detection using the CNT/graphene hybrid material (CNTs/G) was proposed by Badhulika et al., and it demonstrated a linear range of 1-7 mM with a sensitivity of 11.06 $\mu A\, mM^{-1}\, cm^{-2}$ [69].

The carbon-based materials found wide applications in sensor and biosensor systems. Table 2 summarizes another potential use for CNT-based electrochemical and biosensors, along with the detected analyte.

Table 2. *Developments in sensors and biosensors based on carbon nanotubes*

Electrode	Analyte	Ref.
MWCNT	cholesterol in blood	[70]
MWCNT	epinephrine	[71]
Chitosan/CNT	DNA	[72]
Pt/CNT	L-cysteine	[73]
Au/SWNT	rutin	[74]
MWCNT/CoSal	tryptophan	[75]
Teflon/MWCNT/Au/GOx	glucose	[76]
Pt/MWCNT/ChOx	choline	[77]

3.2.2. Metal-based hybrid materials

The characteristic features include high surface areas, high optical and magnetic properties, high electrical qualities, and excellent mechanical and thermal stability [78]. The most used metal nanoparticles can be obtained from gold, silver, copper, iron, or platinum. They present unique electronic, optical, and magnetic features, which make them useful in various applications, for instance, catalysis, sensing, and drug delivery [78]. The examples of metal-based nanomaterials are depicted in Fig. 16.

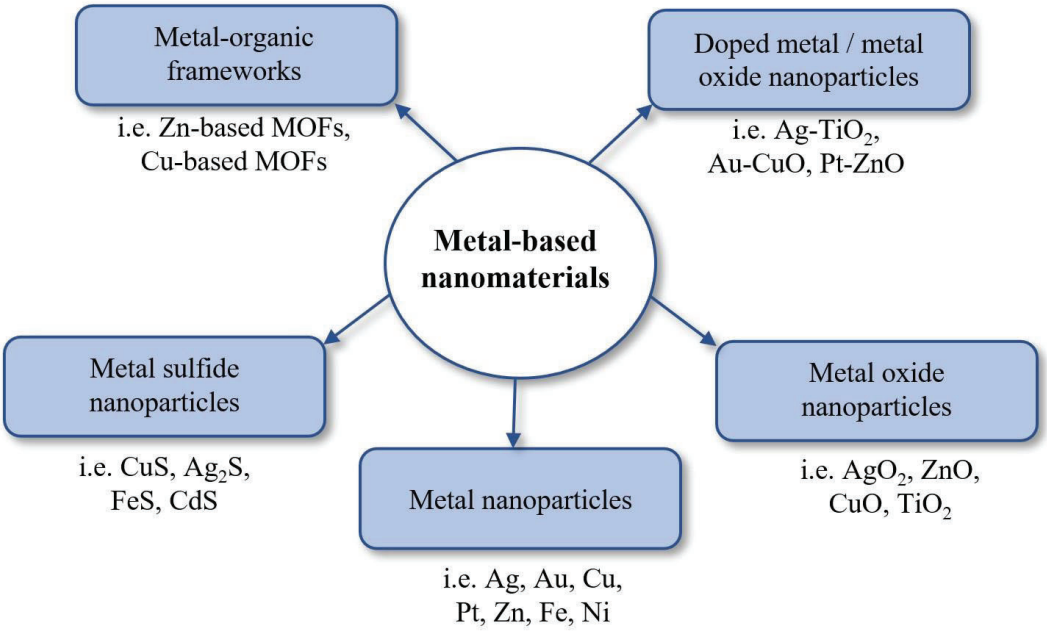


Fig. 16. *Examples of metal-based nanomaterials, based on* [79].

Metal-based nanomaterials find applications in pharmaceuticals, drug delivery, diagnostic imaging, and magnetic resonance imaging (MRI),. These include the creation of sunscreens and stain-resistant clothing [79].

Nanoparticles of hematite (Fe_2O_3) and magnetite (Fe_3O_4) are used in electrochemical detection. Iron oxide nanoparticles were used in publications to modify electrodes for measurements of analytes, including H_2O_2 and glucose [80].

Manganese oxide nanomaterials in various forms, for example, MnO, MnO_2, and Mn_3O_4, are among the most well-researched options for electrode materials in electrochemical sensing. These nanomaterials can be characterized as low-cost, nontoxic, environmentally friendly, abundant in nature, comparatively high energy dense, and highly active in alkaline media. They have significant applications in energy storage, soft magnetism, ion exchange, biosensing, catalysis, and adsorption [80].

A comparison of the advantages and disadvantages of metal-based nanomaterials with carbon materials is shown in Table 3.

Table 3. *Comparison of advantages and disadvantages of carbon vs. metal-based nanomaterials, based on* [81].

Material	Advantages	Disadvantages
SWCNT	• carried with a low load • density • delocalized π-orbitals • electrical conductivity • large surface area to volume ratio (S/V)	• limited surface-to-interface • non-specific protein adsorption • challenging chemical functionalization
MWCNT	• superlative conducting properties • good electrocatalytic properties	• the surface should be functionalized • irreversible agglomerates in aqueous solution
Graphene	• high surface area to volume ratio (S/V) • large active sites • fast electron transfer • high thermal conductivity • better mechanical flexibility • good biocompatibility	• hard to dissolve in water environment • production of graphene often involves harsh chemical processes • its brittleness can limit the overall material's toughness and durability
Metal-based nanoparticles	• effective electron transfer • increase in surface area to volume ratio (s/v) • supplying superior conductivity • biocompatibility • easy to functionalization	• inconsistent upon signal amplification • electrical instability in high salt concentration

A straightforward wet-chemical technique was used by Y. Pan et al. to create hybrid materials of MnO_2 nanoparticles/multi-walled fullerene nanotubes/graphene (MnO_2/MWFNTs/GS) for the H_2O_2 detection of with a linear range of 2.0 μM–8.44 mM [82].

An important element when designing a non-enzymatic sensor is the selection of the receptor layer depending on the material we want to detect due to the different catalytic abilities of these materials.

The analytes detected using magnetite, manganese, and other metal-based nanoparticles are presented in Table 4.

Table 4. *Examples of metal-based nanomaterials and measured substances, based o*n [80].

Metal-based nanomaterials	**Detected substances**
Cu, CuO	acetaminophen, glucose, phenols, dopamine
Ni, NiO	cysteine, ethanol, glucose, glutamate, hydrazine, hydrogen peroxide lactic acid, paracetamol, levodopa
Co_2O_4	glucose, hydrogen peroxide, maltol, xanthine, dopamine
MnO, MnO_2, Mn_3O_4	glucose, hydrogen peroxide, serotonin, thiol, ferulic acid
Fe_2O_3, Fe_3O_4	acetaminophen, ethanol, glucose
ZnO	benzoquinone, hydrazine, trinitrotoluene, hydrogen peroxide

Copper(I) oxide and copper(II) oxide, due to their catalytic properties, are one of the most frequently used structures for electrochemical detection. These two p-type semiconductor copper oxide nanoparticles have numerous possible uses in a variety of industries, like solar energy, catalysis, gas sensors, lithium-ion batteries and optical devices [83].

Copper oxide nanoparticles integrated with carbon materials (i.e., graphene, carbon nanotubes, mesoporous carbons, and carbon nanofibers) were shown to increase the charge transfer between support matrices and analytes.

In the context of imprinted polymer-based sensors, the CuO nanoparticles were utilized to enhance the number of imprinted sites on the electrode, which improved the selectivity and sensitivity of the electrochemical sensors [83].

Efficient non-enzymatic glucose sensors based on CuO nanoparticles were presented by Ashok et al. The authors contributed and characterized various approaches to synthesizing copper nanoparticles and their electrochemical properties in relation to non-enzymatic glucose biosensors [84].

Wide band gap, high exciton, biocompatibility, low cost synthesis, improved electrochemical activities, chemical and photochemical stability, and high electron transmission attributes are only a few of the distinctive enhanced properties that ZnO possesses [85].

Kalambate et al., in their work, present a graphene/zinc oxide/carbon paste electrode (GNS/ZnO/CPE). The zinc oxide was prepared using the precipitation technique. The system was used to detect pyrazinamide. The sensor was sequentially utilized to analyze pyrazinamide in urine and blood serum samples, as well as pharmaceutical formulations [86].

As a consequence of its thermal stability, biocompatibility, and chemical inertness, ZrO_2 was a key component of metal-based nanoparticles. Due to this substance's high affinity for the phosphoric group, it was frequently utilized as a trapping agent for the selective enrichment of free organophosphate insecticides and phosphorylated proteins. Some restricted ZrO_2 nanoparticle orientations with a three-dimensional stage prefer the direct electron transfer between the protein molecules and the conductor surface [87].

Bilirubin oxidase (BOx) was covalently immobilized onto zirconium dioxide/ silica nanoparticles with chitosan (SiO_2/ZrO NPs/Chit) proposed by Batra et al. The proposed composite was electrodeposited onto a gold anode to create the bilirubin sensor [87].

Electrochemical sensing by metal oxide nanoparticles discovered an unavoidable use for cobalt oxide. Due to its high activity and better stability, cobalt oxide garnered attention. The production of a modified electrode for detecting temperature and humidity has frequently used this oxide [88].

The promising material for flavin adenine dinucleotide (FAD) immobilization was Co_2O_3. These materials, after electrodeposition presented by Salimi et al., nitrite reduction over a linear range of 1-30 M with strong catalytic activity and a detection limit of 0.20 μM [89].

Due to their stability, low toxicity, relatively low cost, and catalytic activity, nickel-based nanomaterials are used frequently for molecule immobilization. Through their electrostatic interactions, NiO NPs are compromised by the physical adsorption of enzyme molecules owing to their high isoelectric point [90].

A graphene nanoparticle decorated with nickel oxide (NiO/GNS) on GCE was used to add a non-enzymatic electrocatalyst to the glucose oxidation reaction by Zeng et al. It was proposed that the catalyst obtained in the work could be used to build a biofuel cell with high reliability and current density [91].

3.3. Non-enzymatic and enzymatic electrochemical sensors

Electrochemical sensors have gained applications in many fields like energy, health, environment, food, and pharmaceuticals. Chemical sensors provide analytical data about a specific concentration of a particular chemical species in the immediate environment [92].

According to the International Union of Pure and Applied Chemistry (IUPAC), a chemical sensor is a device that converts data from a chemical process involving an interest species or from a system's physical characteristics into a measurable analytical signal [93].

A sensor contains a receptor component that produces the relevant information and passes it on to a transducer part, which transforms it into an analytical signal [94].

Whenever a target interacts with an electrolyte, an electrochemical sensor measures currents and/or voltages provided by the recognition part [94]. The structure of the electrochemical sensor is shown schematically in Fig. 17.

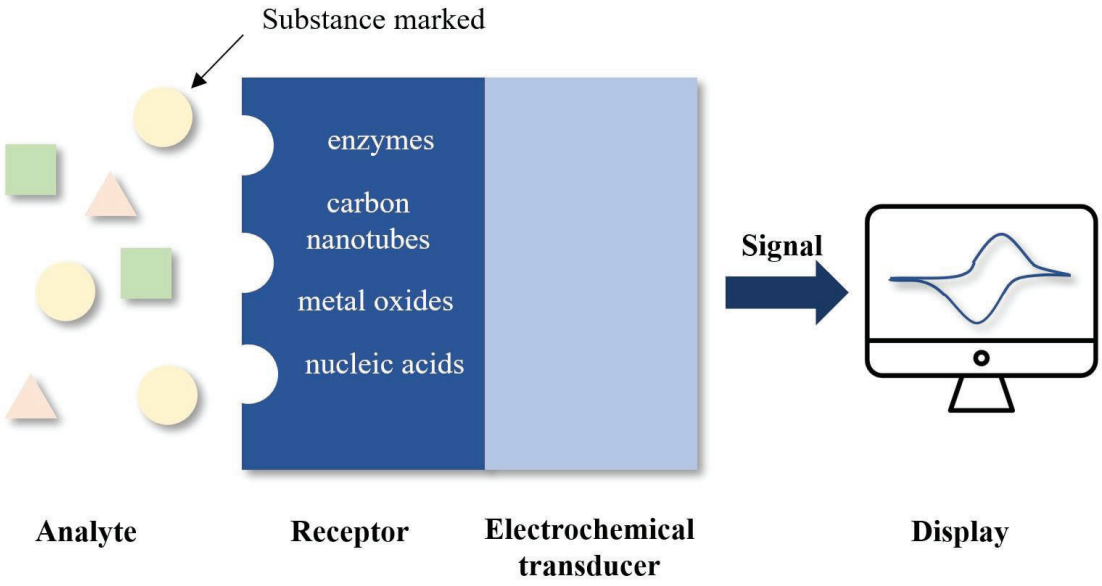


Fig. 17. *The principle of operation of an electrochemical sensor, based on* [94].

Before constructing an electrochemical sensor, it is crucial to take into account the following three factors: (i) **choosing a chemical or biological recognition element**; (ii) **selecting an electrochemical transduction element** (often potentiometric or

amperometric); and (iii) **integrating both recognition and transducer components** [95].

The efficiency of signal transduction is influenced primarily by the electrical conductivity and distribution of additives. The performance of the receptor or sensing efficiency of the sensor depends mainly on the electrocatalytic activity, biocompatibility, and stability [96].

Examples of materials used as a receptor layer in electrochemical sensors are shown in Fig 18.

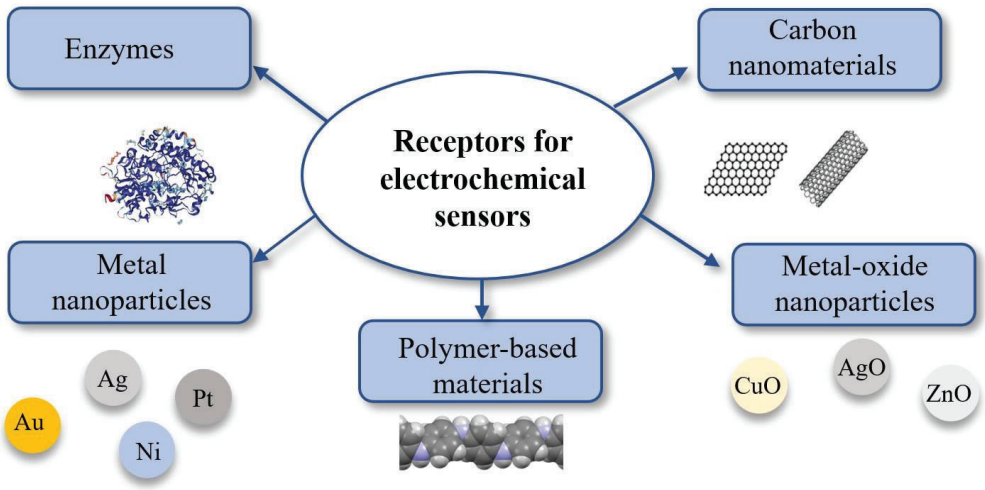


Fig. 18. *Receptor layers in electrochemical sensors, based on* [97].

The most commonly used materials in the receptor layer include enzymes, carbon nanomaterials, metal-oxide nanoparticles, metal nanoparticles, and polymer-based materials [98].

An electrochemical sensor requires possessing a certain number of qualities to function accurately and effectively. These include **independence** from physical constraints (i.e., temperature or pH), **selectivity**, **sensitivity**, the **limit of detection** (LOD), **the limit of quantification** (LOQ), **linear range** (related to accuracy), **stability** (over time), **response time**, and **reproducibility** [98].

Electrochemical sensors can offer significant benefits, including precise, repeatable data on linear responses and individual chemicals. Moreover, they offer a combination of sensitivity, selectivity, rapid response, and versatility that makes them essential tools for diverse industries and applications, ranging from healthcare to environmental protection [99].

The essential features of electrochemical sensors are presented and defined in Table 5.

Table. 5. *Standard characteristics of electrochemical sensors, based on* [99,100].

Characteristic	Definition
Selectivity	Sensor's ability to measure a marked substance in the presence of additional interferences.
Sensitivity	The lowest number of analytes can be accurately detected or recognized at the lowest concentrations to confirm the presence of analyte traces in the sample.
Time stability	The ability of a sensor to produce the same output signal throughout the time when measuring a standard measurement.
Linear range	The accuracy of the measured response (for different analyte concentrations) to a straight line.
Accuracy	The degree to which a sensor's output is near the measured value in reality.
Repeatability	Sensor's capacity to produce the same results each time the same sample is measured.
Limit of detection (LOD)	The smallest amount of an analyte that can be detected.
Limit of quantification (LOQ)	The lowest concentration of an analyte at which an accurate measurement can be made.
Response time	Time required for the sensor to reach a stable value.

In addition, the advantages of these devices made electrochemical sensors widely used in many fields of industry and science. These applications include environmental monitoring, detection of various diseases, drugs, food safety, and many other compounds [101]. The best-known application of sensors is their clinical use in diagnosing blood sugar levels. Blood detection sensors account for about 85% of the sensor market [102].

Moreover, one of the more critical applications of electrochemical biosensors is the detection of biomolecules that are either disease indicators [103] or drug targets [104]. Furthermore, these sensors, due to their precise and accurate measurements, are often used in the fermentation industry [105].

Example applications of electrochemical sensors are shown in Fig. 19.

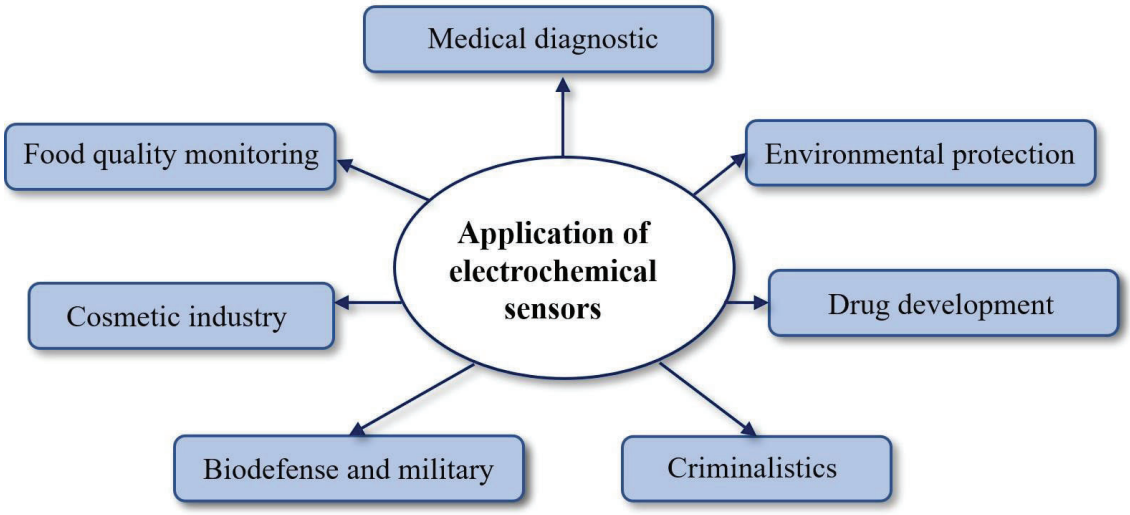


Fig. 19. *Potential applications of electrochemical sensors, based on* [103–106].

Environmental water monitoring is an important area where portable electrochemical sensors are often used to detect hazardous compounds [107]. Furthermore, in the food industry, these detectors control the quality and safety of food products [108].

Moreover, electrochemical CO sensors are vital for detecting this toxic gas in homes and industries. They also help optimize fuel cell performance by monitoring reactant concentrations. These sensors detect hazardous chemical agents for military and homeland security and are used in explosive detection devices for security screening [92,109].

3.3.1. Properties and construction of biosensors

A biosensor is a chemical sensor that contains biological material as a receptor layer, which explicitly recognizes an analyte. Biological particles that can be used as recognition elements are i.e. enzymes, nucleic acids, aptamers, antibodies, or whole cells [110].

The biological material in biosensors is present in close contact with the transducer, and in consequence, the biochemical signal is quickly converted into an electrical signal. An important aspect that should be considered when designing is the ability of the bioreactor to detect only one type of analyte (selectivity) [111]. Examples of biomaterials used in receptor construction are presented in Fig. 20.

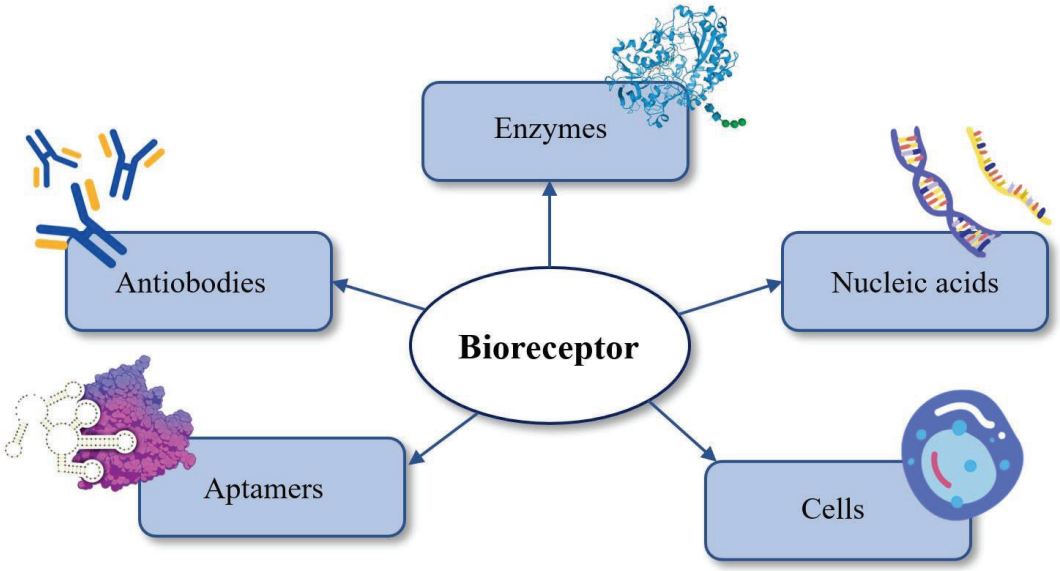


Fig. 20. *Examples of biological materials as a biosensor's receptor layers, based on* [112].

Biosensors can be divided into three generations depending on the method of attaching the biological element to the transducer and the method of electron transport [113].

The concentration of analytes or enzymatic reaction products that diffuse onto the electrode surface and produce an electrical response is measured using first generation biosensors [114]. Based on the glucose biosensor, reduced flavin adenine dinucleotide (FAD) to $FADH_2$ while oxidizing β-D-glucose to β-D-gluconolactone using trapping enzyme -glucose oxidase (GOx). The FAD would then be regenerated using dissolved O_2, which would then be used to produce hydrogen peroxide (H_2O_2). The H_2O_2 on the electrode surface would then undergo oxidation due to the voltage application, creating an electric signal [115]. The principle of operation of the first generation biosensor is shown in Fig. 21.

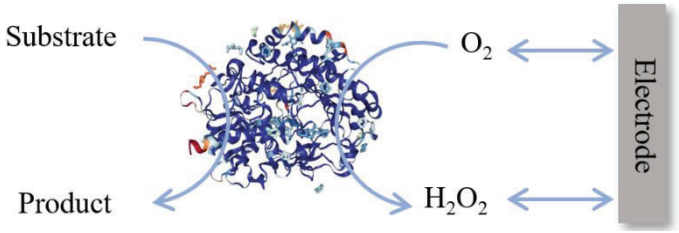


Fig. 21. *Schematic representation of electron transfer in first generation biosensors, based on* [115].

The most significant limitations of the first generation of biosensors include the following: (i) **partial solubility of O_2, the deficit of which at higher concentrations limits the detection**; (ii) **the need to oxidize hydrogen peroxide using very often high voltage**; (iii) **correction of matrix effects which is required related to interference** [116].

The second generation of biosensors, developed due to the problems related to oxygen dependence and electroactive interference observed in the first generation, use external mediators that act as electron carriers [117].

In this case, the O_2 was replaced by a synthetic external mediator. This compound transfers electrons from the buried redox active enzyme to the [118]. A schematic representation of the second generation biosensor demonstrating mediated electron transport (MET) is shown in Fig. 22.

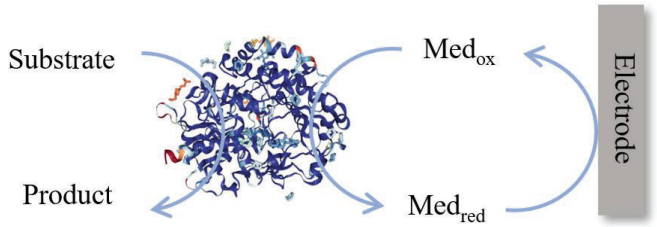


Fig. 22. *Response mechanisms of second generation biosensors where redox mediator transports the electrons, based on* [119].

A mediator is a synthetic substance that helps facilitate the passage of electrons through the transducer for detection. A mediator should (i) **react quickly**; (ii) **have low solubility in aqueous sample environments**; (iii) be **chemically stable in both oxidized and reduced forms**; (iv) **be nontoxic**; and (v) **present electrochemical properties** [120,121].

In the literature, the following compounds were used as mediators for electron transport in second generation biosensors: ferro/ferricyanides [122], quinone derivatives [123], and ferrocene and its derivatives [124,125], methylene blue [126], Prussian blue [127], methyl violet [128], and toluidine blue [129].

Direct electron transfer (DET) between the redox center of the enzyme and the electrode is the main need for third generation biosensors [130]. This generation does not require any extra external mediator for substrate detection. Effective direct electrical communication occurs between the enzyme and the electrode [130]. This provides

effective electron transfer by putting a gap between the enzyme's redox active core and the electrode surface. Furthermore, another factor that affects or prevents electron transfer is the orientation of the enzyme towards the electrode and the denaturation of the enzyme brought on by its adsorption on the electrode surface [131].

Third generation biosensors demonstrated remarkable sensitivity and selectivity. Despite all of these biosensors' advantages, it appears that the limited electron transfer method remains the biggest obstacle to enhancing their effectiveness [132].

Protein-film voltammetry seems to be a promising technique among the different strategies used to achieve the direct electron transfer of protein. In this technology, a proper biochemical signal should be formed under an appropriate applied voltage by immobilizing proteins on the surface of highly conductive support matrices [132]. The scheme of the third generation biosensor is shown schematically in Fig. 23.

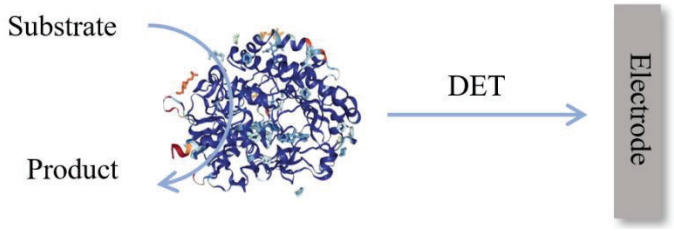


Fig. 23. *Schematic representation of the third generation of glucose biosensors, based on* [102].

Combining biological responses with the generation of an electrochemical signal determines how to evolve new, sensitive, and selective analytical tools characterized by easy operation. Their demanding nature enables both qualitative and quantitative detection [133].

The immobilization of the sensing component with the transducer interface is necessary to develop biosensors. The immobilization of the enzyme allows the initial properties and activity to be retained. This avoids potential loss after each measurement and makes it easier to develop continuous systems, increasing the process's viability. The process is easier to manage as immobilization results in more stable biocatalyst activity [134].

Immobilization methods include: (i) **physical adsorption**; (ii) **cross-linking**; (iii) **covalent attachment**; (iv) **encapsulation**; and (iv) **entrapment** [135,136]. These techniques are shown schematically in Fig. 24.

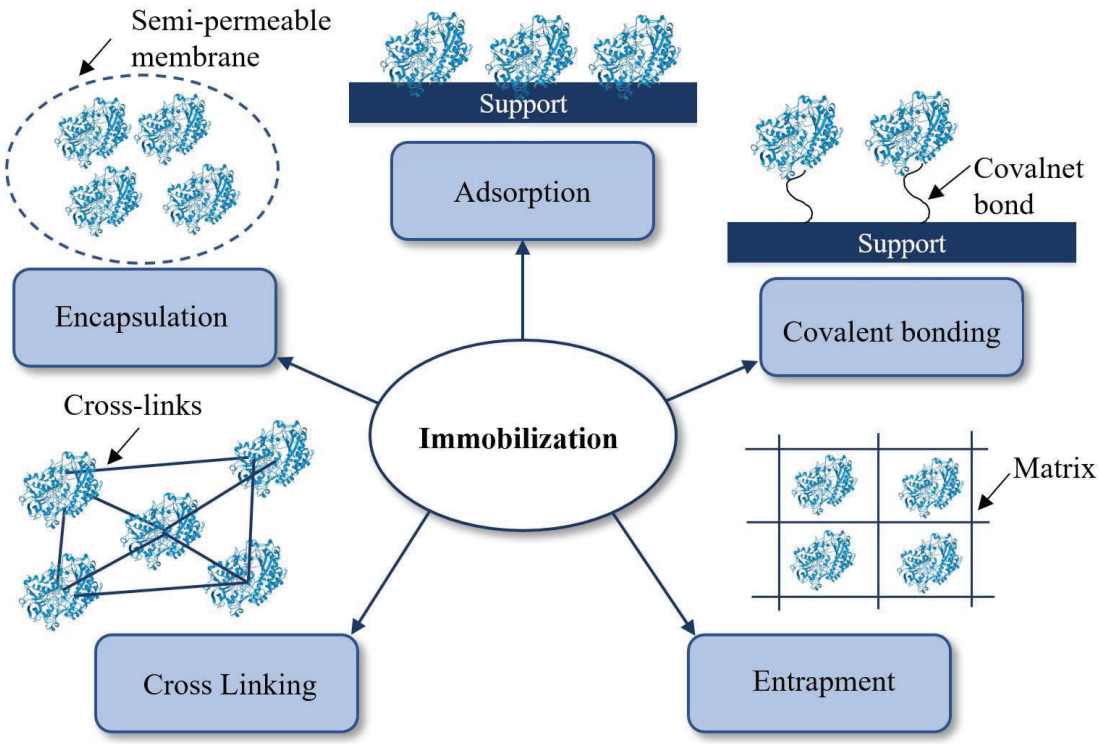


Fig. 24. *Methods of immobilization of biological material, based on* [137].

Immobilization by forming a covalent bond is a direct connection of biological material with the carrier by forming a covalent bond. It is an effective immobilization method based on a chemical reaction between active amino acid groups on the surface of the enzyme and functional groups on the surface of the carrier that uses a binder [138].

Adsorption is a technique for immobilizing an enzyme with interactions between the support and enzyme surfaces, like van der Waals forces and hydrogen bonds. The concentration of the enzyme solution, carrier shape, pore size, specific surface area, pH, ionic strength, and reaction temperature are a few of the many variables influencing the carrier's ability to adsorb [139].

Bifunctional reagents are used in intermolecular processes to cross-link enzymes to the support matrixes. The covalent bonds securely immobilize the biological material, which increases the stability and reusability of the materials. The method of cross-linking can cause them to lose their catalytic abilities [140].

Encapsulation consists of closing the biological material in a spherical capsule made of a semi-permeable membrane [141]. They can be natural and synthetic, often made of silicone, liposomal, nylon, or cellulose derivatives. This method enables the immobilization of many enzymes at the same time. It is based on the dissolution or

dispersion of the enzyme in the solution, followed by the spontaneous formation of a polymer membrane [142].

Entrapment is an enzyme inclusion inside natural or synthetic structures, consisting of trapping cells in a matrix, the size of which must be much larger than the size of the cells [143]. The biological material is mixed with a carrier solution and a cross-linking agent and then subjected to the polymerization process [144]. The inclusion of biological material can be carried out in the gel structure. This immobilization is particularly applicable in the case of easily migrating substances and those that do not cause defects in the gel structure [143].

Since the enzyme-substrate ratio is relatively high in immobilization systems, further substrate consumption can be avoided [134]. A comparison of the enzyme and process characteristics for the native and the immobilized process is shown in Table 6.

Table 6. *The comparison of the native and immobilized enzymes, based on* [134].

Features	**Native enzyme**	**Immobilized enzyme**
Cost-effectiveness	loss of biocatalysts	reused biocatalysts
Enzyme activity	unstable	stable
Temperature, pH tolerance	low	high
Separate ability	difficult	easy
Productivity	low	high
Mass transfer	enable	limited

3.3.2. Features of non-enzymatic sensors

Non-enzymatic sensors are devices that are used to measure the concentration of analyte in a sample without the use of enzymes [145]. **Metals** [146], **alloys** [147], **carbon-based materials** [148], **metal oxides** [149], **polymer-based materials** [150], and **layered double hydroxides** [151] are some of the several types of non-enzymatic electrocatalysts.

Examples of materials used in non-enzymatic sensors are shown in Fig. 25.

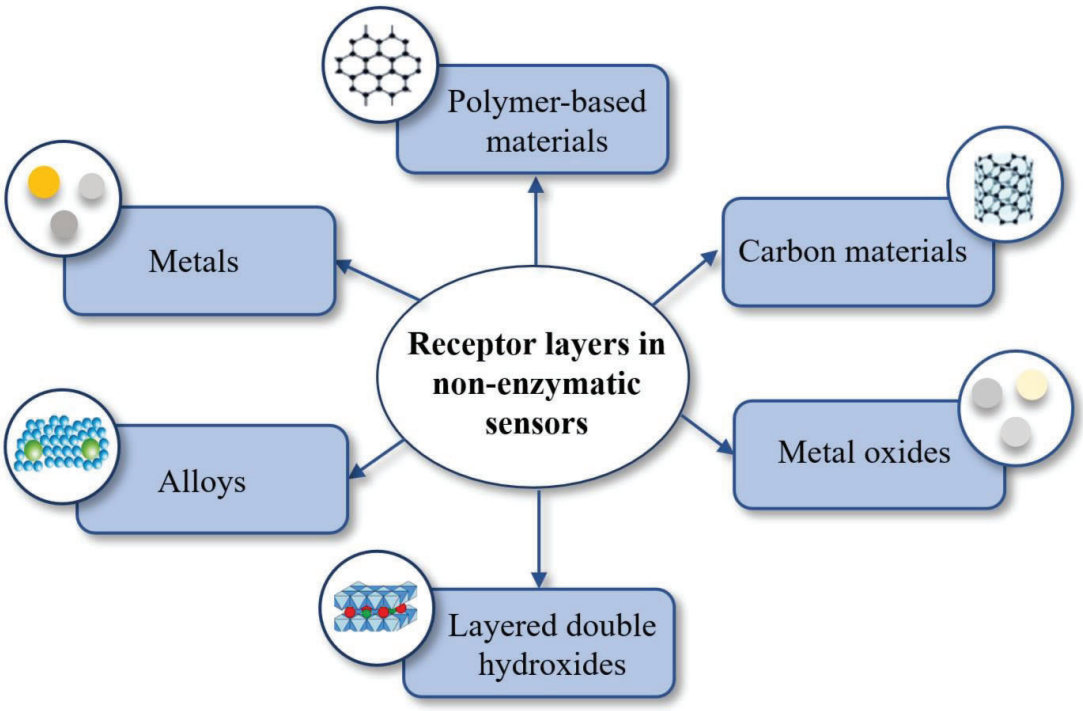


Fig. 25. *Examples of receptor layers in non-enzymatic sensors, based on* [152].

These chemical sensors present several advantages over enzymatic sensors, including: (i) **higher stability**; (ii) **lower cost**; and (iii) **more straightforward construction**. Based on the electrochemical oxidation of glucose under catalytic conditions, non-enzymatic glucose sensors measure glucose levels [153]. A schematic operation of the example of a non-enzymatic glucose sensor is presented in Fig. 26.

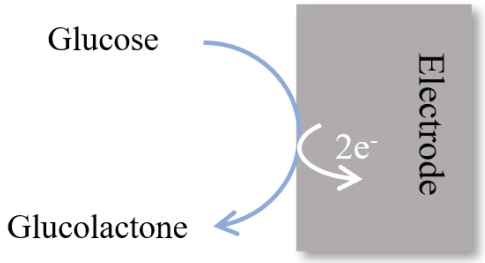


Fig. 26. *Mechanism of a non-enzymatic glucose sensor, based on* [154].

Enzymatic and non-enzymatic electrochemical sensors detect analytes in different fields, including food safety, environmental monitoring, and medical diagnosis. Both sensors have unique advantages and limitations, and their suitability depends on the specific application [155].

Enzymatic electrochemical sensors use enzymes as biorecognition elements to catalyze the reaction of the target analyte. These sensors are sensitive and selective for detecting various analytes, including glucose, cholesterol, or neurotransmitters [156]. The advantages of enzymatic electrochemical sensors include: (i) **high sensitivity**; (ii) **selectivity**; (iii) **specificity**; (iv) **lower limit of detection**; and (v) **the ability to detect analytes in complex matrices**, i.e., blood and urine. However, these sensors can be affected by the **stability and activity of the enzyme**, and the **presence of interfering substances** can affect their accuracy [157].

The advantages of non-enzymatic electrochemical sensors include: (i) **high sensitivity**; (ii) **the ability to detect multiple analytes simultaneously**; and (iii) **fast response time** [158]. However, non-enzymatic sensors can be **affected by the presence of interfering substances** and have **lower specificity** compared to enzymatic sensors [159].

A comparison of the advantages and disadvantages of enzymatic and non-enzymatic sensors is presented in Table 7.

Table 7. *Comparison of advantages and disadvantages of non-enzymatic electrochemical sensors, based on* [159].

	Advantages	**Disadvantages**
Enzymatic sensors	• good selectivity • high sensitivity • biocompatibility • safe and well-functioning operating under physiological conditions • increased stability over time for immobilized biological materials	• high material costs • complex production process • unstable under sterilization • necessary specific storage conditions • short-time strength with native biological material
Non-enzymatic sensors	• high sensitivity • a large number of nanomaterials available at relatively lower cost • a more straightforward method of obtaining • easier storage conditions • relatively stable under sterilization conditions	• low selectivity • alkaline media needed for some catalysts

Furthermore, enzymatic electrochemical sensors are precise, sensitive, and selective, making them suitable for detecting specific analytes in complex matrices. Non-enzymatic electrochemical sensors, on the other hand, are simple, cost-effective, and can detect a wide range of analytes, making them suitable for rapid applications. The choice of sensor type depends on the specific application, the required sensitivity and selectivity, and the complexity of the sample matrix [159].

3.3.3. Methods and assumptions of glucose measurements

Currently, one of the most developing diseases worldwide is diabetes, which has reached epidemic proportions globally, primarily driven by factors, i.e., obesity, poor dietary choices, and insufficient exercise. It is a rapidly growing disease that is managed at an individual level by monitoring and regulating blood glucose levels. Significant complications from diabetes might include lower limb amputations, blindness, and cardiovascular disease [160]. Methods for glucose detection are shown schematically in Fig. 27.

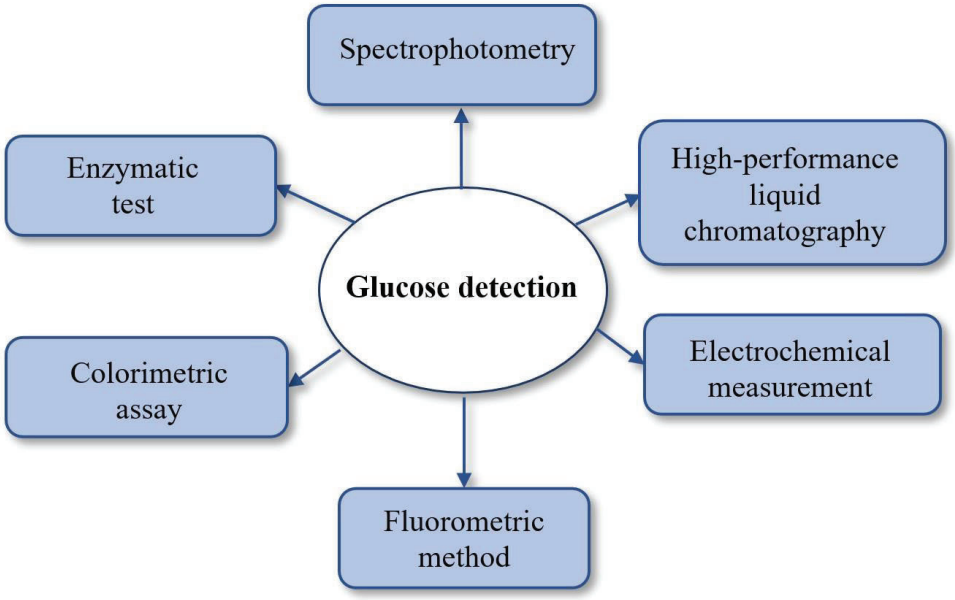


Fig. 27. *Examples of techniques used to detect glucose, based on* [161].

Glucose measurement methods play a pivotal role in modern healthcare, enabling the monitoring and management of diabetes. Methods available for glucose determination include: (i) **enzymatic methods**, which use enzymes to catalyze the conversion of glucose into a measurable signal. Glucose oxidase is a commonly used enzyme in these methods [162]; (ii) **colorimetric methods** use a colorimetric reaction

to measure glucose levels [163]; (iii) **fluorometric methods** use the fluorescence of a compound that is produced during glucose conversion [164]; (iv) **electrochemical methods** measure the electrical current generated by the oxidation of glucose at an electrode [165]; (v) **spectrophotometric methods** measure the absorbance of light at a specific wavelength, which is proportional to the amount of glucose present [166]; (vi) **high-performance liquid chromatography** (HPLC) is a more complex and expensive method that separates glucose from other compounds in a sample using liquid chromatography and then detects and quantifies the glucose using a UV detector [167].

Effective treatment of glucose-related conditions, particularly diabetes, hinges on several crucial elements, i.e., (i) maintaining a balanced and healthy diet; (ii) physical activity; (iii) medications; (iv) patient education; (v) regular monitoring of blood glucose levels.

Diabetic patients must constantly monitor their blood glucose levels. Today's techniques require the patient to take a small blood sample, usually by finger prick. The blood glucose content is determined when blood is applied to a sensor test strip, which a portable electronic reads. These sensors enable quick and precise measures of blood glucose without laboratory analysis because they are based on electrochemical enzymatic assays with screen-printed electrodes [168].

The commercially available method presents drawbacks, i.e., unpleasant sampling, the inability to conduct analyses while the patient is elsewhere engaged, and the ignoring of significant changes in data collection times [169]. Moreover, these errors are caused by manufacturing, storage, calibration problems, aging (both the matrix and the biomaterial or material that catalyzes the reaction), or improper use [124].

4. Motivations, aim and scope of the work

The motivation for the design and synthesis of hybrid materials for biosensors and non-enzymatic sensors was the potential for significant progress in various fields, including healthcare, environmental monitoring and biotechnology.

The main aim of the conducted research was the synthesis of new electroactive hybrid materials for the development of biosensors and sensors, which would be characterized by higher sensitivity, selectivity, and time stability, with reduced potential unit costs of the device, and then measurement. The general idea of the conducted research is shown schematically in Fig. 28.

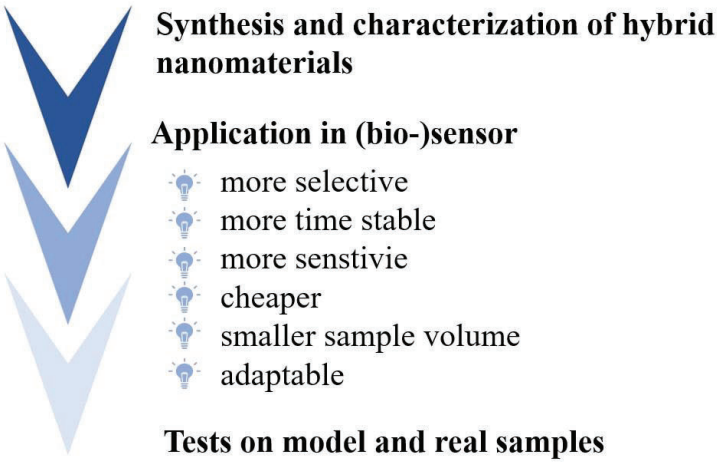


Fig. 28. *General stages carried out during the Ph.D.*

As part of the research, inorganic elements, including magnetite, copper(II) oxide, nickel(II) hydroxide, carbon material (MWCNT), functional biopolymer coatings (polydopamine, and poly(caffeic acid)), and β-cyclodextrins were chosen. Selected materials were used to construct innovative hybrid materials whose properties would demonstrate the synergistic effect of individual components.

It is also important to point out that there were no studies published on the use of hybrid materials based on the selected metal oxides coated with biopolymers (polydopamine, poly(caffeic acid), with attached nanocontainers (β-cyclodextrins) for sensors at the time the research topic was considered.

According to the available knowledge and current literature reports, three research hypotheses were presented.

1. Hybrid materials significantly affect the sensitivity of the sensor.
2. The use of biopolymer coatings will increase the efficiency and amount of enzyme loading.
3. Immobilization of enzymes on the surface of hybrid materials will significantly improve the electrochemical selectivity and time stability of the biosensor.

Based on the research hypotheses and the defined main goal of the doctoral thesis, a methodology for the multi-stage process of obtaining nanomaterials for enzymatic and non-enzymatic sensors was developed.

As a result of the multi-stage interdisciplinary nature of the research, the scope of the Ph.D. research was divided into six parts.

1. Designing new hybrid nanomaterials involving a combination of selected carbon materials, metal oxides/metal hydroxides with excellent electrocatalytic properties, and biopolymer coatings with numerous functional groups will result in a synergistic effect on improving the properties of sensors.
2. Synthesis of electrochemical hybrid materials with the use of carbon materials, metal oxides/metal hydroxides, and biopolymers, which will form the basis for the production of enzymatic and non-enzymatic sensor systems.
3. Performing physicochemical, dispersion-morphological and structural characteristics of the manufactured hybrid materials to determine, among others: (i) thermal and mechanical stability; (ii) electrokinetic properties; (iii) surface morphology; (iv) porosity; and (v) the influence of a changing environment.
4. Immobilization of the enzymes using adsorption immobilization, after proper selection of both carrier material and biocatalyst, will maintain the high catalytic properties of the enzyme and provide adequate carrier stability.
5. Construction of an enzymatic or non-enzymatic sensor using a glassy carbon (GC) or screen-printed electrode (SPE). Performing electrochemical characterization of nanohybrid materials, particularly their electrochemical properties. Based on the electrochemical tests performed (cyclic voltammetry, amperometry), determining the properties of the proposed sensor, i.e. sensitivity, selectivity, linear range, interference effects and temporal stability.

6. Selection and optimization of sensor systems on model and real (analytical-environmental) solutions.

The methodology and scope of the research included several stages, including nanomaterials synthesis for sensors, which are schematically presented in Fig. 29.

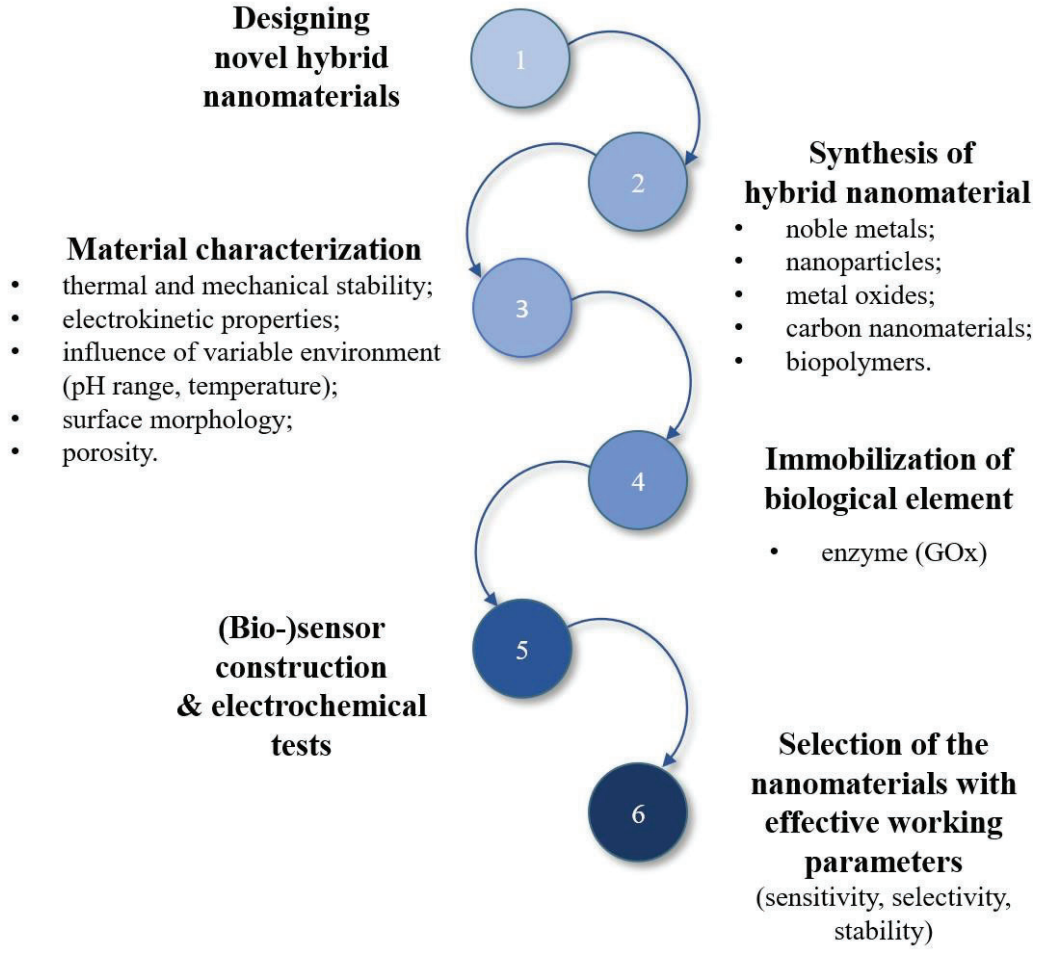


Fig. 29. *Schematic presentation of the scope of research and stages of the Ph.D. thesis.*

5. Description of the selected publications' contents

Research presented in the **Publications 1-6** concentrated on the design and fabrication of hybrid materials and their application in electrochemical biosensors and sensors.

The **Publications 1 and 2** focused on the construction of enzymatic glucose biosensors, which were obtained based on a biopolymer - polydopamine. The proposed nanomaterials were used to immobilize the enzyme glucose oxidase. The key for these studies was to check the role of an external mediator on the biosensor characteristics like limit of detection (LOD), linear range, or sensitivity (the **Publication 1**) and to improve the properties (enzyme loading, wider linear range, more extended time stability) by using β-cyclodextrins in the construction (the **Publication 2**).

The properties of polydopamine showed the potential of catechol-based hybrid materials, therefore, the research was extended to another biopolymer coating poly(caffeic acid). In the **Publications 3-6**, hybrid nanomaterials based on poly(caffeic acid) were obtained, characterized with physico-morphological characteristics and used in non-enzymatic sensors and enzymatic biosensor. As a result of the conducted research, the NADH electrochemical sensor enabling the low potential detection was obtained (the **Publication 3**). The use of PCA in the construction of the hybrid material was intended to functionalize multi-wall carbon nanotubes and detect this analyte at a lower potential, which would be beneficial in potential studies on real solutions containing interfering substances.

The electrochemical potential of poly(caffeic acid) as a biopolymer coating was continued, and the research focuses on designing and production of two non-enzymatic glucose sensors based on the catalytic properties of metal-based compounds (copper(II) oxide, nickel(II) hydroxide in the **Publications 4 and 5,** respectively). The use of these compounds enabled non-enzymatic detection of glucose and potentially reduced costs. Finally, the properties of poly(caffeic acid) were used to construct an enzymatic glucose biosensor with immobilized glucose oxidase (the **Publication 6**). The selection of such components for the construction of the hybrid material was intended to enable glucose detection at lower potentials, reducing the impact of interference and across the entire concentration range specified by the World Health Organization (WHO).

5.1. Results of applied polydopamine-based hybrid materials for sensors

In the **Publications 1 and 2**, polydopamine was used as a catechol-based compound for the construction of the hybrid nanomaterials. These materials were chosen due to the properties of polydopamine and its coating, which can provide several advantages, like improving the sensitivity, time stability, and selectivity of the sensors. In addition, the PDA was used as a matrix for immobilizing enzymes to increase the specificity of the sensors [12].

Moreover, in the **Publications 1 and 2,** the magnetite nanoparticles were obtained by co-precipitation and used as a core for the polydopamine shell, together with PDA, forming a core-shell structure. Magnetite presents several unique properties i.e., biocompatibility and magnetic, which allow easy separation and manipulation of the sensing materials using a magnetic field. This simplifies the sensing process and reduces the interference from the background matrix [170,171].

The **Publication 1** presents a magnetite@polydopamine hybrid nanomaterial. This material was used for the immobilization of the enzyme - glucose oxidase from *Aspergillus niger* and, in the next step, to construct an enzymatic glucose biosensor. The scheme of activities carried out under the **Publication 1** is presented in Fig. 30.

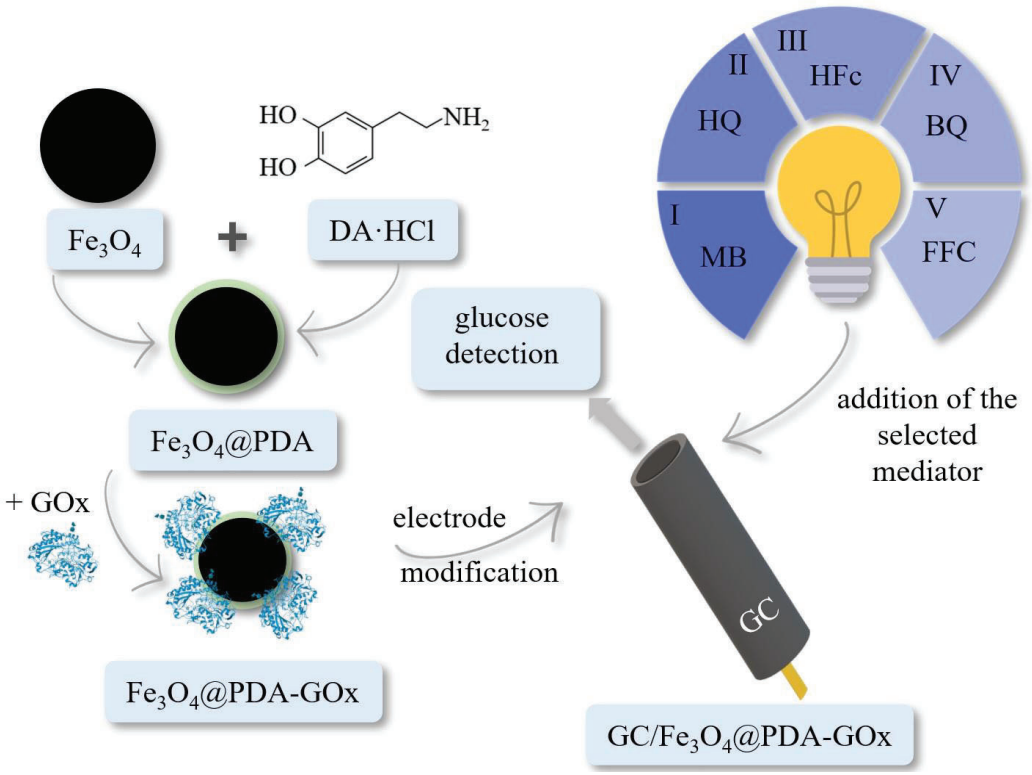


Fig. 30. *Schematic steps of obtaining an enzymatic glucose biosensor based on the magnetite@polydopamine-glucose oxidase hybrid material.*

The Fe_3O_4@PDA hybrid material was characterized by Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and atomic force microscopy (AFM) as well as electrochemical methods (cyclic voltammetry, amperometry).

The effectiveness of obtaining spherical magnetite nanoparticles with a size ranging from 8 to 12 nm was confirmed based on the TEM image. In addition, this technique also valid the coating of nanoparticles with polydopamine, characterized by a layer thickness of 2–3 nm. At a constant pH, electrokinetic analyses of Fe_3O_4 nanoparticles and Fe_3O_4@PDA were carried out. Zeta potential calculations presented that Fe_3O_4 nanoparticles (-45.9 mV) and the Fe_3O_4@PDA hybrid material (-27.6 mV) are stable in the solution.

The adsorption method was used to immobilize the enzyme – glucose oxidase. The Bradford method was applied to quantify the effectiveness of this process. It was determined that 36.3 mg g^{-1} was immobilized, which was more than twice as high as Fe_3O_4 nanoparticles (14.8 mg g^{-1}).

For comparison, the amount of glucose oxidase immobilized on various materials, Lee et al. immobilized GOx on single-walled carbon nanotubes (SWNT), and the efficiency of this process, measured using the Bradford method was 20.7 mg g^{-1} [172]. In different work, Jędrzak et al. proposed a new Fe_3O_4/Lig/PDA hybrid material as a template for GOx immobilization, and it was determined that 29.44 mg g^{-1} of the enzyme was successfully immobilized [173]. This indicates the effective attachment of the enzyme on the surface of the proposed Fe_3O_4/PDA hybrid material.

The efficiency of immobilization results from the polydopamine adhesive and hydrophilic properties, which allow for improved interactions between the enzyme molecules and the PDA surface. Moreover, this biomimetic polymer surface provides a large surface area for enzyme immobilization. This enables efficient mass transfer of the analyte. Furthermore, bare magnetite nanoparticles present a surface that may not provide as many interaction sites for enzyme immobilization as polydopamine [174].

The immobilization efficiency was also confirmed using atomic force microscopy (AFM). This method enabled the characterization of the surface morphology before and after the immobilization process. An increase in the z parameter and a change in the roughness parameter indicates enzyme attachment. Figure 31 shows AFM images before and after the GOx immobilization (24 h, pH 5.5).

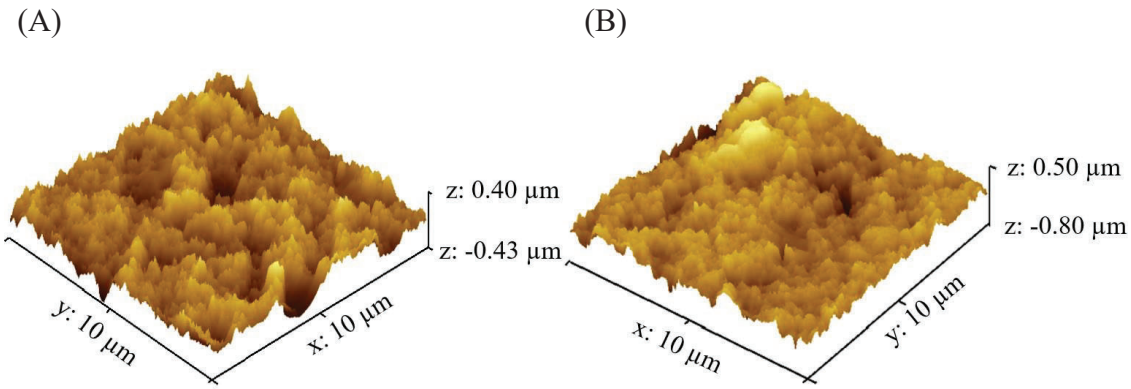


Fig. 31. *Atomic force microscopy of Fe_3O_4@PCA (A); and Fe_3O_4@PCA-GOx (B).*

An integral part of the second generation biosensors is an external mediator enabling the transport of electrons between the electrode and the enzyme active centre. The properties of the mediator (i.e., redox potential, solubility, and stability) can affect the electrochemical sensor's sensitivity, selectivity, and response time. A mediator with a higher redox potential may improve the sensor's sensitivity, while lower solubility may reduce the response time. These compounds may also have different affinities for the analytes, which may affect the sensor's selectivity. Based on this fact, selecting the appropriate mediator is crucial to optimizing the performance of the electrochemical biosensor [175].

The activities undertaken in the **Publication 1** were intended to determine how various external mediators may affect final glucose measurements and the sensor's properties (limit of detection, sensitivity, linear range). In this regard, (hydroxymethyl)ferrocene (HFc), 1,4-benzoquinone (BQ), hydroquinone (HQ), methylene blue (MB), and a mixture of potassium ferrocyanide and potassium ferricyanide (FFC) were tested, and their effect on the electrochemical properties of the sensor was determined. The proposed mediators were selected due to their ability to carry a load and, simultaneously, different properties, i.e., oxidation potential.

A biosensor was constructed during the modification of a glassy carbon electrode (GC). Electrochemical tests were carried out in PBS (pH = 7.4, 50 mM) with the addition of a particular mediator, creating a second generation biosensor. Using cyclic voltammetry (CV), the response of the biosensor to increasing glucose additions was measured, resulting in the oxidation of glucose to gluconolactone. An external mediator mediates this reaction and then the flavin adenine dinucleotide (FAD) is reduced to $FADH_2$. Equations 1 and 2 show the process of glucose oxidation.

$$\text{glucose} + \text{FAD} \rightarrow \text{gluconolactone} + \text{FADH}_2 \quad (1)$$

$$\text{FADH}_2 + \text{Med}_{ox} \rightarrow \text{FAD} + \text{Med}_{red} + 2\text{H}^+ \quad (2)$$

In Fig. 32A, the cathode and anode currents from the oxidation/reduction of hydroxy(methyl ferrocene) are visible. The system's responses were measured for solutions without and with glucose additives in the concentration range from 0 to 300 mM.

Oxidation peaks, depending on the mediator used, occurred at different potentials, which was confirmed in CV studies. Successive additions of glucose resulted in an increase in the anode current for all mediators, suggesting the efficiency of glucose oxidation by GOx and the ability of the mediators to transfer electrons. In addition, using other mediators also affected the linear range of the system.

Based on cyclic voltammetry graphs, the relationship between peak current and glucose concentration was determined (Michaelis-Menten curve), presented in graph 32B. The curves for each mediator show a characteristic, saturating shape corresponding to enzymatic reactions [176]. However, the highest current was recorded for the system with HFc as the mediator.

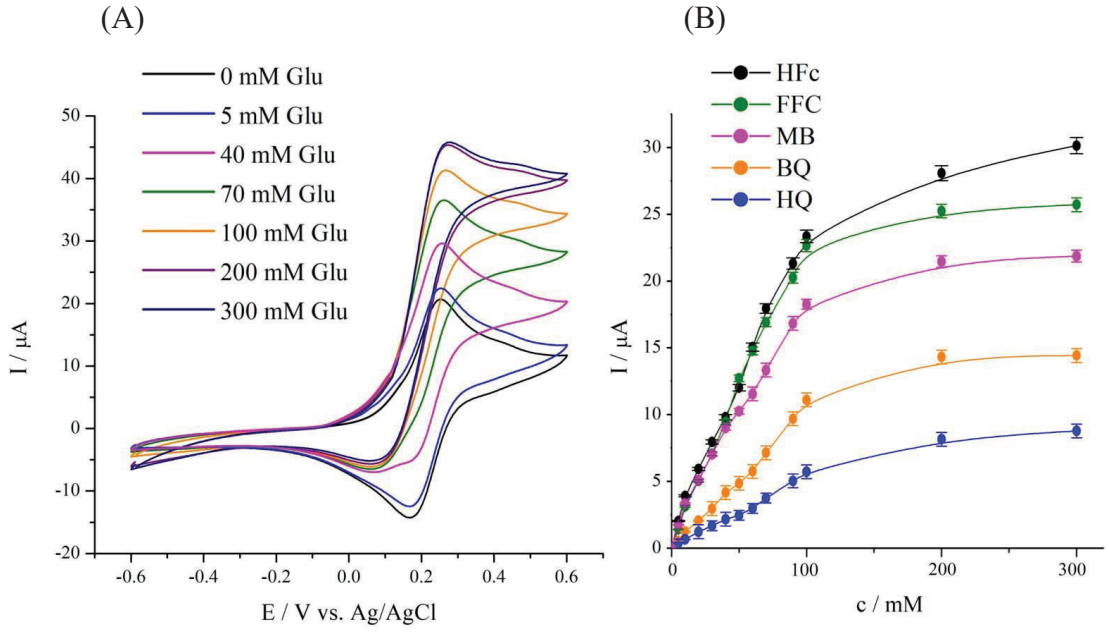


Fig. 32. *Cyclic voltammetry diagrams for GC/Fe_3O_4@PDA-GOx biosensor with increasing glucose concentration (0–300 mM) with HFc as mediator (10 mV s^{-1}, 50 mM PBS pH 7.4) (A); current vs. concentration relationships for tested (hydroxymethyl)ferrocene (HFc), 1,4-benzoquinone (BQ), hydroquinone (HQ), methylene blue (MB), mixture of potassium ferrocyanide and potassium ferricyanide (FFC) mediators (B) (n=3).*

In the next part of the research, a series of amperometric tests were carried out. Amperometry is a technique sensitive to changes in analyte concentration and can achieve low limit of detection. Moreover, amperometry provides quantitative data, allowing for precise determination of analyte concentrations. This is essential in applications where accurate measurements are critical, such as clinical diagnostics or environmental assessments.

The potential used in the research was determined based on previous CV studies. Figure 33A shows the amperometry plot for the GC/Fe_3O_4@PDA-GOx biosensor and HFc as the mediator. Measurements were conducted at a potential of +0.30 V under continuous mixing conditions, with individual additions of 1 mM glucose.

Based on the obtained results, the current-concentration dependencies were determined and shown in Fig. 33B.

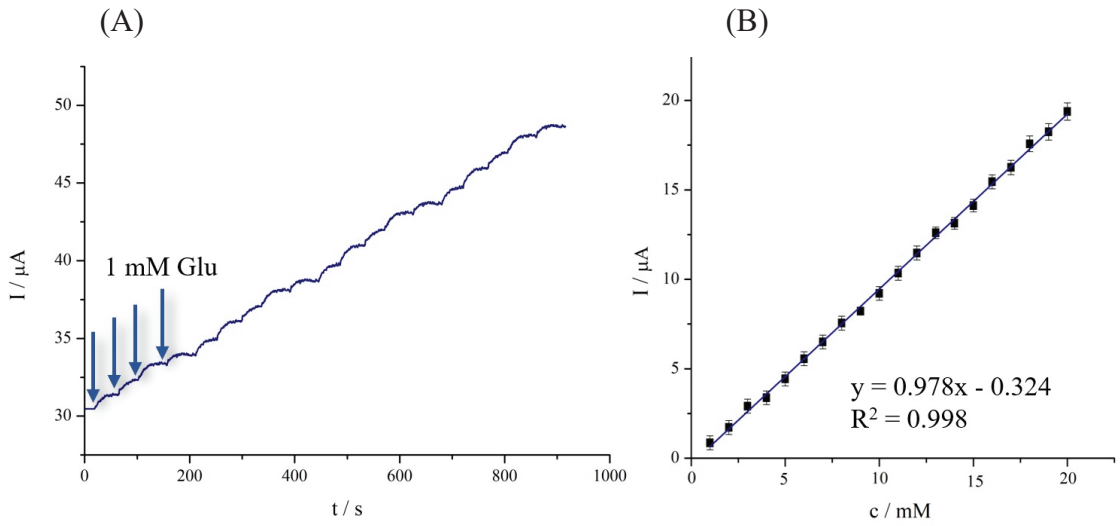


Fig. 33. *Amperometric curve for GC/Fe_3O_4@PDA-GOx biosensor at +0.30 V potential and HFc as a mediator with additions of 1 mM glucose (A); current dependence on glucose concentration (1.0–20.0) (B) (n=3).*

Figure 33B shows that the current rises as the glucose concentration increases, indicating activity with quick mass transport and quick electron transfer capabilities. The current response changed linearly from 1 to 20 mM of glucose concentration. In addition, based on this data, the sensor sensitivity was 139.71 µA mM^{-1} $cm^{-2,}$ and the limit of detection (LOD) as 1.54 µM was determined.

Similar tests were presented for other external mediators. This made it possible to determine the sensitivity, limit of detection (LOD), and linear range for each. The obtained results were compared and summarized in Table 8.

Table 8. *Comparison of the characteristic features of the GC/Fe_3O_4@PDA-GOx biosensor with (hydroxymethyl)ferrocene (HFc), 1,4-benzoquinone (BQ), hydroquinone (HQ), methylene blue (MB), mixture of potassium ferrocyanide and potassium ferricyanide (FFC) tested mediators.*

Mediator	Potential / V	I max / µA	Sensitivity / µA mM^{-1} cm^{-2}	Limit of detection / µM	Linear range / mM
MB	-0.10	29.1	117.5	1.66	2.00–17.0
HQ	+0.10	13.8	124.7	1.74	2.00–17.0
HFc	+0.30	47.2	139.7	1.54	1.00–20.0
BQ	+0.25	16.9	130.4	2.27	2.00–17.0
FFC	+0.25	34.1	125.4	3.11	1.00–19.0

As presented in Table 8, the system with (hydroxymethyl)ferrocene as a mediator was characterized by the highest sensitivity, the broadest linear range, and the lowest limit of detection (LOD). Therefore, it was chosen as an external mediator in further studies.

The choice of mediator in the proposed system was the first step taken to maximize the sensitivity of analytical tools. The environment in which the measurement was carried out also significantly impacts the final result.

Environmental conditions, such as temperature, pH, and mediator concentration, can affect the performance of through changes in sensor properties. Optimizing these conditions can enhance the accuracy and precision of glucose measurements and can potentially reduce the need for expensive components or frequent maintenance, making the biosensor more cost-effective in the long run.

Due to this fact after determining the most effective mediator, the effect of its concentration in the system was examined. Various concentrations up to 0.2 to 1.4 mM were tested. Above 1 mM of mediator, no significant current increase was observed, due to this 1 mM HFc was used for further analysis.

The effect of pH on the biosensor response was studied in the pH range of 4.0 to 10.0. The highest response was recorded at pH 7.4, which was used in further electrochemical tests carried out in the work.

The effect of mediator concentration, buffer pH, and temperature on the final response, biosensor, was determined, as shown in Fig. 34.

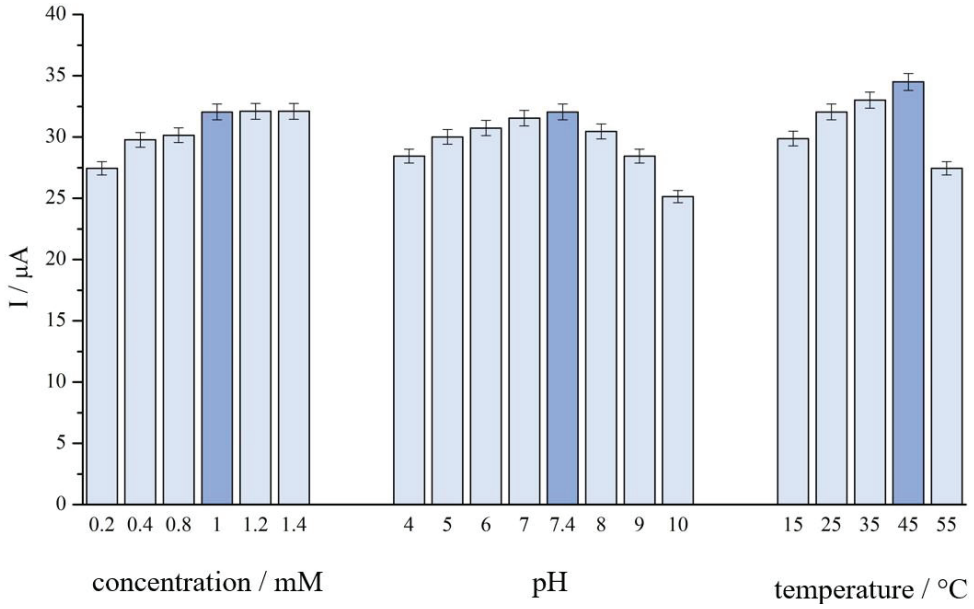


Fig. 34. *Optimization of sensor operating conditions, including temperature, pH, and mediator concentration, on the example of HFc with marked optimal conditions.*

Another parameter whose influence was checked was temperature, the influence of which was examined in the range from 15 to 55 °C. The highest current peak was recorded for a temperature of 45 °C, which is in agreement with the literature [177]. However, due to the commonness of conducting tests at the ambient temperature, it was selected for further measurements.

Electrochemical tests were also used to determine the influence of interfering agents. However, due to the low response (for all tested below 10%), it can be concluded that the proposed GC/Fe_3O_4@PDA-GOx system is selective.

An important analysis was also to check the time stability. The proposed biosensor system was tested for 60 days. After this time, a decrease in the response of 28.0% was observed (compared to the first day).

To compare, Qiu et al. presented CS-Fc/Au NPs/GOx nanocomposite for glucose detection. The system was characterized by a linear range from 0.02 to 8.66 mM, a limit of detection of 5.6 µM, and presents about 81% of its original current after four weeks [178]. The single-walled carbon nanotubes/poly(pyrrole)/glucose oxidase biosensor presented by Valentini et al. showed a linear range from 0.560 to 12.0 mM, and was dedicated to hypoglycemia disease. The limit of detection was calculated as 50 µM, and the biosensor after two weeks showed 75% of the response [179].

Finally, the biosensor system was used for tests on real glucose solutions. For this purpose, four commercially available glucose solutions were tested. The obtained results allowed for glucose detection with a recovery of 93.00 to 99.05%.

The results obtained in the **Publication 1** allowed to conclude that the proposed GC/Fe_3O_4@PDA-GOx biosensor can be a successful alternative for glucose detection, in real solutions. In addition, the scope of research allowed for the characteristics of the system and the selection of an external mediator. However, due to the need to increase the linear range and to improve time stability, it was decided to enrich the proposed system with nanocontainers. Therefore in the **Publication 2**, the presented magnetite@polydopamine hybrid material was enriched with β-cyclodextrins.

β-cyclodextrins (βCD) contain a unique molecular structure consisting of a hydrophobic cavity and a hydrophilic exterior. This structure allows them to form inclusion complexes with various guest molecules, including enzymes, which can be immobilized within the hydrophobic cavity of βCD, protecting them from denaturation and providing a stable microenvironment [180].

Moreover, immobilization with βCD in the structure can improve enzyme stability. It protects enzymes from harsh environmental conditions, for instance, temperature, pH, and exposure to organic solvents. βCD are biocompatible and biodegradable, making them suitable for various biomedical and environmental applications, including biosensors [181].

In the case of biosensors, β-cyclodextrins can enhance the sensitivity by increasing the binding affinity between the target analyte and the receptor. By immobilizing biomolecules, the presented nanocontainers can increase the number of signal-generating elements in the sensing system. This amplification can enhance the detectability and response of the biosensor, allowing for more sensitive and accurate measurements [182].

Based on the properties of βCD, a hybrid nanomaterial magnetite@polydopamine@β-cyclodextrins was proposed. The nanocontainers were attached to the hybrid nanomaterial using a thiol-Michael reaction. In the next step, the obtained system was used to immobilize glucose oxidase via the adsorption method. Then, the resulting nanomaterial was used to modify the GC electrode. The system with (hydroxymethyl)ferrocene as an external mediator was used to measure glucose in the model and real samples (glucose standards, infusion fluids, and a high-glucose diabetic pharmaceutical product).

Graphical representation of the idea and scope of work performed within the **Publication 2** are presented in Fig. 35.

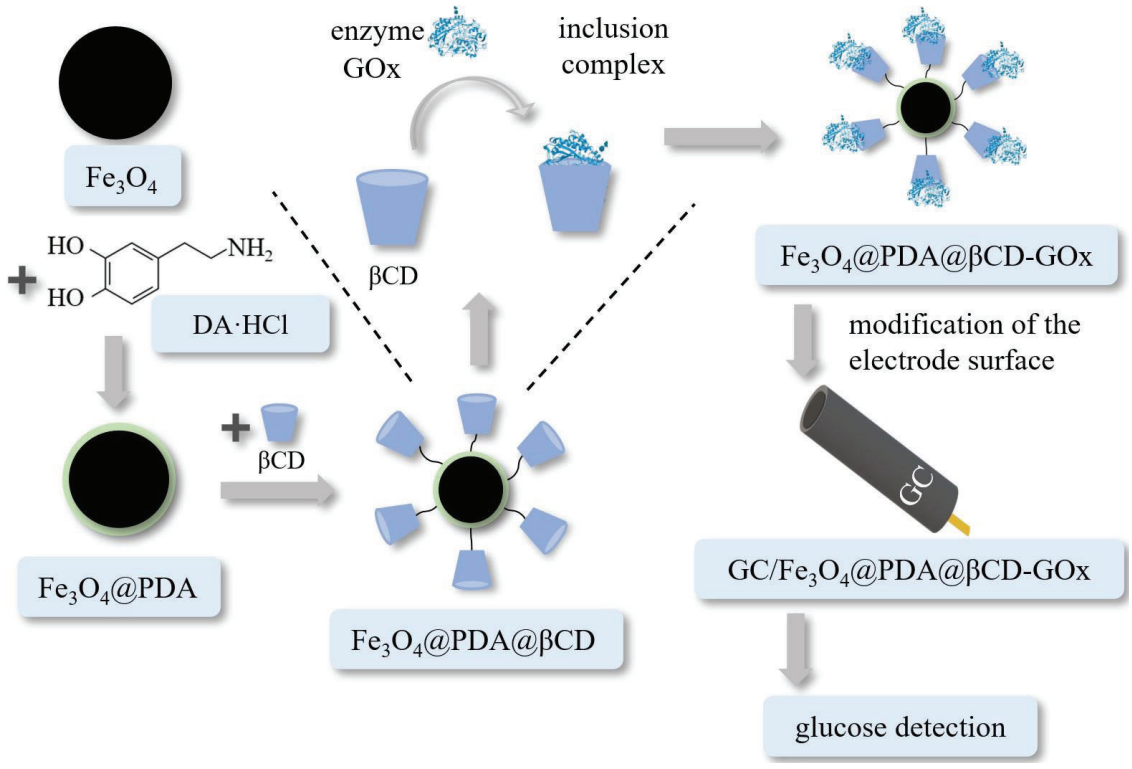


Fig. 35. *Steps for obtaining a time-stable glucose biosensor based on the Fe_3O_4@PDA@βCD-GOx hybrid material.*

To determine the properties of the hybrid nanomaterial, the Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), non-invasive light scattering (NIBS), transmission electron microscopy (TEM), and measurement of the polydispersity index (PdI) were carried out.

The transmission electron microscopy (TEM) micrographs of Fe_3O_4 present individual nanoparticles with a size in the range of 8 to 12 nm. The successful polydopamine coating of the Fe_3O_4 nanoparticles' surface was also demonstrated by the TEM images. The coating of PDA was presented with a thickness of 2–3 nm.

Further studies were performed, due to the inability to confirm the connection of βCD with the magnetite@polydopamine nanomaterial by this technique, due to the same observed sizes with the prior structure. Figure 36 shows TEM images for the Fe_3O_4 (Fig. 36A), Fe_3O_4@PDA (Fig. 36B) and Fe_3O_4@PDA@βCD (Fig. 36C).

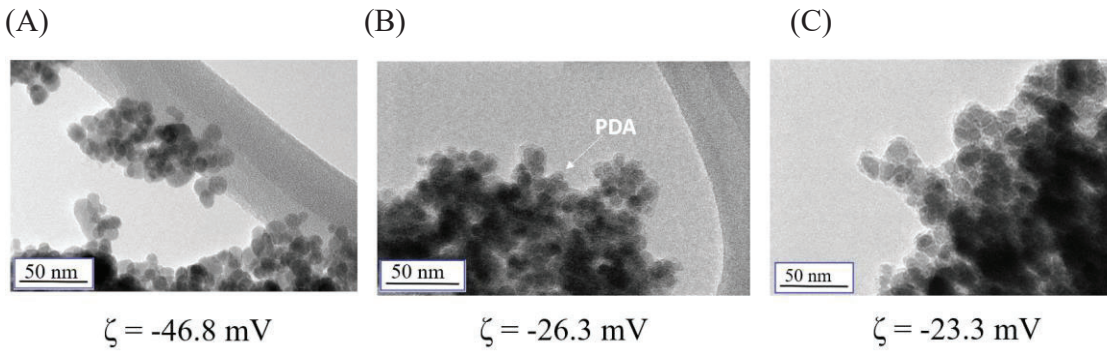


Fig. 36. *TEM photos and zeta potential values for Fe_3O_4 (A); Fe_3O_4@PDA (B); Fe_3O_4@PDA@βCD (C).*

The electrokinetic stability of the dispersion of the produced carrier was possible due to tests with the zeta potential analysis. The Fe_3O_4@PDA and Fe_3O_4@PDA@βCD presented stability dispersion values of -26.3 and -23.3 mV, respectively (Fig. 36). The obtained results indicate the effectiveness of adding β-cyclodextrins to the system and the durability of the obtained nanomaterial. Moreover, it can be inferred that the negative charge is due to the charge derived from the hydroxyl groups isolated in the polydopamine structure [183].

To compare, Liu et al. presented the zeta potential of Fe_3O_4/PDA/Ag as -28.9 mV [184]. In another work, Jędrzak et al. presented Fe_3O_4/Lig and Fe_3O_4/Lig/PDA hybrid materials with zeta potential values of -18.5 and -27.5 mV, respectively [185].

The obtained and characterized nanomaterial was used to immobilize glucose oxidase (GOx). The efficiency of the process was checked using the Bradford technique. In addition, the influence of time and the nanomaterial selection.

As shown in Fig. 37, for three nanomaterials (Fe_3O_4, Fe_3O_4@PDA, Fe_3O_4@PDA@βCD), the highest amount of enzyme was attached to the Fe_3O_4@PDA@βCD matrix (47.6 mg g^{-1}). This suggests the formation of an inclusion complex formed between βCD and guest-enzyme molecules.

Moreover, different times of immobilization (1, 6, 10, 24, 48 h) were checked for each matrix, and the highest efficiency was observed for 24 h, which was used for further research. For a longer process, a decrease in the amount of attached enzyme was observed, resulting from leaching from the surface of the nanomaterial. The comparison of different nanoplatforms with respect to immobilization time is schematically presented in Fig. 37.

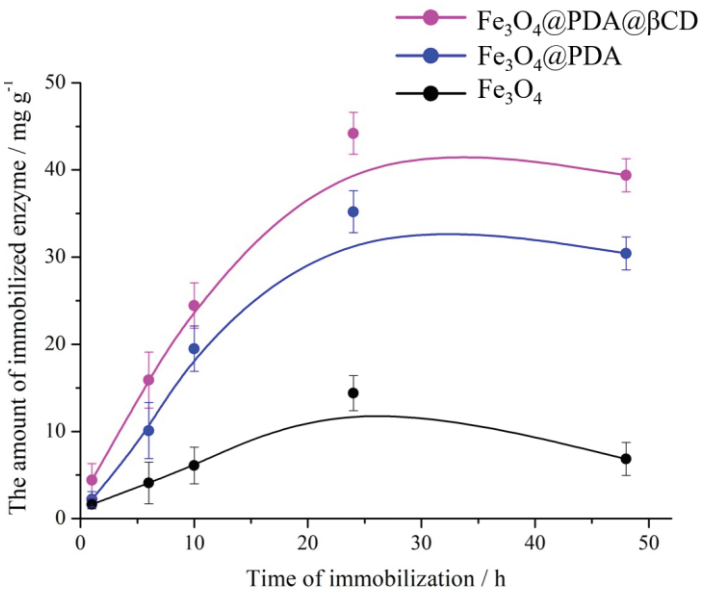


Fig. 37. *Comparison of the amount of adsorbed enzyme - enzyme-glucose oxidase depending on the matrix and immobilization time.*

The electrochemical sensors were constructed by the GC electrode modification, with HFc as an external mediator. The tests were performed in a PBS solution (pH 7.4; 50 mM), corresponding to the pH of human blood (pH 7.35 to 7.45).

Electrochemical tests were conducted to determine the effectiveness of the application potential and to check the response to individual glucose additions. On the graph of cyclic voltammetry (Fig. 38A), characteristic oxidation peaks can be observed at the potential of +0.3 V. The peak comes from the oxidation reaction of (hydroxymethyl)ferrocene used as an external mediator in the system.

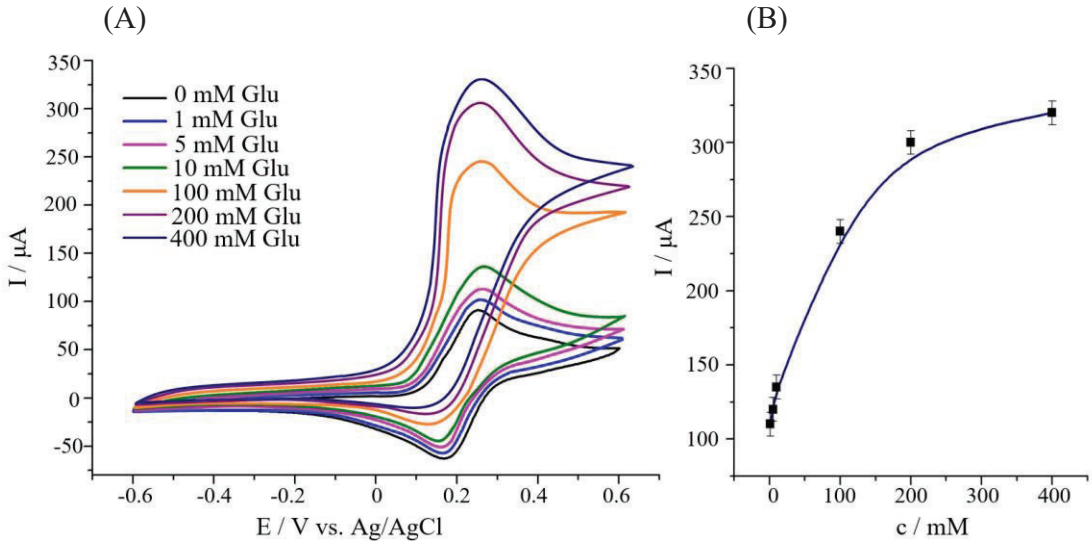


Fig. 38. *Fe₃O₄@PDA@βCD-GOx biosensor cyclic voltammetry response to different concentrations (0-400 mM) of glucose (10 mV s⁻¹, 50 mM PBS pH 7.4) (A); dependence of current on glucose concentration (B) (n=3).*

Based on the obtained cyclic voltammograms, the dependence of the current on glucose concentration was determined (Fig. 38B). For concentrations above 250 mM glucose, the system began to saturate and generate lower and non-linearly currents, which is due to the enzymatic nature of the system [186].

The Km (Michaelis-Menten) constant is a parameter used to describe the affinity between the enzyme and its substrate (the target analyte). It represents the substrate concentration at which the enzyme achieves half its maximum catalytic activity. It indicates how effectively the enzyme can convert the substrate into a product at different substrate concentrations. Based on the obtained cyclic voltammetry, the Michaelis-Menten constant (Km) was determined, which was 23.2 mM, suggesting that relatively high substrate concentrations are required to achieve maximum enzyme activity.

To compare, the Km value of the PNE/GOD/AuNPs@PNE/Au electrode presented by Liu et al. was determined to 6.8 mM [187]. Another Km value (19 mM) was presented by Kong et al., which attached GOx to ZnO nanotubes [188]. The Michaelis–Menten constant was also presented by Zare et al. on the chitosan film and determined as 11.3 mM [189].

To determine the important features of the biosensor tests were carried out at a potential of +0.3 V, under constant mixing conditions. As part of the research, the increase in current for increasing glucose concentrations was checked (additions of 1 mM and 2 mM). The results are shown as current versus time in Fig. 39A.

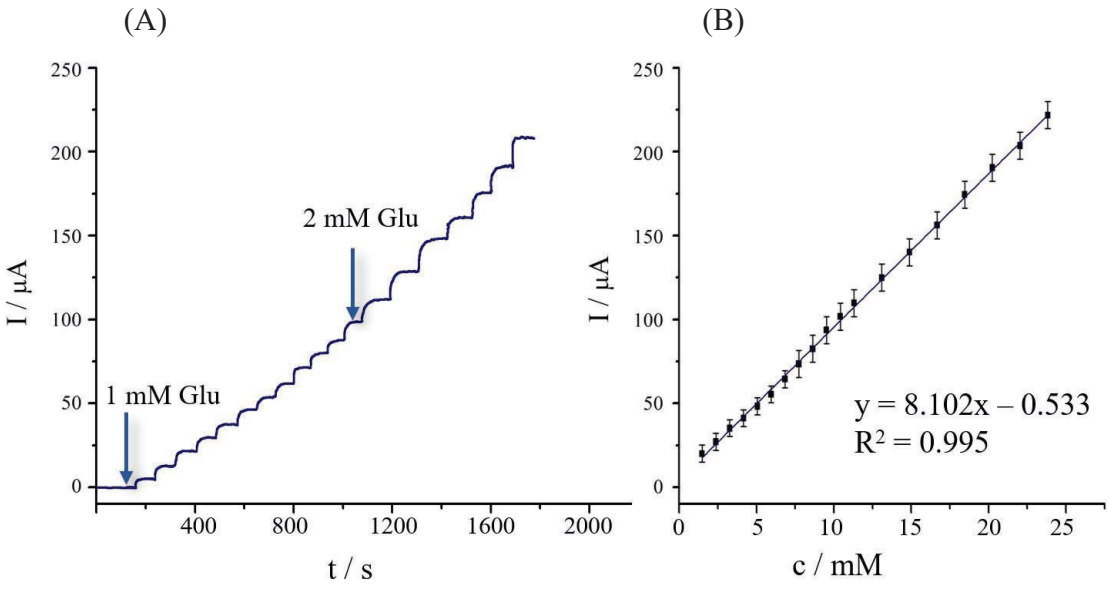


Fig. 39. *The amperometric response of the Fe_3O_4@PDA@βCD-GOx biosensor to glucose additions (1.0-26.0 mM) at +0.30 V potential and HFc as mediator (PBS pH 7.4) (A); the dependence of the current vs. concentration (B).*

Plotting the current-concentration relationship (Fig. 39B) allowed the determination of the limit of detection (LOD) was 1.55 μM, linear range from 1 to 26 mM, and sensitivity as 115.74 μA mM^{-1} cm^{-2}.

The characteristics of the obtained glucose biosensors in the **Publication 1** and the **Publication 2** were compared with other second generation glucose biosensors, these properties are summarized in Table 9.

Table. 9. *Comparison of second generation glucose biosensors using an external ferrocene-based mediator*

Electrode material	**Mediator**	**Linear range / μM**	**Sensitivity / μA mM^{-1} cm^{-2}**	**LOD / μM**	**Ref.**
AMWNTs-GOx	ferrocene monocarboxylic acid	0.01–4.2	10.56	3.4	[190]
GOx/PEI	ferrocene	0–5.0	18.0	-	[191]
GNPs/CD/GOx	ferrocene	0.08–11.5	-	15.0	[192]
Fe_3O_4/Lig/PDA/GOx	ferrocene	1.0–90.0	-	-	[173]
Nylon-6 NFs-GOx	ferrocene methanol	1.0–10.0	-	6.0	[193]
Fe_3O_4@PDA-GOx	**(hydroxymethyl) ferrocene**	**1.0–20.0**	**139.71**	**1.54**	**Publ. 1**
Fe_3O_4@PDA@βCD-GOx	**(hydroxymethyl) ferrocene**	**1.0–26.0**	**115.74**	**1.55**	**Publ. 2**

In addition, electrochemical tests were used to determine the influence of the interfering agents, which, as a result of the selection of the selective enzyme, all responses were below 10% relative response to glucose.

Time stability is an essential feature of biosensors because it ensures the reliability and accuracy of their measurements over an extended period. These properties directly impact the accuracy and precision of biosensor measurements. If a biosensor is not time stable, its response may change, leading to inaccurate readings. A stable biosensor, on the other hand, maintains a consistent and reliable response, enabling precise and accurate quantification of analytes. Commercial biosensors are often produced in large quantities and may need storage before use [194].

One of the main goals of adding β-cyclodextrins to the system was to extend the time stability through the unique properties of the nanocontainers. As shown in Fig. 40,

the sensor system without βCD showed time stability for five months, after which the system's response was less than 50% of the relative response.

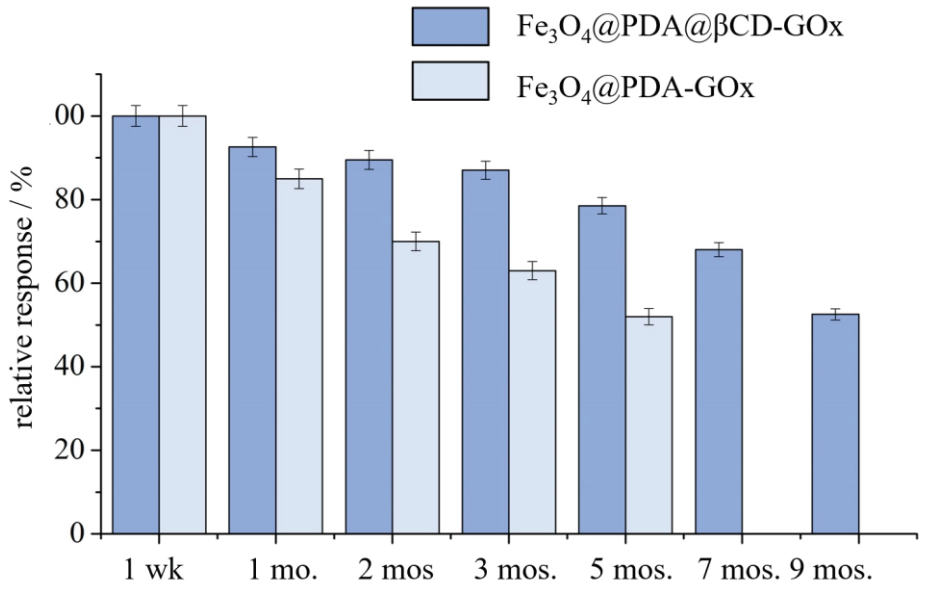


Fig. 40. *Time stability of the glucose biosensor without and with β-cyclodextrins (n=3).*

Furthermore, the enrichment of the system with nanocontainers resulted in extending the stability up to nine months. After this time, the system operated with a relative response of 52% compared to the initial property.

A comparison of the time stability of the obtained biosensor with other glucose sensors is presented in Table 10.

Table 10. *Comparison of the glucose biosensors time stability.*

Electrode	**Retained activity / %**	**Storage time / days**	**Ref.**
FMC/AMWNTs/GOx	91.0	30	[190]
Au/GOx	96.0	10	[195]
Nafion/GOx–GNPs	90.0	14	[196]
Pt nanoflowers/GOx/PU-PEG	90.0	23	[197]
Fe_3O_4@PDA-GOx	**82.0**	**90**	**Publ. 1**
Fe_3O_4@PDA@βCD-GOx	**74.4**	**150**	**Publ. 2**

Real solution studies were performed using four samples, including glucose standards, infusion fluids, and a high glucose diabetic pharmaceutical product.

The tests were carried out using the standard addition technique. The results are presented in Table 11.

Table 11. *Measurements of the real glucose samples with the use of Fe_3O_4@PDA@βCD-GOx biosensor.*

Sample	**Reported concentration / mM**	**Reported concentration / mg mL^{-1}**	**Found / mM**	**Recovery / %**
Glucose Standard I	3.88	69.9	3.45 ± 0.05	88.9
Glucose Standard II	7.00	126.1	6.55 ± 0.07	93.5
Glucose 5% Infusion	5.67	102.1	5.15 ± 0.09	90.8
1WW	3.27	58.9	3.01 ± 0.05	92.0

The proposed system showed a recovery in the range of 88.91 to 93.57%, which can be concluded that it is suitable for testing on real solutions in the food and pharmaceutical industries.

The advantages of the proposed biosensor based on the magnetite@polydopamine@β-cyclodextrines-glucose oxidase hybrid material include: (i) **an increased amount of the enzyme immobilized**; (ii) **a wider linear range compared to the system without βCD**; (iii) **lower limit of detection (LOD)**; (iv) **a longer period of time stability**.

5.2. Results of applied poly(caffeic acid)-based hybrid materials for sensors

The effectiveness and results obtained using the polydopamine-based biosensor led to the use of another biopolymer. Poly(caffeic acid) was chosen due to its numerous functional groups and more significant electrochemical activity. Polydopamine and poly(caffeic acid) are both polymeric materials derived from naturally occurring compounds and share some similarities, primarily due to their structural components. Both polymers are known for their biocompatibility, making them suitable for various biomedical and bioengineering applications. They are generally considered nontoxic and biologically compatible. However, polydopamine's catechol groups enable it to interact with metal ions, facilitating metal coating and surface modification. Poly(caffeic acid), except for catechol groups, contains carboxylic acid groups that can be involved in hydrogen bonding and other chemical interactions.

Research conducted with poly(caffeic acid) was described in the **Publications 3-6**. This catechol compound was used for the construction of a non-enzymatic sensor for the detection of NADH and glucose and also for the construction of an enzymatic glucose biosensor.

Poly(caffeic acid) exhibits natural electrochemical activity, allowing redox reactions due to functional quinone groups. This moiety can undergo reversible oxidation and reduction reactions, allowing the detection and quantification of various analytes. This property makes it possible to use PCA as an active material in electrochemical sensors.

In addition, poly(caffeic acid) derived from a natural phenolic compound can be qualified as biocompatible. This property is beneficial for the development of biosensors, which use biopolymer film as a platform to immobilize the enzyme. The biocompatibility of this compound ensures minimal interference with biological components, allowing for sensitive and selective detection [198,199].

In the **Publications 3-5,** multi-walled carbon nanotube (MWCNT) were used to create a hybrid nanomaterial. MWCNT exhibits electrical conductivity, crucial for efficient electron transfer during electrochemical reactions, enables rapid charge transfer and promotes electrochemical signal amplification, improving sensor performance.

Moreover, MWCNT can be easily functionalized by introducing different chemical groups or modifying their surfaces. Functionalization provides opportunities for tailoring the properties of MWCNT, like enhancing selectivity, promoting specific

analyte interactions, or facilitating the attachment of specific molecules for sensing applications [200,201].

In the **Publication 3**, a synthesis of the hybrid system multi-walled carbon nanotube@poly(caffeic acid) was proposed. The presented system was used as a non-enzymatic sensor to detect nicotinamide adenine dinucleotide (NADH). The individual steps and the genre idea of the publication are schematically presented in Fig. 41.

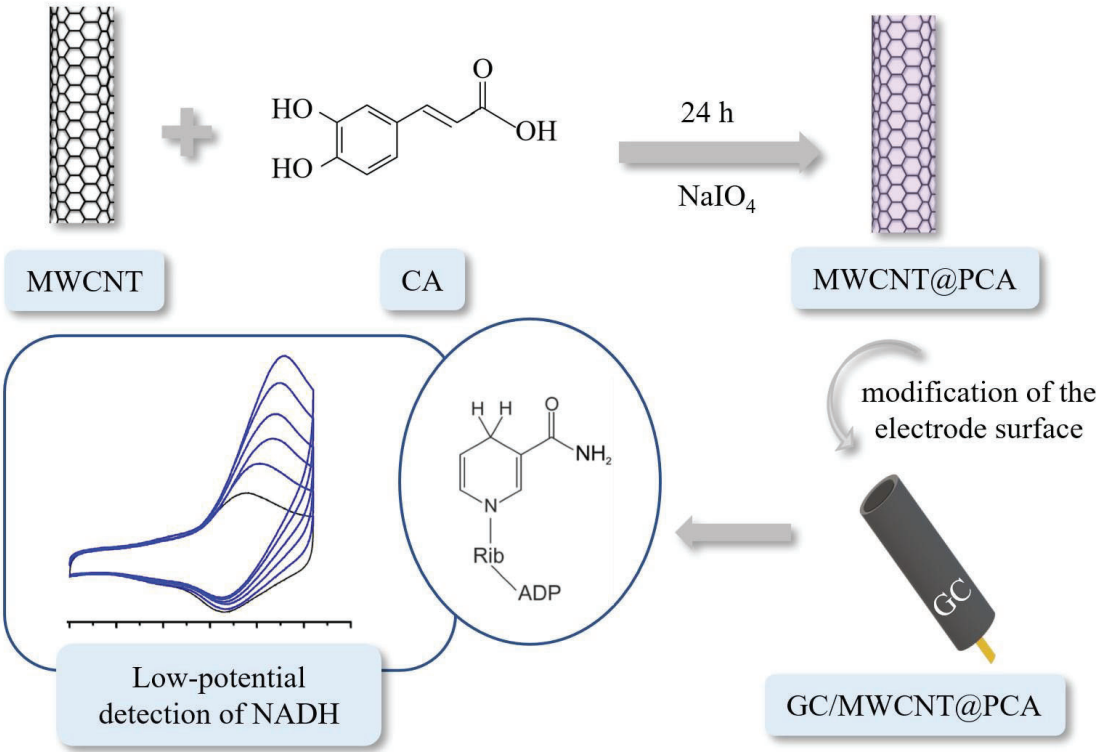


Fig. 41. *The stages of obtaining the PCA@MWCNT hybrid nanomaterial and the design of the electrochemical sensor for NADH detection.*

The synthesis of the hybrid material was based on a multi-walled carbon nanotube (MWCNT) placed in a solution of caffeic acid (CA). In the next step, an oxidant (sodium periodate, $NaIO_4$) was added, and the system was stirred without oxygen for the specified time.

The cyclic voltammetry was used to examine the electrochemical characteristics of the materials that were produced at varied polymerization periods in PBS solution (pH = 7.4, 50 mM). The CVs for PCA@MWCNT, with different polymerization times, are presented in Fig. 42A. As shown by the results of cyclic voltammetry, polymerization time significantly affects the electrochemical properties of materials.

The modified electrode showed a well-defined redox couple at $E^{0'}$ = +0.18 V and a small, irreversible redox couple at $E^{0'}$ = -0.10 V after 10 h of polymerization. Both the PCA_{12h}@MWCNT (curve b) and GC/PCA_{16h}@MWCNT (curve c) electrodes were distinguished by two redox couples with comparable current intensities. Moreover, the PCA_{24h}@MWCNT electrode exhibited a dominating pair at $E^{0'}$ = -0.10 V and smaller peaks at $E^{0'}$ = +0.18 V (curve d). After 48 h of polymerization (PCA_{48h}@MWCNT), both redox couples' intensities and locations essentially stayed consistent. Figure 42B presents the structure and mechanism of the proposed redox transitions.

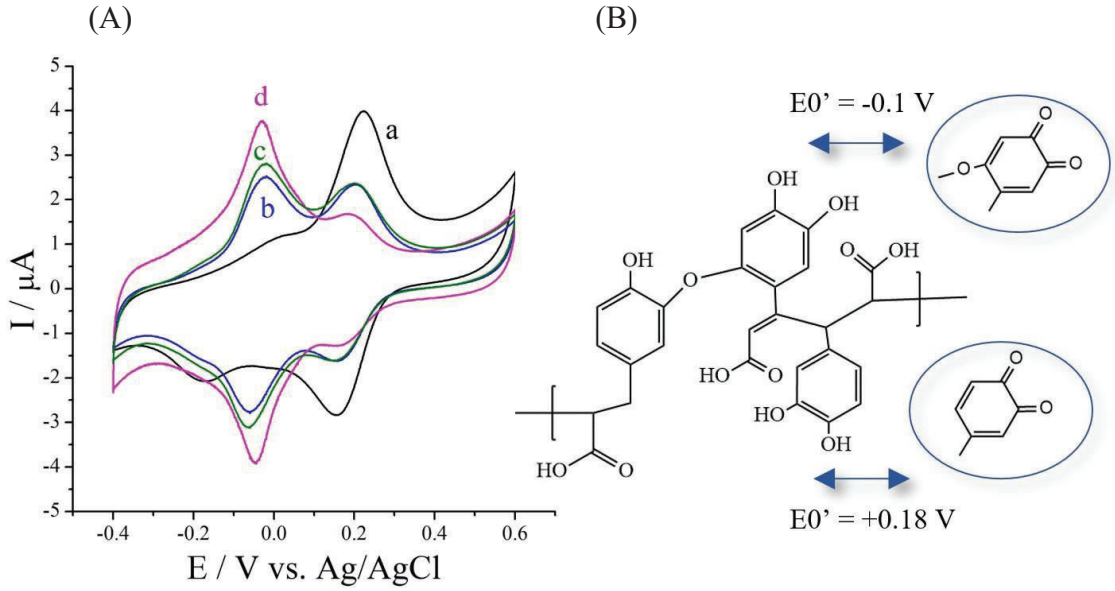


Fig. 42. *CVs for PCA_{10h}@MWCNT (a), PCA_{12h}@MWCNT (b), PCA_{16h}@MWCNT (c), PCA_{24h}@MWCNT (d) PCA_{48h}@MWCNT (e) recorded in PBS solution (50 mM, pH = 7.4) (A); proposed structure of PCA polymerization and redox transitions taking place on PCA_{24h}@MWCNT modified electrode (B).*

Due to the presented electrochemical potential and the observed peaks, the material, after 24 h of polymerization, was selected for further tests and a more detailed physicochemical characterization.

The MWCNT morphology was characterized before and after PCA coating using transmission electron microscopy (TEM) and atomic electron microscopy (AFM). As the obtained AFM images showed, the PCA layer (thickness 3-4 nm) is uniform and smooth on MWCNT, which proves the strong interaction of PCA with the surface of carbon nanotubes. AFM images of MWCNT before (A) and after PCA polymerization (B) are shown in Fig. 43.

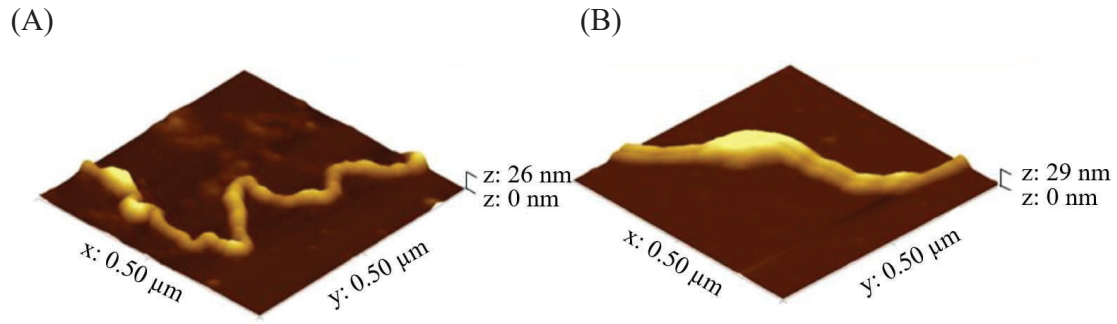
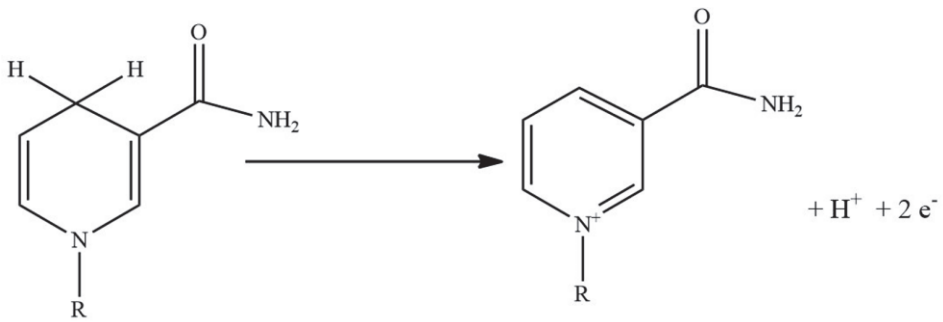


Fig. 43. *AFM images of MWCNT (A); and MWCNT@PCA (B) hybrid material.*

Electrochemical measurements of nicotinamide adenine dinucleotide (NADH) are important in various fields, including biochemistry, biotechnology, and medical diagnostics. NADH is a crucial coenzyme involved in numerous enzymatic reactions, particularly in cellular energy metabolism. It participates in the electron transfer process, playing a key role in cellular respiration and energy production. Measuring NADH levels, gain insights into the metabolic activity and redox status of cells or biological systems [202].

The electrochemical measurement of NADH involves the use of electrodes to detect the changes in current or potential resulting from the oxidation of NADH. The electrode is typically functionalized with suitable materials or enzymes to enhance the electrochemical response. NADH is oxidized at the electrode surface, leading to electron transfer, which is transformed into measurable signals like current or potential.

To check the effectiveness of the proposed sensor, electrochemical tests were carried out. The electro-oxidation of NADH involves the transfer of electrons from NADH to an electrode surface. The mechanism of NADH electro-oxidation can be described as follows [203]:



1,4-NADH NAD^+ (3)

The PCA@MWCNT, which was created by chemically polymerizing of CA after 24 h presents the maximum oxidation current and a strong oxidation peak at -0.02 mV, as presented in Fig. 44A. As a result, the PCA@MWCNT electrode showed a considerable improvement in electrocatalytic performance.

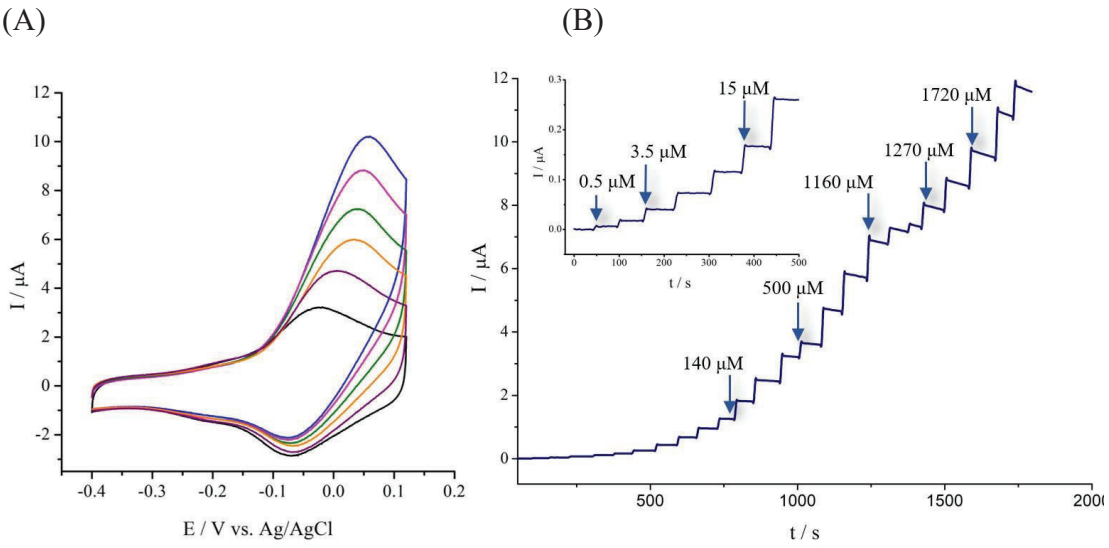


Fig. 44. *The PCA@MWCNT system with NADH additions (0, 1, 2, 3, 4, 5 mM) (A); amperometric response for different concentrations (B).*

As shown in Fig. 44A, the electro-oxidation process was observed at the potential of 0 V. The possibility of detection at lower potentials enables the reduction of the influence of interfering agents, which is a beneficial effect when conducting measurements in real solutions. In addition, such values mean that the proposed electrically shielding material can be used in biocells [204].

In comparison, the potential of NADH oxidation presented by Manusha P. et al. on the SCPE/AgRNPs/PTZ electrode was +0.4 V [205]. Eryiğit M. et al. presented Au/ERGO/PB material, which uses the +0.85 V potential for NADH detection [206]. Another material Si/CNTf, was presented by Barrio M. et al. and allowed to conduct tests at a potential of +0.2 V [207].

The amperometric response of the sensor was recorded in PBS (pH 7.4, 50 mM) at a potential of +0.1 V and presented in Fig. 44B. Individual additions of NADH caused a rapid increase in current, which stabilized over time. These results suggest the catalytic capabilities of the proposed sensor and the ease of NADH oxidation at a relatively low potential. The dependence of the current on the concentration of NADH allowed to obtain calibration curves and determine the linear range from 0.5 to

1270 µM. The limit of detection for the proposed sensor was 0.12 µM, with a sensitivity of 85.7 µA mM^{-1} cm^{-2}.

A comparison of the characteristics of the proposed PCA@MWCNT sensor with other electrochemical ones for NADH detection is presented in Table 12.

Table. 12. *Comparison of electrochemical sensors for NADH detection.*

Electrode	**Potential / V**	**Linear range / µM**	**Sensitivity / µA mM^{-1} cm^{-2}**	**LOD / µM**	**Ref.**
SCPE/AgRNPs/PTZ	+0.40	1.90–89.0	68.9	0.52	[205]
Au/ERGO/PB	+0.85	1.00–100.0	1.74	0.23	[206]
Si/CNTf	+0.20	50.0–1600.0	54.0	-	[207]
Cro/MWCNT	+0.22	0.50–100.0	0.25	0.10	[208]
GC/MWCNTs/Flu	+0.10	15.0.0–84.0	40.0	5.00	[209]
PCA@MWCNT	**+0.10**	**0.50–1270.0**	**85.7**	**0.12**	**Publ. 3**

An important stage of the conducted activities was the detection of NADH in human fluids (human serum). Measurements were carried out by the standard addition method. The results obtained for the two concentrations of 30 and 60 µM resulted in a recovery of 103.6 % and 103.8 %, respectively.

The proposed in the **Publication 3** hybrid nanosystem enriched with poly(caffeic acid) enabled (i) **modification of multi-walled carbon nanotubes by significantly increasing electroactive functional groups**; (ii) **oxidation of NADH at reduced electrocatalytic potentia**l; (iii) **improvement of properties (sensitivity, linear range) in relation to PCA obtained by electropolymerization**. In addition, satisfactory results in the direction of NADH oxidation allowed to show the potential of the produced material for the construction of enzymatic biosensors based on dehydrogenases (i.e., alcohol dehydrogenase, glucose dehydrogenase, lactate dehydrogenase).

Poly(caffeic acid) has shown promise as an electroactive material due to its ability to undergo redox reactions, as a result in the **Publications 4-5,** the development of non-enzymatic sensors for glucose detection were performed. Non-enzymatic glucose sensors employ alternative methods to detect and quantify glucose levels, these sensors typically rely on specific chemical reactions or physical properties of glucose to achieve their purpose.

Non-enzymatic sensors can be considered as an alternative to enzymatic sensors for glucose detection due to (i) **stability** (enzymes used in enzymatic sensors are susceptible to degradation over time); (ii) **cost-effectiveness** (enzymes used in enzymatic sensors can be expensive to produce, which contributes to the overall cost of the sensor); (iii) **time stability and reliability** (non-enzymatic sensors, being less dependent on a biological component, can offer long-term stability and consistent performance). These properties were the motivation for this doctoral dissertation to attempt to design and obtain non-enzymatic glucose sensors.

The **Publication 4** uses the electrocatalytic properties of copper(II) oxide (CuO) for the electrochemical oxidation of glucose as a result of decorating the previously proposed hybrid material poly(caffeic acid)@multi-walled carbon nanotubes (PCA@MWCNT).

The individual stages performed as part of the **Publication 4** are shown in Fig. 45.

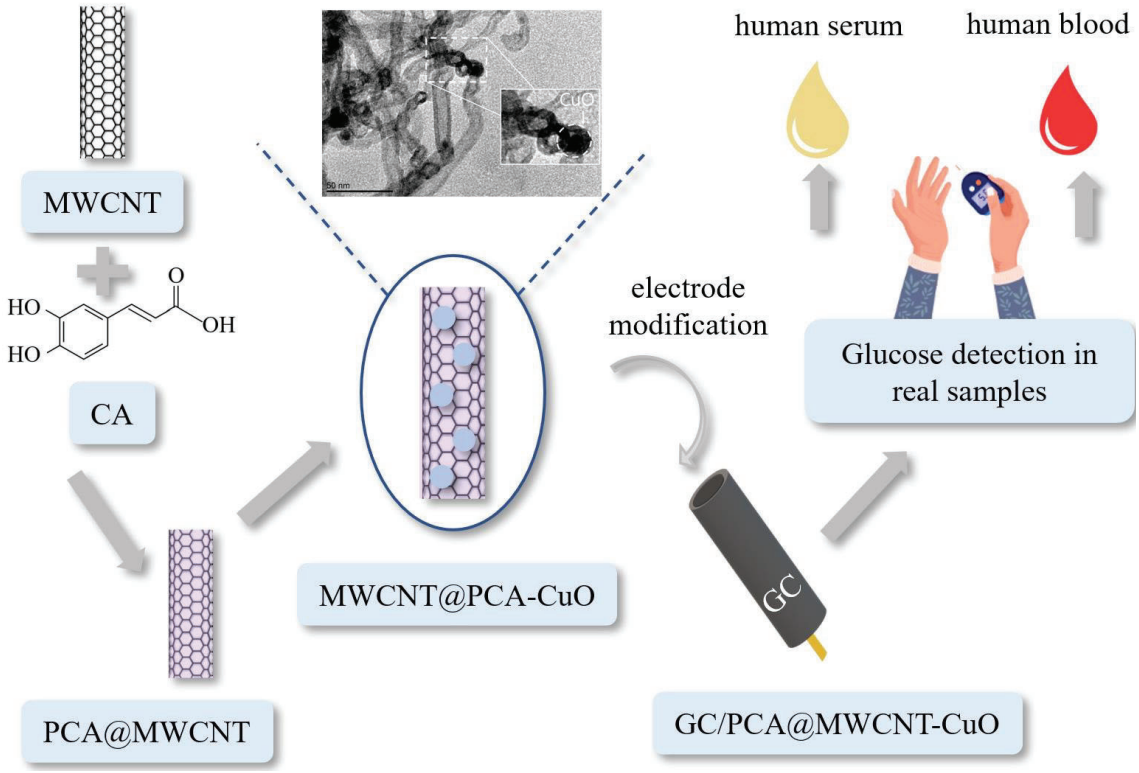


Fig. 45. *Preparation of a non-enzymatic glucose sensor based on the hybrid PCA@MWCNT-CuO nanomaterial.*

Copper(II) oxide (CuO) is a promising material for non-enzymatic electrochemical glucose detection due to its electrocatalytic properties, making it suitable for glucose sensing applications. CuO-based electrodes can be easily fabricated through various methods, for instance, electrodeposition, sputtering, or hydrothermal synthesis.

Copper(II) oxide acts as an efficient catalyst for the oxidation of glucose. It provides a favorable surface for glucose molecules to interact and undergo oxidation, thereby facilitating the electrochemical reaction [210].

On the previously (in the **Publiaction 4**) characterized PCA@MWCNT material, copper(II) oxide nanoparticles were deposited in an electrochemical process. In the first stage, the electrode was immersed in a solution of copper(II) sulfate(VI) ($CuSO_4$). The optimum range for the $CuSO_4$ concentration was checked using different concentrations (0.001-0.1 M), and 0.1 M was chosen as the optimum. Additionally, the duration of immersion ranged was tested from 1 to 20 min, with 15 min being the most beneficial accumulation time.

The PCA@MWCNT containing chelated copper ions was added to the PBS solution, where it was reduced by cyclic voltammetry sweep between potentials of +0.2 and -0.6 V (with the scan rate 10 mV s^{-1}) to produce Cu nanoparticles. The resulting electrode was then submerged in a 0.1 M NaOH solution after being washed with distilled water. The electro-oxidation procedure was carried out using a +0.6 V for 5 min.

The obtained nanomaterial was then characterized using various techniques including: energy-dispersive X-ray spectroscopy (EDS), atomic force microscopy (AFM), transmission electron microscopy (TEM), Raman spectroscopy, and inductively coupled plasma mass spectrometry (ICP-MS).

The morphological characteristic (TEM, AFM) allowed to defined obtained CuO particles as roughly spherical, with sizes of 10-15 nm. TEM and AFM images for the hybrid nanomaterial PCA@MWCNT decorated with CuO are presented in Fig. 46.

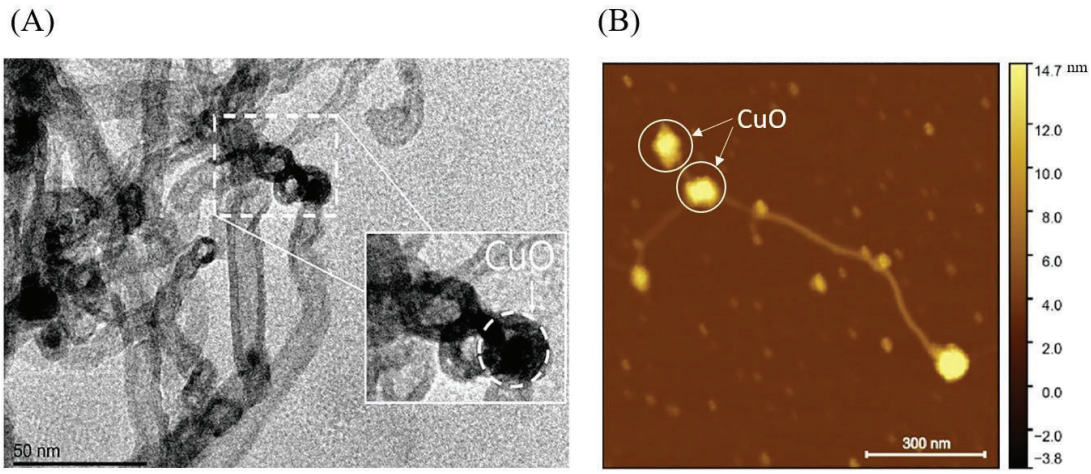


Fig. 46. *TEM (A); AFM pictures (B) of PCA@MWCNT-CuO hybrid nanomaterial.*

The calculation of the quantitative elemental composition found that copper made up 4.6% of the total (EDS). Mass spectrometry combined with inductively induced plasma (ICP-MS) was a second technique used to determine the total quantity of copper present in the PCA@MWCNT-CuO material. The amount of copper in the sample was 4.3% (by weight).

Electrochemical tests (cyclic voltammetry, amperometry, and electrochemical impedance spectroscopy), as in the case of enzymatic systems, were used to check the response of the proposed system to individual additions of glucose.

Glucose can be electrochemically oxidized using copper oxide as a catalyst. The existence of the active redox pair Cu^{2+} and Cu^{3+} in an alkaline environment serves as the foundation for the non-enzymatic mechanism of glucose oxidation. Glucose electro-oxidation is a two-step procedure, in the first one, Cu^{2+} is converted to Cu^{3+} during the anodic reaction when the proper voltage is supplied. The regeneration of CuO occurs in the second stage, which involves an oxidation/reduction reaction between glucose and CuO(OH). The following is a presentation of the mechanism [211,212]:

$$CuO + OH^- \rightarrow CuO(OH) + e^- \quad (4)$$

$$2CuO(OH) + \text{glucose} \rightarrow 2CuO + \text{gluconolactone} + H_2O \quad (5)$$

In the next phase, gluconolactone undergoes a hydrolysis reaction to become gluconic acid [213]. The cyclic voltammetry tests presented in Fig. 47A show that the glucose oxidation current increases in proportion to the concentration. In addition, the oxidation peak was observed at +0.45 V. The current dependence on glucose

concentration (Fig. 47B) shows the linear increase in current after the addition of glucose in the range of glucose concentrations from 1 to 15 mM. Additional electrochemical tests determine the kinetics of the proposed sensor as diffusion by examining the change in the scanning rate. Moreover, to determine the characteristic parameters of the sensor, tests were carried out using amperometry at a potential of +0.45 V.

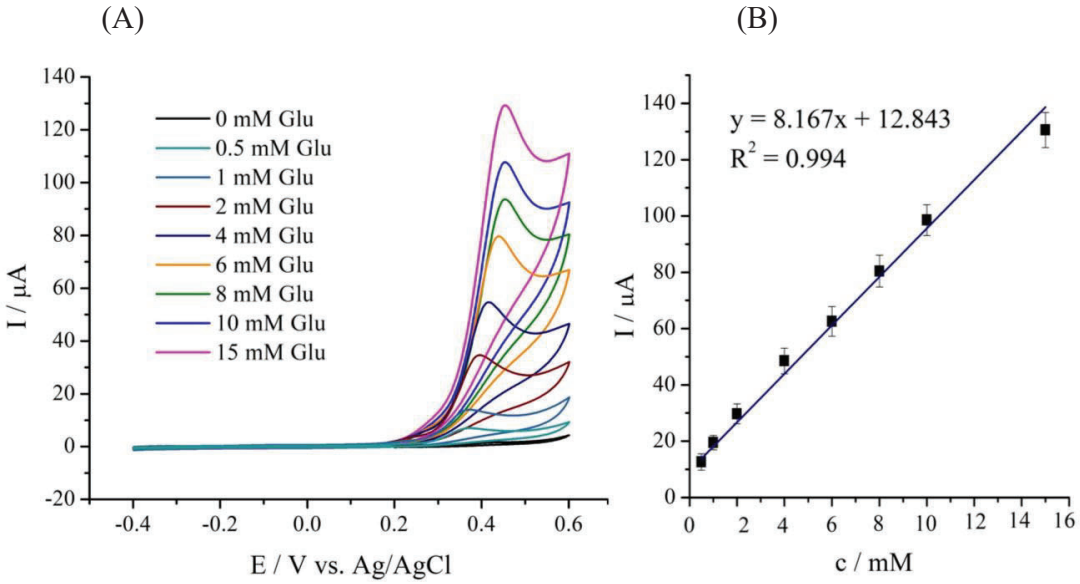


Fig. 47. *Cyclic voltammetry response to increasing concentrations (0-15 mM) of glucose (10 mV s^{-1}, 0.1 M NaOH) (A); current vs. glucose concentration (B) (n=3).*

Using various quantities of glucose (1-10000 µM) in 0.1 M NaOH, the amperometric response of the GC/PCA@MWCNT-CuO electrode was measured under stirring conditions at a constant potential of +0.45 V (Fig. 48A). The electrode demonstrated linearity in a wide range of concentrations (2 - 9000 µM), as shown by the calibration curve displayed (Fig. 48B), Based on this data the sensitivity was calculated as 2412.0 µA mM^{-1} cm^{-2}, with a limit of detection (LOD) of 0.43 µM, and a limit of quantitation (LOQ) of 1.31 µM.

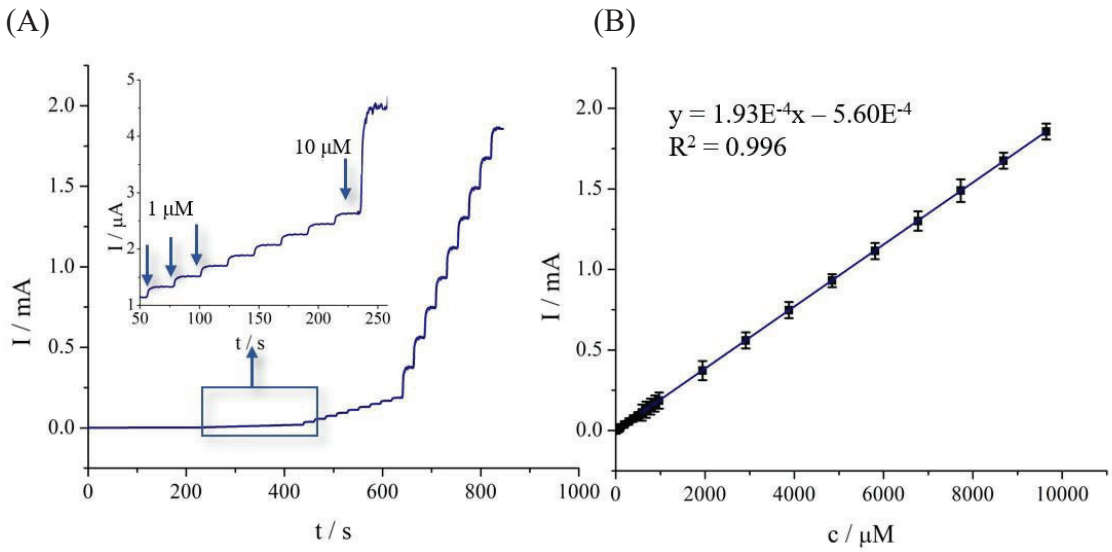


Fig. 48. *Amperometric response of the proposed PCA@MWCNT-CuO sensor to increasing glucose (2 - 9000 µM) concentrations (+0.45 V, 0.1M NaOH) (A); current vs. glucose concentration (B).*

In the next stage, the influence of the interfering agents (Fig. 49) on the proposed system was checked. Interferences were selected for the study, along with the appropriate concentrations that can be found in human fluids. For amperometric tests, 0.01 mM dopamine, 0.04 mM uric acid, 0.1 mM ascorbic acid, and 0.01 mM L-cysteine in 0.1 M NaOH were recorded at +0.45 V relative to 4 mM glucose measurements.

In the case of the tested interferents, the current changes are below 10%, the values of which are considered acceptable for the electrochemical sensor or biosensor selectivity [214]. In addition, the fact that the influence of the interferents is estimated at 3% compared to the relative glucose response indicates a good anti-interference ability of the presented interferents.

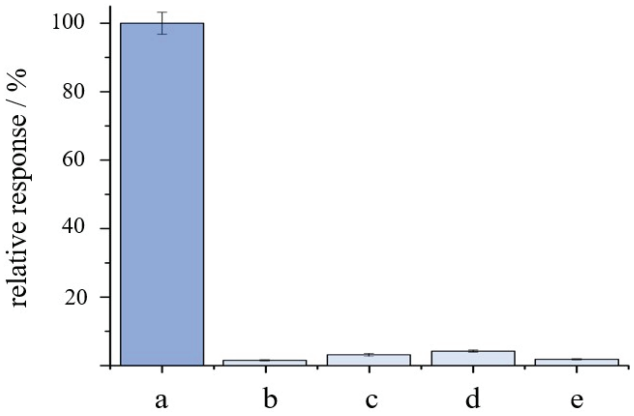


Fig. 49. *Effect of various interference 0.01 mM dopamine (b), 0.04 mM uric acid (c), 0.1 mM ascorbic acid (d), and 0.01 mM L-cysteine (e) on sensor performance compared to glucose measurement (a)(+0.45 V, 0.1 M NaOH) (n=3).*

Further electrochemical studies of the biosensor allowed to check its time stability. The designed GC/PCA@MWCNT-CuO electrode in the presented study was tested for three months and kept at 4 °C between measurements. According to the amperometric studies, the electrode kept 93% of the initial signal after one month and 82% after three months.

To compare the time stability, the non-enzymatic sensor presented by Wang F. et al. after 30 days, a Co_3O_4/CuNPs/Pt sensor's current intensity for glucose was reduced by 5.8%. Moreover, the proposed system was characterized with linear range from 0.5 to 336 μM, and a limit of detection (LOD) of 0.43 μM [214].

Another research was performed by Ahmad R. et al., in their work, they presented CuO nanoleaves. The sensor was characterized with linear range from 0.005 to 5.89 mM), and a limit of detection 12 nM, it also exhibited a 3.25% drop in responsiveness after 21 days [215].

Finally, after checking the electrochemical response to each glucose addition, the constructed PCA@MWCNT-CuO sensor was used for non-enzymatic detection of glucose in human fluids, i.e., human blood or human serum. The summarized results are presented in Table 13.

Table 13. *Determination of glucose in real solutions using the PCA@MWCNT-CuO non-enzymatic sensor system.*

	Glucose dopped / μM	**GC/PCA@MWCNT-CuO sensor /mM**	**Recovery / %**
Human serum	0	0	-
	10.0	9.95±0.02	99.5±0.01
	20.0	19.69±0.05	98.5±0.01
	40.0	38.49±0.08	96.2±0.01
	Hospital method / mM	**GC/PCA@MWCNT-CuO sensor /mM**	**Recovery / %**
Human blood	3.14	2.84±0.04	90.4±0.01
	6.28	6.09±0.09	96.9±0.01
	14.80	14.06±0.14	95.0±0.01

The suggested hybrid material made it possible to create a reliable non-enzymatic glucose sensor that could find glucose in real solutions.

The most significant advantages of the obtained system include: (i) **glucose detection without the use of enzymes, which reduced the unit costs of the system**; (ii) **lower limit of detection (LOD) compared to earlier sensor systems**; (iii) **the higher calculated sensitivity value**; (iv) **the ability to detect lower concentrations**

than in the case of enzyme-based sensor systems. In addition, the proposed method allowed for glucose-sensitive measurements using the electrocatalytic properties of copper(II) oxide.

The effectiveness of non-enzymatic glucose detection using CuO was the motivation to develop a new electrochemical sensor using the electrocatalytic properties of nickel nanoparticles. Some metals-based hybrid materials may offer higher catalytic activity toward glucose oxidation. Switching to a more catalytically active metal can achieve better sensor sensitivity, allowing for the measurement of lower glucose concentrations.

In the **Publication 5**, a non-enzymatic glucose sensor based on a hybrid material poly(caffeic acid)@multi-walled carbon nanotubes - nickel hydroxide (PCA@MWCNT-$Ni(OH)_2$) was constructed. Replacing copper(II) oxide with nickel(II) hydroxide was intended to increase the time stability and increase the linear range of the system.

Nickel exhibits higher electrocatalytic activity toward glucose oxidation compared to copper [216]. This means that nickel can facilitate the electrochemical reaction of glucose more efficiently, leading to improved sensitivity and limit of detection. Moreover, nickel-based electrodes demonstrate reduced susceptibility to interference from other electroactive species present in complex samples. This selectivity enables accurate and reliable glucose detection even in the presence of potential interfering substances. Nickel-based electrodes generally offer better time stability compared to copper [216].

The construction process of the non-enzymatic sensor was carried out in the similar way as in the case of the CuO system. In the first step, the electrode was altered with the PCA@MWCNT hybrid material to adorn it with nickel(II) hydroxide. After that, a $NiSO_4$ solution was added to the modified electrode to accumulate Ni^{2+}. Different $NiSO_4$ concentrations (0.001 to 0.1 M) and immersion times (from 0.2 to 10 min) were tried to determine the best conditions for electrodeposition. The individual steps of obtaining the sensor are shown in Fig. 50.

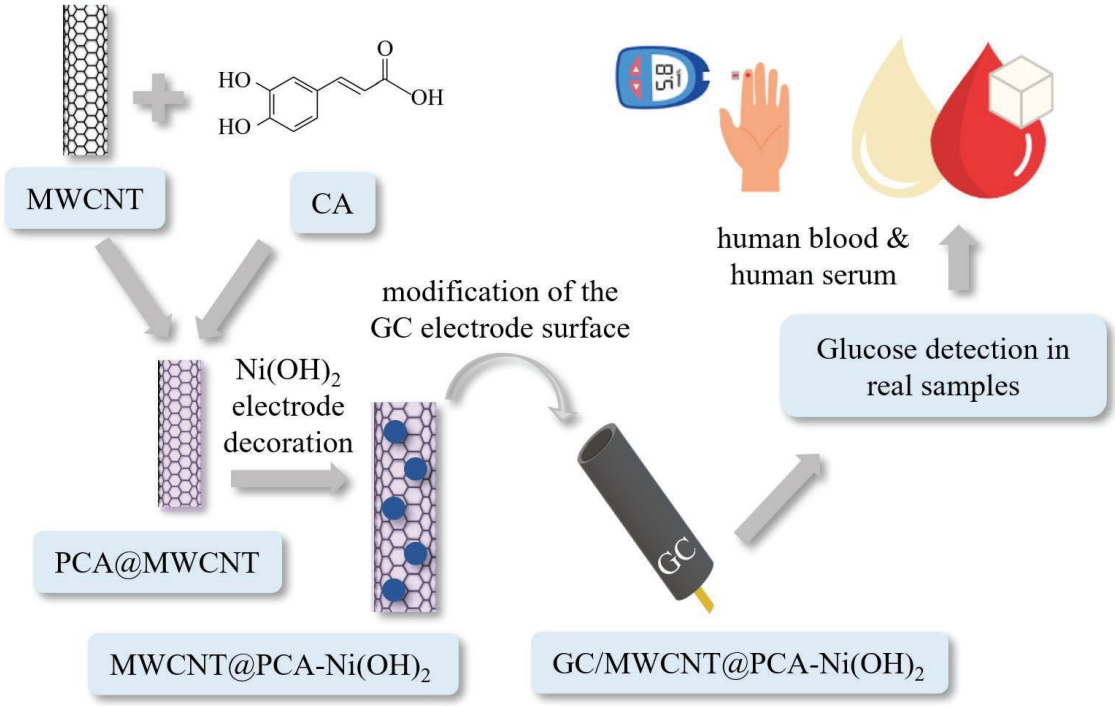


Fig. 50. *Individual stages of research conducted to obtain the PCA@MWCNT-Ni(OH)₂ sensor.*

To confirm the effectiveness of the individual syntheses, physicochemical tests were performed - including high-resolution transmission electron microscopy (HR-TEM), energy-dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM). The TEM images shown in Fig. 51A and AFM presented in Fig. 51B show the efficiency of electrodeposition of $Ni(OH)_2$ on the surface of the PCA@MWCNT hybrid nanomaterial.

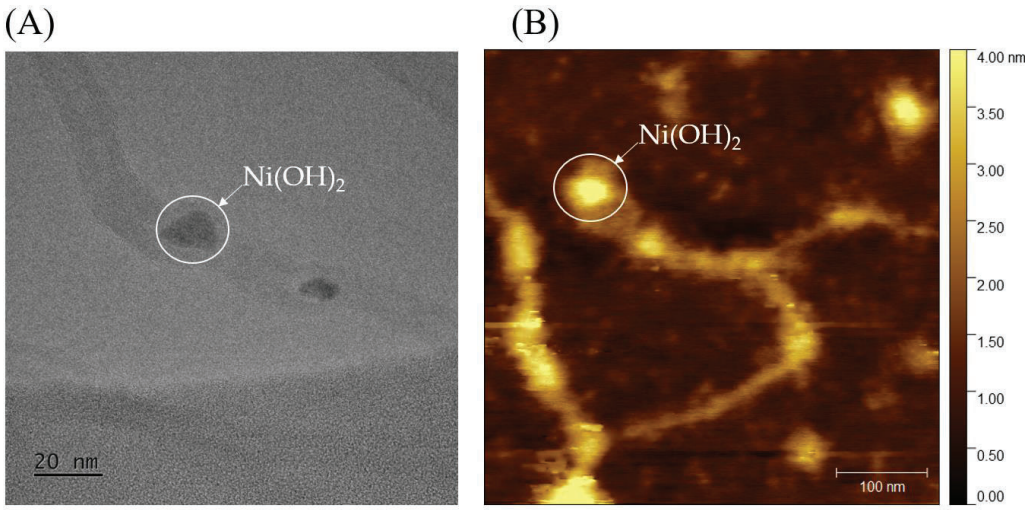


Fig. 51. *HR-TEM (A); and AFM (B) images of the PCA@MWCNT-Ni(OH)₂ hybrid nanomaterial.*

By analyzing X-ray photoelectron spectra (XPS), the chemical valence and elemental makeup of the MWCNT@PCA-$Ni(OH)_2$ hybrid material were confirmed. The survey spectrum demonstrates the coexistence of the elements C, Ni, and O. This is consistent with the EDS mapping-based conclusions, which also allowed to determine the content of nickel in the proposed hybrid nanomaterial 3.78%.

At first, the electrocatalytic oxidation of glucose was tested with the use of cyclic voltammetry. As a result of the conducted tests, an increased peak anode current was observed at a potential close to the potential of the $NiOOH/Ni(OH)_2$ redox pair. In an alkaline solution, the electrogenerated NiOOH oxidized glucose to gluconolactone while also reducing it to $Ni(OH)_2$. As a result, the reverse scan's oxidation peak current increased while the reduction peak current decreased. The electrochemical glucose oxidation reaction mechanism can be presented as:

$$Ni(OH)_2 + OH^- \rightarrow NiOOH + H_2O + e^- \quad (6)$$

$$NiOOH + glucose \rightarrow Ni(OH)_2 + glucolactone \quad (7)$$

Figure 52A displays the amperometric response of GC/PCA@MWCNT-$Ni(OH)_2$ (at a constant potential +0.5 V) in 0.1 M NaOH following additions of various glucose concentrations.

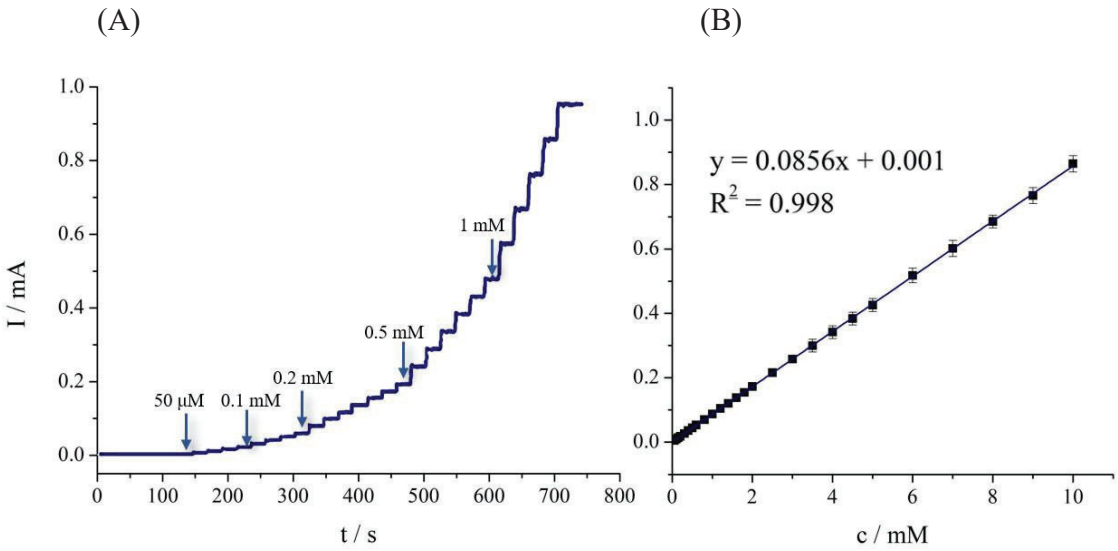


Fig. 52. *Amperometric response of the PCA@MWCNT- $Ni(OH)_2$ sensor to increasing (50 µM - 1 mM) glucose concentrations (0.1 M NaOH, +0.5 V) (A); current vs. glucose concentration (B) (n=3).*

From 50 µM to 1 mM, the current rises linearly and proportionately as the glucose concentration. Based on the obtained amperogram, the dependence of current on

concentration was determined (Fig. 52B). As observed, the system was characterized by a linear increase in current proportional to the concentration of glucose in the range from 0.05 to 10 mM glucose. Moreover, the obtained curve allowed to calculate the sensitivity at 232.7 μA mM^{-1} cm^{-2} and the limit of detection at 0.29 μM.

Interference is a significant factor that can influence the performance of non-enzymatic glucose sensors and refers to the presence of other substances or analytes in the sample that can interfere with the detection and measurement of glucose, leading to inaccurate or unreliable results. In particular, this aspect was necessary because the applied potential in the case of the proposed nickel-based sensor was the highest in comparison to the systems analyzed as part of the Ph.D. thesis. The influence of individual 0.1 mM interferents was examined and compared their relative responses to 1 mM glucose. As a result of the conducted research, it was found that for all interfering agents, the relative response was below 8%, which suggests relatively good selectivity and resistance of the proposed sensor to interfering compounds that may be present in real samples.

The obtained main characteristics of the proposed PCA@MWCNT sensor were compared with PCA@MWCNT-CuO and the properties of other non-enzymatic glucose sensors are listed in Table 14.

Table 14. *Comparison of copper- and nickel-based non-enzymatic sensors.*

Electrode	**Sensitivity / μA mM^{-1} cm^{-2}**	**LOD / μM**	**Linear range / mM**	**Ref.**
Cu-MOF/CF	30.0	0.076	0.001–0.95	[217]
Cu_2O nanoclusters	1082.0	12.0	0.190–1.00	[218]
MWCNT-CuBTC	14.9	10.0	0.20–1.00	[219]
Fc-CHIT/CNT@Cu	1.25	13.5	0.20–22.0	[220]
$Ni(OH)_2$/nanoflowers	265.3	0.50	0.10–1.10	[221]
$Ni(OH)_2$@oPPyNW	1049.0	0.30	0.001–3.86	[222]
NiNP/SMWNTs	1438.0	0.50	0.001–0.10	[223]
NiO–MWCNTs/CPE	122.1	31.0	0.50–9.0	[224]
PCA@MWCNT-CuO	**2412.0**	**0.43**	**0.002–9.0**	**Publ. 4**
PCA@MWCNT-$Ni(OH)_2$	**232.7**	**0.29**	**0.05–10.0**	**Publ. 5**

Time stability is an important characteristic of non-enzymatic glucose sensors as it determines the long-term performance and reliability of the sensor over time.

Time stability is a critical characteristic for any sensor, as it ensures that the measurements remain consistent and reliable over the sensor's useful lifetime.

As in the case of the previously presented sensor systems, temporal stability tests were performed using the amperometric technique. Measurements were performed over 5 months, and changes in relative response over time are shown in Fig. 53.

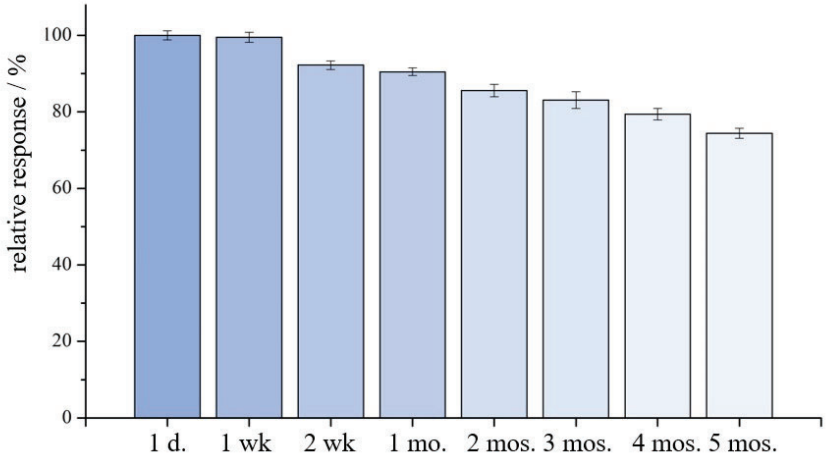


Fig. 53. *Time stability of the proposed PCA@MWCNT- Ni(OH)$_2$ sensor (n=3).*

The presented GC/PCA@MWCNT-Ni(OH)$_2$ electrode retained a relative response of 93.5% one month after structuring and 74.4% after five months, indicating good time stability. Between individual measurements, the electrode was stored in a dry environment at 4 °C. The time stability of the proposed non-enzymatic sensor and the proposed PCA@MWCNT-CuO sensor (the **Publication 4**) is compared with other non-enzymatic sensor systems in Table 15.

Table 15. *Comparison of time stability of proposed non-enzymatic sensors with Ni-based-, and Cu-based reported electrodes.*

Electrode	**Retained activity / %**	**Storage time / days**	**Ref.**
Nafion/CuO/ZnO	92.8	15	[225]
Co_3O_4-CuNPs/Pt	94.2	30	[220]
Ni-NPs/TiO_2 nanotubes	80.3	20	[226]
NiO-APTS@SBA/CNT	95.0	24	[227]
PCA@MWCNT-CuO	**82.0**	**90**	**Publ. 4**
PCA@MWCNT-Ni(OH)$_2$	**74.4**	**150**	**Publ. 5**

The final stage of the conducted research was tested on real glucose solutions, the research was carried out using human serum and human blood. In studies aimed at detecting glucose in human serum, three different concentrations (2.4, 6.6, 8.5 mM) were tested, resulting in recoveries of 96.3 to 98.2%. The same sugar was tested in human blood solutions. Three different concentrations were also tested (3.08, 5.88, and 14.35 mM), showing recovery in the range of 93.3 to 97.6%.

Compared to the sensor system based on the hybrid nanomaterial PCA@MWCNT-CuO, the use of $Ni(OH)_2$ instead of CuO allowed for (i) **a shorter response time**; (ii) **increased time stability (from 3 to 5 months)**; (iii) **lower limit of detection** (LOD) compare to PCA@MWCNT-CuO.

The higher detection potentials of the obtained non-enzymatic sensors (the **Publication 4 and 5**) and the resulting influence of interferents resulted in the desire to construct a more selective sensor, which led to the decision to use the enzyme - glucose oxidase. Moreover, it was decided to use the physicochemical properties of the poly(caffeic acid) coating and check its ability to load the enzyme. An additional motivation for the actions taken was the construction of a biosensor operating in accordance with concentrations consistent with the World Health Organization (WHO).

As a result, in the **Publication 6**, an enzymatic biosensor system was proposed, which was used for the selective detection of glucose in real solutions like human blood, and human serum.

Poly(caffeic acid) can be used to create films or coatings on various surfaces, this can be achieved through chemical or electrochemical polymerization methods. Moreover, PCA surfaces can effectively immobilize enzymes through various mechanisms. Poly(caffeic acid) films have shown promising results in retaining the activity of immobilized enzymes. The film matrix provides a protective environment for the enzyme, shielding it from denaturation or degradation. This allows for the time stability and reusability of the immobilized enzyme [38,199].

In the presented work, magnetite nanoparticles obtained by co-precipitation were coated with poly(caffeic acid). The proposed system was then used as a matrix for the immobilization of the enzyme - glucose oxidase.

In the **Publication 6,** a screen-printed electrode (SPE) was used to construct the electrochemical sensor, which made it possible to ensure the miniaturization of the system, which often requires smaller sample volumes for analysis than in electrolytic cell used for research using a GC electrode.

The small electrode area of SPEs allows for efficient utilization of the sample, reducing the volume required for testing. This is particularly advantageous in applications where sample volumes are limited or precious, like point-of-care diagnostics or field testing.

Moreover, the miniaturized nature of SPE-based biosensors promotes faster mass transport and shorter diffusion pathways for analytes. This facilitates rapid response times, enabling real-time monitoring of target analytes. The reduced dimensions of the electrodes also contribute to faster electron transfer kinetics, further enhancing the sensor's response time.

The general idea of the conducted research is schematically presented in Fig. 54.

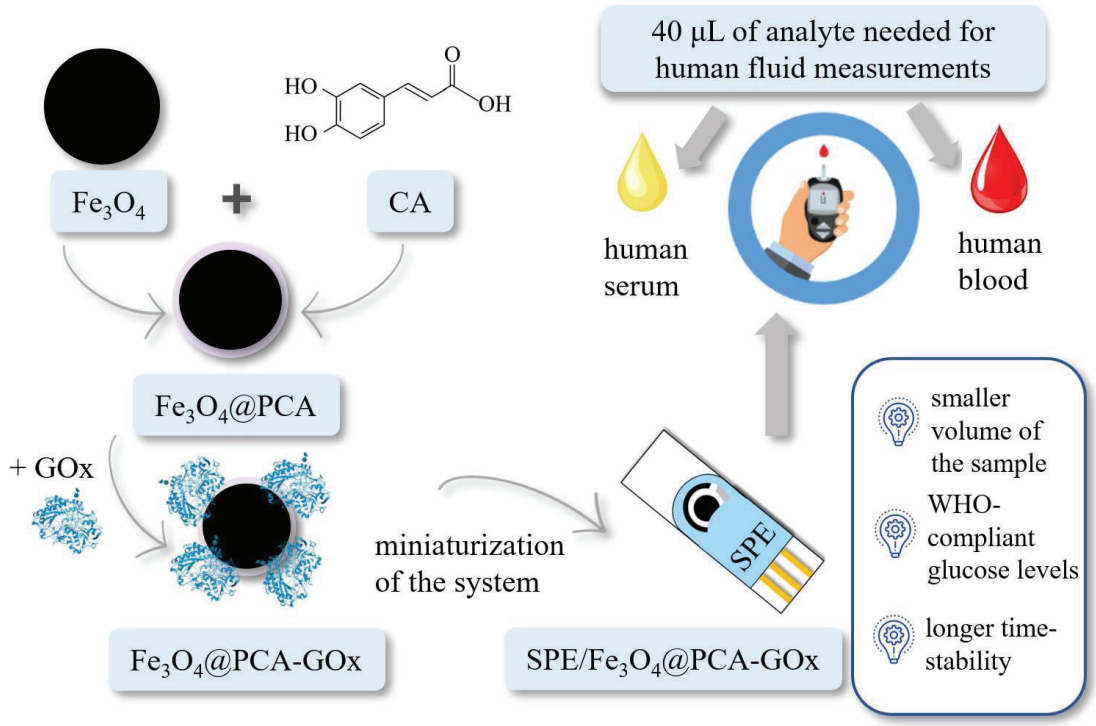


Fig. 54. *Individual activities carried out to obtain the Fe_3O_4@PCA-GOx biosensor for glucose detection.*

To determine the effectiveness of coating magnetite nanoparticles with poly(caffeic acid), tests using high-resolution transmission electron microscopy (HR-TEM) were performed. This technique made it possible to characterize the morphology of the proposed system and also to determine the thickness of the PCA layer of the coating at 3-4 nm. HR-TEM images for the PCA@Fe_3O_4 hybrid nanomaterial are shown in Fig. 55.

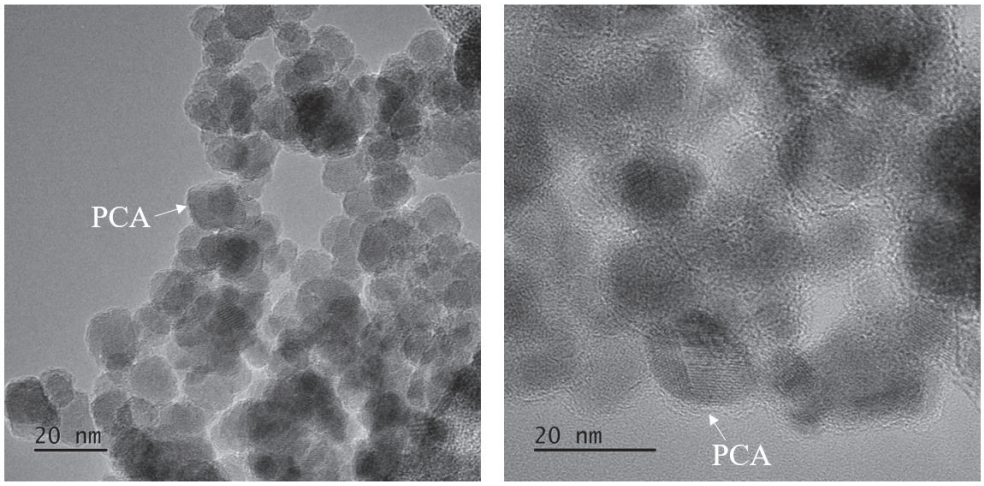


Fig. 55. *HR-TEM images of the PCA@Fe_3O_4 hybrid nanomaterial.*

Catechol-based polymers have gained significant attention in the field of enzyme immobilization due to their unique adhesive properties and the ability to form robust coatings on various surfaces. The efficiency of glucose oxidase immobilization on the Fe_3O_4@PCA nanomaterial was checked using the Bradford method. The use of poly(caffeic acid) as a nature-inspired coating enabled the attachment of 38.1 mg (24 h; pH 5.5) of enzyme per 1 g of the tested carrier. The comparison of a proposed carrier for enzyme immobilization was compared with other works presented in Table 16.

Table. 16. *Amounts of glucose oxidase (GOx) immobilized on various matrix.*

Sample	Immobilized GOx /mg g^{-1}	Ref.
Fe_3O_4@PNE	37.8	[124]
Silica	12.9	[125]
Ga_2O_3/Lig	24.7	[228]
ZrO_2/Lig	27.1	[228]
Fe_3O_4@PDA	**36.3**	**Publ. 1**
Fe_3O_4@PDA@βCD	**47.6**	**Publ. 2**
Fe_3O_4	**16.4**	**Publ. 6**
Fe_3O_4@PCA	**38.1**	**Publ. 6**

The effectiveness of the enzyme immobilization process - GOx of the Fe_3O_4@PCA hybrid nanomaterial was also indirectly confirmed using the atomic force microscopy (AFM). As shown in Fig. 56, an increase in the z parameter can be observed after the immobilization process and smoothing of the surface.

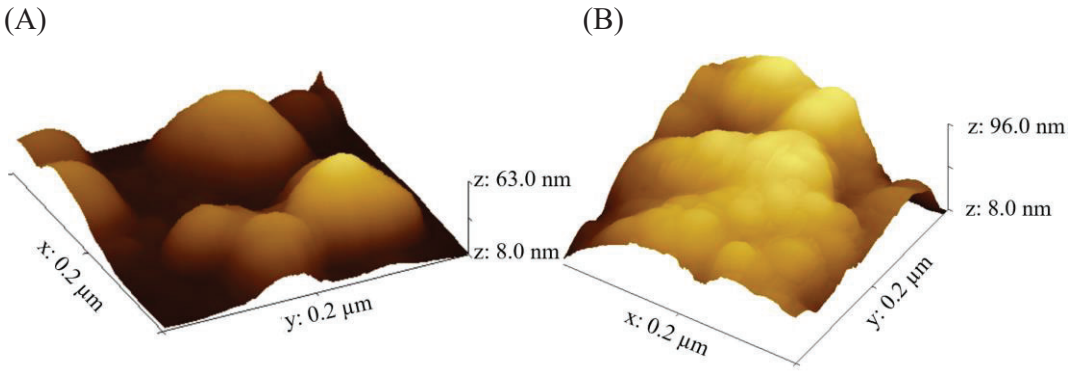


Fig. 56. *AFM images for Fe_3O_4@PCA nanomaterial before (A); and after (B) GOx immobilization.*

In the case of amperometric tests on the SPE-based biosensor, they were carried out without constant mixing conditions, at a potential of +0.1 V, and with an HFc solution as an external mediator. The amperogram results are summarized in Fig. 57A.

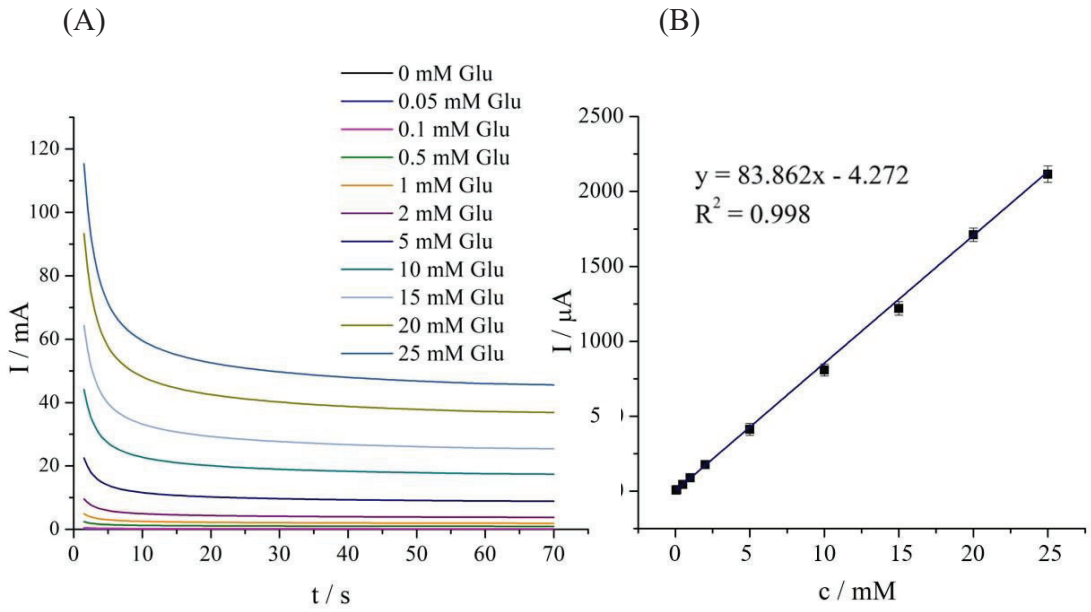


Fig. 57. *Amperometric detection of different ranges of glucose (0 - 25 mM) in the presence of 10 mM HFc (+0.1 V, 50 mM PBS pH 7.4) (A); dependence of current on glucose concentration (B) (n=3).*

The use of SPE electrodes made it possible to conduct tests with 40 µL of the tested solution, which allowed for the miniaturization of the system compared to the previously constructed system with a GC electrode. This solution has its advantages, including: (i) **less amount of used reagents, which reduces the overall cost of experiments and analyses**, (ii) **less waste production, which is environmentally friendly and reduces disposal costs,** (iii) **shorter diffusion distances in smaller samples can accelerate reactions, leading to quicker results**, (iv) **miniaturization**

helps conserve precious materials, (v) **smaller volumes are less invasive and can be obtained with less discomfort to patients.**

Amperometric tests allowed to plot the dependence of current on concentration. The obtained results allowed to determine that the proposed one generates a current response proportional to the concentration (Fig. 57B). In addition, the sensor was determined to generate a linear response in the range of 0.05 to 25 mM. It is worth emphasizing that this enables the detection of glucose in all blood concentrations (respectively, hypoglycemia: < 3.5 mM; normal: 3.5-5.5 mM; early diabetes: 5.5-6.9 mM; diabetes: > 6.9 mM) [229].

A summary and comparison of the most important features of biosensors (sensitivity, LOD, and linearity range) presented in this doctoral dissertation and other ones based on glucose oxidase are presented in Table 17.

Table 17. *Glucose biosensor based on glucose oxidase enzyme.*

Electrode	**Sensitivity/ μA mM^{-1} cm^{-2}**	**LOD / μM**	**Linear range / mM**	**Ref.**
PGA/GCE/GOx	2.13	120.0	0.50–5.50	[230]
CNS-Nafion-GOx	7.31	39.1	0.08–2.04	[231]
ZnO TPs/MXene/GOx	29.0	17.0	0.05–0.70	[232]
PAMAM-Fc/GOx	6.54	0.48	1.00–22.0	[233]
Fc-PLL/GOx	6.55	23.0	0–10.0	[234]
Fe_3O_4@PNE-GOx	97.30	6.10	0.2–24.0	[124]
Fe_3O_4@PDA-GOx	**139.71**	**1.54**	**1.0–20.0**	**Publ. 1**
Fe_3O_4@PDA@βCD-GOx	**115.74**	**1.55**	**1.0–26.0**	**Publ. 2**
Fe_3O_4@PCA-GOx	**1198.0**	**5.23**	**0.05–25.0**	**Publ. 6**

An important stage of the conducted research was to check the influence of interfering agents on glucose measurements (Fig. 58). As part of the research, the effect of 1 mM of individual interferents (maltose, fructose, sucrose, L-cysteine, dopamine, uric acid, ascorbic acid) was compared to the response of 1 mM glucose. As shown schematically in Fig. 58, the presented biosensor is selective for particular additions of interfering compounds. The use of a low potential for detection meant that even a slight response was registered for ascorbic acid, uric acid, and dopamine are biomolecules that in electrochemical sensors oxidize and produce incorrect signals [235].

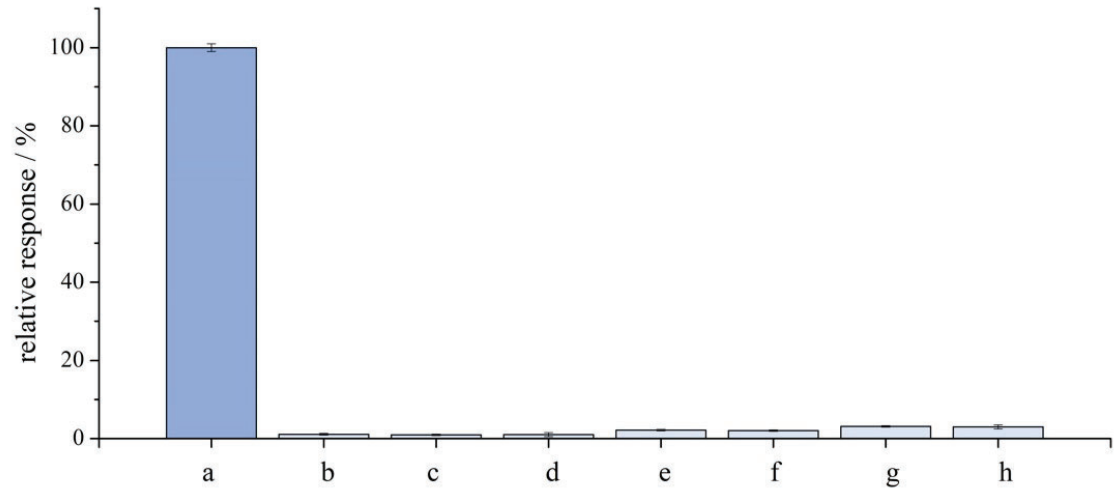


Fig. 58. *Relative response of 1 mM individual interferents: maltose (b), fructose (c), saccharose (d), L-cysteine (e), uric acid (f), dopamine (g) ascorbic acid (h) compare to 1 mM glucose (a).*

The proposed SPE/Fe_3O_4@PCA-GOx biosensor was examined for time stability. Amperometry was used in the investigation, and a potential of +0.1 V was used. The electrode system was studied for ten months, and during this time, between tests the electrode was kept at 4 °C. Figure 59 presents the relative response dependent on the biosensor's lifetime.

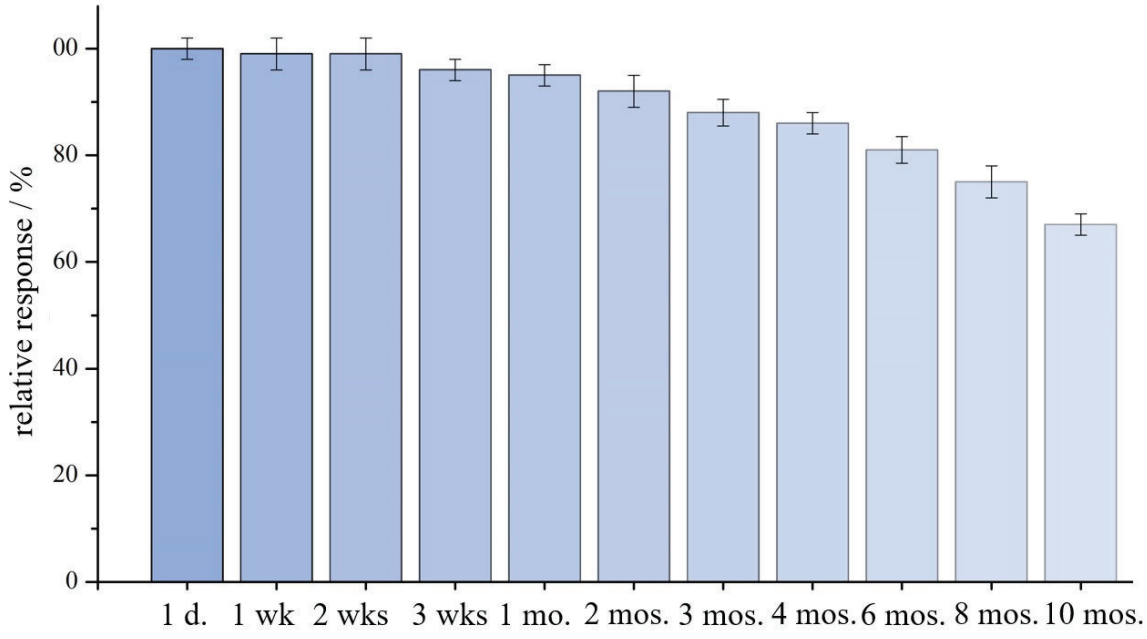


Fig. 59. *Time stability of the presented enzymatic Fe_3O_4@PCA-GOx glucose biosensor.*

The proposed system after 10 months, still showed a linear response, proportional to the individual additions of glucose. In addition, the proposed method showed a 67.5% relative response to system performance at design time. In addition, Table 18

compares the presented time stability with biosensors also presented earlier in this dissertation and other available ones based on GOx.

Table 18. *Comparison of time stability of glucose biosensors based on glucose oxidase (GOx).*

Electrode	Retained activity / %	Storage time / days	Ref.
PPy/GOx	76.0	60	[236]
Au/SWCNT/PPy–GOx	30.0	30	[179]
PPy/pTSA/GOx	90.0	14	[237]
Fe_3O_4/CS/CD/MWCNTs/GOx	93.4	25	[238]
SPE/Fe_3O_4@PNE-GOx	75.1	140	[124]
Fe_3O_4@PDA-GOx	**82.0**	**90**	**Publ. 1**
Fe_3O_4@PDA@βCD-GOx	**74.4**	**150**	**Publ. 2**
Fe_3O_4@PCA-GOx	**67.5**	**300**	**Publ. 6**

Finally, the system was tested to detect glucose in real solutions. For this purpose, studies were carried out using human serum and human blood, with different glucose concentrations were tested. The measurement results are summarized in Table 19.

Table 19. *The effectiveness of the SPE/Fe_3O_4@PCA-GOx biosensor for glucose detection in real samples (human serum and human blood) (n=3).*

Sample	Glucose concentration / mM	Glucose concentration / mg mL^{-1}	SPE/Fe_3O_4@PCA-GOx	
			Find /mM	Recovery / %
Human serum	2.80	50.4	2.74±0.05	97.9±1.79
	6.90	124.2	6.84±0.05	99.1±0.72
	9.20	165.6	9.09±0.10	98.8±1.08
Human blood	3.20	57.6	3.14±0.04	98.2±1.25
	6.30	113.4	6.21±0.06	98.6±0.95
	14.7	264.6	14.4±0.05	98.2±0.34

Individual recoveries for real cancers ranged from 97.9 to 99.1%, and the individual results were confirmed by measuring the same samples in a hospital analytical laboratory. The obtained results allow us to conclude that the proposed system can be an alternative to the insertion of glucose in human fluids.

The use of poly(caffeic acid) for the construction of the Fe_3O_4@PCA-GOx biosensor system enabled the selective and sensitive detection of glucose. The biosensor

proposed in the **Publication 6** compared to the previously presented glucose biosensors, was characterized by (i) **increased selectivity**, **due to the use of glucose oxidase and conducting research at lower potential values**; (ii) **a wider linear** range that **was in line with World Health Organization (WHO) guidelines**; (iii) **miniaturization of the system**, **which allowed for tests with a sample volume of several µL**; (iv) **sample recovery results (for human serum and human blood testing) that were similar to those obtained in the hospital**.

6. Summary

The presented doctoral dissertation concerned the topic of synthesis, characterization, and applications based on the electroactive hybrid materials for non-enzymatic and enzymatic sensors.

As part of the presented research, several syntheses of hybrid materials were carried out, including nature-inspired biopolymers (polydopamine, poly(caffeic acid)). The presented materials were used for enzyme immobilization or decoration with metal oxides/hydroxides. They were characterized in terms of morphology and physicochemical properties. Finally, they were used to construct enzymatic and non-enzymatic electrochemical sensors. The obtained sensor systems were discussed and summarized in the **Publications 1-6**.

Enzymatic biosensors based on the biomimetic polymer – polydopamine (PDA) were obtained and discussed in the **Publications 1-2**. The proposed biopolymer was used to modify magnetite nanoparticles obtained by co-precipitation. To increase the linearity and time stability in **Publication 2**, the system was additionally enriched with a nanocontainer – β-cyclodextrins. The glucose oxidase enzyme used was adsorption immobilized and the process was optimized.

It was shown that the addition of PDA to the system more than doubled, and the addition of PDA and βCD more than three times the amount of the enzyme-glucose oxidase immobilized on the obtained nanomaterial. In addition, the **Publication 1** optimized the selection of an external mediator responsible for the transport of electrons between the electrode and the enzyme. The tests showed that the best parameters (sensitivity, LOD, linearity range) were obtained using (hydroxymethyl)ferrocene as an external mediator. The choice of mediator ultimately depends on factors, for instance, the target analyte, the enzyme used, and the desired performance characteristics of the biosensor.

Therefore, due to glucose deduction with GOx, it was also decided to use it in the **Publications 2 and 6**. However, it was decided to enrich the system with the addition of nanocontainers that would increase the loading of enzymes through encapsulation.

The addition of βCD allow to increase the amount of attached enzyme (over three times as compared to bare magnetite nanoparticles) and extend the range of linearity and time stability of the system from five to nine months.

Finally, both systems were used to detect glucose in real solutions, which were pharmaceutical samples and standard glucose. Both systems were characterized by relatively high results recovery, which were compared to the chemical analyzer.

This showed the detection efficiency of the proposed biosensors and their application potential. Therefore, the research conducted in the **Publications 1-2** allowed for selecting the most effective external charge transfer mediator and the extension of the time stability of the glucose biosensor. Subsequent studies focused on increasing electrochemical activity by changing the biopolymer used to poly(caffeic acid) (PCA).

The articles 3-6 uses another nature-inspired polymer - poly(caffeic acid), which modify multi-walled carbon nanotubes (the **Publications 3-5**) and magnetite **(**the **Publication 6)**.

The proposed PCA@MWCNT system, after detailed characterization, was tested for the detection of NADH **(**the **Publication 3**). The proposed sensor made it possible to conduct amperometric tests at a relatively low potential (+0.1 V) relative to pure multi-walled carbon nanotubes. Moreover, tests conducted in real solutions enabled the detection of NADH in human serum, indicating the potential application of this system.

The electrochemical properties of poly(caffeic acid) and its application potential were continued in further research for the construction of a non-enzymatic glucose sensor in the **Publications 4 and 5**. The ability to oxidize glucose was used by decapping the hybrid material with copper(II) oxide (the **Publication 4**) and nickel(II) hydroxide (the **Publication 5**). The proposed systems were characterized by relatively high sensitivity. An important stage of the research was to check the response to the addition of interferents, due to the lack of a selective element, which is an enzyme. It was shown that the system for individual compounds present in human blood and/or human serum, at the real concentration level also present in these real samples, does not show a significant response, and for each of the interfering agents it was below 10%, therefore the systems can be considered stable. It was also shown that the proposed non-enzymatic systems (the **Publications 4 and 5**) are characterized by a lower limit of detection (LOD) and linearity in lower concentration ranges than in the case of the proposed enzyme systems (the **Publications 1, 2, and 6**). An important stage of the conducted research was the performance of tests on real solutions (human blood, human serum), which made it possible to determine the effectiveness of glucose detection in human fluids and the application potential of the proposed systems.

In order to test the potential of poly(caffeic acid) as an enzymatic component of the glucose biosensor and to expand the linearity range, subsequent studies focused on the immobilization of glucose oxidase. Moreover, the aspect of miniaturization of the biosensor through the use of the screen-printed electrode (SPE) was discussed in the **Publication 6.** The magnetite@poly(caffeic acid) nanomaterial used was used to immobilize the enzyme - GOx. The proposed system allowed glucose detection in a wide range of concentrations (from hypoglycemia to diabetes), corresponding to WHO guidelines. In addition, changes in the test setup enabled glucose detection in much smaller volumes (several μL). In addition, the proposed system showed the longest time stability (ten months) compared to the proposed enzymatic and non-enzymatic glucose biosensors.

The most important detection parameters (modifying material, type of electrode, potential, mediator) and the most important features of the proposed sensors (linearity range, sensitivity, LOD, time stability) are listed in Table 20.

Table 20. *Comparison of the properties of enzymatic and non-enzymatic sensors obtained as part of this doctoral dissertation*

Publication no.	Hybrid nanomaterial	Analyte	Electron mediator	Electrode	Applied potential / V	Sensitivity / μA mM^{-1} cm^{-2}	LOD / μM	Linear range / mM	Time stability / days
1	Fe_3O_4@PDA-GOx	glucose	HFc		0.30	139.71	1.54	1.0–20.0	60
			BQ		0.25	130.43	2.27	2.0–17.0	
			HQ	GC	0.10	124.71	1.74	2.0–17.0	
			MB		-0.10	117.57	1.66	2.0–17.0	
			FFC		0.25	125.43	3.11	1.0–19.0	
2	Fe_3O_4@PDA@βCD-GOx	glucose	HFc	GC	0.30	115.74	1.55	1.0–26.0	210
3	MWCNT@PCA	NADH	-	GC	0.10	85.70	0.12	0.0005-1.27	-
4	MWCNT@PCA-CuO	glucose	-	GC	0.45	2412.0	0.43	0.002–9.0	90
5	MWCNT@PCA-$Ni(OH)_2$	glucose	-	GC	0.50	232.70	0.29	0.05 – 10.0	150
6	Fe_3O_4@PCA-GOx	glucose	HFc	SPE	0.10	1198.0	5.23	0.05 – 25.0	300

7. Future outlook

The hybrid nanomaterials proposed in this research confirmed their effectiveness as materials for enzyme immobilization and the effective production of sensor systems.

The doctoral dissertation concerned the design, synthesis, and characterization of electroactive hybrid materials and their application in biosensors and sensor systems. They were successfully used for detection in real solutions, like food samples, infusion solutions, human blood, or human serum.

In the course of the research, new challenges emerged and research problems to be solved, which would be worth exploring.

Based on the obtained results, it can be concluded that further research in the field of hybrid materials for sensor applications.

1. Immobilization of enzymes from the classes of dehydrogenases, enabling the analysis of various analytes, i.e., ethanol and glucose.
2. Protein synthesis with the possibility of direct electron transport (DET) and the construction of third generation biosensors.
3. Electropolymerization and polymerization of poly(caffeic acid) and other catechol-based compounds on carbon-based materials, i.e. graphene oxide.
4. Attaching other biological materials, i.e., aptamers, nucleic acids, and, as a result, detecting the concentration of, i.e., biomarkers.
5. Design and optimization of alternative hybrid nanomaterials for non-enzymatic glucose detection.

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9. Scientific activity

Publications

1. A. Jędrzak, T. Rebiś, **M. Kuznowicz**, T. Jesionowski; Bio-inspired magnetite/lignin/polydopamine-glucose oxidase biosensing nanoplatform. From synthesis, via sensing assays to comparison with others glucose testing techniques; *International Journal of Biological Macromolecules*, 127 (2019) 677–682.
2. A. Jędrzak, T. Rebiś, **M. Kuznowicz**, A. Kołodziejczak-Radzimska, J. Zdarta, A. Piasecki, T. Jesionowski; Advanced Ga_2O_3/lignin and ZrO_2/lignin hybrid microplatforms for glucose oxidase immobilization: Evaluation of biosensing properties by catalytic glucose oxidation; *Catalysts*, 9 (2019) 1044-1063.
3. **M. Kuznowicz**, A. Jędrzak, A. Leda, T. Rębiś, T. Jesionowski; Measurements of working parameters of external mediators for biodetectors based on the polydopamine@magnetite nanoparticles; *Measurement*, 184 (2021) 109950.
4. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Biomimetic magnetite/polydopamine/β-cyclodextrins nanocomposite for long-term glucose measurements; *Biochemical Engineering Journal*, 174 (2021) 108127.
5. T. Rębiś, **M. Kuznowicz**, A. Jędrzak, G. Milczarek, T. Jesionowski; Design and fabrication of low potential NADH-sensor based on poly(caffeic acid)@multi-walled carbon nanotubes, *Electrochimica Acta*, 386 (2021) 138384.
6. A. Jędrzak, **M. Kuznowicz**, T. Rębiś, T. Jesionowski; Portable glucose biosensor based on polynorepinephrine@magnetite nanomaterial integrated with a smartphone analyzer for point-of-care application, *Bioelectrochemistry*, 145 (2022) 108071.
7. **M. Kuznowicz**, T. Rębiś, A. Jędrzak, G. Nowaczyk, M. Szybowicz, T. Jesionowski; Glucose determination using amperometric non-enzymatic sensor based on electroactive poly(caffeic acid)@MWCNT decorated with CuO nanoparticles, *Microchimica Acta*, 189 (2022) 159.
8. A. Jędrzak, **M. Kuznowicz**, T. Jesionowski; Mobile-assisted diagnostic biosensor for point-of-care glucose detection in real human samples with rapid response and long-live stability, *Journal of Applied Electrochemistry*, 2023 (2023) 1-12.

9. **M. Kuznowicz**, T. Rębiś, A. Jędrzak, G. Nowaczyk, T. Jesionowski; Facile Fabrication of a Selective Poly(caffeic acid)@MWCNT-Ni(OH)$_2$ Hybrid Nanomaterial and Its Application as a Non-Enzymatic Glucose Sensor, *Chemosensors*, 11 (2023) 452.
10. **M. Kuznowicz**, A. Jędrzak, T. Jesionowski; Sensitive glucose electrochemical biosensor based on magnetite@poly(caffeic acid) nanomaterial for real samples analysis, *Molecules, manuscript ID: 2647041, under review.*

Book chapters

1. T. Jesionowski, **M. Kuznowicz**, A. Jędrzak, T. Rębiś; Sensing Materials: Biopolymeric Nanostructures, This chapter is a printed in Encyclopedia of Sensors and Biosensors, vol. 2 (2023) 286-304, ISBN 978-0-12-822549-3. Imprint Elsevier.

International conferences contributions

Oral presentations

1. A. Jędrzak, T. Rębiś, **M. Kuznowicz**, T. Jesionowski; Biosensor based on magnetite/lignin/polydopamine hybrid material combined with glucose oxidase for amperometric glucose biosensor, 6th International Conference and Exhibition on Materials Science and Chemistry, Rome, Italy; 17-18.05.2018.
2. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; An optimization of mediated biosensor systems based on hybrid material; Conference of Biosensors and Bioelectronics 2020, Paris, France, 20.07.2020.
3. A. Jędrzak, **M. Kuznowicz**, T. Rębiś, T. Jesionowski; Hybrid microplatform for glucose biosensor and its comparison with other testing techniques, Conference of Biosensors and Bioelectronics 2020, Paris, France, 20.07.2020.
4. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; The role of the mediated biosensor based on bioinspired nanoplatforms, Ist PSSC, On-line Conference, Poland, 26-27.09.2020.
5. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Timestable glucose biosensor based on multi-component nano-platform; International Conference on Nanomaterials and Biomaterials, Porto, Portugal, 3-4.12.2021.

6. A. Jędrzak, **M. Kuznowicz**, T. Rębiś, T. Jesionowski; Design and syn-thesis of nano- and microplat-forms for biosensors; International Conference on Nanomaterials and Biomaterials, Porto, Portugal, 3-4.12.2021.
7. A. Jędrzak, **M. Kuznowicz**, T. Jesionowski; Glucose biosensors and their potential application in the point-of-care testing. International Conference on Nanoscience, Nanotechnology and Advanced Materials, Mississauga, Canada, 28-29.07.2022.
8. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski, Electrochemical non-enzymatic sensor based on PCA@MWCNT-CuO nanomaterial for glucose determination, ElecNano10, Paris, France, 10-12.05.2023.
9. A. Jędrzak, **M. Kuznowicz**, T. Jesionowski, Amperometric portable biosensor based on hybrid nanomaterial for point-of-care testing application, ElecNano10, Paris, France, 10-12.05.2023.
10. **M. Kuznowicz**, A. Jędrzak, T. Jesionowski, Nonenzymatic glucose sensor based on PCA@MWCNT-CuO nanomaterial, Baltic Conference Chemistry, Gdansk, Poland, 27-28.05.2023.
11. A. Jędrzak, **M. Kuznowicz**, T. Jesionowski, Portable biosensor with hybrid nanomaterial for POCT application, Baltic Conference Chemistry, Gdansk, Poland, 27-28.05.2023.

Poster presentations

1. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Multicomponent system magnetite/lignin/polydopamine-glucose oxidase as functional biosensor for glucose detection; 8th Intercollegiate Biotechnology Symposium, Warsaw, Poland; 17-19. 05.2019.
2. A. Jędrzak, **M. Kuznowicz**, T. Rębiś, T. Jesionowski; Glucose biosnesor based on the hybrid microplatform and its comparision with other testing techniques on commercial food-samples; 8th Intercollegiate Biotechnology Symposium, Warsaw, Poland; 17-19.05.2019.
3. **M. Kuznowicz**, A. Jędrzak, T. Rebiś, T. Jesionowski; An enzymatic biosensor composed of hybrid material for glucose detection; The XIVth Summer School for Graduate Students and Young Researchers ‘Interfacial Phenomena in Theory and Practice’; Sudomie, Poland; 24-28.06.2019.

4. **M. Kuznowicz**, A. Jędrzak, T. Rebiś, T. Jesionowski; Amperometric biosensor for glucose detection based on multicomponent hybrid system, Conference of Biosensors and Bioelectronics 2020, Paris, France, 20.07.2020.
5. A. Jędrzak, **M. Kuznowicz**, T. Rębiś, T. Jesionowski; Direct electron transfer of glucose oxidase and biosensing for glucose, Conference of Biosensors and Bioelectronics 2020, Paris, France, 20.07.2020.
6. A. Jędrzak, **M. Kuznowicz**, T. Rębiś, T. Jesionowski; An efficient DET amperometric biosensor for glucose determination, Ist PSSC, On-line Conference, 26-27.09.2020.
7. **M. Kuznowicz**, A. Jędrzak, T. Jesionowski; Poly(caffeic acid)@Carbon Nanotube Decorated with CuO for Glucose Detection; XXVIth International Symposium on Bioelectrochemistry and Bioenergetics, Cluj-Napoca, Romania, 9-13.05.2021.
8. A. Jędrzak, **M. Kuznowicz**, T. Jesionowski; Accurate and Rapid Sensor based on Polydopamine Nanomaterial; XXVIth International Symposium on Bioelectrochemistry and Bioenergetics, Cluj-Napoca, Romania, 9-13.05.2021.
9. A. Jędrzak, **M. Kuznowicz**, S. Jurga, T. Jesionowski; An electrochemical highly sensitive and long term nanobiosensor for glucose detection; NanoTech Poland 2021, Poznan, Poland, 9-11. 06.2021.
10. **M. Kuznowicz**, A. Jędrzak, T. Jesionowski; Ultra- highly sensitive sensor for glucose detection in real samples; Biosensors 2020/2021, The 31st Anniversary World Congress on Biosensors, Busan, Korea, 26-29.07.2021.
11. A. Jędrzak, **M. Kuznowicz**, T. Jesionowski; Novel selective and sensitive amperometric biosensors based on hybrid nanoplatforms, Biosensors 2020/2021, The 31st Anniversary World Congress on Biosensors, Busan, Korea, 26-29.07.2021.
12. A. Jędrzak, **M. Kuznowicz**, T. Jesionowski; Point-of-care glucose biosensor based on hybrid materials; NanoTech Poland 2022, Poznan, Poland, 1-3.06.2022.
13. A. Jędrzak, **M. Kuznowicz**, G. Nowaczyk, T. Jesionowski; Mobile-based electrochemical enzymatic and non-enzymatic sensors for glucose monitoring, NanoTech Poland 2023, Poznan, Poland, 14-16.06.2023.

Domestic conferences contributions

Oral presentations

1. A. Jędrzak, T. Rębiś, **M. Kuznowicz**, T. Jesionowski; Konstrukcja biosensora enzymatycznego do detekcji glukozy w oparciu o układ magnetyt/lignina/polidopamina/oksydaza glukozowa, II Ogólnopolskie Sympozjum Nauk Przyrodniczo-Rolniczych, Poznan, Poland; 7-8.04.2018.
2. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Badanie i optymalizacja roli mediatorów w biosensorach drugiej generacji; Ogólnopolska Konferencja Interdyscyplinarna pn: "OMNIBUS cz. II" Nauki Interdyscyplinarne, Krakow, Poland, 3-4.04.2020.
3. A. Jędrzak, **M. Kuznowicz**, T. Rębiś, T. Jesionowski; Bezpośredni transfer elektronów w biosensorze opartym o nanoplatformę hybrydową; Ogólnopolska Konferencja Interdyscyplinarna pn: "OMNIBUS cz. II" Nauki Interdyscyplinarne, Krakow, Poland, 3-4.04.2020.
4. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Enzyme based amperometric biosensor and its comparison with other techniques; III Studencka Konferencja Nauk Ścisłych, Krakow, Poland, 14.11.2020.
5. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Długotrwała stabilność amperometrycznego biosensora glukozy opartego na nanomateriale hybrydowym; Ogólnopolska Studencka Konferencja Naukowa “Bliżej chemii” Krakow, Poland, 9-10.01.2021.
6. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Novel and rapid glucose biosensor for long-term glucose meas-urements; IV Studencka Konferencja Nauk Ścisłych im. prof. Anto-niego Hoborskiego, Krakow, Poland, 15.11.2021.

Poster presentations

1. A. Jędrzak, **M. Kuznowicz**, T. Rębiś, T. Jesionowski; Amperometryczny biosensor oparty o funkcjonalny materiał hybrydowy Fe_3O_4/Lig/PDA-GOx oraz jego ewaluacja; BioOrg 2019, III Ogólnopolskie Sympozjum Chemii Bioorganicznej, Organicznej i Biomateriałów; Poznan, Poland, 7.12.2019.
2. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Optymalizacja i miniaturyzacja układów biosensorowych opartych na bioinspirowanej nanoplatformie Fe_3O_4/PDA/β-CD/GOx; BioOrg 2019, III Ogólnopolskie

Sympozjum Chemii Bioorganicznej, Organicznej i Biomateriałów; Poznan, Poland, 7.12.2019.

3. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Mediowany transport elektronów w biosensorze II generacji opartym o hybrydową nanoplatformę; XVII Konferencja Elektroanaliza w Teorii i Praktyce; Krakow, Poland, 19-20.11.2020.
4. A. Jędrzak, **M. Kuznowicz**, T. Rębiś, T. Jesionowski; Amperometryczny biosensor jako alternatywna metoda detekcji glukozy; XVII Konferencja Elektroanaliza w Teorii i Praktyce; Krakow, Poland, 19-20.11.2020.
5. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Detekcja glukozy w próbkach rzeczywistych w oparciu o nieenzymatyczny sensor PCA@MWCNT-CuO; I Ogólnopolska Konferencja PUTChemikon; Poznan, Poland, 06.05.2023.

Research projects

1. OPUS 14 (2017/27/B/ST8/01506); Poznan University of Technology, NCN; Prof. T. Jesionowski – principal investigator, **M. Kuznowicz** – investigator; origin topic "*Zaawansowane platformy hybrydowe MxOy/ biopolimer do biosensorów enzymatycznych. Projektowanie, charakterystyka i zastosowanie*"; ("Advanced hybrid platforms MxOy/biopolymer for enzymatic biosensors. Design, characteristics and application") 01.05 – 30.09. 2020.
2. STER – Mobility I; Poznan University of Technology, NAWA; **M. Kuznowicz** – investigator; Novel hybrid nanomaterials as (bio)sensing platforms. 1.09-30.11.2022.

Internships

1. **NanoBioMedical Centre**, Adam Mickiewicz University in Poznan, Poland, 1.06-31.08.2021
2. **University of Catania,** NanoHybrid Biointerfaces Laboratory (NHBIL), Catania, Italy, 1.09-30.11.2022

Awards

1. **Laureate of the Polish Academy of Sciences (PAN)** for the best creative publication published by Ph.D. student (technical sciences) in 2022.

2. **Award for the best oral communication at the conference** "IV Studencka Konferencja Nauk Ścisłych" Krakow, 15.11.2021.
3. **Award for the best oral communication at the conference** "III Studencka Konferencja Nauk Ścisłych" Krakow, 14.11.2020.

11.Statements of co-authorships

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Poznan, 22.09.2023

STATEMENT

I confirm the participation in following publications:

1. **M. Kuznowicz**, A. Jędrzak, A. Leda, T. Rębiś, T. Jesionowski; *Measurements of working parameters of external mediators for biodetectors based on the polydopamine@magnetite nanoparticles*; Measurement, 184 (2021) 109950.

I contributed to the preparation of the hybrid material, the immobilization process, construction of the biosensor, performed electrochemical tests, wrote part of the manuscript, and drew up charts and tables.

2. **M. Kuznowicz**, A. Jędrzak, T. Rębiś, T. Jesionowski; Biomimetic magnetite/polydopamine/β-cyclodextrins nanocomposite for long-term glucose measurements; Biochemical Engineering Journal, 174 (2021) 108127.

My participation in this publication included synthesis of the hybrid nanomaterial, enzyme immobilization, and whole electrochemical tests. I described the analysis results, wrote the manuscript, drawing up charts and tables.

3. T. Rębiś, **M. Kuznowicz**, A. Jędrzak, G. Milczarek, T. Jesionowski; *Design and fabrication of low potential NADH-sensor based on poly(caffeic acid)@multi-walled carbon nanotubes*, Electrochimica Acta, 386 (2021) 138384.

I declare that in this publication I designed the methodology of the synthesis, obtained the hybrid material, and described part of the manuscript and the results.

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4. **M. Kuznowicz**, T. Rębiś, A. Jędrzak, G. Nowaczyk, M. Szybowicz, T. Jesionowski; *Glucose determination using amperometric non-enzymatic sensor based on electroactive poly(caffeic acid)@MWCNT decorated with CuO nanoparticles*, Microchimica Acta, 189 (2022) 159.

My participation in this publication included synthesis of the nanomaterial, construction of a non-enzymatic sensor, and electrochemical research, I wrote the manuscript, drew up charts, tables, and drawings, and discussed with reviewers.

5. **M. Kuznowicz**, T. Rębiś, A. Jędrzak, G. Nowaczyk, T. Jesionowski; *Preparation of poly(caffeic acid)@MWCNT- $Ni(OH)_2$ hybrid nanomaterial and its application as non-enzymatic glucose sensor*, Chemosensors, 11 (2023) 452.

My participation in the publication included the synthesis of the hybrid nanomaterial, conducting electrochemical tests, describing the results, writing the manuscript, preparing graphs and tables, and discussing with the reviewers.

6. **M. Kuznowicz**, A. Jędrzak, T. Jesionowski; *Nature-inspired biomolecular corona-based on poly(caffeic acid) as a low potential and time-stable glucose biosensor*, Molecules, sent to the journal.

I declare that my participation consisted in receiving the hybrid material, conducting and assessing the effectiveness of immobilization, performing electrochemical tests, writing part of the manuscript, preparing graphs and tables.

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STATEMENT

I declare that I took an active part in the preparation of the following publications and that I am the corresponding author in them:

1. M. Kuznowicz, A. Jędrzak, A. Leda, T. Rębiś, **T. Jesionowski**; *Measurements of working parameters of external mediators for biodetectors based on the polydopamine@magnetite nanoparticles*; Measurement, 184 (2021) 109950.
2. M. Kuznowicz, A. Jędrzak, T. Rębiś, **T. Jesionowski**; Biomimetic magnetite/polydopamine/β-cyclodextrins nanocomposite for long-term glucose measurements; Biochemical Engineering Journal, 174 (2021) 108127.
3. T. Rębiś, M. Kuznowicz, A. Jędrzak, G. Milczarek, **T. Jesionowski**; *Design and fabrication of low potential NADH-sensor based on poly(caffeic acid)@multi-walled carbon nanotubes*, Electrochimica Acta, 386 (2021) 138384.
4. M. Kuznowicz, T. Rębiś, A. Jędrzak, G. Nowaczyk, M. Szybowicz, **T. Jesionowski**; *Glucose determination using amperometric non-enzymatic sensor based on electroactive poly(caffeic acid)@MWCNT decorated with CuO nanoparticles*, Microchimica Acta, 189 (2022) 159.
5. M. Kuznowicz, T. Rębiś, A. Jędrzak, G. Nowaczyk, **T. Jesionowski**; *Preparation of poly(caffeic acid)@MWCNT- $Ni(OH)_2$ hybrid nanomaterial and its application as non-enzymatic glucose sensor*, Chemosensors, 11 (2023) 452.

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6. M. Kuznowicz, A. Jędrzak, **T. Jesionowski**; *Nature-inspired biomolecular corona-based on poly(caffeic acid) as a low potential and time-stable glucose biosensor*, Molecules, manuscript ID: 2647041, under review.

My participation in the preparation of the publications included designing experiments, substantive supervision, writing, editing, and reviewing the manuscript.

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STATEMENT

I confirm the participation in following publications:

1. M. Kuznowicz, A. Jędrzak, A. Leda, **T. Rębiś**, T. Jesionowski; Measurements of working parameters of external mediators for biodetectors based on the polydopamine@magnetite nanoparticles; Measurement, 184 (2021) 109950.
2. M. Kuznowicz, A. Jędrzak, **T. Rębiś**, T. Jesionowski; Biomimetic magnetite/polydopamine/β-cyclodextrins nanocomposite for long-term glucose measurements; Biochemical Engineering Journal, 174 (2021) 108127.

My precipitation in these publications included formal analysis, writing – review and editing of the manuscript.

3. **T. Rębiś**, M. Kuznowicz, A. Jędrzak, G. Milczarek, T. Jesionowski; Design and fabrication of low potential NADH-sensor based on poly(caffeic acid)@multi-walled carbon nanotubes, Electrochimica Acta, 386 (2021) 138384.

I declare that my participation consisted in designing, performing and analyzing electrochemical experiments and writing the manuscript.

4. M. Kuznowicz, **T. Rębiś**, A. Jędrzak, G. Nowaczyk, M. Szybowicz, T. Jesionowski; Glucose determination using amperometric non-enzymatic sensor based on electroactive poly(caffeic acid)@MWCNT decorated with CuO nanoparticles, Microchimica Acta, 189 (2022) 159.
5. M. Kuznowicz, **T. Rębiś**, A. Jędrzak, G. Nowaczyk, T. Jesionowski; Preparation of poly(caffeic acid)@MWCNT- $Ni(OH)_2$ hybrid nanomaterial and its application as non-enzymatic glucose sensor, Chemosensors, 11 (2023) 452.

My participation in the presented publications included the concept, conducting some electrochemical tests, review and editing of the manuscript.

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Poznan, 22.09.2023

STATEMENT

I confirm the participation in following publications:

1. M. Kuznowicz, **A. Jędrzak**, A. Leda, T. Rębiś, T. Jesionowski; *Measurements of working parameters of external mediators for biodetectors based on the polydopamine@magnetite nanoparticles*; Measurement, 184 (2021) 109950.
2. M. Kuznowicz, **A. Jędrzak**, T. Rębiś, T. Jesionowski; Biomimetic magnetite/polydopamine/β-cyclodextrins nanocomposite for long-term glucose measurements; Biochemical Engineering Journal, 174 (2021) 108127.

My participation in the publications included conceptualization, physicochemical research, and participation in writing the manuscripts (original draft, review and editing).

3. T. Rębiś, M. Kuznowicz, **A. Jędrzak**, G. Milczarek, T. Jesionowski; *Design and fabrication of low potential NADH-sensor based on poly(caffeic acid)@multi-walled carbon nanotubes*, Electrochimica Acta, 386 (2021) 138384.

I declare that my participation consisted of performing the physicochemical characterization of the materials.

4. M. Kuznowicz, T. Rębiś, **A. Jędrzak**, G. Nowaczyk, M. Szybowicz, T. Jesionowski; *Glucose determination using amperometric non-enzymatic sensor based on electroactive poly(caffeic acid)@MWCNT decorated with CuO nanoparticles*, Microchimica Acta, 189 (2022) 159.

My participation in this publication included physicochemical characteristics of the materials, and participation in writing the manuscript (original draft, review, and editing).

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5. M. Kuznowicz, T. Rębiś, **A. Jędrzak**, G. Nowaczyk, T. Jesionowski; *Preparation of poly(caffeic acid)@MWCNT- Ni(OH)$_2$ hybrid nanomaterial and its application as non-enzymatic glucose sensor*, Chemosensors, 11 (2023) 452.

My participation in the presented publication included formal analysis and participation in writing the manuscript (original draft, review, and editing).

6. M. Kuznowicz, **A. Jędrzak**, T. Jesionowski; *Nature-inspired biomolecular corona-based on poly(caffeic acid) as a low potential and time-stable glucose biosensor*, Molecules, manuscript ID: 2647041, under review.

I declare that my participation consisted of developing methodology, performing physicochemical tests, writing the original manuscript, editing and reviewing.

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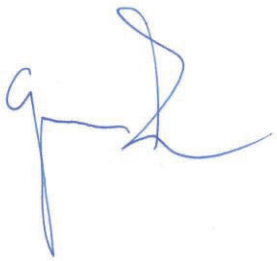
Poznan, 04.09.2023

STATEMENT

I confirm my participation in the following publications:

1. M. Kuznowicz, T. Rębiś, A. Jędrzak, **G. Nowaczyk**, M. Szybowicz, T. Jesionowski; Glucose determination using amperometric non-enzymatic sensor based on electroactive poly(caffeic acid)@MWCNT decorated with CuO nanoparticles, Microchimica Acta, 189 (2022) 159.
2. M. Kuznowicz, T. Rębiś, A. Jędrzak, **G. Nowaczyk**, T. Jesionowski; Preparation of poly(caffeic acid)@MWCNT- $Ni(OH)_2$ hybrid nanomaterial and its application as non-enzymatic glucose sensor, Chemosensors, 11 (2023) 452.

I declare that my participation consisted of part of the morphology characterization using high-resolution transmission electron microscopy (HR-TEM).



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STATEMENT

I declare that in the work "*Glucose determination using amperometric non-enzymatic sensor based on electroactive poly(caffeic acid)@MWCNT decorated with CuO nanoparticles*" Maria Kuznowicz, Tomasz Rębiś, Artur Jędrzak, Grzegorz Nowaczyk, **Mirosław Szybowicz**, Teofil Jesionowski; Microchimica Acta, 189 (2022) 159. I declare that my participation consisted of the part of the morphology characterization – Raman Spectroscopy.

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Poznań, 15.09.2023

STATEMENT

I declare that in the work " Design and fabrication of low potential NADH-sensor based on poly(caffeic acid)@multi-walled carbon nanotubes" Tomasz Rębiś, Maria Kuznowicz, Artur Jędrzak, Grzegorz Milczarek, Teofil Jesionowski; Electrochimica Acta, 386 (2021) 138384, my participation consisted of the writing and the revision of the manuscript. At the same time, I consent to the use of this publication in the doctoral dissertation of Maria Kuznowicz, M.Sc. Eng.

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Poznan, 04.09.2023

STATEMENT

I declare that in the work "*Measurements of working parameters of external mediators for biodetectors based on the polydopamine@magnetite nanoparticles*" Maria Kuznowicz, Artur Jędrzak, **Amanda Leda**, Tomasz Rębiś, Teofil Jesionowski; Measurement, 184 (2021) 109950, my participation consisted in carrying out part of the electrochemical research.

Amanda Leda