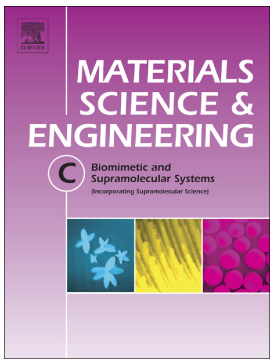

Application of cold plasma corona discharge in preparation of laccase-based biosensors for dopamine determination

Cecylia Wardak, Beata Paczosa-Bator, Szymon Malinowski

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Application of cold plasma Corona Discharge in preparation of laccase-based biosensors for dopamine determination

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Declarations of interest: none

Highlights

The Corona Discharge was used for bio-sensing layer deposition.

A voltammetric laccase-based biosensor was obtained.

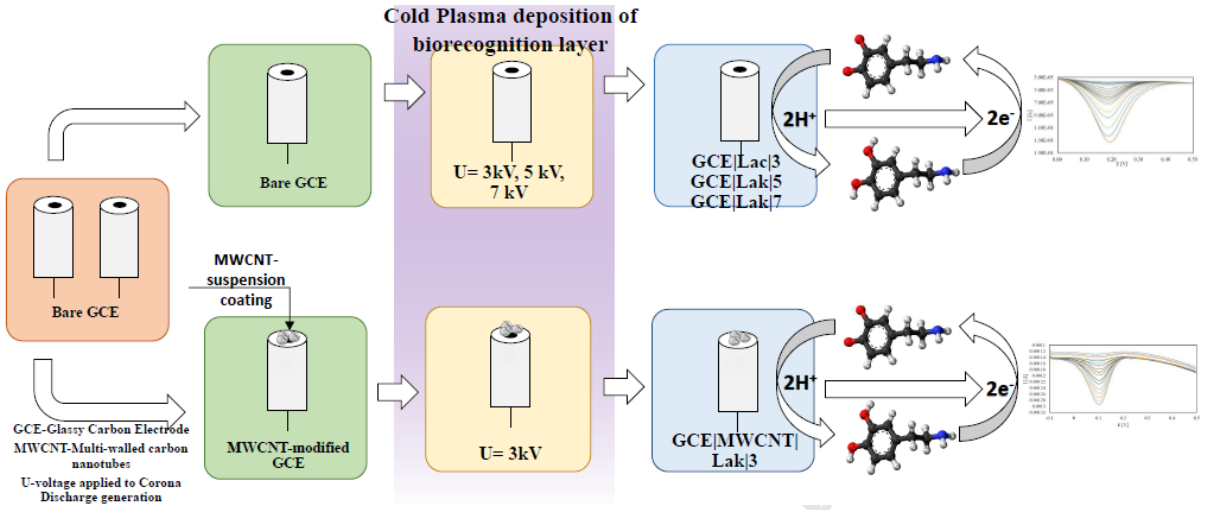
Bare and MWCNT-modified glassy carbon electrodes were used in biosensor construction.

The laccase-based biosensors were used for dopamine determination.

Abstract

Laccase-based biosensors were successfully prepared using innovative, cheap, one-step Soft Plasma Polymerization technique by deposition of a bio-recognition layer on glassy carbon electrode and MWCNT (Multi-walled Carbon Nanotubes)-modified glassy carbon electrode. The Soft Plasma Polymerization technique is based on corona discharge of cold atmospheric plasma with close to room temperature. The presented work includes study of biosensor working conditions, optimization of the voltage value applied for corona discharge generation as well as applicability and interference studies for dopamine determination. The biosensor constructed under optimal conditions (corona discharge generated at a voltage of 3 kV and in 30 sec time deposition, helium flow rate 10 L/min, laccase solution flow rate 200 µL/min) has two linear ranges from 0.1 µmol/dm^3 to 10 µmol/dm^3 and from 10 µmol/dm^3 to 50 µmol/dm^3 with dopamine detection sensitivities of 3.63 µA*dm^3/µmol and 1.33 µA*dm^3/µmol. Application of the MWCNT interlayer allows the dopamine detection sensitivity to be significantly increased to 22.35 µA* dm^3/µmol for a linear range from 0.1 µmol/dm^3 to 6 µmol/dm^3. Additionally, the studied biosensors have stable and anti-interference ability. Both biosensors were successfully applied for dopamine determination in pharmaceutical preparation.

Graphical abstract



Keywords

Dopamine; laccase; cold plasma; plasma polymerization; multi-walled carbon nanotubes.

1. Introduction

Dopamine (DA), 3,4-dihydroxyphenethylamine, is a catecholamine neurotransmitter [1,2] existing in the human body [3] responsible for the transmission of pulses between cells [4]. DA is a primary amine belonging to the biogenic amine class [5] and plays an important role in the functioning of the central nervous, renal, hormonal [1], cardiovascular and endocrine systems [6]. A low level of DA in the human body can contribute to the development of Parkinson's, schizophrenia and Huntington's diseases [4,7].

Sensitive and selective determination of DA is a very important issue in modern science and technology both the point of view of human physiology and the pharmaceutical industry. Moreover, the quantification of the DA level in biological fluids is helpful in the diagnosis of Alzheimer's and Parkinson's diseases [5] because it acts as a biomarker [8]. Many different methods, including capillary electrophoresis [9], chromatography [10], spectrophotometry [11,12] and chemiluminescence [7,13,14] can be employed for the quantitative determination of DA. Despite their high sensitivity and selectivity these methods are high-cost, time-consuming and require long and complex sample pre-treatment. Therefore, in the last few decades electrochemical methods of DA determination have received great attention and many electrodes and bio-electrodes have been developed [7]. Due to its

simplicity as well as rapid and cost-effective measurements, electrochemical determination of DA has become very popular. In recent years, increased application of biosensors for DA determination has been particularly seen. Biosensors are small devices able to transform chemical information into useful analytical signal, which are composed of a bio-recognition layer and a transducer element. The bio-recognition layer can involve enzymes, proteins, antibodies, or nucleic acids; however in DA bio-sensing, enzyme-based biosensors have found the widest application. Many different enzymes are used to construct them including the laccase enzyme.

The laccase enzyme (oxygen oxidoreductase, EC 1.10.3.2) has been studied since the end of the 19th century when it was firstly isolated by Yoshida in 1883. Laccase belongs to a large groups of enzymes containing a four-copper active center [15], identified in many fungi, higher plants as well as bacterial organisms [16]. Due to its redox properties, this enzyme is biotechnologically attractive [17–19] especially in the construction of biosensors for the determination of many different chemical compounds i.e. dihydroxybenzene isomers, rutin as well as dopamine. In the preparation of sensors and biosensors many different materials and nanomaterials have been used to modify bare glassy carbon electrode (GCE), notably carbon-based nanomaterials, metal nanoparticles or conducting polymers [20] improving their electrochemical responses [5]. Carbon-based nanomaterials such as graphene, graphene oxide (GO), carbon nanotubes (CNTs) and their derivatives are very attractive materials due to their special structural, physicochemical and electronic properties [13]. Their importance in sensor and biosensor construction is clearly seen in almost 30-fold increase in the number of papers dealing materials with these over the last 18 years [21]. CNTs are especially useful in electrochemical sensor and biosensor construction because of their high electrical conductivity, large specific surface area as well as good mechanical and chemical stability. Moreover they are easy to immobilization of bio-recognition probes [22]. So far, CNTs were successfully applied for tyrosinase-based [23], 3-αhydroxysteroid dehydrogenase-based [24], glucose oxidase-based [25] and laccase-based biosensors.

In this work, laccase-based biosensors were constructed by innovative, one-step and eco-friendly Soft Plasma Polymerization (SPP) technique and applied to quantitative DA determination. One-step means that polymerization/crosslinking and deposition of the biorecognition layer occur

simultaneously inside the plasma zone (Scheme 1). Two types of biosensors were prepared and studied: biosensors obtained by direct deposition of the laccase bio-recognition layer on bare glassy carbon electrode and MWCNT modified glassy carbon electrode, respectively. It was observed that the bio-electrodes prepared by SPP technique in considerably shorter time show similar or better analytical performance than bio-electrodes known from literature. It is particularly important in relation to trends in scientific research aimed at reducing the biosensor preparation time without losing their applicability.

2. Materials and Methods

2.1 Chemicals

All chemicals used in experiments without further purification were analytical grade. Dopamine hydrochloride, sodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), acetic acid (CH_3COOH), sodium acetate (CH_3COONa), ammonia water solution (NH_3*H_2O), ammonium chloride (NH_4Cl), glutaric acid ($C_5H_8O_4$), lactose ($C_{12}H_{22}O_{11}$), glucose ($C_6H_{12}O_6$), glycine ($C_2H_5NO_2$), mannitol ($C_6H_{14}O_6$), saccharose ($C_{12}H_{22}O_{11}$), starch, sodium chloride (NaCl), ascorbic acid ($C_6H_8O_6$), and multi-walled carbon nanotubes (MWCNT, diameter: 6-9 nm, length: 5 μm, purity ≥98%) were purchased from Sigma Aldrich. The stock solution of dopamine at concentration 1 mmol/dm^3 was prepared by the dissolution of an appropriate amount of dopamine hydrochloride in freshly distilled water. The working solutions of dopamine were prepared by diluting the stock solution as required.

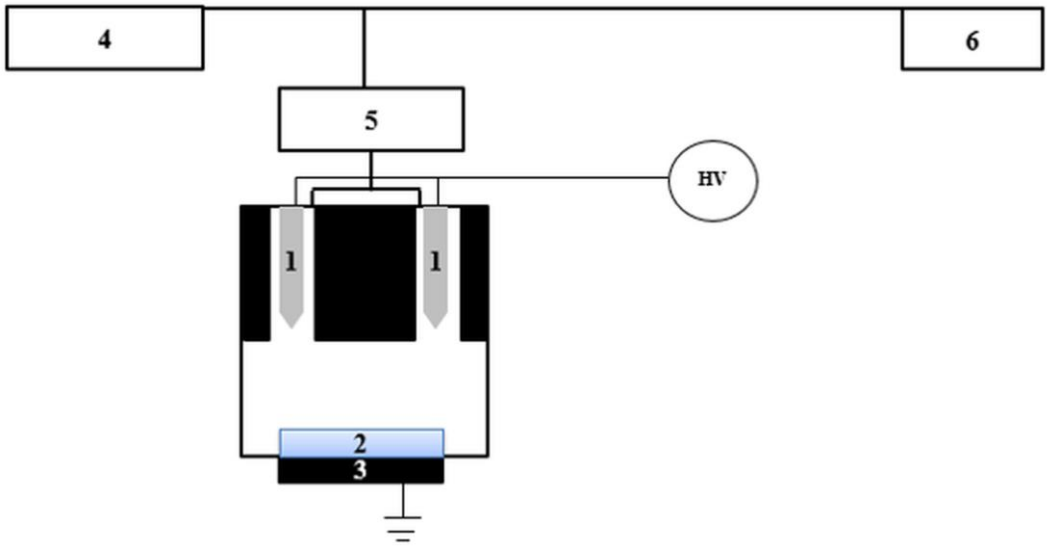
The biological precursor in the form of Laccase enzyme was obtained from Cerrena unicolor C-139 and isolated according to the procedure described in the work [26]. The liophylisate with a concentration $C_{Laccase}$=178 mg/mL and activity 186 000 nkat/L was dissolved in 1 mL of freshly deionized water to obtain enzyme stock solution. Then, the working solution of Laccase was prepared by 10-fold dilution of the stock solution. Concentration of Laccase enzyme was measured spectrophotometrically using syringaldazine as substrate.

MWCNT modified glassy carbon electrode were prepared according to the procedure reported in the work [27]. The suspension of MWCNT in THF (tetrahydrofuran) at a concentration of 1 mg/mL

was dropped and left to completely dry. Afterwards, the laccase bio-recognition layer was deposited according to the procedure presented below in section 2.2.

2.2 Deposition of the laccase bio-recognition layer by Soft Plasma Polymerization technique

Laccase-based biosensors were constructed using Soft Plasma Polymerization (SPP) technique. This technique utilizes low energy corona discharges of cold plasma with temperature the not exceeding 40^{o}C generated under atmospheric pressure. The discharge was generated in a pin-to-plane configuration between two tungsten needle electrodes in presence of helium carrier gas. The above properties of corona discharges applied allowed the laccase solution to be introduced directly into the plasma reaction zone in the form of vapour or microdroplets . As was reported in our previous paper during the plasma process laccase molecules are polymerized/cross-linked inside the corona discharge and deposited onto the conductive material in the form of bio-recognition layer. The biosensors were constructed under conditions optimized in our earlier paper, 30-second plasma deposition process [28] with an enzyme solution flow rate of 200 µL/min [29]onto Glassy Carbon Electrode (GCE) using corona discharge generated at a helium flow rate of 10 L/min [29] and three different voltages, 3 kV, 5 kV and 7 kV. The as-fabricated biosensors were named GCE|Lac|3, GCE|Lac|5 and GCE|Lac|7, respectively. Analogously the laccase-based biosensor obtained for GCE with MWCNT as an intermediate layer at 3kV voltage was named GCE|MWCNT|Lac|3. Between electrochemical measurements the biosensors were stored in fridge at 4^{o}C.



Scheme 1. Scheme of the set-up applied for laccase bio-recognition layer deposition (1-pointed tungsten electrodes, 2-conductive solid support, 3-grounded electrode, 4-laccase enzyme solution, 5-atomizer, 6-helium gas).

2.3 Electrochemical measurements

Electrochemical measurements were carried out using the square-wave voltammetric method by a μAutolab analyzer (Utrecht, the Netherlands). The electrochemical cell involved a three-electrode system where the studied biosensor was applied as a working electrode, Ag|AgCl (3 mol/dm^3 KCl) electrode as the reference electrode and a platinum electrode as an auxiliary electrode. First, the working electrode was polarized by applying a potential impulse at -1.4 V within 10 s. At such a negative potential, the species adsorbed during the previous measurement step are desorbed from the biosensor surface. Next , after 5 s of solution equilibration, the potential was changed from 0 to 0.5 V square-wave voltammograms were recorded. Other parameters were as follows: pulse amplitude 100 mV, frequency 40 Hz, scan increment 5 mV. All electrochemical measurements were carried out at room temperature (25.0-0.5^{o}C) in non-de-aerated, unstirred solution using an electrochemical cell volume of 10.00 mL.

2.4 Pharmaceutical sample preparation

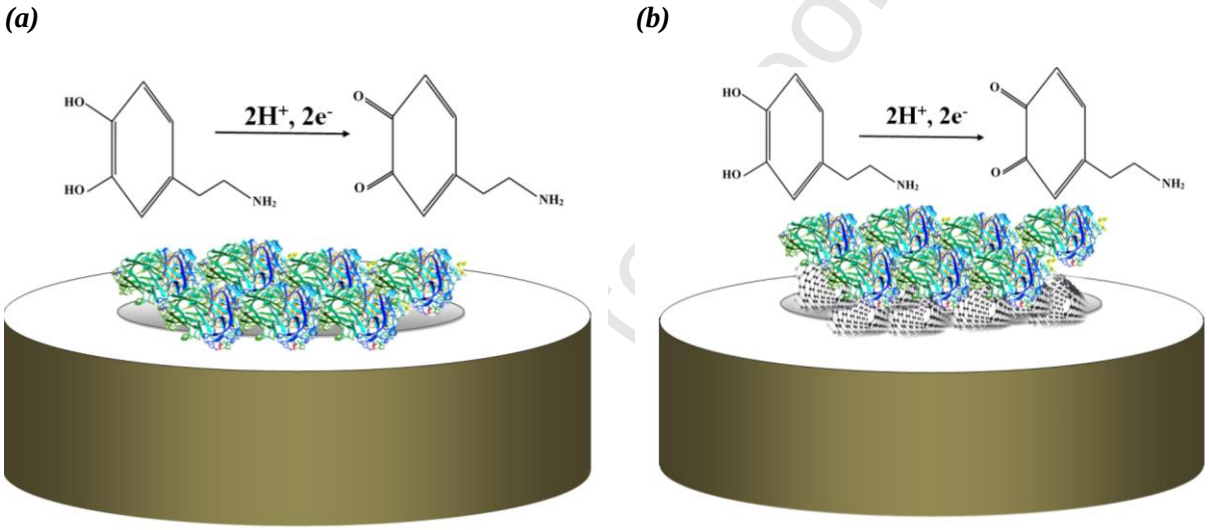
A pharmaceutical sample in the form of injection solution containing DA (0.1 % dopamine hydrochloride) as well as disodium edetate and sodium pyrosulfite was obtained from a local drugstore. It was analysed after 50 fold dilution with deionized water. Aliquots of the pharmaceutical sample solution were transferred to the a cell containing the supporting electrolyte and quantified using the standard addition method.

3. Results and Discussion

3.1 Mechanism of electrochemical DA determination

The electrochemical determination of DA by laccase-based biosensors is based on redox reaction occurring between the analyte molecule and the active centre of the enzyme deposited onto glassy carbon electrode and MWCNT modified glassy carbon electrode (Scheme 2). The complex DA oxidation reaction described by electron transfer-chemical reaction-electron transfer (ECE) has been

studied for many years and it involves oxidation and intramolecular cyclization of the DA molecule [30]. According to the ECE mechanism the DA molecule is first oxidized to dopaminequinone (DAQ) in a the two-electron oxidation reaction [31] and dissociated to relatively reactive deprotonated form. Then, the deprotonated form of DAQ is undergoes the follow-up ring closure to form 5,6-dihydroxyindole (DHI) [31] as a result of Michael addition reaction [30]. The DHI formation involves rearrangement of two bi-products: leucodopamine chrome (LDAC) and dopaminechrome (DAC). Finally, the DHI is oxidized to indole-5,6-quinone (IQ) [31] as a result of disproportionation or laccase-catalyzed reaction [30].



Scheme 2. Electrochemical reaction occurring during DA determination by the bioelectrode constructed using Soft Plasma Polymerization technique onto (a) glassy carbon electrode and (b) MWCNT modified glassy carbon electrode

At the beginning, the electrochemical signal for bare GCE and GCE covered with the laccase bio-recognition layer in the solution containing 5µM DA was recorded and it is presented in Fig. 1. As it was expected the peak current significantly increased after modification of the GCE surface with laccase. The recorded signals were 3.125 µA and 24.84 µA for GCE and GCE|Lac|3, respectively. The observed increase of peak currents confirms the development of the electrode surface area and electrochemical activity enhancement towards DA determination after GCE modification by the laccase bio-recognition layer. Moreover, GCE modification also affects the shape of generated

analytical signals. Unmodified GCE electrode generates SQW voltamperogram in the form of a broad and unclear line which depicts poor electrocatalytic ability toward DA, whereas GCE modified by the laccase bio-recognition layer generates a sharp and well-shaped current signal at a potential of 0.15 V.

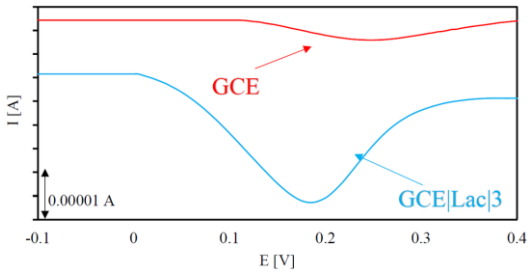


Fig. 1. SQW curves for 5 µmol/dm^3 DA in ammonia buffer environment using bare GCE electrode and GCE|Lac3; I (current), E (potential).

3.2 Effect of scan rate

In order to understand the nature of the electrocatalytic reaction occurring during DA determination by biosensors constructed using SPP technique, the effect of scan rate on DA peak current was studied. The measurements were carried out at different scan rates ranging from 150 mV/s to 450 mV/s for a constant DA concentration of 5 µmol/dm^3. The obtained results are shown in Fig. 2a, where it can be seen that the peak current of DA increased linearly with scan rate increase according to the following equation: $\Delta I\ [\mu A] = 0.0123\upsilon\ [mV/s] + 22.8$ with the correlation coefficient R^2=0.986. This linear dependency indicates that the electrode process of DA determination is surface-controlled [32]. The nature of the electrochemical process in DA determination by GCE|Lac|3 can also be confirmed by the dependency of $\log(\Delta I)=f(\log\upsilon)$, presented in Fig. 2b. It is well known that [13] diffusion-controlled redox reaction occurs when the slope of the linear relationship of $\log(\Delta I)=f(\log\upsilon)$ is about 0.5. The obtained dependency (Fig. 2b) indicates that the slope of the $\log(\Delta I)=f(\log\upsilon)$ relation determined for GCE|Lac|3 is 0.129, which clearly confirms the adsorption-controlled electrochemical reaction process. This indicates DA adsorption onto the laccase bio-recognition layer immediately before the electrochemical reaction. Moreover, SQW responses recorded at different scan rates showed slight peak potential shift towards more positively values, pointing to the reversible process of electron transfer (Fig. 2c).

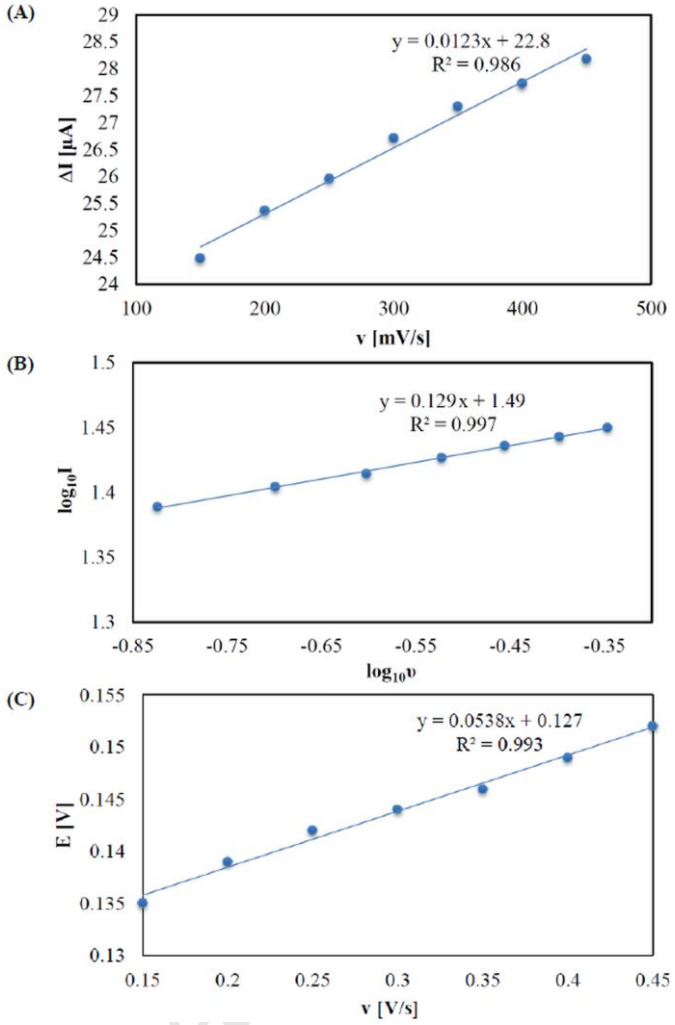


Fig. 2. (A) Dependency of peak currents of GCE|Lac|3 towards DA determination on scan rate ranging from 150 mV/s to 450 mV/s; (B) plot of decimal logarithm of peak current vs. decimal logarithm of scan rate and (C) the variation of peak potential vs scan rate.

3.3 Effect of supporting electrolyte buffer, pH buffer and concentration buffer on analytical signal generated by laccase-based biosensors constructed using SPP technique.

To gain the highest sensitivity of DA determination by the studied biosensors, some experimental parameters were optimized. First, the influence of supporting electrolyte type on analytical signal of GCE|Lac|3 was investigated. To select supporting electrolyte, the analytical responses of the biosensor were recorded for a constant dopamine concentration of 5 μmol/dm^3 in an environment of 0.05 mol/dm^3 acetic buffer (pH=4.60), 0.05 mol/dm^3 phosphorous buffer (pH=7.60) and 0.05 mol/dm^3 ammonium buffer (pH=8.50). DA determination in an environment of acetic buffer is not possible, because the GCE|Lac|3 does not generate analytical signal in low pH supporting electrolyte. A low DA

peak current of 2.62 µA was recorded only in an environment of acetic buffer at pH=6.13. In contrast to acetic buffer, application of the GCE|Lac|3 for DA determination in an environment of phosphorous and ammonium buffer allowed generation of significantly higher analytical signals of 4.62 µA and 9.91 µA respectively (Fig. 3a). As was reported in the paper [33], an increase of peak current generated in an environment of different buffers is directly related with the mobility and size of ions occurring in different supporting electrolytes. Therefore, the further studies of electrochemical DA determination were carried out in an environment of phosphorous and ammonium buffer.

A more important factor, influencing generation of analytical signal by sensors and biosensors is supporting electrolyte pH. To find its optimal value, electrochemical response of GCE|Lac|3 was determined in a solution containing 5 µmol/dm^3 DA with supporting electrolytes at pH ranging from 4.00 to 8.50. As was mentioned in the above paragraph, the bio-electrode did not generate analytical signal in an environment of acetic buffer at pH ranging from 4.00 to 5.60. A peak of 2.62 µA was only observed in the acetate buffer medium with pH=6.13. As for application of GCE|Lac|3 for DA electrochemical determination in solutions at higher pH i.e. pH=7.00, pH=7.60 (phosphorous buffer) and pH=8.50 (ammonium buffer), DA peak currents of 6.99 µA, 10.75 µA and 18.60 µA, respectively, were observed. Summarizing, the greatest peak current for electrochemical determination of DA was generated by the GCE|Lac|3 in an environment of ammonium buffer and therefore this supporting electrolyte was chosen for further studies. The obtained results demonstrate that an alkaline solution is significantly better for DA electrochemical determination because it is rich in OH^- ions making DA molecules easier to oxidize [3,8,33].

Proton participation in DA redox reaction on GCE|Lac|3 can be confirmed by the gradual shift of the peak potential (E_P) towards more negative values with increasing supporting electrolyte pH [8]. In the SQW responses recorded in the supporting electrolyte at a pH of 6.10, 7.05, 7.60 and 8.50 maximum current peaks (in brackets) were generated at potentials of 0.293 V (2.62 µA), 0.210 V (6.99 µA), 0.181 (10.75 µA) and 0.127 V (18.60 µA), respectively. Shown in Fig. 3b, the linear relationship between Ep [mV] and supporting electrolyte pH is described by the following regression equation: E [mV]= -50.3pH+329 with the correlation coefficient R^2=0.983. The slope (dEp/dpH) close to the Nernst equation value of -59 mV/pH indicates that the same number of protons and electrons

participate in DA oxidation electrochemical reaction [7],which was also reported for DA determination using other electrochemical analytical devices [34,35].

Furthermore, the optimization of phosphorous and ammonium buffer concentration was carried out. Therefore, the peak current for the constant DA concentration ($C_{dopamine}$=5µmol/dm^3) was recorded for the GCE|Lac|3 in an environment of 0.01 mol/dm^3, 0.05 mol/dm^3 and 0.1 mol/dm^3 phosphorous buffer (pH=7.60) and 0.01 mol/dm^3, 0.05 mol/dm^3, 0.1 mol/dm^3 ammonia buffer. In general, higher peak currents were generated by the GCE|Lac|3 in an environment of less concentrated supporting electrolytes. Considering the phosphorous buffer concentration effect, the highest peak current of 10.75 µA was generated in the 0.01 mol/dm^3 electrolyte solution. On the other hand, a 5-fold increase in phosphorous buffer concentration caused a decrease in the generated peak current to 4.62 µA. Higher values of the analytical signal were found when ammonia buffer was used. The greatest peak current of 18.60 µA was observed during DA determination in 0.01 mol/dm^3 ammonium buffer, whereas 5- and 10-fold increase in its concentration caused a decrease of generated signals to 9.91 µA and 7.14 respectively.

Comparison of the above results indicates that supporting electrolyte and its concentration play a critical role in DA determination. The obtained results show that GCE|Lac|3 generates the highest peak in an environment of ammonia buffer at pH=8.50 and a concentration of 0.01 mol/dm3 and therefore this supporting electrolyte was chosen for further studies.

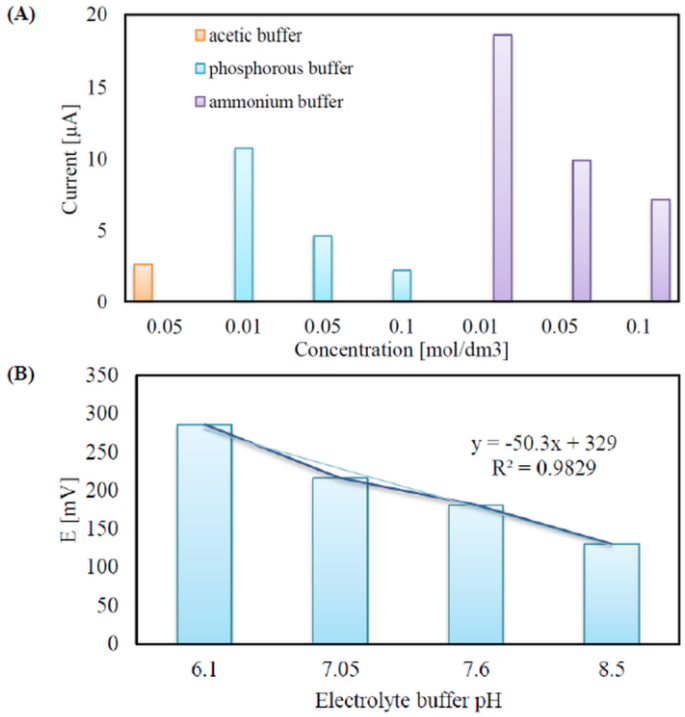


Fig. 3. (A) Effect of supporting electrolyte type (acetic buffer pH=6.13, phosphorous buffer pH=7.6, ammonium buffer pH=8.5) and concentration on analytical signal generated by GCE|Lac|3 in solution containing constant DA concentration 5µmol/dm^3 ; (B) Related plot of potentials *vs.* solution pH.

3.4 Response time of laccase-based biosensors constructed by Soft Plasma Polymerization technique

Response time is an important factor influencing biosensor applicability. It is defined as time elapsed from analyte dosing to an electrochemical cell until the moment when the signal is recorded to obtain the maximum response current. To evaluate GCE|Lac|3 response time, the analytical responses were recorded for 5 minutes with 1-minute intervals in an environment of ammonia buffer at optimized pH and DA concentration of 5 µmol/dm^3. The obtained results showed that the maximum response was recorded just after 60 seconds after dosing of analyte to electrochemical cell, and this period of time was applied in further electrochemical measurements.

3.5 Optimization of voltage applied for corona discharge generation

Voltage applied to generate corona discharge influences its properties i.e. energy density and plasma power. Therefore, in this paper influence of voltage applied to generate corona discharge on analytical parameters of biosensors fabricated by SPP technique has been investigated. For this purpose, three different biosensors were prepared using corona discharge generated at a voltage of 3

kV, 5kV and 7 kV. In voltage optimization, three analytical parameters of biosensors, i.e. dynamic range, sensitivity and biosensor lifetime were considered. According to the previous results, presented in sections 3.2 and 3.3, the biosensors constructed by SPP technique were applied for DA determination in an environment of 0.01 mol/dm^3 ammonium buffer at a pH of a 8.5. Voltage optimization was done based on the analytical response after 60 seconds from DA dosing to the electrochemical cell in the concentration range from 0.01 to 200 µmol/dm^3. The measurements were repeated three times. On the basis of the obtained results, the linear part of calibration dependencies with the correlation coefficient $R^2 \geq 0.990$ and its slope were determined.

Fig. 4a-c shows the SQW responses of GCE|Lac|3, GCE|Lac|5 and GCE|Lac|7 and indicates that voltage applied for cold plasma corona discharge has a significant impact on their dynamic ranges, sensitivities and stability of the constructed laccase-based biosensors. In case of GCE|Lac|3, the peak current and DA concentration exhibit linear correlation in two ranges: from 0.1 to 10 µmol/dm^3 and from 10 to 50 µmol/dm^3, with the following corresponding equations, respectively: ΔI [µA]= $3.80C_{DA}$ [µmol/dm^3]+4.56 (R^2=0.9946) and ΔI [µA]= $1.31C_{DA}$ [µmol/dm^3]+31.2 (R^2=0.9946). As is seen in the Fig. 4e, the peak current generated by GCE|Lac|5 was linearly proportional to DA concentration in the range from 0.1 to 10 µmol/dm^3 with the equation of ΔI [µA]= $1.39C_{DA}$ [µmol/dm^3]+0.623 (R^2=0.9923). Fig. 4f indicates that GCE|Lac|7 showed two linear ranges of analytical response, from 0.1 to 3 µmol/dm^3 and from 3 to 10 µmol/dm^3, according to the following equations respectively: ΔI [µA]= 3.19_{DA} [µmol/dm^3]+0.577 (R^2=0.9942) and ΔI [µA]= $1.80C_{DA}$ [µmol/dm^3]+4.67 (R^2=0.997). For DA concentration higher than 10 µmol/dm^3, no linear response was observed for both GCE|Lac|5 and GCE|Lac|7.

As is observed in the Fig. 4d-f, the calibration curve slopes of the studied bio-electrodes are also different. It means that the biosensors obtained using Corona Discharge generated at different voltages have different sensitivities. The electrochemical measurements showed that the sensitivity of GCE|Lac|3 was 3.80 and 1.31 µA*dm^3/µmol for the 1st and 2nd linear range, respectively. The sensitivity of GCE|Lac|5 was 1.39 µA*dm^3/µmol and the sensitivity of GCE|Lac|7 was 3.19 and 1.80 µA*dm^3/µmol for 1st and 2nd linear range, respectively.

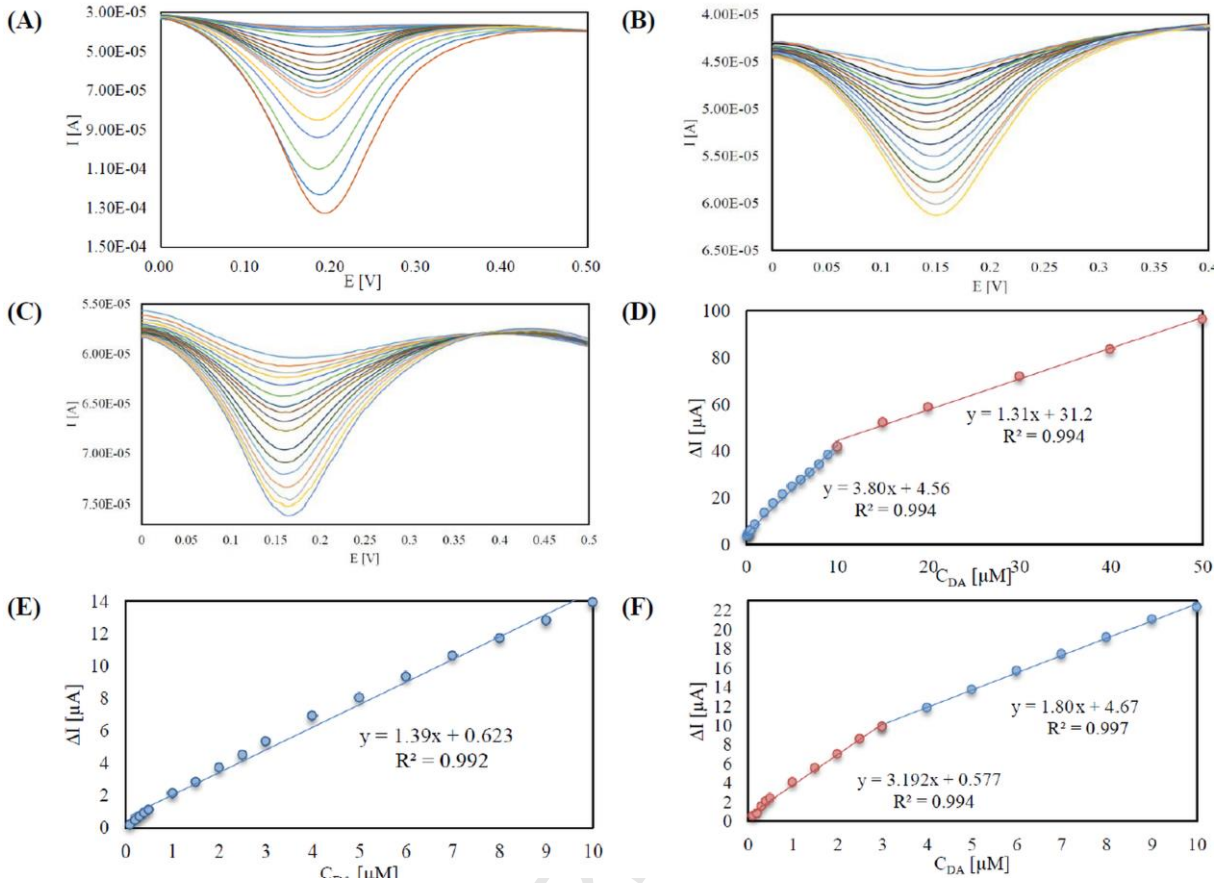


Fig. 4. SQW voltammograms for DA determination by the (A) GCE|Lac|3 (for the following concentrations: 0 - 50 μmol/dm^3), (B) GCE|Lac|5 (for the following concentrations: 0-10 μmol/dm^3) and (C) GCE|Lac|7 (for following concentrations: 0- 10 μmol/dm^3); as well as the dynamic ranges of (D) GCE|Lac|3, (E) GCE|Lac|5 and (F) GCE|Lac|7 .

The third studied parameter was biosensor lifetime. Fig. 5 indicates that voltage applied in corona discharge generation significantly influences analytical signal stability of bio-electrodes obtained by SPP technique. The biosensor lifetime was estimated based on the peak current generated by the bio-electrode (Fig. 5a) and the relative decrease of analytical signal ($\Delta I_{\%}$, Fig. 5b) defined as a ratio of the peak current decrease after 25 days to the initial peak current generated just after laccase bio-recognition layer deposition. As is seen in the Fig. 5a, the signals generated by all the sensors tested gradually decreased but to a different extent. This is caused by partial enzyme deactivation, which is a well-known phenomenon in biosensor technology. GCE|Lac|3 generates the highest and stable analytical signal with $\Delta I_{\%}$ almost 23%. The biosensors constructed using Corona Discharge generated at higher voltages generate more stable, but lower analytical signals. The $\Delta I_{\%}$ of GCE|Lac|5 and

GCE|Lac|7 was about 18% and 16% respectively. As is seen in Fig. 5b, $\Delta I_{\%}$ decreases linearly with increasing voltage according to the corresponding equation $\Delta I_{\%}$ [%]=-3.09U [kV]+25.4 (R^2=0.931). Corona discharge generated at higher voltage has a higher energy density and a larger amount of active plasma species i.e. ions or electrons. The amount of these species in corona discharge is responsible for the degree of bio-recognition layer polymerization/cross-linking and its biological activity, as was reported in our earlier work [29]. A higher amount of active plasma species in the plasma reaction zone led to deposition of the laccase bio-recognition layer with a higher degree of polymerization/cross-linking, which is observed based on lower $\Delta I_{\%}$ during the biosensor lifetime. However, application of too energetic corona discharge led to interference into the four-copper active centre of laccase, which is responsible for losing the biological activity of the bio-recognition layer. Therefore, the peak currents generated by GCE|Lac|5 and GCE|Lac|7 were significantly lower than the peak currents generated by GCE|Lac|3. The stability of the recorded signals observed during the 25 – day experiment confirms that the biorecognition layer deposited using SPP technique is firmly fixed on the GCE surface and no fallout enzyme layer was observed.

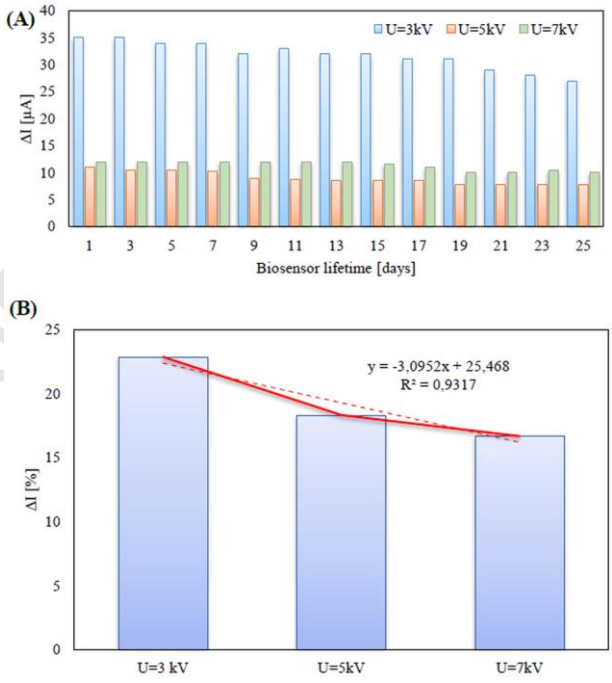


Fig. 5. (A) Lifetime of biosensors constructed using Soft Plasma Polymerization technique, (B) Influence of voltage used for Corona Discharge generation on relative peak current.

Furthermore, the electrochemical active surface (A) of GCE|Lac|3, GCE|Lac|5 and GCE|Lac|7 was calculated. In order to calculate A, the following Randles-Sevcik equation $Ip=(2.69x10^5)n^{3/2}AD_0^{1/2}Cv^{1/2}$ has been used where, n is the number of electrons participating in DA electrochemical determination, v is scan rate (V/s), A is area of electrode (cm^2), C is the concentration of bulk solution (mol/cm^3), D is DA diffusion coefficient (cm^2/s) and Ip is peak current generated by the bioelectrode. Based on the D value for DA equal to $3.68x10^{-6}$ cm^2/s [36], the A values of GCE|Lac|3, GCE|Lac|5 and GCE|Lac|7 were estimated to be 11.88 cm^2, 8,43 cm^2 and 10.77 cm^2 , respectively. The obtained A values indicate that voltage applied to generate corona discharge does not significantly influence the electrochemically active surface of the deposited laccase bio-recognition layer. It means that the worse analytical parameters of the biosensors fabricated using corona discharge generated at higher voltage values result from the interference of active cold plasma species (i.e. ions, atoms, electrons) in the 4-copper active centre of the biologically active precursor of laccase.

3.6 Application of MWCNT in laccase-based biosensor preparation

In order to evaluate the influence of MWCNT on the analytical parameters of the biosensors constructed using Soft Plasma Polymerization technique, the laccase bio-recognition layer was deposited onto GCE modified by MWCNT using Corona Discharge generated at a voltage of 3 kV. Fig. 6 shows comparison of voltammograms recorded for GCE|Lac|3 and GCE|MWCNT|Lac|3 in a solution containing 5 µmol/dm^3 DA. As it can be seen, GCE|MWCNT|Lac|3 exhibited a significantly higher peak current slightly shifted to the more positive potential values compared to the GCE|Lac|3 response.

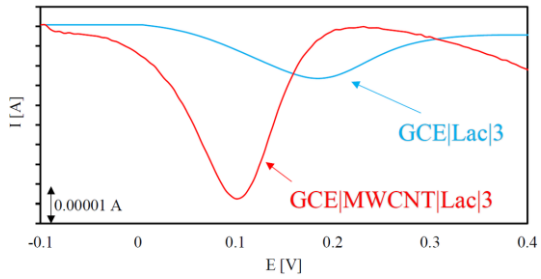


Fig. 6. SQW curves of 5 µmol/dm^3 DA in ammonia buffer environment using GCE|Lac3 and GCE|MWCNT|Lac|3.

Thus the calibration curve for this biosensor was determined in next measurements. The obtained results are presented in Fig. 7 where it can be seen that the GCE|MWCNT|Lac|3 showed a linear relationship between the generated current [µA] and the DA concentration ranging from 0.1 to 6 µmol/dm^3 with the corresponding equation ΔI [µA]= 22.3C_{DA} [µmol/dm^3]+0.355 (R^2=0.992). Then, the sensitivity of the GCE|MWCNT|Lac|3 was 22.3 µA*dm^3/µmol. The comparison of the analytical parameters of GCE|Lac|3 and GCE|MWCNT|Lac|3 showed that application of MWCNT in laccase-based biosensor construction caused a slight shortening of the dynamic range and about a 6-time increase of its sensitivity. GCE|MWCNT|Lac|3 has also a longer lifetime in comparison to the GCE|Lac|3. Its $\Delta I_{\%}$ value after 25 days from construction using SPP technique was 18%. Application of MWCNT in the construction of laccase-based biosensors by the cold plasma also caused ~2-time increase in the A value calculated following Randles-Sevcik equation, and for GCE|MWCNT|Lac|3 this value was 27.42 cm^2.

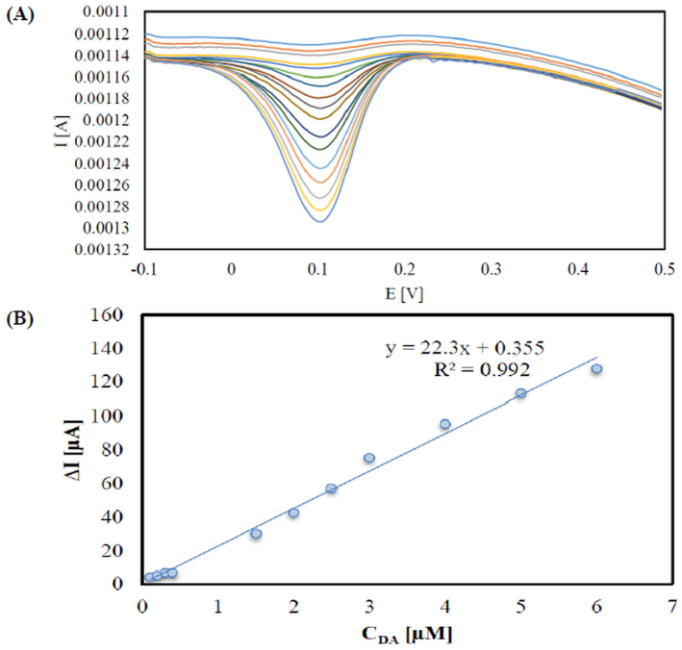


Fig. 7. (A) SQW voltammograms for DA determination by the GCE|MWCNT|Lac|3 (for the following concentrations: 0-6 µmol/dm^3) and (B) its dynamic range.

A change in the analytical parameters of the biosensor constructed using glassy carbon electrode and glassy carbon electrode modified with a MWCNT interlayer comes from different interactions occurring between the laccase enzyme and the electrode surface. Laccase-based bio-recognition layer

can be deposited onto MWCNT-modified GCE via polymerization/crosslinking reactions [28] and/or adsorption by van der Waals interactions, hydrophobic interactions, amphiphilic interaction or electrostatic interaction [37]. The surface of glassy carbon electrode is completely smooth and therefore the bio-recognition layer can be deposited only via polymerization/crosslinking reactions [28]. The strongest interaction between the deposited enzyme and the electrode surface causes the deposited bio-recognition layer to have more laccase molecules and hence it has higher bio-activity directly responsible for sensitivity. The second factor directly influencing on biosensor sensitivity is electrode specific surface area. Application of MWCNT as an interlayer causes considerable development of the electrode surface area, which is clearly seen by approximately a 2-fold increase in A value in GCE|MWCNT|Lac|3 compared to GCE|Lac|3. It allows for deposition of more laccase biomolecules responsible for higher biosensor sensitivity. However, the development of the electrode specific surface may hinder dopamine access to the 4-copper active centre of the laccase enzyme. Therefore, not all enzyme particles building the bio-recognition layer take part in the redox reaction with DA, which results in a slight shortening of the dynamic range of GCE|MWCNT|Lac|3 in comparison to GCE|MWCNT|Lac|3. Moreover, application of MWCNT in laccase-based biosensor construction enhances electron-transfer kinetics and promotes electron-transfer reactions, responsible for generation useful analytical signals [21].

Table 1 presents comparison of the analytical parameters the biosensors fabricated by cold plasma corona discharge to other sensors and biosensors. The analytical parameters such as dynamic range and sensitivity of the GCE|Lac|3 and GCE|MWCNT|Lac|3 are comparable with values earlier reported by other research groups. It shows that application of corona discharge to deposition of laccase bio-recognition layer allows biosensors to be constructed in significantly shorter time without losing their applicability.

Table 1. Summary of previously published results for DA detection by using different electrodes and bioelectrodes.

Bioelectrode	Dynamic range	Sensitivity [µA*dm^3/µ	Ref.

	[μmol/dm^3]	mol]	
WS_2 NS_2-CNF_s	0.5-60	5.36	[4]
PdAu/RGO/GCE	2.5-66.25	0.7066	[34]
AG-NA/GCE	0.5-35	2.672	[6]
ZnO NSB/GF	1-80	1.52	[38]
p-GLY/GO	0.1-105	0.534	[1]
NiO-CuO/GR	0.5-20	0.3095	[7]
Fe_3O_4-SnO_2-Gr	0.02-2.8	12.494	[13]
Laccase/MWCNR modified GC	1.0-30.0	0.61	[30]
Laccase-based biosensor obtained using MWCNT modified GCE	2.99-38.5	0.639	[39]
SPE/rGO-CDBA-Lac	0.1-70	0.1041	[40]
GCE|Lac|3	0.1-10	3.808	This work
	10-50	1.3185	
GCE|MWCNT|Lac|3	0.1-6.0	22.344	

WS_2 NS_2-CNF_s: WS_2 nanospheres-carbon nanofibers electrode

PdAu/RGO/GCE: reduced graphene oxide-bimetallic PdAU nanocomposites modified GCE

AG-NA/GCE: activated graphene-Nafion modified GCE

ZnO NSB/GF:ZnO nanosheets balls anchored onto graphene foam

p-GLY/GO: poly(glucine)/graphene oxide modified GCE

NiO-CuO/GR: nickel and copper oxides-decorated graphene composite

Fe_3O_4-SnO_2-Gr: Fe_3O_4 and SnO_2 decorated graphene nanocomposite

SPE/rGO-CDBA-Lac: laccase immobilized on β-cyclodextrin and benzaldehyde complex placed on reduced ographene oxide screen-printed electrode

Laccase/SiO_2-PA modified GCE: laccase biosensor based on silica nanoparticles modified with phytic acid

3.7 Interference studies of GCE|Lac|3

In order to evaluate the anti-interference ability of GCE|Lac|3 constructed by Soft Plasma Polymerization technique, the influence of some possible interference with the DA determination was studied. The electrochemical measurements were carried out by analysing of a standard DA solution at a concentration of 5 μmol/dm^3 in 0.01 μmol/dm^3 ammonium buffer at pH=8.5 in the presence of coexisting and potentially interfering substances at equal and 5-fold higher concentration. The recorded analytical signals are seen in Fig. 8 (A). The impact of interfering species on the DA oxidation peak current generated by GCE|Lac|3 constructed using corona discharge was evaluated based on selectivity coefficients (SC) calculated using the following formula: $SC=(I_{A+I}/I_C)*100\%$, where I_{A+I} and I_C are currents generated for DA in the presence and absence of coexisting substances, respectively [41]. The tolerance limit was taken as the maximum concentration of foreign chemical compounds changing the analytical signal generated by GCE|Lac|3 by maximum ±5% (SC<5%). Fig. 8 (B) indicates that glutamic acid, lactose, glucose, glycine, mannitol, saccharose, starch, NaCl and ascorbic acid had no significant impact on the electrochemical response of DA generated by GCE|Lac|3. The SC of GCE|Lac|3 for electrochemical DA determination was below 5% in the presence of interfering substances in both: an equal and 5-fold higher concentration. From the SC values, it can be proven that GCE|Lac|3 is completely selective in detecting DA. Therefore, the presence of the studied interfering substances in real samples will not influence DA voltammetric determination.

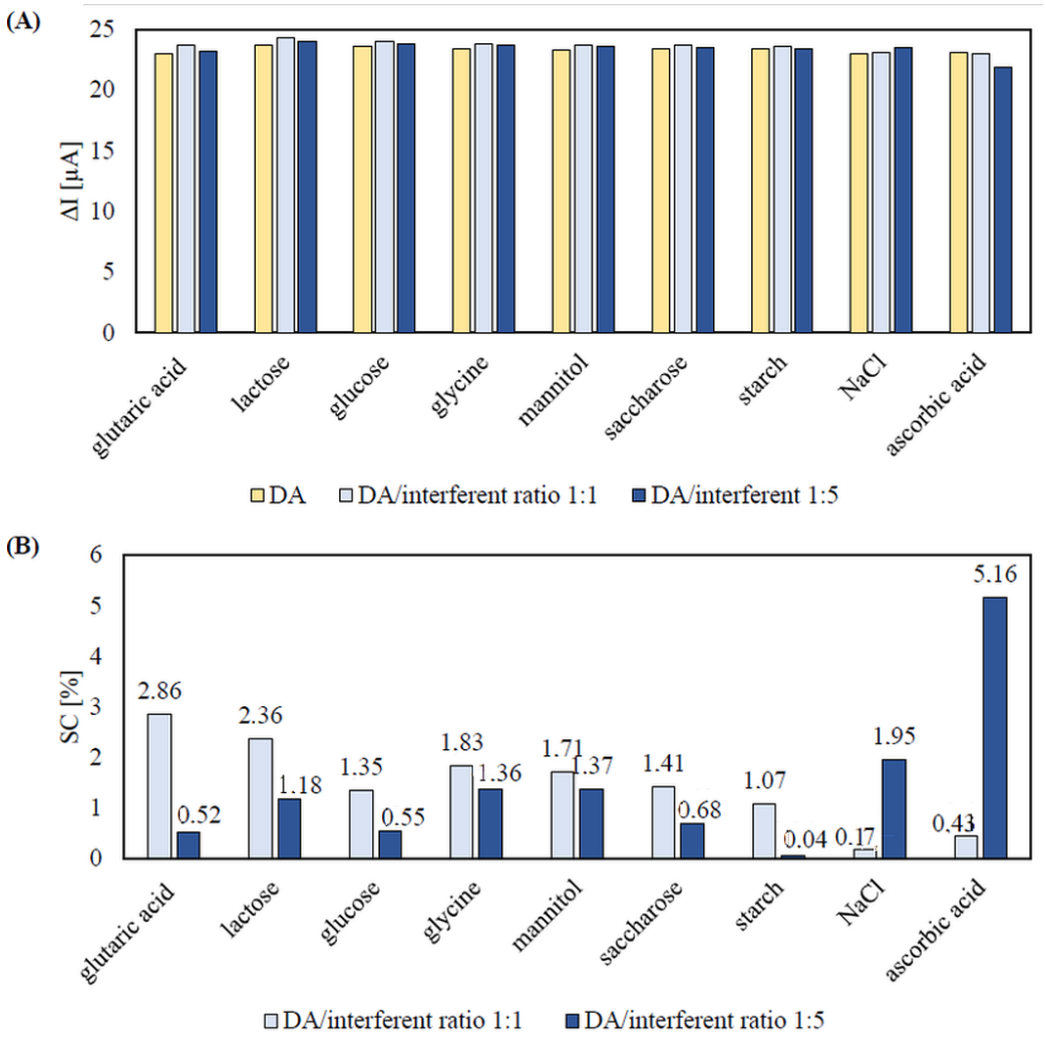


Fig. 8. (A) Electrochemical signal and (B) calculated selectivity coefficients of GCE|Lac|3 in 0.01 mol/dm^3 ammonium buffer (pH=8.5) containing 5 μmol/dm^3 od DA and different foreign substances at an equal and 5-fold higher concentration.

3.8 Real sample analysis

To evaluate the applicability the proposed bio-electrodes, they were used for dopamine determination in pharmaceutical preparation. It was purchased as an injectable solution and analysed without preliminary preparation after 50-fold dilution with distilled water using the standard addition method. The obtained results were compared to the declared content (10 mg/mL) to calculate recovery values expressed as: recovery [%]= (found concentration/declared concentration)*100% (Table 2). As it can be seen in Table 2, quantitative recovery was found to be at a level of 94.60% and 98.00 % for GCE|Lac|3 and the GCE|MWCNT|Lac|3, respectively. This means that the matrix of a real

pharmaceutical sample does not influence analytical signals generated by the studied electrodes and evidences that the biosensor with laccase bio-recognition layer deposited by Soft Plasma Polymerization technique can be successfully applied for DA determination in real pharmaceutical samples with satisfactory results.

Table 2. Results of DA determination in pharmaceutical preparation (n=4)

Sample	Bioelectrode	Added [mg/mL]	Found [mg/mL]	Recovery [%]
Dopamine hydrochloride solution for injection	GCE|Lac|3	10	9.46±0.29	94.60
	GCE|MWCNT|Lac|3	10	9.80±0.19	98.00

4. Conclusions

Electrochemical determination of DA is a huge area of research as is indicated by thousand publications in this area. This study demonstrates that laccase-based biosensors obtained by innovative Soft Plasma Polymerization technique are an attractive tool for DA determination by square wave voltammetry method. The characterization of working conditions reveals that the highest analytical signal was generated by the biosensor in an environment of 0.01 mol/dm^3 ammonium buffer at pH=8.50. Generation of the highest peaks in physiological pH is promising for a real in vivo measurement of DA. The electrochemical measurements carried out under optimum experimental conditions showed that the proposed bio-electrode is characterized by a wide dynamic range, high sensitivity and good long-term stability. Among the three studied biosensors deposited onto bare, unmodified glassy carbon electrode, the device constructed using corona discharge generated at a voltage of 3 kV has superior analytical parameters. The GCE|Lac|3 has two linear ranges from 0.1 to 10 µmol/dm^3 and from 15 to 50 µmol/dm^3 with a sensitivity of 3.80 µA*dm^3/µmol and 1.31µA*dm^3/µmol respectively. Due to the development of the surface area and electron transfer enhancement, application of MWCNT in construction of a laccase-based biosensor by Soft Plasma

Polymerization technique allows the sensitivity to be significantly increased to 22.3μA*dm^3/μmol for the linear range from 0.1 μmol/dm^3 to 6 μmol/dm^3. Because GCE|Lac|3 is highly selective towards many chemical compounds, it has potential application for DA determination in real pharmaceutical samples with satisfactory results. This work presents a simple, cheap and one-step way of laccase-based biosensor fabrication which is very competitive to the currently known "wet" methods. Application of Cold Plasma in biosensors construction allows the time of their fabrication to be reduced without worsening the analytical parameters.

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