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New Amperometric Glucose Biosensors Based On Nafion Nanofibers

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Research Article

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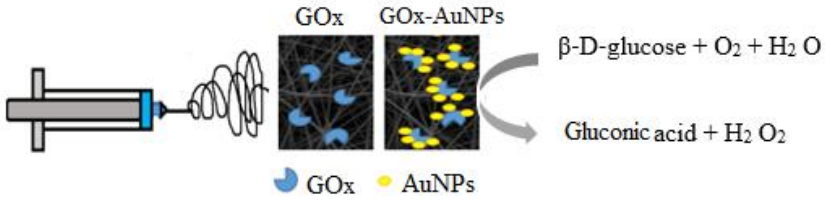
Abstract



In this study, we present new amperometric glucose biosensors based on Nafion nanofibers. Nafion possesses a number of desirable properties, including stability, thermal, mechanical, physio-chemical and biocompatibility, which make it a competitive polymer for use in glucose biosensors. Nafion nanofibers were produced using a low-cost electrospinning method, and their morphology was characterized via scanning electron microscopy (SEM). The biosensors were fabricated by loading glucose oxidase (GOx) and GOx-AuNPs (10 nm) on the Nafion-Cellulose Acetate nanofibers (Nafion-CA Nfs) (461.53 ± 30.34 nm) on the surface of a platinum electrode, followed by cross-linking using glutaraldehyde. The biosensors operating at 0.6 V demonstrated lower LODs with wider linear ranges, enhanced stability, elevated sensitivity, and improved selectivity. For Pt/Nafion-CA NFs/GOx biosensor demonstrated remarkable sensitivity, with values of 68.67 $\mu A.mM^{-1}cm^{-2}$ and 18.38 $\mu A.mM^{-1}.cm^{-2}$, respectively, and a limit of detection (LOD) of 1.36 µM. Meanwhile, the Pt/Nafion-CA NFs/ GOx-AuNPs biosensor achieved sensitivities of 55.56 µA.mM-1.cm² and 28.49 µA/mM ·cm², respectively. It also exhibited a broad linear range of up to 12.81 mM and an LOD of 10.8 µM, with no observable interference effects. The present studies highlight the potential of Nafion nanofibers as a suitable matrix for immobilizing GOx and GOx-AuNPs, thereby demonstrating their viability as interference-free, easy-to-use sensors with good sensitivity, LOD and stability for commercialization.

1. Introduction

Nanofibers (NFs) and nanoparticles (NPs) represent the fundamental building blocks of nanotechnology. Nanofibers exhibit a range of morphologies, with diameters spanning from micrometers to hundreds of nanometers and lengths that can extend to several meters. Nanofibers have been produced by a number of different processing techniques, including

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drawing (Ondarcuhu et al., 1998), template synthesis (Feng et al., 2002; Martin, 1996), phase separation (Ma and Zhang, 1999), self-assembly (Liu et al., 1999; Whitesides and Grzybowski, 2002) and electrospinning (Deitzel et al., 2001; Islam et al., 2019). The history of electrospinning (ES) as a scientific technique spans approximately one century, it is only in recent decades that this process has gained popularity in both academic and industrial contexts (Nascimento et al., 2015). ES has proven to be a simple, versatile, and low-cost approach to fabricating electrospun nanofibers using a range of materials, including natural and synthetic polymers, liquid crystals, solid particle suspensions, ceramics, and emulsions (Pakravan et al., 2011).

Nanofibers have attracted considerable interest from a number of academic disciplines for a multitude of applications, including regenerative medicine and tissue engineering (Schiller and Scheibel, 2024; Guarino et al., 2018), drug delivery (Muratoglu et al., 2024; Ozkizilcik et al., 2018), sensors (Shooshtari, 2025; Yıldız et al., 2024; Liu et al., 2018), power generation and energy storage (Sarangika, et al., 2024; Yuriar-Arredondo et al., 2018), filtration (Akduman, 2021; Feng et al., 2024), catalysis (Din et al 2018; Gupta et al., 2018), textiles (Barhoum et al., 2018; Yan et al., 2023), defense and security (Suja and Mathiya, 2024 ; Mondal et al., 2023).

The excellent properties of these nanofibers, including straightforward production process control, a large surface area (Wang et al., 2021; Zhang et al., 2023), flexible surface characteristics, a small and adjustable pore size, high and interconnected porosity (Yue et al., 2024), intrinsic 3D topography and superior mechanical properties (Zhao et al., 2023), high bio-immobilization activity (Smith et al., 2020) and faster electron transfer compared to nanoparticle-based films of the same material, make them highly versatile and valuable materials (Choi et al., 2023; Vargas-Molinero et al., 2023).

Nanoparticles have been described as “breakthrough materials”. The material offers a vast surface area within the constraints of the nanoscale, providing an optimal platform for a multitude of chemical and biological detection applications, including drug delivery, biolabeling, protein detection, gene delivery, and body probing. Furthermore, DNA, molecule purification (Hynes et al., 2021), and the potential to address numerous health concerns are additional areas of interest. A substantial body of research has been conducted to develop nanoparticles comprising noble metals (e.g., gold, silver, platinum, and palladium), oxides (e.g., copper oxide, copper oxide, nickel oxide, and iron oxide), and bimetallic systems (e.g., gold-platinum and copper-silver) for use in biological, medical, and biosensing applications (Lipińska et al., 2021).

At present, gold nanoparticles (AuNPs) are the subject of considerable interest in the field of glucose biosensors, largely due to the favorable characteristics they possess. These include the capacity to be modified by altering their shape, size, aggregation, and surface electron enrichment, which collectively affords a large specific surface area and a conducting label for the detection of the target. Furthermore, it facilitates a specific modification of the electrode surface, resulting in a notable enhancement of the response current. AuNPs exhibit a strong interaction with various functional groups, including amino acids in the outer and exposed areas of proteins. This interaction occurs through the binding of the AuNPs to the sulfhydryl (SH) group of cysteine (Maguteeswaran et al., 2024), as well as its oxidized S–S cystine group (Shokri et al., 2017). Additionally, AuNPs can interact with CH– NH groups (Lyu et al., 2024; Hernández et al., 2018), such as those found in amino acids. Additionally, the NH groups (Hernández et al., 2018; Dadadzhanov et al., 2020), carboxylates (Courrol and Matos, 2016), glutamic and aspartic acids have the capacity to regulate the negatively charged glucose oxidase (GOx), and there are π interactions between the aromatic rings of phenylalanine and tyrosine (Roy, 2017). Furthermore, the impact of AuNPs on the enzymatic activity of GOx has been meticulously examined, along with the dimensions and expansion of AuNPs in the oxidation of glucose catalyzed by the GOx enzyme (Guo et al., 2023; Ramanavicius et al., 2017). Glucose oxidase is an enzyme that is widely employed in the design of biosensors for the

detection of glucose in the blood of diabetic patients and in the food industry. It exhibits relatively high stability, catalytic turnover, and specificity (Tu et al., 2019). It has been demonstrated that the enzyme can maintain its activity within a pH range of between 2 and 8, depending on the strain of origin and the extent to which it can be immobilized on solid surfaces (Hecht et al., 1993; Yan et al., 2022), allowing it to bind and spread. The enzyme is a homodimer, comprising two flavin adenine dinucleotide (FAD) cofactors (Samukaite-Bubniene et al., 2020; Tu et al., 2024) which catalyze the reduction of molecular oxygen through the oxidation of β-D-glucose to form hydrogen peroxide and gluconic acid.

$$\beta\text{-D-glucose} + O_2 + H_2O \rightarrow \text{gluconic acid} + H_2O_2$$

Among the materials explored to optimize glucose oxidase immobilization and activity, Nafion has emerged as a critical component due to its unique chemical and physical characteristics.

Nafion is a perfluorinated anionic polymer that exhibits excellent biocompatibility, thermal, mechanical and chemical stability, as well as high water-saturated proton conductivity. Consequently, it has been explored in a number of applications, including chlor-alkali electrolysers (Carvela et al., 2021; Thummar et al., 2022), sensors (Shen et al., 2023; Choo et al., 2022), super acid catalysts (Rong et al., 2022; Chen et al., 2023) and in particular, as a polymer and ionomer electrolyte membrane in hydrogen fuel cells (Song et al,, 2024; Yurova et al., 2021). It has been employed as an electrode modifier to immobilize cationic redox species or electrocatalysis systems, and as a matrix to immobilize GOx in glucose biosensors (Fortier et al., 1992). The low viscosity, inadequate chain entanglement and the presence of aggregates in the micellar structures in a typical solution render the formation of pure Nafion nanofibers by electrospinning a challenging process (Welch et al., 2012). It is therefore necessary to add a secondary polymer to the Nafion solution in order to prevent the formation of aggregates, increase chain entanglement and promote the successful electrospinning of Nafion nanofibers (Dong et al., 2010). Commonly, polyacrylic acid (PAA) (Chen et al., 2008; Hwang et al., 2019), polyethylene oxide (PEO) (Ballengee and Pintauro, 2011; Choi et al., 2010), polyacrylonitrile (PAN) (Han et al., 2015; Sharma et al., 2014), polyvinyl alcohol (PVA) (Zizhou et al., 2021) polyvinyl prolidone (PVP) (Sun et al., 2019) and Cellulose Acetate (CA) (Bostancı et al., 2019) are employed as carrier polymers for Nafion electrospinning. The utilization of Nafion nanofibers has recently been demonstrated in the development of DNA biosensors and a biosensor for the electrochemical detection of hydrogen peroxide (Devados et al., 2013). Despite the successful production of Nafion nanofibers and their pervasive deployment in biosensors, their applications remain constrained in biosensors designed for the detection of analytes such as glucose, urea, and cholesterol (Ding et al., 2010; Gideon et al., 2024; Singh et al., 2022).

This study introduces new amperometric glucose biosensors utilizing Nafion nanofibers as an innovative immobilization platform for both glucose oxidase (GOx) and GOx-AuNPs. To enhance the sensitivity of traditional biosensors based on Nafion films; this research integrated the enzyme with gold nanoparticles (AuNPs) prior to immobilization within the Nafion matrix. This strategy led to the development of sensors exhibiting a high surface area-to-volume ratio, increased enzyme loading capacity, and faster response times. This work focused on the systematic investigation of GOx-AuNPs (10 nm) immobilization onto Nafion nanofibers. The synthesis of Nafion nanofibers involved incorporating cellulose acetate (CA) into a Nafion solution, where CA acted as the carrier polymer. The nanofibers were fabricated through an optimized electrospinning process. Subsequently, biosensors were prepared by immobilizing a solution containing GOx and GOx-AuNPs onto the synthesized Nafion nanofibers. The performance of the fabricated biosensors was assessed under conditions conducive to hydrogen peroxide production. Amperometric measurements were employed to

determine key performance parameters, including the linear range, sensitivity, operational stability, and limit of detection (LOD). A comparative analysis was conducted between Pt/Nafion-CA NFs/GOx and Pt/Nafion-CA NFs/GOx-AuNPs biosensors to elucidate the influence of AuNPs on the catalytic activity of the GOx enzyme.

2. Experimental

2.1. Materials and instrumentation

15 wt % Cellulose Acetate powder (Mn ~30.000 CA), Nafion (~ 5 wt % in mixture of lower aliphatic alcohols and water solution), Glucose oxidase (b-D-Glucose: oxygen 1-oxidoreductase) from Aspergillus niger (type X-S, lyophilized powder, 135 200 U/g), glutaraldehyde (50 wt% in H_2O), D-glucose (99%), sucrose (99%), citric acid (99%), ascorbic acid (99%), sodium dodecyl sulfate (SDS) (ACS reagent, 99.0%), lactic acid (99%), fructose (99%), uric acid (99%), NaCl, KCl, disodium hydrogen phosphate, sodium dihydrogen phosphate, acetone (99% purity) and colloidal gold nanoparticles (10nm, stabilized in PBS) were purchased from Sigma-Aldrich. Blood serum samples were obtained from a local clinic. The ultrapure water (18.2 mX/cm was from a Direct-Q_ Water Purification System (Merck Millipore). All other chemicals were of analytical grade and used without any purification.

2.2. Electrospinning of Nafion-Cellulose Acetate (Nafion-CA) nanofibers

Cellulose Acetate (CA) with a weight ratio of 15% was dissolved in acetone under gentle stirring. Subsequently, the previously received Nafion solution was added to the CA/acetone solution, maintaining a Nafion/CA volume ratio of 1:4, and magnetically stirred overnight until a suitable homogeneous and transparent solution was obtained. The solution was electrospun into a nanofiber structure using the optimal electrospinning parameters, namely 20 kV, 1 mL/h, and 20 cm. For morphological characterization, the nanofibers were electrospun onto an aluminum foil. For biosensor construction, a platinum disk electrode was utilized. The morphology of the Nafion-CA nanofibers was characterized using scanning electron microscopy (SEM), with analysis conducted on a Zeiss LEO 1430 scanning electron microscope. All chronoamperometric measurements were conducted using the GAMRY potentiostat (600 ™). The experiment was conducted using a Gamry Instruments Inc. 600 potentiostat and the resulting data were analyzed using the Gamry Echem Analysis Software. The three-electrode cell configuration comprised a platinum disk electrode acting as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl (3 KCl saturated with AgCl as an internal solution, BASi) reference electrode.

2.3. Preparation of Pt/Nafion- CA NFs/GOx and Pt/Nafion- CA NFs/GOx-AuNPs enzyme electrodes

After electrospinning on the surface of the Pt disk electrode and subsequently drying, enzyme was immobilized by cross-linking. For Pt/Nafion- CA NFs/GOx biosensor glucose oxidase solution (5 µL in ultrapure water) equivalent to 47.72 U was dropped on the surface of electrode and allowed to dry at room temperature. For Pt/ Nafion- CA NFs/GOx-AuNPs biosensor enzyme electrode has been prepared by mixing GOx enzyme in 35 µL AuNPs(10nm) solution ,then 5 µL of GOx-AuNPS (10 nm) (equivalent to 47.72U GOx and 3.0 10^{10} gold particles) was dropped onto surface of Pt electrode and allowed to dry at room temperature. Both enzyme electrodes was cross-linked by dropping 10µ L of 2.5% glutaraldehyde solution and left to dry again at room conditions. Prepared biosensors were stored in buffer solution PBS at pH 7 and in the refrigerator at +4 °C to be used later in electrochemical analyzes.

2.4. Amperometric studies on the Pt/Nafion-CA NFs /GOx and Pt/Nafion-CA NFs /GOx-AuNPs biosensors

Some operational parameters such as the pH value of buffer solution and applied potential can affected the performance of biosensors. Therefore, before the amperometric measurement optimum pH and using potential for the biosensors must be determined. For this purpose, for the Pt/Nafion-CA NFs /GOx biosensors buffer solutions at different pH values (in the 5.5–7.5) range were used in order to determine the optimum pH by measuring the current response to a 0.02 mM glucose solution at 0.6 V. Figure 1(a) shows the effect of the pH value on the Pt/Nafion-CA NFs /GOx biosensors, where the maximum response was determined at a pH of 7 which is consistent with that of most GOx-based glucose biosensors. Figure 1(b) shows the effect of applied potential on the Pt/Nafion-CA NFs /GOx biosensor, where the maximum current response was achieved at 0.6 V; at pH 7. Hence, the potential of 0.6 V and pH 7 were found to be the optimum conditions for Pt/Nafion-CA NFs /GOx biosensors.

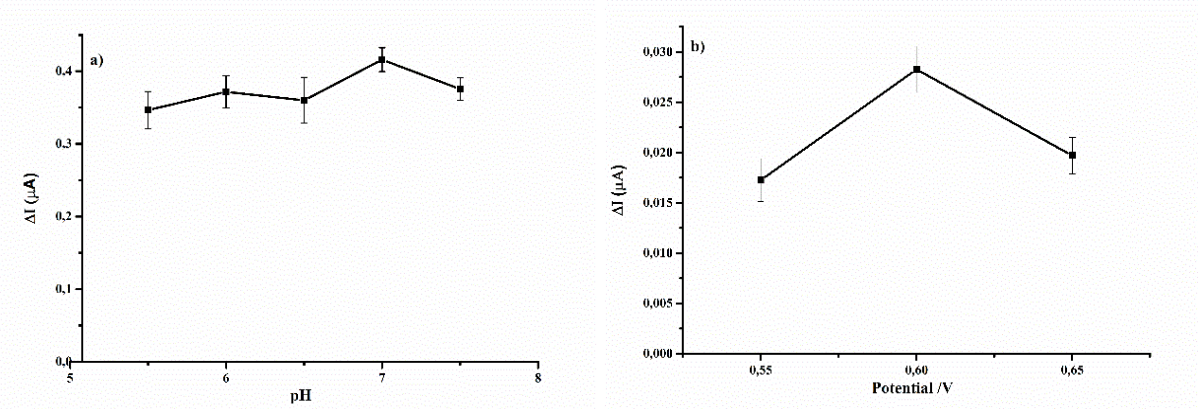

Figure 1. The effect of (a) pH and (b) potential on the response of the Pt/Nafion-CA NFs/GOx biosensor to 0.02mM of glucose in 0.1M PBS at 25°C

For the Pt/Nafion-CANFs /GOx-AuNPs biosensors a buffer solution at different pH values (in the 5.5–8) range were used in order to determine the optimum pH by measuring the current response to a 0.5 mM glucose solution at 0.6 V. Figure 2(a) shows the effect of the pH value on the Pt/Nafion-CA NFs /GOx-AuNPs biosensors, where the maximum response was determined at a pH of 7.5. Immobilized GOx can retain its activity under wide pH conditions, indicating that nanofibers and AuNPs provide a biocompatible microenvironment conducive to GOx survival (Zheng et al., 2011). The presence of gold nanoparticles ensures the stability of biosensors. This effect should be attributed to the fact that gold nanoparticles can strongly adsorb the enzyme and thus prevent enzyme leakage (Luo et al., 2004). The binding of enzymes on the nanoparticles surface involves electrostatic forces and therefore depends on both the pH of the suspension and the isoelectric point of the enzyme (Sotnikov et al., 2019). To determine the appropriate pH for optimal enzymes-gold conjugation, various approaches have been used such as spectrophotometric determination of absorption isotherms, isoelectric focusing and radio assay (Teichroeb et al., 2009). The effect of pH onto GOx-AuNPs was studied by Wang et al. (2011). They showed that the increases of pH value of the GOx-AuNPs related to the adsorption and co-adsorption in template monolayer to multilayer of the enzyme on the gold nanoparticles (Wang et al., 2016). The shift from monolayer to multilayer protein immobilization can be either a consequence of a change in the sorption properties of the nanoparticles, or a spontaneous aggregation of the proteins with an increase in the pH of the medium (Sotnikov et al., 2019). Figure 2(b) shows the effect of applied potential on the Pt/Nafion-CA NFs /GOx-AuNPs biosensor, where the maximum current response was

achieved at 0.6 Vat pH 7.5. Hence, the potential of 0.6 V and pH 7.5 were found to be the optimum conditions for Pt/Nafion-CA NFs /GOx-AuNPs biosensors.

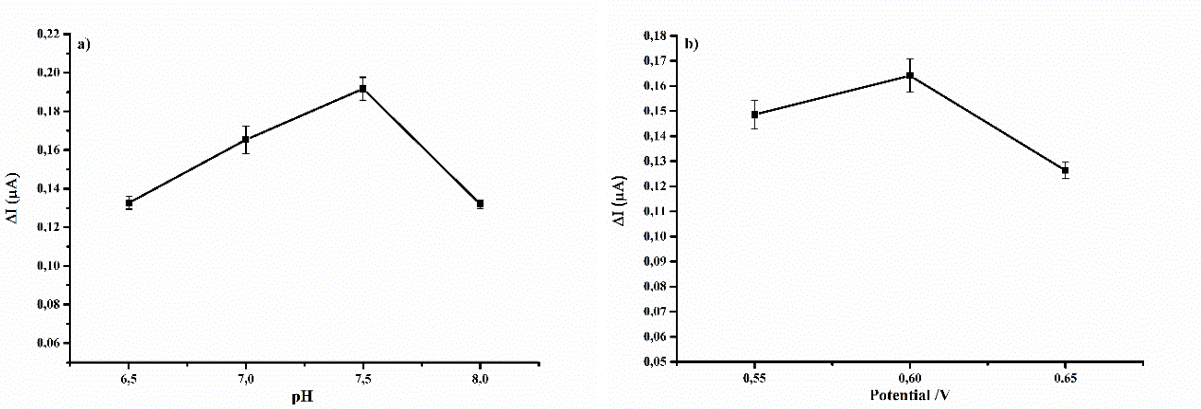

Figure 2. The effect of (a) pH and (b) potential on the response of the Pt/Nafion-CA NFs/GOx-AuNPs biosensor to 0.5mM of glucose, in 0.1M PBS at 25°C

2.5. Principles of measurements

For prepared biosensors all chronoamperometric measurements were carried out under controlled magnetic stirring (200 rpm) under conditions optimized for the detection of glucose (0,6 V vs Ag/AgCl). Increasing concentrations of glucose were added under steady state conditions in working medium (10 mL, 0.1 M PBS) until the slope between the substrate concentration and the current response deviated from the straight line. The LOD was calculated using the 3Sb/m criterion, (m: slope of the calibration curve and Sb: standard deviation of the responses at the minimum concentration (n = 10) (Shrivastava and Gupta, 2011)). Interference studies were carried out with an interfering compound: glucose ratio of 1:10. The applicability of the developed biosensors in real samples was tested by measuring glucose levels in two samples of human serum without pretreatment or dilution.

3. Results and Discussion

3.1. Morphology characterization of Nafion- CA nanofibers

Figure 3 represents the SEM images of Nafion-CA nanofibers. As can be seen, all the nanofibers have a well-defined, bead free, homogeneous morphology with optimum diameters was around 461,53l ±30,34nm. Obtained nanofibers were founded smaller than Nafion-PEO nanofibers 700nm (Okafor et al., 2014) 900nm (Ballengee and Pintauro, 2010). We notice that the diameters of the Nafion nanofibers depend on the nature of the carrier polymers as well as on the ratio of the electrospun solution (Bostancı et al., 2019) and the electrospinning parameters.

3.2. Chronoamperometric measurements

Chronoamperometric measurements on the Pt/Nafion-CA NFs /GOx

As it is known, the working principle of the first generation glucose biosensors is generally based on monitoring the oxidation of the H_2O_2 product formed as a result of the enzymatic reaction or the reduction of the O_2 consumed in this enzymatic reaction. A comparative study was conducted to understand the effect of gold nanoparticle use on the enzymatic action of GOx enzyme in amperometric biosensors based on Nafion film and nanofibers. An important part of the literature studies are studies in which enzymatic reactions are followed, and linear

range and sensitivity values are calculated in a significant part (Table 1). Nafion film has been used frequently in several fields for a long time, but in biosensor for glucose detection is very rare (Fortier et al., 1992; Tsai et al., 2005). Nafion film was used as a matrix to immobilize the enzyme glucose as well modified enzyme with nanoparticles for detection of glucose (Zhao et al., 2006; Thibault et al., 2008).

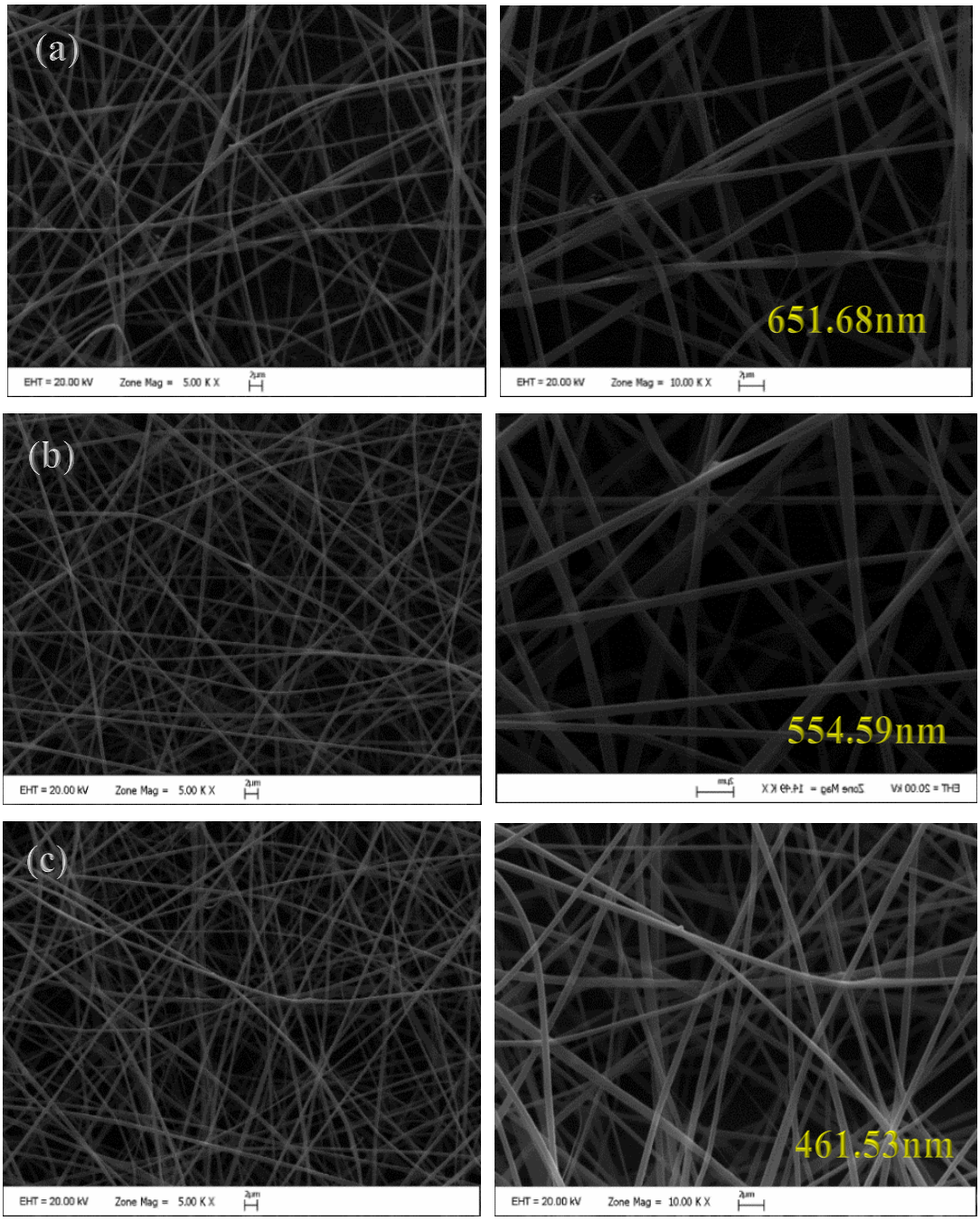

Figure 3. SEM images of Nafion-CA nanofibers with Potential, Distance, Flow rate: (a) 18 kV, 15 cm, 1 ml/h. (b) 18 kV, 20 cm, 1 ml/h. (c) 20 kV, 20 cm, 1 ml/h

In our study Nafion nanofibers were used to prepare amperometric glucose biosensors. Figure 4(a) shows the amperometric response of the Pt/Nafion-CA NFs /GOx biosensor on the successive addition of glucose (from 0.01 mM to 20.39 mM). The response current increased upon an increase in the glucose concentration and the biosensor became saturated at about

9.39 mM (Figure 4(b)). Two linear ranges of the calibration curve were identified: 0.01–0.2 mM and 0.3–9.93 mM, with sensitivities calculated as 68.67 µA mM^{-1} ·cm^2 and 18.38 µA mM^{-1} ·cm^2, respectively. The Pt/Nafion-CA NFs /GOx biosensor reached a steady state in less than 10 s.

Table 1. Comparison of glucose enzymatic and non-enzymatic electrodes

Biosensor	LOD	Sensitivity	Linear range	Reference
Nafion-GOx	–	–	5µM -10 mM	Wang et al., 2016
MWNT-Nafion-GOx	4 µM	330 nA mM^{-1}	2 mM	Fortier et al., 1992
Nafion/GOx-AuNPs/GC	0.34 µM	6.5 µA mM^{-1} cm^{-2}	6 mM	Tsai et al., 2005
Nafion/GOx-AuNPs/GC	370 µM	0.4 µA mM^{-1} cm^{-2}	20 mM	Zhao et al., 2006
PEDOT-NFs/GOx-3	2.9 µM	74.22 µA.mM^{-1}.cm^2	0.01-1.7 mM	Thibault et al., 2008
PPy-NFs/GOx-1	7.8 µM	68.95 µA.mM^{-1}.cm^2	0.01–3.5mM	Çetin and Camurlu, 2017
Pristine PAN	4.2 µM	39.5 ± 0.5 µA.mM^{-1}.cm^2	0.01 - 2 mM	Çetin and Camurlu, 2018
PAN/Fc/MWCNT-COOH	4.0 µM	27.167 µA.mM^{-1}.cm^2	8 m M	Apetrei and Camurlu, 2020
FTO-CNTs/PEI/GOx	–	63.38 µA.mM^{-1}.cm^2	70–700 µM	Lin et al., 2022
MN array w/Au/Fc-PAMAM/GOx	660 µM	0.1622 µA.mM^{-1}.cm^2	1–9 mM	Dervisevic et al., 2022
Electrospun fiber w/PEDOS: PSS	3.31 mM	0.386 µA mM^{-1} cm^{-2}	0–30 mM	Seufert et al., 2024
Pt/Nafıon-CA NFs/ GOx	1.36 µM	68.67 µA. mM^{-1}.cm^{-2} 18.38 µA.mM^{-1}.cm^2	0.01-0.2mM 0.3-9.39 mM	**This work**
PVA/BTCA/β-CD/ GOx/AuNPs	10 µM	47.2 µA mM^{-1}	0.1- 0.5 mM	Guven et al., 2021
MWCNT–AuNano/GCE	10.0	0.55 ± 0.03 µA.mM^{-1}.cm^2	0.1–25 mM	Branagan and Breslin, 2019
PB/GO*x*-AuNPs-CS	1.62 µM	40.41µA ·mM^{-1} ·cm^{-2} 8.90µA ·mM^{-1} ·cm^{-2}	0.025–2.00 mM 2.00–6.50 mM	Peng et al., 2023
AuNR/GCE	1.58 mM	13.7 µA mM^{-1}.cm^2	5–160 µM	Nazish et al., 2024
Electrospun electrode w/AuNFs/PB	1.01 mM	31.94 µA $(lg(mM))^{-1}$ cm^{-2}	1–30 mM	Tang et al., 2024
GOx/AuNPs/PMMA/PET	330 ± 50 µM	3.10 ± 0.06 µA.mM^{-1}.cm^2	8 mM	Aldea et al., 2021
Pt/Nafıon- CA Nfs /GOx-AuNPs	10.8µM	55.78µA.mM^{-1}.cm^2 28.49µA.mM^{-1}.cm^2	0.01-1.11 mM 1.51-12.89 mM	**This work**

This demonstrated the fast electron exchange and good electrocatalytic oxidative behavior of the nanofibers. By comparing the results obtained for the biosensors based on Nafion nanofibers and that based on Nafion film (Fortier et al., 1992; Tsai et al., 2005), we find that the generated Pt/Nafion-CA NFs/GOx biosensors have the good results in terms of sensitivity and LOD value. The lowest LOD data of the Pt/Nafion-CA Nfs/GOx biosensor, which was examined by monitoring H_2O_2, stood out as (1.36 µM) and high linear range data (0.01-0.2 mM and 0.3-9.39 mM) respectively. The low LOD values in question were found to be better than the other results of biosensor based on nanofibers (Çetin and Camurlu, 2017; Çetin and Camurlu, 2018; Apetrei and Camurlu, 2020; Guven et al., 2021) and good sensitivity values.

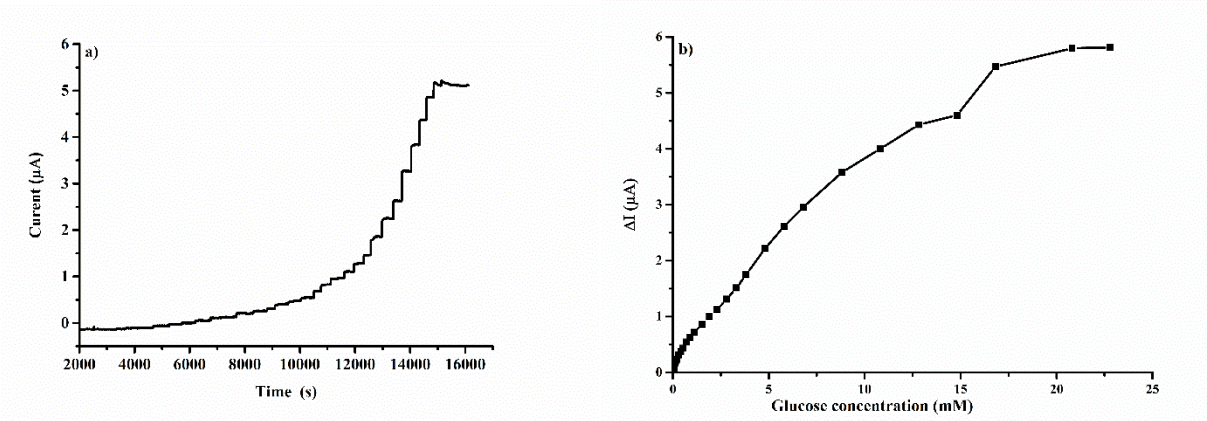

Figure 4. (a) Amperometric response of the Pt/Nafion-CA NFs /GOx biosensor to the addition of glucose and (b) The calibration curve.

Chronoamperometric measurements on the Pt/Nafion-CA NFs /GOx-AuNPs

Figure 5(a) illustrates the amperometric response of the Pt/Nafion-CA NFs/GOx-AuNPs biosensor upon successive additions of glucose, ranging from 0.01 mM to 56.81 mM. A consistent increase in the response current was observed with increasing glucose concentration, with sensor saturation occurring at approximately 12.81 mM (Figure 5(b)). The calibration curve exhibited two distinct linear ranges: 0.01-1.11 mM and 1.51-14.81 mM, with calculated sensitivities of 55.56 µA $mM^{-1} \cdot cm^2$ and 28.49 µA $mM^{-1} \cdot cm^2$, respectively. The Pt/Nafion-CA NFs/GOx-AuNPs biosensor demonstrated rapid response times, reaching a steady-state within 7 seconds. This rapid response time signifies efficient electron transfer and excellent electrocatalytic oxidative behavior of the GOx enzyme, facilitated by the presence of AuNPs and the unique structure of the Nafion nanofibers. The limit of detection (LOD) was determined to be 10.8 µM (signal-to-noise ratio of 3, with five replicates), establishing it as the lowest LOD reported among glucose biosensors employing GOx-AuNPs immobilized on nanofibers. For instance, Kim and Kim (2020) reported a PVA/BTCA/β-CD/GOx/AuNP nanofiber hydrogel biosensor with a sensitivity of 47.2 µA mM^{-1} cm^{-2} a narrow linear range of 0.1 mM - 0.5 mM, and an LOD of 10 µM. Similarly, Aldea et al. (2021) described a GOx/Au/PMMA/PET biosensor characterized by a significantly lower sensitivity of 3.10 ± 0.06 µA mM^{-1} cm^{-2} and a linear range extending up to 8 mM. In comparison to these reported results, the newly developed Pt/Nafion-CA NFs/GOx-AuNPs biosensor based on Nafion nanofibers emerges as a superior platform for the immobilization of GOx-AuNPs.

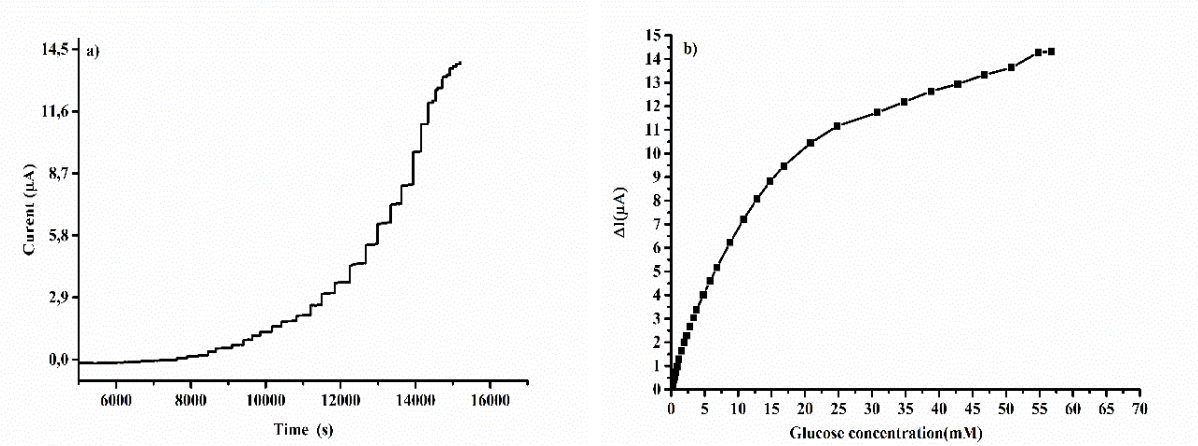

Figure 5. (a) Amperometric response of the Pt/Nafion-CA NFs /GOx-AuNPs biosensor to the addition of glucose and (b) The calibration curve.

Comparison between Pt/ Nafion- CA Nfs /GOx and Pt/Nafion- CA Nfs /GOx-AuNPs biosensors

The generated glucose biosensors Pt/Nafion-CA Nfs/GOx and Pt/Nafion-CA Nf/GOx-AuNPs were compared to evaluate the sensor sensitivity of the glucose biosensor in the presence and absence of gold nanoparticles with the same amount of enzyme. The sensor containing gold nanoparticles demonstrated a broader glucose concentration range, as shown in Figure 6(a, b). This effect can be attributed to the expanded linear range and amplified response current, resulting from the high conductivity of gold nanoparticles. Additionally, the nanoparticles enhanced the specific modification of the enzyme on the platinum electrode surface, thereby improving the glucose oxidase (GOx) target detection capability. As showed the speed of response of the biosensor with the GOx enzyme modified with gold nanoparticles is the fastest (< 7 s) biosensor due to the specificity and their ability to intensify direct electrons transfer between the active site of the enzyme and the surface of the electrode. Concerning the sensitivity of the biosensors, it was noticed that the presence of AuNPs makes it possible to increase the sensitivity of the second liner range with a slight decrease in the first sensitivity of the primary liner range.

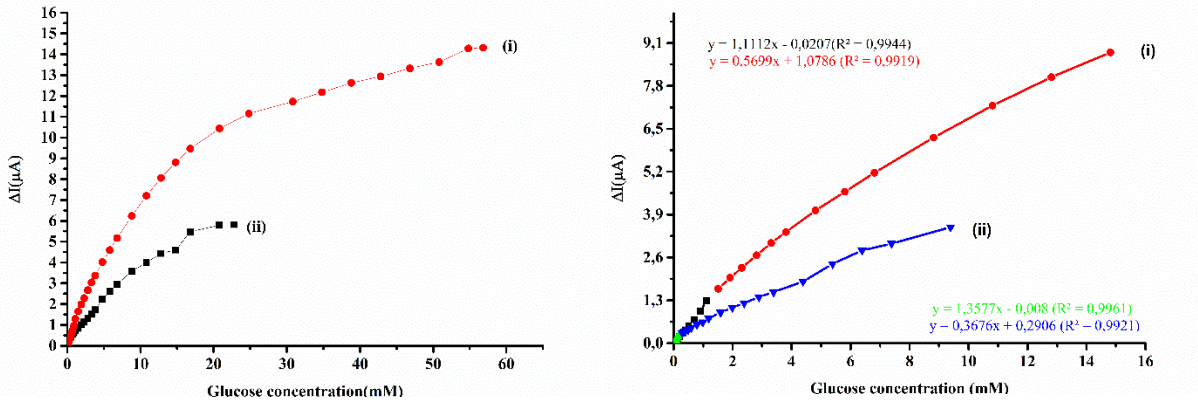

Figure 6. Evolution of the observed current values of (i) Pt/Nafion-CA Nf/GOx-AuNPs (0.01-56.81 mM) and (ii) Pt/Nafion-CA Nf/GOx (0.01-20.39 mM) with (a) the glucose concentration and (b) their linear ranges

3.3. Interference study

To assess the selectivity of the biosensors, an interference study was conducted on both Pt/Nafion-CA NFs/GOx and Pt/Nafion-CA NFs/GOx-AuNPs biosensors. Potential interferents, including ascorbic acid (AA), sucrose (Su), fructose (Fru), citric acid (CA), lactic acid (LA), uric acid (UA), urea, NaCl, KCl, and paracetamol (Parac), were evaluated using 0.5 mM solutions. Figures 7 (a,b) illustrate the amperometric responses of the Pt/Nafion-CA NFs/GOx biosensor to glucose, Su, Fru, CA, and AA. The results demonstrated minimal or negligible interference from these common interferents compared to the well-defined glucose signal, aligning with previous studies (Thota, Ganesh, 2014; Jia et al., 2009). The biosensor exhibited insignificant interference from Su, Fru, CA, UA, urea, NaCl, KCl, and paracetamol. However, the Nafion-CA NF/GOx biosensor demonstrated a response to 2.5% AA (Figure 7(a)) and 10.78% LA (Figure 7(b)). In contrast, the Pt/Nafion-CA NFs/GOx-AuNPs biosensor exhibited negligible interference from all tested substances (Figure 8). However, a slight response to 9.83% LA was observed in the presence of glucose.

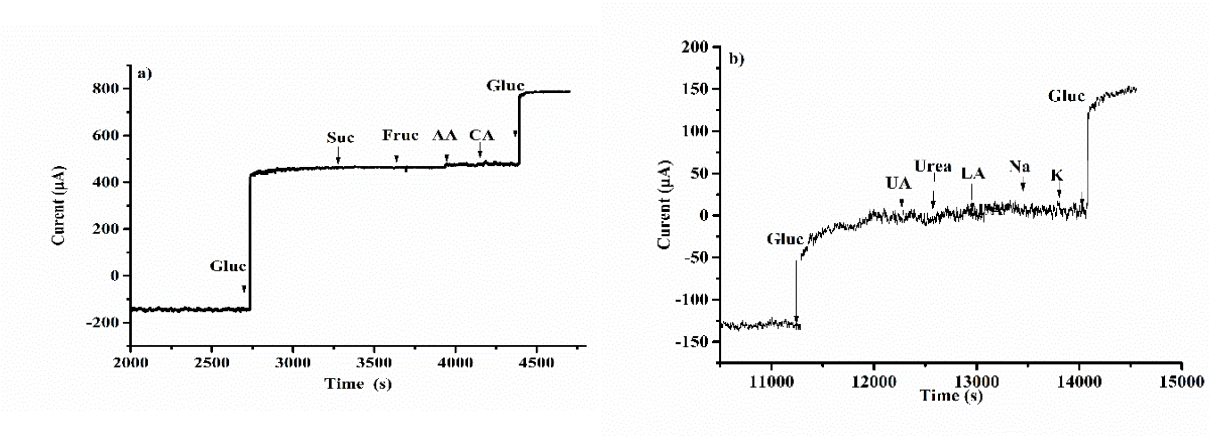

Figure 7. (a,b) Interference effect of Pt/Nafion- CA Nfs /GOx biosensor

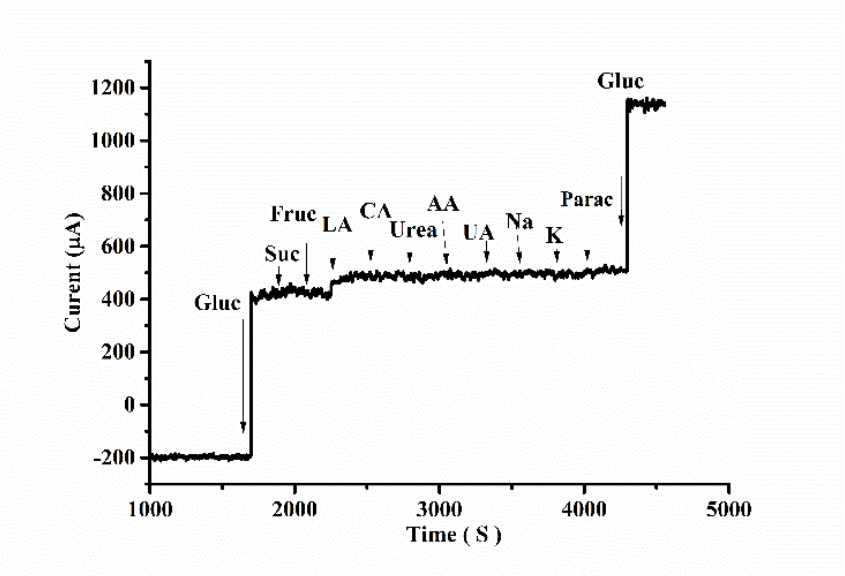

Figure 8. Interference effect of Pt/Nafion- CA Nfs /GOx AuNPs biosensor

3.4. Stability of biosensors

To evaluate operational stability, both Pt/Nafion-CA NFs/GOx and Pt/Nafion-CA NFs/GOx-AuNPs biosensors were subjected to repeated measurements with 0.5 mM glucose. Figure 9(a) demonstrates that the Pt/Nafion-CA NFs/GOx biosensor retained approximately 86.5% of its initial response after 14 consecutive measurements, exhibiting a standard deviation of ±0.024 and a relative standard deviation (RSD) of 8.38%. The reusability of Pt/Nafion-CA NFs/GOx-AuNPs biosensors was assessed under ambient conditions (25°C) by performing 14 measurements within a single day. The results revealed a standard deviation of ±0.073 and an RSD of 11.89%. As shown in Figure 9(b), the Pt/Nafion-CA NFs/GOx-AuNPs biosensor maintained 80.6% of its initial activity after the 14th measurement. Both Pt/Nafion-CA NFs/GOx and Pt/Nafion-CA NFs/GOx-AuNPs biosensors exhibited commendable operational stability, likely attributed to the biocompatibility of the porous Nafion-CA nanofibers. This porous structure may effectively preserve the structural integrity of the GOx molecules and GOx-AuNPs, thereby minimizing enzyme deactivation.

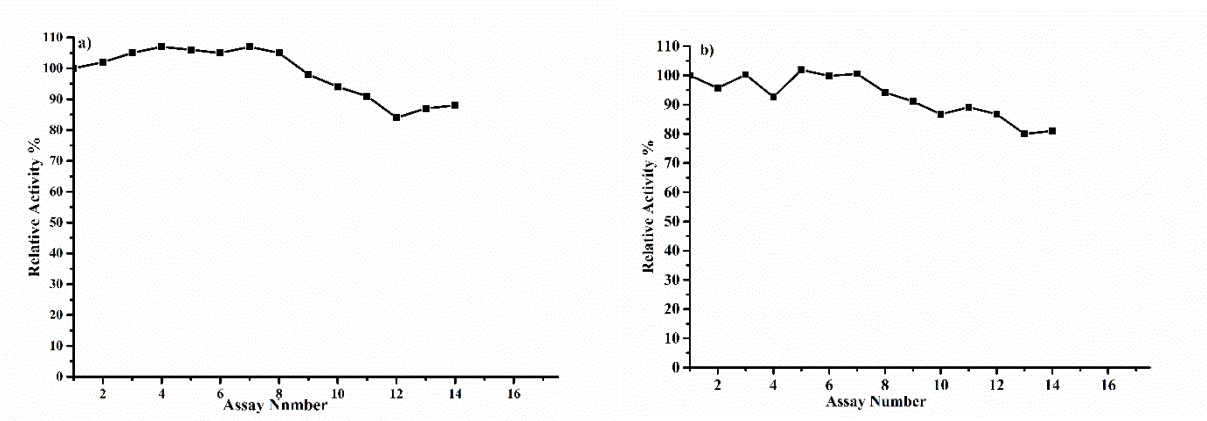

Figure 9. (a) Relative activity of Pt/Nafion-CA NFs/GOx and (b) Pt/Nafion- CA Nfs/GOx AuNPs biosensors.

3.5. Determination of glucose in real samples

To assess the applicability of the Pt/Nafion-CA NFs/GOx and Pt/Nafion-CA NFs/GOx-AuNPs biosensors in real-world scenarios, the sensors were employed to quantify glucose levels in spiked human blood samples. Recovery assays were performed to evaluate the accuracy of the biosensor measurements. Spiked blood serum samples at two different concentrations were directly introduced into a 0.1 M PBS buffer without any dilution steps. Following each sample addition, the corresponding current changes were measured and correlated to glucose concentration using the pre-established calibration curves. Table 2 summarizes the results, which represent the average of three consecutive measurements for each sample, alongside the corresponding data obtained from high-performance liquid chromatography (HPLC) analysis.

Table 2. Recovery rates of the developed biosensors for glucose detection in human blood serum samples

Biosensor	Human blood serum concentration (mg/dL)	Recovery (%)
Pt/Nafion- CA Nfs /GOx	89 mg/dL	104
Pt/Nafion- CA Nfs /GOx-AuNPs	126 mg/dL	103.7

4. Conclusion

This study describes the generation of a new amperometric biosensor based on bead-free, homogeneous Nafion nanofibers with a diameter of 461.31 ± 36 nm. The biosensors were constructed by loading GOx and GOx-AuNPs onto the Nafion-CA nanofibers and then employing glutaraldehyde to cross-link the enzyme and nanoparticles within the nanofiber matrix. The biosensors were employed for the measurement of hydrogen peroxide production at an operating potential of 0.6 V. Calibration curves were constructed for each biosensor, plotting the current response (ΔI) versus glucose concentration (mM). From these, the sensitivity, linear range and LOD values were determined. The stability of all sensors was also investigated. Nafion's nanofiber-based biosensors exhibit good sensitivity, low LOD values and reproducibility, which are superior to those of Nafion's film-based glucose biosensors. This is attributable to the intrinsic characteristics of Nafion's nanofibers, which facilitate the provision of a biocompatible milieu for the enzyme, thereby ensuring the preservation of its intrinsic activity and structural integrity. Conversely, the incorporation of gold with the enzyme immobilized on Nafion nanofibers has been observed to enhance the measurement range and linear range of glucose, as evidenced by an increase in the intensity of the current response. This is attributed to the favorable conductivity of gold and its capacity to enhance analytical detection without compromising the morphology and activity of the enzyme. Nafion nanofibers have demonstrated efficacy as a novel matrix for immobilizing GOx and GOx-AuNPs, yielding favorable outcomes in amperometric glucose detection biosensors relative to nanofiber-based biosensors. With regard to this topic, the electrochemical sensors developed based on Nafion nanofibers are particularly well-suited for monitoring other enzymatic reactions, exhibiting excellent sensitivity and stability.

Authorship Contribution Statement

Each author made significant contributions to the study. SG conducted the experimental work, was responsible for the data collection and analysis, drafted the manuscript, and contributed to the methodology. EMŞ led the conceptualization and methodology. PÇ supervised the manuscript preparation and review. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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