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Amperometric glucose biosensor with the IrNPs/Ludox — modified enzyme matrix

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Aim. To develop an amperometric biosensor based on glucose oxidase (1.1.3.4) from Aspergillus niger immobilized in the IrNPs/Ludox/GOx matrix for glucose detection. **Methods.** To achieve a highly selective and sensitive glucose detection, the enzymatic membrane was functionalized with Ir nanoparticles (IrNPs) and silica composite Ludox. The enzymatic selective layer was formed on the surface of a platinum disk electrode using immobilization in glutaraldehyde vapor. **Results.** The voltamperometric characteristics of the transducers with modified IrNPs/Ludox/GOx matrix were studied. Enzyme immobilization on the surface of amperometric transducers was optimized to perform sample analysis. Modified transducers improved biosensor sensitivity. The analytical characteristics of amperometric transducer were determined: detection limit is 0.1 μM (s/n = 3), linear working range is 0.05–3.2 mM, sensitivity is 106 mA × M^{-1} × cm^{-2}. **Conclusions.** Application of the matrix modified with Ir nanoparticles and silica composite Ludox was investigated for the amperometric glucose biosensor as the most studied model of biosensors. A significant increase in the biosensor sensitivity was obtained using the new approach of glucose oxidase immobilization; therefore application of the matrix modified with mesoporous silica composite and nanometals opens new possibilities to obtain a bioselective membrane of high sensitivity and stability at the development of new electrochemical biosensors.

Keywords: amperometric biosensor, Ir nanoparticles, silica, glucose oxidase

Introduction

Glucose detection is of great importance due to its wide application in many areas (food processing, clinical diagnostics, environmental monitoring, fuel cells). Among a large variety of reported approaches, the electrochemical methods for glucose determination are the most popular, because of their portability, high sensitivity, selectivity, and low cost [1].

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Electrochemical glucose sensors are usually based on enzyme glucose oxidase (GOx) [2]. The GOx-based sensors exhibit high sensitivity and selectivity for glucose being rather a stable enzyme . However, most enzymes are very unstable and require complicated immobilization procedures to exhibit good performance and stability during storage. Moreover, the sensing abilities of biosensors are affected by pH, temperature and interfering agents such as ascorbic acid, heavy metals, *etc.* [3]. As a result, increasing attempts have been undertaken to improve the performance of glucose enzymatic sensors using new immobilization methods for GOx and new materials, such as nanoparticles of noble metals, alloys, oxides (NiO, CuO, Cu_2O, Co_3O_4, MnO_2, ZnO), and the composites with carbon nanotube or graphene.[4−7].

Mesoporous silicon dioxide (MPSs) is promising as an immobilization matrix due to its mechanical, thermal, chemical stability and large surface area.

A considerable drawback of MPSs is its low conductivity, but the use of platinum group metal nanoparticles can overcome [9, 10]. Additionally, the biocompatible nanoparticles of palladium group metals can help to maintain the activity and stability of the immobilized enzyme [8].

Thus, on the basis of results obtained with the GOx biosensor we propose the usage of the IrNPs/Ludox/enzyme matrix for the development of highly efficient amperometric biosensors.

Materials and Methods

Materials

Glucose oxidase (1.1.3.4) from *Aspergillus niger* with activity of 108 U/mg, iridium atomic adsorption standard solution in 10 % HCl (IrNPs) and Ludox HS-40 colloidal silica were from "Sigma" (Germany); 99 % ethanol was from "Fluka" (Germany); bovine serum albumin (BSA) and 25 % aqueous solution of glutaraldehyde (GA) were from "Sigma-Aldrich Chimie S.a.r.l." (France); D-glucose, $Na_2HPO_4 \cdot 7H_2O$ and $KH_2PO_4 \cdot H_2O$ were from "helicon" (Russia); 3 % solution of hydrogen peroxide was from "Fargomed" Ltd. (Teteriv, Kiev region, Ukraine); $Na_2SO_4 \cdot 10H_2O$ was from Mikhailovsky chemical reagent factory (Russia). All chemicals were of analytical grade.

The scheme of measuring setup

Amperometric measurements were carried out in 3 ml electrochemical cell at a constant potential using the potentiostat/galvanostat PalmSens and multichannel multiplexer of Palm Instruments BV (Netherlands) production controlled by the PalmSens PC programme. All electrochemical experiments were performed using a conventional three-electrode system with the 0.5mm platinum disk working electrode, platinum auxiliary and Ag/AgCl reference electrodes [11]. The electrodes were previously tested with regard to their reproducibility and reliability. The cyclic voltamperometry in the potential range of 0–1000 mV (speed of potentil involute 50 mV/s) was used. The experiment was carried out in 0.1 M phosphate buffer, pH 7.2.

Procedur of functionalization of the amperometric transducer surface by IrNPs/Ludox/GOx matrix immobilised in glutaraldehyde vapour

To form the bioselective membrane, we mixed 0.025 % Ludox solution with IrNPs in the

ratio of 1:25 in an ultrasonic bath for 30 min. The obtained mixture was then stirred with 10 % GOx solution in the ratio of 1:1. A drop of the obtained solution was deposited onto the surface of working electrodes, which were next placed for 10 min into a crystallizer with the atmosphere of saturated glutaraldehyde vapor at room temperature and then was air dried for 10 min.

Procedure of measuring substrates in model solutions

The measurements were performed in 3 ml of 100 mM phosphate buffer, pH 7.2, at room temperature in an open vessel with intensive stirring. Before operation, the transducers were kept for a while in the buffer solution until the stable signal (baseline) was obtained. The glucose concentration was changed by adding certain aliquots of stock solution. After each measurement, the biosensor was placed into the buffer solution for 3 min to wash from the substrate residues.

Statistics

Statistical package Microsoft Excel 10 was used for statistical analysis of the results, the average values and standard deviations were calculated; the results were considered as reliable at $p < 0.05$.

Results and Discussion

Using nanoparticles at immobilization provides freer enzyme orientation. The combination of nanoparticles with polymers offers an additional pathway, thus facilitating the electron transfer, which increases the biosensor sensitivity to an analyte. This can be explained by the interaction of electrons in dπ orbitals of metal centers and in π or π* orbitals of the conjugated polymer [12].

The proposed method of immobilization produced a three-dimensional matrix in which the enzyme is trapped. Thus, the biomolecule is maintained on the electrode surface and the electrical communication between the recognition element and the electrode surface is provided.

Electrochemical characteristics of amperometric transducer

Platinum electrodes with IrNPs as a modification of the electrode surface were tested regarding reproducibility and reliability of the results. Cyclic voltamperometry was used. Experiments were carried out in 0.1 M phosphate buffer, pH 7.2, in the potential range of 0–1000 mV (see Fig. 1) (scan rate was 50 mV/s). The data obtained were compared with the results obtained for the sensors without modification.

Next step was to confirm our suggestion about an increase of the biosensor response as

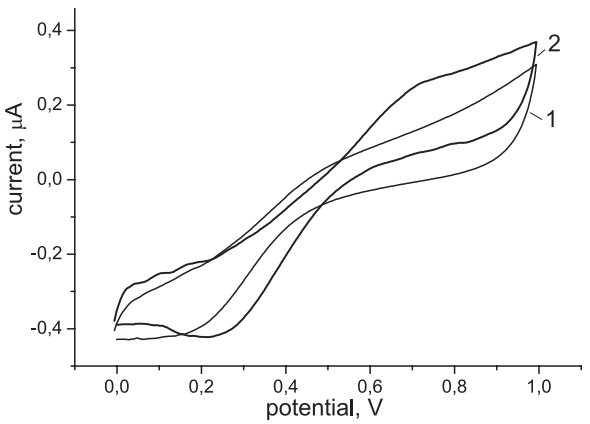

Fig. 1. Cyclic voltamperometry for platinum electrode, 1 — response before modification with IrNPs/Ludox, 2 — response after modification with IrNPs/Ludox. Measurement was carried out in 100mM phosphate buffer, pH 7.2

a result of the electrode modification with IrNPs/Ludox/GOx matrix. The results shown in Fig 2 demonstrate the improvement of analytical characteristics of the created biosensor.

The optimized biosensor had the following characteristics: linear working range of glucose determination is 0.05÷3.2 mM, sensitivity 106 mA • M^{-1} • cm^{-2}, detection limit 0.1 μM (s/n = 3).

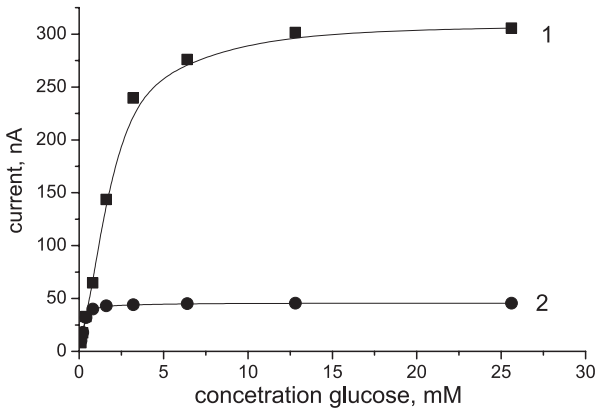

Fig. 2. Dependence of response of amperometric glucose biosensor on : 1 — modified with IrNPs/Ludox/GOx matrix matrix and 2 — without modification. Measurement was carried out in 100mM phosphate buffer, pH 7.2 at potential of +0.7 V versus reference electrode.

Investigation of dependence of biosensor response on concentration of background electrolyte and buffer solution

The basic working characteristics of biosensors depend on environment, in which the experiments are conducted. As blood, sweat and beverages may contain salt in different concentrations it was necessary to examine an effect of buffer capacity and ionic strength on the biosensor performance. The dependence of biosensor response on ionic strength and buffer concentration is shown in Fig. 3.

As seen, the solution ionic strength and buffer capacity slightly affect a response value of the developed amperometric biosensor, thus it can be used to analyse real samples of biological fluids.

pH effect on biosensor operation

It is known that the speed of enzymatic reactions in homogenous solutions strongly depends on pH value. It is due to the fact that all

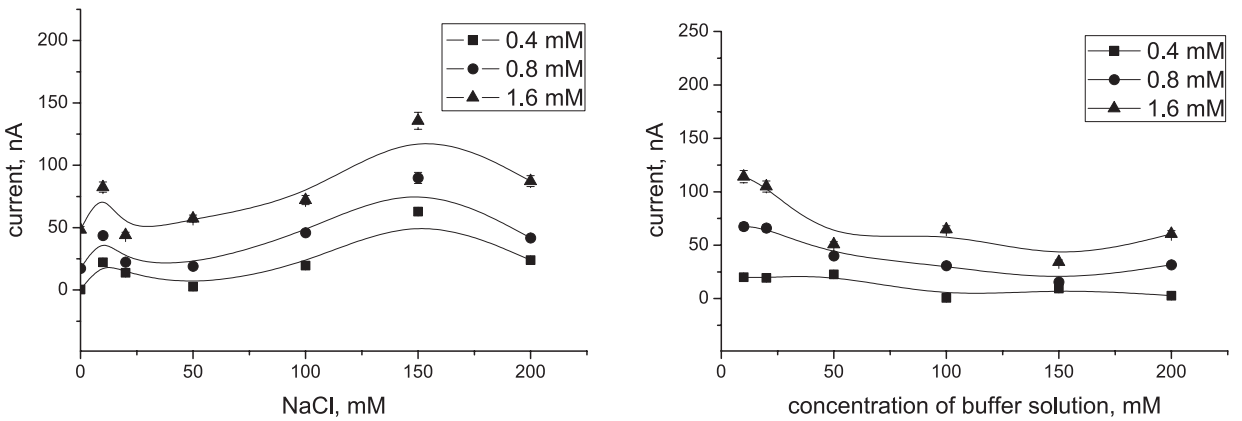

Fig. 3. Dependence of response of amperometric glucose biosensor modified with IrNPs/Ludox/GOx matrix on concentration *a* — of background electrolyte in 100 mM phosphate buffer, pH 7.2; *b* — of buffer solution, pH 7.2, at potential of +0.7 V versus reference electrode.

the protein functional groups capable of protonation/deprotonation (depending on the solution pH) take part in the catalysis whereas only one of these forms is active. Noteworthy, according to the producer, pH optimum for native GOx is 6.5.

We showed (Fig. 4) that optimum working pH of amperometric glucose biosensor modified with IrNPs/Ludox matrix is 6.7, which is close to the native enzyme. Therefore, pH 6.7 was taken as an optimal value for the biosensor operation.

Biosensor stability

The research of biosensor stability showed that after operation during 20 days the sensor retained 65 % of its activity towards GOx, which ensures reliable measurements. (see Fig. 5).

The calibration curve of the amperometric glucose biosensor modified with IrNPs/Ludox/GOx matrix developed for glucose determination (Fig. 6) was obtained under the optimal working conditions.

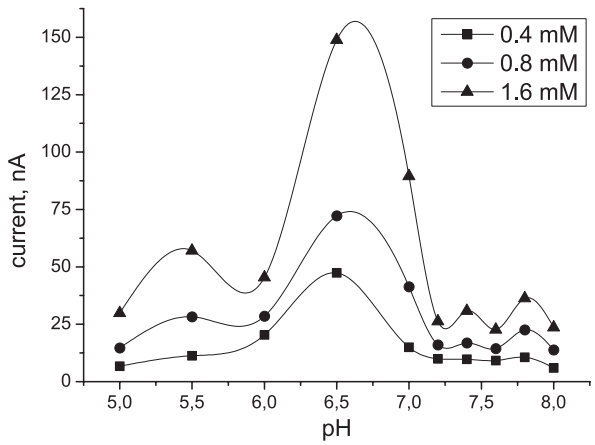

Fig. 4. Dependence of responses of amperometric glucose biosensor modified with IrNPs/Ludox/GOx matrix on pH. Measurements were carried out in 100 mM phosphate buffer, at potential of +0.7 V versus reference electrode.

As seen, the optimized biosensor has the following characteristics: linear working range of glucose determination is 0.05÷3.2 mM, sensitivity 106 mA • M^{-1} • cm^{-2}, detection limit 0.1 μM (s/n = 3). The response time is 5s.

Conclusion

The performed research resulted in the improvement of the glucose biosensor, selected

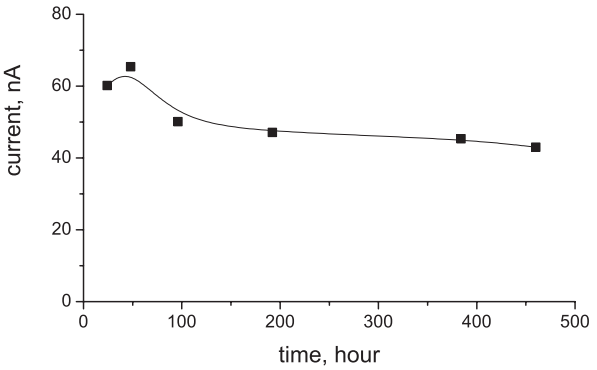

Fig. 5. Stability of amperometric glucose biosensor modified with IrNPs/Ludox/GOx matrix. Measurement at potential +0.7 V versus intrinsic reference electrode. Storing at +4°C. Substrate concentration 0.8 mM

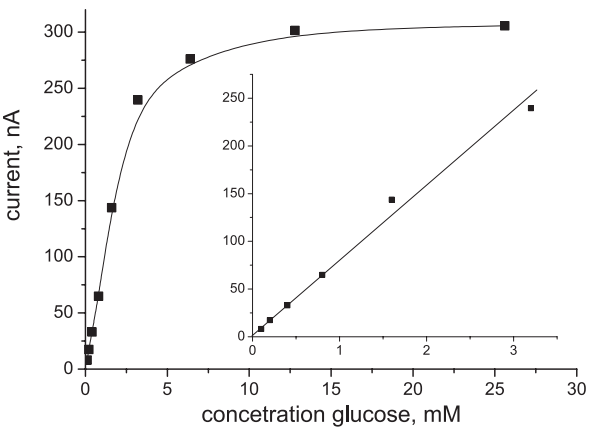

Fig. 6. Calibration curve of amperometric glucose biosensor modified with IrNPs/Ludox/GOx matrix. Measurements were carried out in 100 mM phosphate buffer, pH 6.7, at potential of +0.7 V versus intrinsic reference electrode.

as a model for our research, sensitivity and selectivity due to the functionalization of amperometric transducer with mesoporous silica composite and IrNPs. The analytical characteristics of the developed biosensor based on platinum amperometric electrodes were studied; a slight effect of the environmental conditions on its activity was found. A high reproducibility of the results and a good storage stability were shown. The method of the enzyme immobilization on the surface of amperometric transducers was optimized to meet the conditions of functioning in real samples. An increase of the biosensor sensitivity by 5 times compared to the electrode without modification with IrNPs/Ludox/GOx matrix was shown.

The biosensor demonstrated the fast response (5s), high sensitivity and selectivity, linear working range of glucose determination 0.05÷3.2 mM, sensitivity 106 mA • M^{-1} • cm^{-2}, detection limit 0.1 μM (s/n = 3).

Using the matrix of mesoporous silica composite and nanometals opens new possibilities for the enzyme immobilization and the development of new electrochemical biosensors.

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Амперометричний глюкозний біосенсор з IrNPs/Ludox модифікованою ферментною матрицею

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Мета. Розробити амперометричний біосенсор на основі іммобілізованої глюкозооксидази (1.1.3.4) з *Aspergillus niger*, іммобілізованій в матриці IrNPs/Ludox/GOx, для виявлення глюкози в реальних рідинах. **Методи.** Для отримання високоселективного та чутливого визначення концентрації глюкози ферментна мембрана була функціоналізована наночастинками Ir (IrNP) та кремнієвим композитом Ludox. Ферментний селективний шар був утворений на поверхні платинового дискового електроду шляхом іммобілізації в парах глутарового альдегіду. **Результати.** Вивчено вольтамперометричні характеристики перетворювачів, модифікованих матрицею IrNPs/Ludox/GOx, досліджено їх роботу. Оптимізовано метод іммобілізації ферменту на поверхню амперометричних перетворювачів для можливості проведення аналізів в реальних зразках. При порівнянні роботи немодифікованих та модифікованих перетворювачів, показано поліпшення чутливості біосенсора. Досліджено аналітичні характеристики амперометричних перетворювачів: межа виявлення — 0,1 мкМ (s/n = 3), лінійний робочий діапазон — 0,05–3,2 мМ, чутливість — 106 мА × $М^{-1}$ × $см^{-2}$. **Висновки.** Розроблений біосенсор продемонстрував високу чутливість та може бути використаний у подальших експериментах з реальними зразками. Застосування матриці, модифікованої кремнієвим композитом та нанометалами, відкриває нові можливості для іммобілізації ферментів при розробці нових електрохімічних біосенсорів.

Ключові слова: амперометричний біосенсор, Ir наночастинки, кремній, глюкозооксидаза

Амперометрический глюкозный биосенсор с IrNPs/Ludox модифицированной ферментной матрицей

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Цель. Разработать биосенсор на основе глюкозооксидазы (1.1.3.4) из *Aspergillus niger*, иммобилизованной в матрице IrNPs/Ludox/GOx, для определения глюкозы в реальных жидкостях. **Методы.** Для получения высокоселективного и чувствительного определения концентрации глюкозы ферментная мембрана была функционализирована наночастицами Ir (IrNP) и кремниевым композитом Ludox. Ферментный селективный слой был образован на поверхности платинового дискового электрода путем иммобилизации в парах глутарового альдегида. **Результаты.** Изучены вольамперометрические характеристики преобразователей, модифицированных матрицей IrNPs/Ludox/GOx, исследована их работа. Оптимизирован метод иммобилизации фермента на поверхность амперометрических преобразователей для возможности проведения анализов в реальных образцах. При сравнении работы немодифицированных и модифицированных преобразователей, показано улучшение чувствительности биосенсора. Иссследованы аналитические характеристики амперометрических преобразователей: предел обнаружения — 0,1 мкм (s/n = 3), линейный рабочий диапазон — 0,05-3,2 мм, чувствительность — 106 мА × $М^{-1}$ × $см^{-2}$. **Выводы.** Разработанный биосенсор показал высокую чувствительность и может быть использован в дальнейших экспериментах с реальными образцами. Применение матрицы, модифицированной кремниевым композитом и нанометаллами, открывает новые возможности для иммобилизации ферментов при разработке новых электрохимических биосенсоров.

Ключевые слова: амперометнрический биосенсор, Ir наночастицы, кремний, глюкозооксидаза

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