Molecular and Cell Biotechnology

ISSN 1993-6842 (on-line); ISSN 0233-7657 (print)
Biopolymers and Cell. 2024. Vol. 40. N 2. P 96–108
http://dx.doi.org/10.7124/bc.000AB0

UDC 620.3:577.15

Nanoparticles of prussian blue analogues as peroxidase mimetics for nanozyme – oxidase – based biosensors

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Aim. This work aims to develop new H_2O_2-sensing elements and nanozyme-oxidase-based sensors for the detection of hydrogen peroxide, glucose, and galactose. **Methods.** Chemical synthesis. Assay of enzymatic activity. Scanning electron microscope. **Results.** Hexacyanoferrates of several transient metals were synthesized and tested for their nanozymes (HCF NZs) (certificial peroxidase) activity in solution. The best representative nanozymes (NZs) were used for the construction of H_2O_2-sensitive sensors and nanozyme-oxidase-based biosensors. The NZs-based sensors: nAuHCF/GE and nPtHCF/GE demonstrated higher sensitivity (7.5 and 9.4-fold) than the biosensor with natural peroxidase. The developed biosensors GOX/nPtHCF/GE and GaOx/nPtHCF/GE for the detection of glucose and galactose showed enhanced sensitivity: 900 and 540 $A \cdot M^{-1} \cdot m^{-2}$, respectively, broad linear range (0.02–0.2 mM). Along with a broad linear range of detection these biosensors possess low limits of detection — 4.0 μM for glucose and 6.0 μM for galactose. Additionally, these biosensors exhibited improved stability when compared to the control. **Conclusions.** The novelty of the presented work is related to the synthesis of new peroxidase-like NZs and the evaluation of their functionality as the chemosensors on H_2O_2 and as the sensing components in the oxidase-based-biosensors for assay of glucose and galactose. The nPtHCF and nAuHCF NZs exhibit high sensitivity for the target analytes, a broad linear range, and satisfactory storage stability. The main advantages of the proposed nanozyme-oxidase-based biosensors are the simple architecture of the sensing layer and the ability to operate at low working electrode potentials.

Keywords: Prussian blue analogues, nanozymes, artificial peroxidase, glucose oxidase, galactose oxidase, amperometric biosensor.

Introduction

Glucose and galactose are two the most important low molecular weight metabolites whose levels are monitored for diagnostic purposes. The glucose level in the blood is an important indicator of the metabolism of carbohydrates and the main energy substrate of the body [1]. Galactose is also essential for the production of energy, galactosylation of endogenous and exogenous proteins, ceramides, myelin sheath metabolism, and others [1, 2]. The concentrations of glucose and galactose in blood are used as the biomarkers of diabetes or galactosemia [1–3], which is important in reducing the incidence and mortality rate. Early detection of galactose in newborns is very rewarding, because specific diet correction prevents mental retardation, liver cell failure, renal tubular acidosis, and neurological sequelae, and may lead to the resolution of cataract formation. In addition, the accurate detection and quantification of glucose and galactose are important in various industries, including food and beverages. Therefore, it is important to detect glucose and galactose in some products, especially in fruit juices and beverages, and in biological liquids as the essential metabolites in medicine [1–6].

The most perspective devices for analysis of these compounds are based on electrochemical biosensors [2–7]. In most cases, oxidases are used as signal generation enzymes [4, 6, 7]. This class of enzymes acts by oxidizing the substrate and then returning to their original active state by transferring the electrons to molecular oxygen, so that the final products of these enzymes are the oxidized form of the substrate and, as a side product, hydrogen peroxide. The detection of H_2O_2 is the most progressive way to ensure the operation of the corresponding biosensors [8]. In contrast to the commonly used platinum electrode, which requires a relatively high potential (0.6 vs. Ag/AgCl) for the detection of hydrogen peroxide, it was shown that Prussian Blue can be used as a selective electrocatalyst for H_2O_2 reduction allowing its low-potential (0.0 V vs. Ag/AgCl) detection in the presence of oxygen [9].

The Prussian Blue can simultaneously serves as a carrier of bioelement as well as a mediator and an artificial substitute for the natural enzyme peroxidase (nanozyme) [10]. The nanozymes (NZs) have essential advantages over natural enzymes, namely low preparation costs, stability, high surface area, and biocompatibility. NZs, especially PO-like NZs or "nanoperoxidases" (nanoPOs), are promising substitutes for natural PO, and have wide potential practical applications as catalysts in biosensors, fuel-cell technology, environmental biotechnology, and medicine [11–13].

In the current paper, we describe the synthesis and characteristics of new nano-synthesized hexacyanoferrates based on transient metals which possess PO-like activity. The synthesized NZs were applied for construction of a new nanozyme-oxidase-based biosensors for the detection of glucose and galactose. The combination of highly sensitive PO-mimetics with microbial oxidases results in significantly improved operational stability of the developed biosensors. One of the constructed sensors was tested for glucose assay in juice.

Materials and Methods

Synthesis and characterization of HCF PO-like NZs

Bi- and trimetallic NPs were synthesized by the chemical reduction method [18]. To obtain: nFeHCF, nZnHCF, nCuHCF, nCoHCF, nNiHCF, nMnHCF, nAgHCF, 5 ml 50 mM solution of the appropriate salt ($FeCl_3$, $ZnSO_4$, $CuSO_4$, $CoCl_2$, $NiSO_4$, $MnCl_2$ or $AgNO_3$) was mixed with 5 ml 50 mM $K_4Fe(CN)_6$ and 0.1 ml 100 mM ascorbic acid was added, by intensively stirring an reaction mixture on magnetic stirrer for 15 min.

To synthesize nPtHCF, nPdHCF, and nAuHCF, 2 mL 1 mM H_2PtCl_6, $PdCl_2$, or $HAuCl_4$ solutions were preliminarily reduced by the addition of 0.2 mL 100 mM ascorbic acid. After heating at 100 °C for 10 min with stirring, 8 mL of 50 mM $K_4Fe(CN)_6$ solution were added and incubated for one day without stirring [14]. The synthesized NPs were concentrated by centrifugation, washed with water, and tested on pseudo-peroxidase activity.

Determination of PO-like activity of the synthesized NZs in solution

To evaluate the PO-like activity of the synthesized NZs in solution, the colorimetric method with *o*-dianisidine as a chromogenic substrate in the presence of hydrogen peroxide was applied [13]. One unit (U) of PO-like activity was defined as the number of NZs releasing 1 μmol H_2O_2 per 1 min at 30 °C under the standard assay conditions.

Constructed amperometric sensors

The constructed sensors were characterized using amperometry in a three-electrode configuration with an Ag/AgCl/KCl (3 M) reference electrode, a Pt-wire counter electrode, and a working GE. To fabricate the H_2O_2-sensitive amperometric sensors, peroxidase-like NZs were placed by dropping onto the surface of a 3.05 mm (diameter) GE, followed by drying at room temperature. The dried film was covered with 5 μL 1 % Nafion solution, and stored at 4 °C until use. To prepare 1 % Nafion solution, the stock 5 % solution was diluted with the appropriate buffer: with 50 mM PB, pH 7.0. H_2O_2-sensing ability of the electrodes was studied in 50 mM acetic buffer, pH 4.5, and the profiles of amperometric outputs at increasing concentrations of H_2O_2 were compared. The most active NPs that have the highest PO-like electrochemical activity were chosen for further investigation.

For development of the NZs-based electrode, 5–10 μL of NZs solution with PO-like activity of 1 U/mL were dropped onto the surface of bulk GEs. After drying for 10 min at room temperature, the layer of NZs on the electrode was covered with 10 μL of GOX or GaOX. The dried composite was covered by a Nafion membrane. The coated bioelectrode was rinsed with water and stored in phosphate buffer, pH 7.0, until being used.

All experiments were carried out three times (n = 3) and measurements were performed in two parallels. The statistical parameters and all figures were calculated and built using Origin 8.5 Pro. Sensitivity ($A \times M^{-1} \times m^{-2}$) was calculated as follows: Sensitivity = B/S, where B — the slope for the dependence of current on the analyte concentration in linear range ($A \times M^{-1}$); S — the surface area of the working electrode (m^2). The limit of detection (LOD) was calculated by using the standard deviation (SD) of the blank current signals and

the B value according to the formula: LOD = (3 × SD/B).

Determination of glucose in real samples

The commercial juices by "Sadochok" (LtD Sandora, Odessa, Ukraine) — multifruit; by "Galicia" (T.B. Fruit Company, Horodok, Ukraine) — apple with pear were used for analysis of glucose. All samples were analyzed using a standard addition test (SAT). Before assay, all samples were diluted stepwise in 50 mM phosphate buffer, pH 6.0. Each assay was performed for two dilutions of the sample and repeated three times.

Results and Discussion

Synthesis and catalytic characterization of hexacyanoferrate NZs with oxidoreductase activity

The aim of current work was to perform synthesis and screening of HCF NPs for pseudo-oxidoreductase activity as well as to select NZs that exhibit peroxidase-like activity. The obtained HCF NZs with high activity have been used to construct NZs-based biosensors coupled with one of oxidases (GOX and GaOX), for the detection of glucose or galactose in medical diagnostics.

The synthesized NPs of Prussian blue (PB) and ten PB-analogues containing ions of transition and noble metals: Nickel, Cobalt, Copper, Manganese, Zinc, Platinum, Argentum, Palladium, Cerium, and Aurum with the general formula $M_4[Fe(CN)_6]_z\ xH_2O$ were studied for their oxidoreductase activity in solution (acetate buffer, pH 4.5). As shown in Table 1, seven HCF NPs possess only peroxidase activity: nAuHCF, nPtHCF, nPdHCF, nAgHCF, nFeHCF, nCu/FeHCF, nPt/CeHCF. The nAuHCF NPs have the highest peroxidase activity (6.7 units×mg^{-1}), whereas the NPs nPtHCF, nPdHCF, and nAgHCF have lower activity (1.6 units×mg^{-1}). The rest of the NPs shows the lowest activity measuring less than 1 unit×mg^{-1}.

For seven NPs (nAuHCF, nPtHCF, nPdHCF, nAgHCF, nFeHCF, nCu/FeHCF and nPt/CeHCF), which have significant peroxidase activity, we investigated the catalytic parameters (K_M, V_{max}, k_{cat}, and k_{cat}/K_M) from the data

Table 1. **Pseudoperoxidase activity of HCF NPs obtained by chemical synthesis**

HCFs NZs	Pseudoperoxidase activity, units·mg^{-1}	K_M, mM	V^*_{max}, mM·c^{-1}	k_{cat}, µmol s^{-1}·mg^{-1}	k_{cat}/K_M s^{-1}·ml·mg^{-1}
nAuHCF	6.71 ± 0.01	0.50	10.83×10^{-5}	0.135	0.27
nPtHCF	1.85 ± 0.02	3.40	1283×10^{-5}	0.054	0.015
nPdHCF	1.68 ± 0.01	3.08	1000×10^{-5}	5.0	1.62
nAgHCF	1.61 ± 0.02	16.68	345×10^{-5}	32.08	1.92
nFeHCF	1.10 ± 0.01	41.32	41.33×10^{-5}	0.689	0.016
nCu/FeHCF	0.87 ± 0.02	16.60	13.33×10^{-5}	0.067	0.004
nPt/CeHCF	0.85 ± 0.01	4.17	1241×10^{-5}	4.85	1.163
PO	-	3.7	8.71×10^{-5} (2.5×10^{-11} M)	3.48×10^{3}	17

* For HCF, V_{max} was determined at concentration 1 mg×ml^{-1}

on dependence of the reaction rate on the concentration of the principal substrate H_2O_2. As shown in Table 1, for three types of NZs (nPtHCF, nPdHCF, and nPt/CeHCF) the values of K_M for H_2O_2 (3.4; 3.08; and 4.17) are very close to the value of K_M for horseradish peroxidase (PO) (3.7 mM). For other NZs (nFeHCF, nCu/FeHCF, and nAgHCF), the values of K_M are the following: 41; 16.6, and 16.7 mM, respectively, which are higher than the K_M for PO, but close to the value of K_M of PO from rice *Oryza sativa* (23.3 mM) and *Mycobacterium avium* ssp. (30 mM) [15]. The K_M values of the synthesized NZs nCu/FeHCF and nAgHCF (16.6 mM and 16.7 mM) are very close to the K_M value of PB (14.7 mM) and CuO nanosheets (15.8 mM) [16].

Among the synthesized NZs, nAuHCF has the highest affinity for the H_2O_2 substrate: its K_M is 0.5 mM, which is lower than the K_M of horseradish PO (3.7 mM). The NPs based on iron oxide modified with HCF (PB-γ-Fe_2O_3) and palladium NPs have higher values of both K_M (324 mm and 1064 mm). The most catalytically effective were nPdHCF, nAgHCF, and nPt/CeHCF (the highest kcat /Km value).

The obtained NPs (nAuHCF, nPtHCF, nPdHCF, nAgHCF), as catalytically active NZs, which have a high affinity for H_2O_2 as PO mimetics, can be used as sensitive elements to H_2O_2 in chemosensors or oxidase-based biosensors.

Development and characterization of the H_2O_2-sensitive sensors based on PO-like NZs

The best selected PO-like NZs (nAuHCF, nCu/FeHCF, nPtHCF, nPdHCF, nCu/FeHCF, and nAgHCF) were studied as promising H_2O_2-sensitive elements of amperometric biosensors. Bioanalytical properties of such sensors determined by using chronoamperometry analysis and calibration graphs at –200 mV are shown in Table 2.

The linear ranges, limits of detection (LOD), I_{max} values (for an electrode area of 7.06 mm^2), and sensitivities were calculated. As shown in Fig. 1, the nAuHCF/GE and nPtHCF/GE sensors have the highest sensitivity (2650 and 3300 $A\times M^{-1}\times m^{-2}$, respectively) in comparison with the similar chemosensors and biosensors based on native PO (Table 2). The PO-like HCF-based (nAuHCF/GE and nPtHCF/GE) sensors reveal a significantly higher sensitivity (up to 1.8–2.7-fold) than the Ni–FePBA/DBD, MnPBA/GCE, gCuPBA-based sensors [13], and the sensitivity was 7.5-fold and 9.4-fold higher compared with PO/GE (Table 2), respectively.

For comparison, a PB-modified glassy carbon electrode (GCE) demonstrated a lower sensitivity (2000 $A\times M^{-1}\times m^{-2}$) [10]. The chemosensor Ni-PBA/GCE with sensitivity (3500 $A\times M^{-1}\times m^{-2}$) was described, which is close to the sensitivity of the currently prepared nPtHCF/GE (3300 $A\times M^{-1}\times m^{-2}$). So, our results and data, published in the literature prove that PB-analogues have better H_2O_2-sensitivity compared with PB, and they may be used as sensitive elements in the oxidase-based biosensors.

Amperometric biosensors based on oxidases and PO-like NZs for assaying glucose and galactose

A characteristic feature of all oxidases is the ability to catalyze oxidation of specific substrates by molecular oxygen-producing hydro-

Table 2. **Analytical characteristics of the developed amperometric H_2O_2-sensitive chemosensors of the architecture HCF/GC**

H_2O_2-selective layer	Sensitivity, $A \times M^{-1} \times m^{-2}$	Linear range, up to mM	I^*_{max}, μA	K_M^{app}, mM	References
nAuHCF/GE	2650 ± 2.15	1.25	37.8 ± 3.2	0.9 ± 0.3	This work
nPtHCF/GE	3300 ± 2.33	0.4	28.71 ± 0.70	1.59 ± 0.13	
nPdHCF/GE	458 ± 3.7	0.5	63.73 ± 2.91	19.01 ± 0.93	
nAgHCF/GE	407 ± 3.8	0.6	34.86 ± 0.75	12.42 ± 0.65	
nCu/FeHCF/GE	369 ± 3.4	1.0	15.57 ± 0.21	5.62 ± 0.25	
gCuPBA/GE	1620 ± 1.32	10–800	-	-	[13]
gPdHCF/GE	697 ± 5.6	0.8	62.4 ± 3.0	33.1 ± 3.9	[13]
PO/GE	352 ± 3.2	0.4	5.0 ± 0.2	4.9 ± 1.1	[13]

GE — graphite electrode; PO — peroxidase;* — I_{max} for an electrode area of 7.06 mm^2

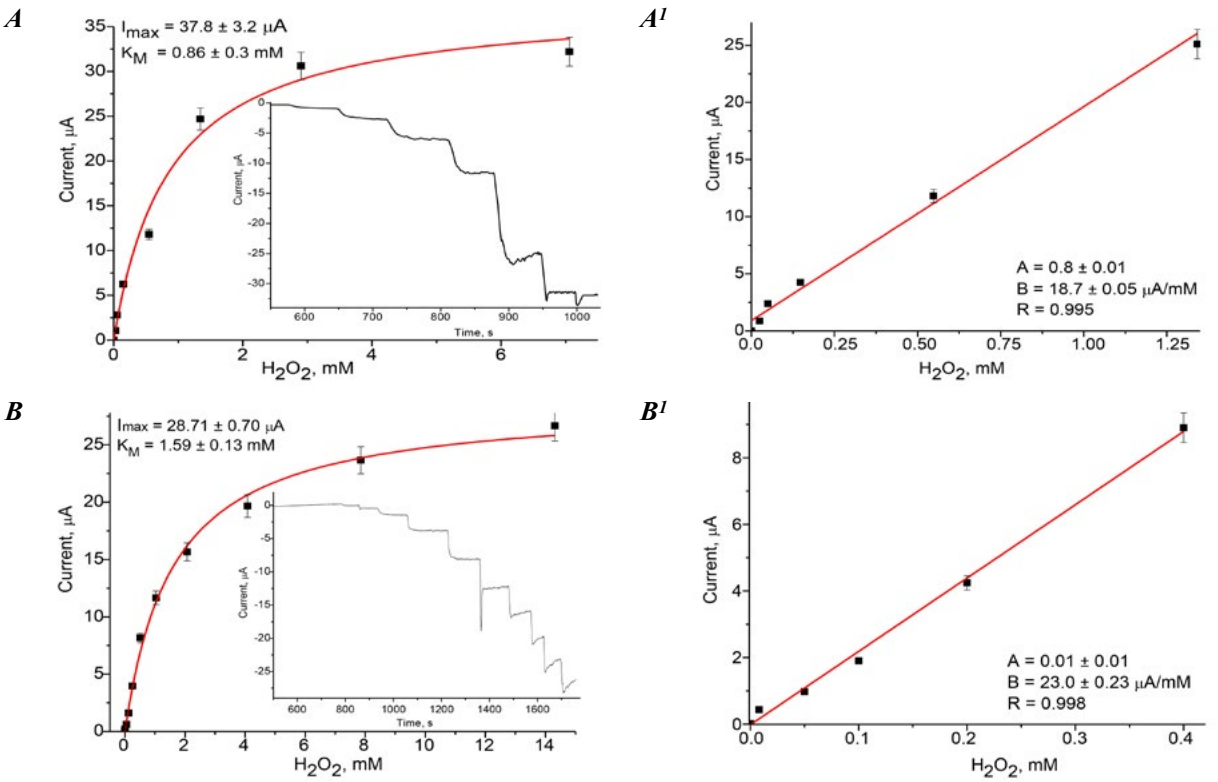


Fig. 1. Amperometric characteristics of the electrodes: nAuHCF/GE (*A*, *A*[1]) and nPtHCF/GE (*B*, *B*[1]): *A*, *B* — chronoamperogram (inserted) and dependence of the amperometric signal on the concentration of H_2O_2; *A*[1], *B*[1] — calibration graphs for H_2O_2 determination. Conditions: working potential –200 mV vs. Ag/AgCl/3 M KCl in 50 mM PB, pH 6.0.

gen peroxide (H_2O_2) as a byproduct. This redox-active compound can be easily monitored amperometrically. We constructed the biosensors using each of the oxidases (GOX or GaOX) as an analyte-sensitive element and PO-like NZs (nAuHCF and nPtHCF) for the analysis of glucose and galactose, respectively. The constructed NZ–oxidase biosensors (GOX/nAuHCF/GE, GOX/nPtHCF/GE, GaOX/nAuHCF/GE, and GaOX/nPtHCF/GE) have been tested with cyclic voltammogram (CV) and chronoamperometry, at added increased concentration of the correspondent analyte upon stirring. CV for all types of NZ-oxidase biosensors demonstrated that a reduction peak caused by H_2O_2 decomposition occurred at potential 0 to –300 (vs. Ag/AgCl) (Fig. 2). In order to obtain sensitive and reproducible glucose or galactose detection, the applied potential was investigated in a range between 0 and –300 mV by chronoamperometry, using the analites. It was not observed a significant difference in signal intensities, within the errors, by using different applied potential (not shown). Therefore, –50 mV was chosen as the working potential because it allows a satisfactory sensitivity.

For further experiments, the potential of –50 mV was taken as the optimal one. It should be noted that nPtHCF/GE and nPtHCF/GE was also tested as a control electrode and no amperometric signals were observed under injected glucose or galactose. The main bioanalytical characteristics of the developed biosensors are shown in Table 3 and Fig. 3.

As shown, nPtHCF as a PO-like NZ for two types of biosensors (GOX/nPtHCF/GE and GaOx/nPtHCF/GE) revealed the highest sensitivity: 900 and 540 $A \times M^{-1} \times M^{-2}$ glucose and galactose, respectively. However, the sensitivity of other biosensors (GOX/nAuHCF/GE and GaOx/nAuHCF/GE), where nAuHCF NZ is used, is slightly lower compared to nPtHCF-base analogue: 1.5- and 1.2-fold. The developed NZ-oxidase biosensors exhibit improved analytical characteristics in comparison with the correspondent bi-enzyme biosensor that contained natural PO. The developed glucose

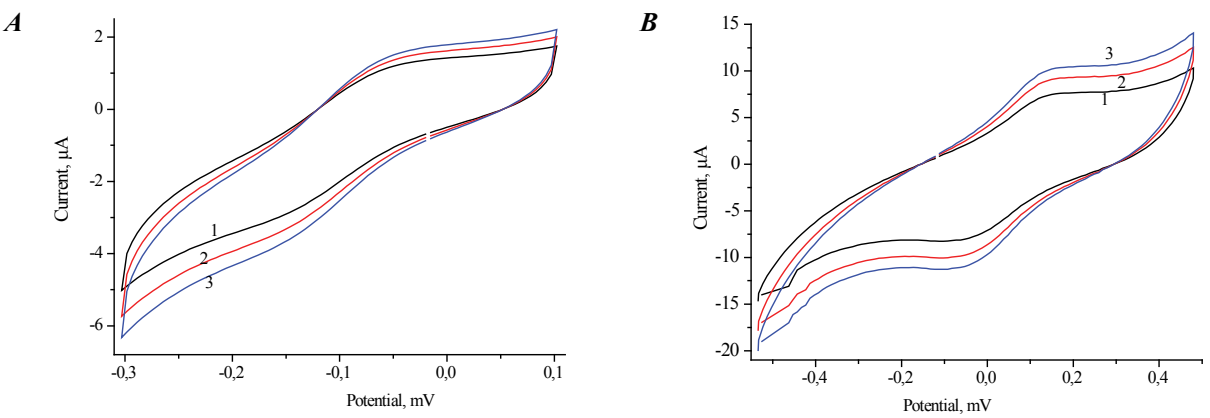


Fig. 2. Cyclic voltammograms (CV) of the GaOx/nPtHCF/GE (*A*) and GOX/nPtHCF/GE (*B*). CV profiles (1–3) as outputs upon addition of galactose or glucose up to concentrations: (1) 0, (2) 0.2, (3) 0.2 mM. Conditions: scan rate 50 $mV \times s^{-1}$; Ag/AgCl (reference electrode) in 50 mM PB, pH 6.5.

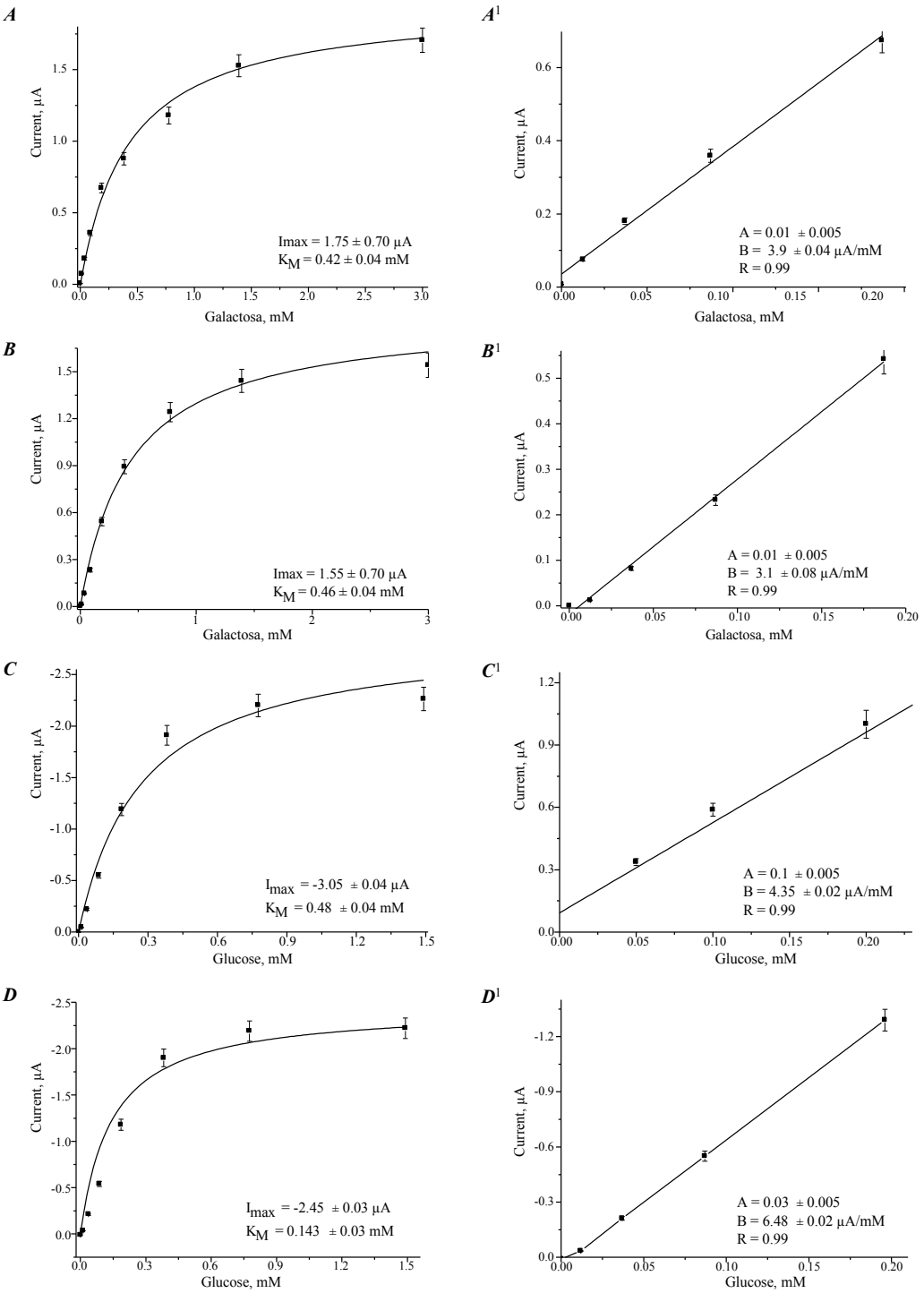
Table 3. **Analytical characteristics of the fabricated nanozyme–oxidase-based biosensors**

Bioelectrode	Sensitivity, $A \cdot M^{-1} \cdot m^{-2}$	Linear range, mM	I_{max}., μA	K_M^{app}, mM	Reference
GOX/HCF/GE					
GOX/nPtHCF/GE	900 ± 3	0.02–0.2	2.45 ± 0.03	0.14 ± 0.03	This work
GOX/nAuHCF/GE	600 ± 4	0.03–0.2	3.05 ± 0.04	0.48 ± 0.04	
GOX/nAgHCF/GE	240 ± 3	0.04–0.2	0.8 ± 0.03	0.3 ± 0.03	
GOX/nCuFeHCF/GE	162 ± 9	0.04–0.2	0.51 ± 0.03	0.3 ± 0.03	
GOX/nPdHCF/GE	272 ± 12	0.03–0.35	1.46 ± 0.04	0.5 ± 0.03	
GOX/PO/GE	44 ± 3	0.5–5	-	5.23	[11]
GO/gCuHCF/GE	710 ± 3	200	3.22	0.35	[13]
GOX/PB-NiHCF NP	530 ± 3	0.1–2	-	-	[19]
GOX/PB	340 ± 4	0.1–2	-	-	[19]
GaOx/HCF/GE					
GaOx/nPtHCF/GE	550± 3	0.03–0.2	1.75 ± 0.70	0.42 ± 0.04	This work
GaOx/nAuHCF/GE	450 ± 3	0.03–0.2	1.55 ± 0.70	0.46 ± 0.04	
GalOx/Co_3O_4/MWCNTs/GC	104 ± 3	0.009	-	-	[20]
GalOx/Poly-GMA-Co-Fc/Pt	2300 ± 19	2–20	-	-	[20]

biosensor GOX/nPtHCF/GE has 2.6- and 1.7-fold higher sensitivity in comparison with GOX/PB, GOX/PB-NiHCF, and GO/gCuHCF/GE [13].

The constructed biosensor (GaOX/nPtHCF/GE) for the detection of galactose has a higher sensitivity compared with the sensors based on Prussian blue [4]. To evaluate the storage stability of the biosensors (GOX/nPtHCF/GE and GaOX/nPtHCF/GE) during five days, we measured the current responses for injected 0.1 mM glucose and galactose, respectively, daily under the same conditions. These responses were then compared with those of a bi-enzyme sensor based on natural peroxidase. The results showed that after five days, the responses of the nanozyme-based biosensors to the analyte were more than 50 % of the initial value, and they possessed the improved stability by 1.5 times compared to bi-enzymatic. This indicates to an increase in storage stability for the nanozyme-based biosensors.

The selectivities of the proposed GOX/nPtHCF/GE and GaOX/nPtHCF/GE biosensors to the target analyte (glucose or galactose) are of great importance, especially for analysis of real samples. The concentration of glucose or galactose is normally about 3–8 mM and 0.28 mM respectively, which is higher than that of main interferences [2]. The selectivity of the constructed sensors was estimated in relative units (%) as a ratio of the detected signal to the value of the highest current response: no signals were observed for most of the tested other interfering species (ascorbic acid, uric acid, urea, sucrose, and lactose). The fabricated sensors had excellent selectivity toward glucose or galactose, respectively.



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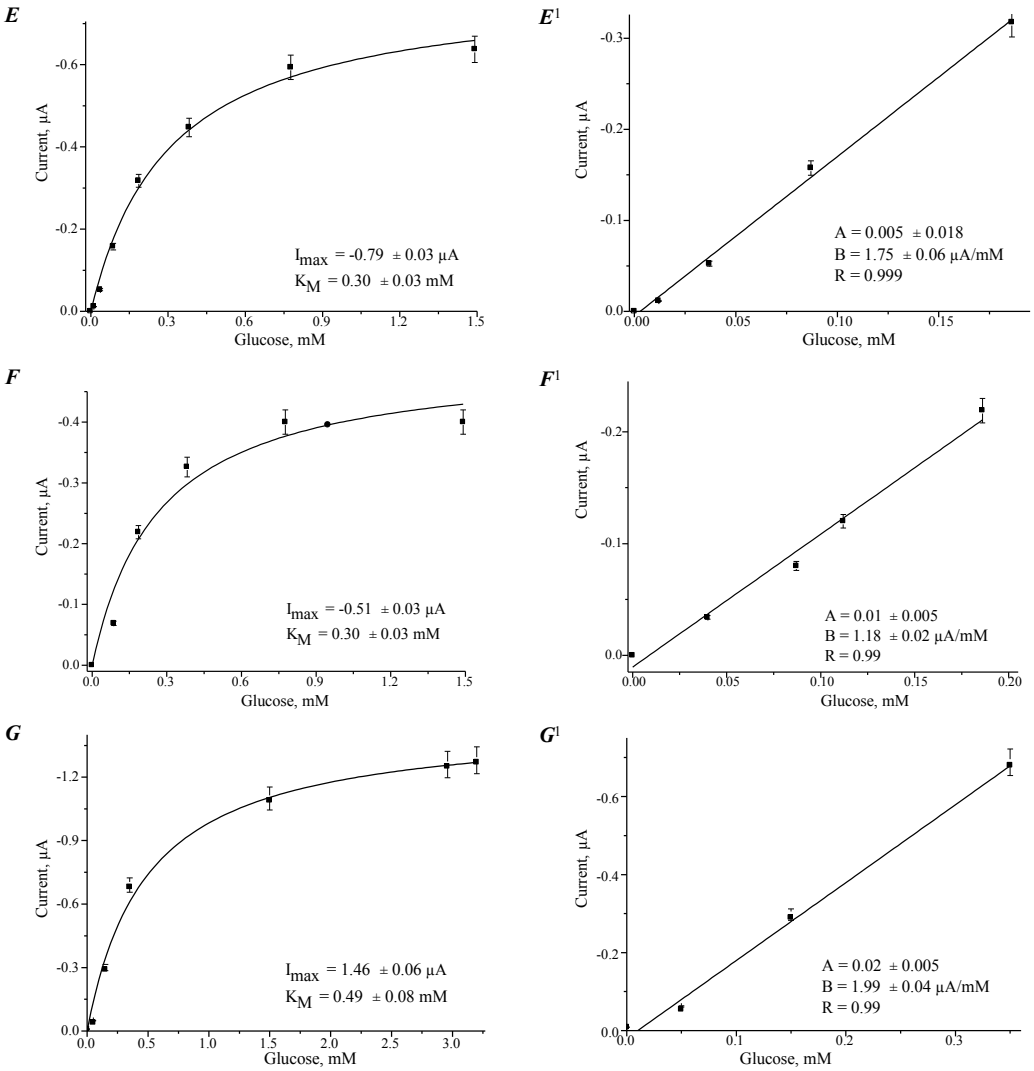

Fig. 3. Amperometric characteristics of the electrodes: GaOx/nPtHCF/GE (*A*, *A*[1]) and GaOx/nAuHCF/GE (*B*, *B*[1]), GOX/nPtHCF/GE (*C*, *C*[1]), GOX/nAuHCF/GE (*D*, *D*[1]), GOX/nAgHCF/GE (*E*, *E*[1]), GOX/nCuFeHCF/GE (*F*, *F*[1]), GOX/nPdHCF/GE (*G*, *G*[1]): *A*, *B*, *C*, *D*, *E*, *F*, *G* — dependence of the amperometric signal on the concentration of galactose and glucose, respectively ; *A*[1], *B*[1], *C*[1], *D*[1], *F*[1], *E*[1], *G*[1] — calibration graphs for galactose and glucose determination, respectively.

The repeatability of the assay using biosensors GOX/nPtHCF/GE and GaOX/nPtHCF/GE was investigated by detecting 0.1 mM glucose and 0.1 mM galactose, respectively, five times in succession. The oxidation current of sensors was approximately steady in the five measurements. The relative standard deviation (RSD) was 4.2 % and 4.5 %, indicating good repeatability. Five different GOX/nPtHCF/GE and GaOX/nPtHCF/GE elec-

trodes were developed for 0.1 mM glucose and 0.1 mM galactose concentration detection through the method of chronoamperometry to determine the reproducibility of the glucose biosensor. The relative standard deviation for the five modified electrodes was 2.6 %, confirming that the biosensor exhibited good reproducibility (data not shown). Thus, the developed biosensors may be advantageous for practical applications due to the easiness of their fabrication, reproducibility, and stability.

To test the practical feasibility of the constructed biosensor, the sensor GOX/nAuHCF/GE was used for glucose analysis in three fruit juice samples using the standard addition method. Table 4 demonstrates the results of glucose determination in the juices by the proposed biosensor and by an enzymatic kit. The determined average glucose concentration correlates well with the data obtained using the reference method, with a difference of less than 10 %. The determined average glucose concentration correlates well with the data obtained using the reference method, are in good agreement ($p > 0.05$) with strong ($R = 0.993–0.999$) correlations.

To expand the scope of application, the proposed sensor was used for non-invasive analysis of glucose in the urine of patients with diabetes. When monitoring diabetes, the level of glucose in the urine of patients can vary from 5.5 to 110 mM [2]. To check accuracy, urine samples were also tested using the breathalyzer spectrophotometric method. All obtained results are presented in Table 5. The determined average glucose concentration correlates well with the data obtained using the reference method, are in good agreement ($p > 0.05$) with strong ($R = 0.993–0.999$) correlations. The proposed sensors are universal in clinical research and in the food industry. The obtained results prove the accuracy of the biosensor approaches for glucose assay (differences are less 10.0 %) and can be used for control of food quality.

Conclusion

We offered the advanced nanozyme-oxidase biosensors based on Prussian blue analogues

Table 4. **Results of glucose assay (mM) in the fruit juices, n = 3**

The fruit juices	Biosensor	Enzymatic method	Difference, %
Multi vitamin ("Sadochok")	190 ± 17 p > 0.05	205 ± 15 p > 0.05	9.2
Apple-pear («Galicia")	121 ± 10 p > 0.05	133 ± 12 p > 0.05	9.0
Apple fresh	187 ± 16 p > 0.05	200 ± 18 p > 0.05	9.3

Values are expressed as mean ± SD Student's test (p) was performed for values obtained by the biosensor approach compared to the reference one

Table 5. **Detection of glucose (mM) concentrations in real urine samples, n = 3**

Samples	Biosensor	Enzymatic method	Difference, %
Urine 1	8.4 ± 1.7 p > 0.05	8.9 ± 1.7 p > 0.05	9.4
Urine 2	7.4 ± 1.5 p > 0.05	7.8 ± 1.5 p > 0.05	9.5
Urine 3	9.3 ± 1.6 p > 0.05	9.7 ± 1.6 p > 0.05	9.6

Values are expressed as mean ± SD. Student's test (p) was performed for values obtained by the biosensor approach compared to the reference one

(hexacyanoferrates of transient metals as peroxidase mimetics as well as oxidases, enabling the assay of glucose or galactose). The synthesized HCF NZs are catalytically active: their peroxidase-like activity in a solution is in the range 1.61–6.7 Units×mg. As they are stable and highly sensitive to hydrogen peroxide, we have used them in the construction of oxidase-based sensors for the assay of glucose and galactose. H_2O_2-sensitive electrodes nAuHCF/GE and nPtHCF/GE reveal the best sensitivity 2650 and 3300 $A \times M^{-1} \times m^{-2}$ at electrodes low applied potential around –0.050 mV versus Ag/AgCl. The developed nanozyme-oxidase biosensors exhibit improved analytical characteristics in comparison with the corresponding natural PO-based sensors. As an example, the nPtHCF-oxidase biosensors GOX/nPtHCF/GE and GaOx/nPtHCF/GE have the following sensitivity: 900 and 540 $A \times M^{-1} \times M^{-2}$ in the assay of glucose or galactose, respectively. Thus, the developed monoenzymatic nanozyme-oxidase-based biosensors are promising for fast and simple assay of analytes in biological fluid and food.

Funding

This work was supported by the IIE Scholar Rescue Fund, the Simons Foundation award, US National Science Foundation (NSF) grant CBET-2235349 including IMPRESS-U supplement (project STCU 7113), and the National Research Foundation of Ukraine (projects No. 2021.01/0010).

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Аналоги пруської блакиті, як міметики пероксидази для нанозим – оксидазних біосенсорів

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Мета. Метою роботи була розробка нових H_2O_2-чутливих елементів і нанозим-оксидазних біосенсорів для аналізу гідроген пероксиду, глюкози та галактози. **Методи.** Хімічний синтез. Ензиматичний та біосенсорний аналіз. Сканувальна електронна мікроскопія. **Результати.** Синтезовано гексаціаноферратні нанозими (HCF NZs) як каталітично активні аналоги Пруської блакиті, які мають високу пероксидазоподібну активність у розчині і можуть слугувати для побудови H_2O_2-чутливих сенсорів і нанозим-оксидазних біосенсорів. Сенсори на основі: nAuHCF/GE та nPtHCF/GE виявляють вищу чутливість (у 7,5 та 9,4 раза), в порівнянні з аналогом, на основі пероксидази. Розроблені біосенсори GOX/nPtHCF/GE, GaOx/nPtHCF/GE для глюкози та галактози мають вищу чутливість (900 і 540 $А \times М^{-1} \times м^{-2}$, відповідно). Крім того, ці біосенсори мають широкий лінійний діапазон виявлення, що досягає 0,2 мМ, і вони мають низькі межі виявлення — 4,0 мкМ для глюкози та 6,0 мкМ для галактози. Крім того, ці біосенсори продемонстрували кращу стабільність порівняно з контролем. **Висновки.** Новизна представленої роботи пов'язана із синтезом нових пероксидазоподібних НЗ та оцінкою їхньої функціональності як хемосенсорів на H_2O_2 та каталітичних компонентів біосенсорів для аналізу аналізу глюкози та галактози. NZs nPtHCF і nAuHCF у складі створених сенсорів показують високу чутливість до цільових аналітів, широкий лінійний діапазон і задовільну стабільність при зберіганні, а головною їхньою перевагою є проста архітектура чутливого шару та здатність функціонувати при низьких потенціалах.

Ключові слова: аналоги Пруської блакиті, нанозими, штучна пероксидаза, глюкозооксидаза, галактозооксидаза, амперометричний біосенсор.

Received 30.12.2023