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# Molecularly Imprinted Polypyrrole based Sensor for the Detection of SARS-CoV-2 Spike Glycoprotein

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## Abstract

This study describes the application of a polypyrrole-based sensor for the determination of SARS-CoV-2-S spike glycoprotein. The SARS-CoV-2-S spike glycoprotein is a spike protein of the coronavirus SARS-CoV-2 that recently caused the worldwide spread of COVID-19 disease. This study is dedicated to the development of an electrochemical determination method based on the application of molecularly imprinted polymer technology. The electrochemical sensor was designed by molecular imprinting of polypyrrole (Ppy) with SARS-CoV-2-S spike glycoprotein (MIP-Ppy). The electrochemical sensors with MIP-Ppy and with polypyrrole without imprints (NIP-Ppy) layers were electrochemically deposited on a platinum electrode surface by a sequence of potential pulses. The performance of polymer layers was evaluated by pulsed amperometric detection (CA). According to the obtained results, a sensor based on MIP-Ppy is more sensitive to the SARS-CoV-2-S spike glycoprotein than a sensor based on NIP-Ppy. Also, the results demonstrate that the MIP-Ppy layer is more selectively interacting with SARS-CoV-2-S glycoprotein than with bovine serum

albumin. This proves that molecularly imprinted MIP-Ppy-based sensors might be applied for the detection of SARS-CoV-2 virus proteins.

**Keywords:** COVID-19; SARS-CoV-2 Spike glycoprotein; Polypyrrole (Ppy); conducting polymers; molecularly imprinted polymers (MIPs); electrochemical determination of virus proteins; thin layers.

## 1. Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) induced COVID-19 pandemic that began in 2019 has caused drastic changes in the world. 197 countries were affected [1]: lockdowns [2], quarantine, economic problems hit the most significant part of the world, people's emotional health has deteriorated. Even at the beginning of the 2021, this pandemic is still not adequately controlled. Although the vaccines became available to society, this viral infection is still very active and the virus is rather rapidly mutating and appears in new even more infectious forms. Therefore, a much deeper understanding of the virus SARS-CoV-2 is required and rapid analytical methods that are suitable for the diagnosis of COVID-19 and/or detection of virus or their parts are demanded to overcome and defeat this infection. Thus, various aspects of the virus itself [3], genome [4-7], research of the structure, function of proteins, and nucleocapsid, envelope, spike, and membrane protein interactions with drugs [8-11], and some other aspects [12, 13] were investigated. Better and easier detection methods could improve the diagnosis of viral infection and enable more efficient ways of defeating the COVID-19 pandemic. Recently, label-free protein detection has become relevant in research and clinical practice [14, 15]. The discovery and detection of biomarkers during the diagnosis of human diseases is required for biomedical purposes [15, 16].

In biosensors the analyte recognition elements are typically based on bio-macromolecules such as enzymes, antibodies, DNA, aptamers, etc. However, such bioanalytical systems have some limitations due to operating conditions and expensive production. Therefore, the development of artificial biorecognition-systems based on synthetic receptors and molecularly imprinted polymers (MIPs) has attracted a great interest as a potential alternative [14, 17]. Researchers have been focused on the development of a system that replicates the natural recognition process. Therefore, the interest in the development of MIPs has grown during recent years [15, 16, 18-25]. The technique of molecular imprinting allows the formation of specific molecular recognition sites that operate on the principle of

complementarity between the imprinted sites and the analyte. Therefore, MIPs can selectively bind the analytes of interest, which were used as templates during formation of these MIPs [14, 16, 26-28]. MIPs also have some other benefits including low-cost, easy way of preparation, advanced storage stability, and rather good specificity [14, 29]. In previous studies, it was reported that various types of small molecules can be imprinted within polymers [22, 27, 30, 31]. In some researches, it was demonstrated that high molecular mass biomolecules including proteins [15, 20, 21, 32-41] can be also molecularly imprinted within polymers. Polypyrrole (Ppy) is among several other polymers that can be very efficiently applied for the design of MIP-based sensors [22, 27, 30, 31, 42-45]. This is a conducting polymer, which can be easily electropolymerized and used as a polymeric matrix of MIPs for the detection of low and high molecular weight analytes [15, 42]. Electrochemical methods like cyclic voltammetry, differential pulse voltammetry, and electrochemical impedance spectroscopy were used for the detection of the proteins both on the polypyrrole modified with molecular imprints and on the unmodified in previous studies [15, 46-52]. Meanwhile, there is only few reports on the application of chronoamperometry for determination of virus-proteins [42]. In chronoamperometry the changes in the current appear in response to increase or decrease of the diffuse layer thickness at the surface of the working electrode. Therefore, the application of chronoamperometry (in pulsed amperometric mode) and the analysis of data gathered by this method using Cottrell or Anson plots are providing interesting and useful insights into the evaluation of interaction between analytes and the electrode.

At the moment, there are some explorations reported that are already applying MIP technology for SARS-CoV-2 [53, 54]. The development of so called 'monoclonal-type plastic antibodies' based on MIPs was described [53]. Such 'antibodies' were able to selectively bind a spike protein of the novel coronavirus SARS-CoV-2 to block its function. The obtained nanoparticles were analyzed by SDS-PAGE electrophoresis. The results of the electrophoretic analysis demonstrated promising results in the formulation of 'free-drug therapeutics' due to their ability to bind the virus spike glycoprotein and, thus, to block the infection process. According to reported results it was concluded that the 'monoclonal-type plastic antibodies' could be potentially used as free-drug therapeutics in the treatment of infection 2019-nCoV. In another research, SARS-CoV-2 nucleoprotein (ncovNP) was qualitatively and quantitatively determined by MIP-based layer on poly-m-phenylenediamine (PmPD), which was deposited on the Au-TFE electrode [54]. Cyclic voltammetry (CV) was applied for the characterization of the preparation steps of the sensor. Meanwhile, the rebinding of SARS-CoV-2 nucleoprotein on the sensors was studied by differential pulse voltammetry (DPV) in the solution of 1 M KCl

containing a redox probe  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ . The obtained results demonstrated the linear increase of the sensor response with increasing ncovNP concentration. The feasibility of sensor performance in clinical samples was tested. For this purpose they analyzed the samples prepared from nasopharyngeal swab specimens. Genetically engineered receptor-binding domain of SARS-CoV-2-RBD protein was imprinted in ortho-phenylenediamine and deposited on a macroporous gold screen-printed electrode [55].

The aim of recent research was to design the MIP-based sensor for the determination of SARS-CoV-2-S glycoprotein. For this purpose, Ppy layers were deposited on the working platinum electrode from the polymerization mixture containing SARS-CoV-2-S glycoprotein and pyrrole dissolved in phosphate buffered saline (PBS) solution, pH 7.4. The performance of the electrode modified by the deposited MIP-Ppy layer imprinted with SARS-CoV-2-S glycoprotein was investigated and compared with that of non-imprinted (NIP-Ppy) layer.

## **2. Materials and methods**

### **2.1. Chemicals and instrumentation**

Pyrrole 98% (*Alfa Aesar*, Germany),  $H_2SO_4$  (96 %) (*Lachner*, Czech Republic),  $HNO_3$ , NaOH (*Merck*, Germany),  $H_2PtCl_6$  (*Merck*, Germany), and bovine serum albumin (BSA) (*Carl Roth*, Germany) were used as received.  $KH_2PO_4$  (*Honeywell Riedel-de Haen*, Germany), NaCl, KCl, and  $Na_2HPO_4$  (*Roth*, Germany) salts were used for the preparation of buffer. The detailed description of expression and purification of SARS-CoV-2-S spike glycoprotein is presented in supporting material.

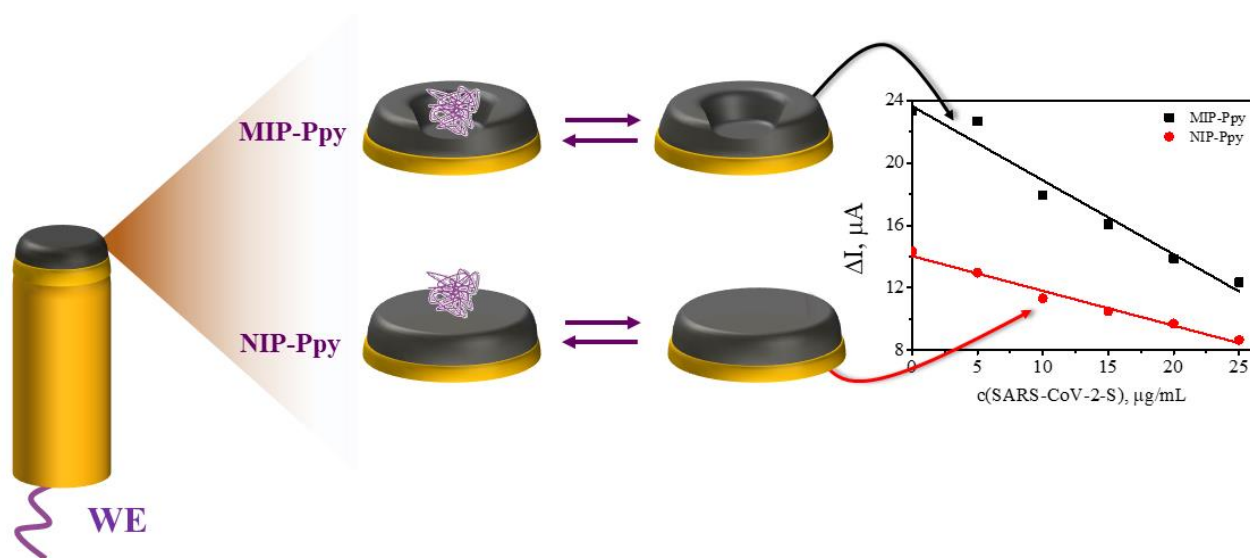
Experiment was performed using potentiostat/galvanostat Metrohm AutoLAB model  $\mu$ AutolabIII/FRA2  $\mu$ 3AUT71079 controlled by NOVA 2.1.3 software (*EcoChemie*, The Netherlands). All measurements were done in a home-made cell. The total volume of the cell was 250  $\mu$ L. Three-electrode system consisted of Pt disk with 1 mm diameter sealed in glass as the working electrode, Ag/AgCl in 3M KCl solution electrode as a reference electrode (Ag/AgCl), and Pt disk of 2 mm diameter as a counter electrode.

### **2.2. Pretreatment of working electrode**

The working electrode was pretreated before electrochemical deposition of Ppy following the procedure described in previous studies [42, 56]. All solutions were thoroughly degassed just before use with a stream of  $N_2$ . According to this procedure, the Pt electrode was rinsed with concentrated  $HNO_3$  solution in an ultrasonic bath for 10 min, then rinsed with water and polished with alumina paste. Later, it was rinsed with water again and then with 10 M solution

of NaOH, thereafter – with 5 M solution of H<sub>2</sub>SO<sub>4</sub> in an ultrasonic bath for 5 min. Electrochemical cleaning of the electrode was carried out in 0.5 M H<sub>2</sub>SO<sub>4</sub> by cycling the potential for 20 times in the range between –100 mV and +1200 mV vs Ag/AgCl at a sweep rate of 100 mV s<sup>-1</sup>. The identification of the bare electrode surface was made possible by a stable indication of the cyclic voltammogram. To improve the adhesion of the Ppy layer to the electrode surface, a layer of ‘platinum black’ was deposited over the working electrode [56]. Deposition of Pt clusters was performed in 5 mM solution of H<sub>2</sub>PtCl<sub>6</sub> containing 0.1 M of KCl by 10 potential cycles in the range between +500 mV and –400 mV vs Ag/AgCl at a sweep rate of 10 mV s<sup>-1</sup>.

### 2.3. The electrochemical deposition of MIP and NIP and evaluation of sensor signal



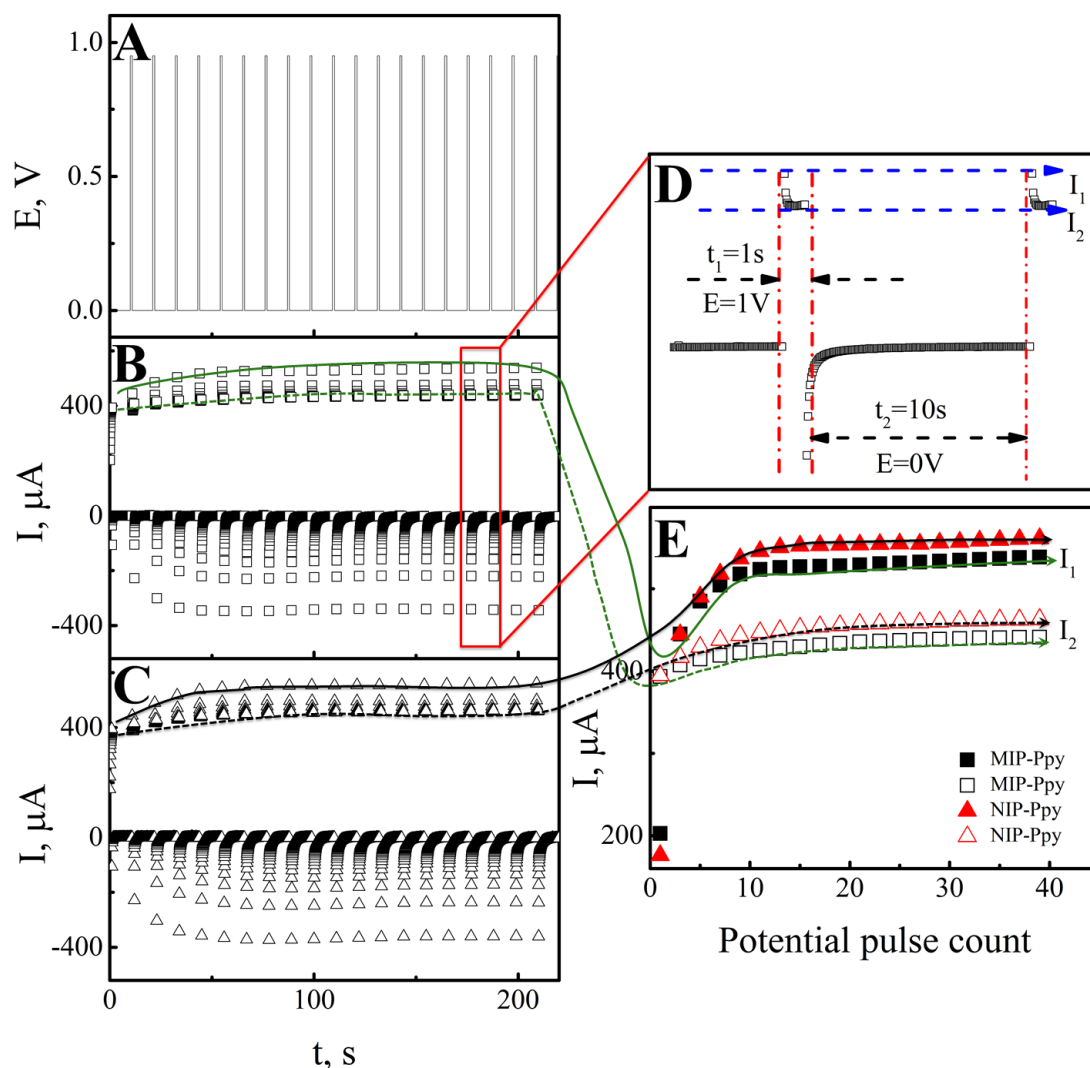
**Fig. 1.** Schematic representation of evaluation by chronoamperometry of Pt electrode modified with non-imprinted polypyrrole (NIP-Ppy) and with molecularly imprinted polypyrrole (MIP-Ppy) with SARS-CoV-2-S glycoprotein imprints. Electrochemical measurements were performed in phosphate-buffered saline (PBS) solution, pH 7.4.

The electrochemical deposition of the polypyrrole layer was performed in the same electrochemical cell. NIP-Ppy was electrochemically deposited from the polymerization solution containing 0.5 M solution of pyrrole in PBS. The preparation of MIP-Ppy was carried out in two steps. Step I: deposition of polymeric layer was carried out from the polymerization solution containing 0.5 M solution of pyrrole and 50 μg/mL of SARS-CoV-2-S glycoprotein all

dissolved in PBS solution. The polymeric layers were formed by a sequence of 20 potential pulses of +950 mV for 1 s, between these pulses 0 V potential for 10 s was applied [42, 56]. Step II: the MIP-Ppy was formed when the imprinted protein molecules were extracted by incubation in 0.05 M H<sub>2</sub>SO<sub>4</sub> for 10 min. In the same way as MIP-Ppy, NIP-Ppy was also exposed to 0.05 M solution of H<sub>2</sub>SO<sub>4</sub>. MIP-Ppy and NIP-Ppy were analyzed using pulsed amperometric detection by the sequence of 10 potential pulses of +600 mV vs Ag/AgCl lasting for 2 s, between these pulses 0 V vs Ag/AgCl was applied for 2 s (Fig. 1).

### **3. Results and discussions**

Electrochemical polymerization of the two types of Ppy layers was performed by a sequence of potential pulses (Fig. 2). The profile of potential pulses sequence is represented in figure 2A. Figures 2B and 2C demonstrate the currents registered during the electrochemical deposition of Ppy layer from polymerization solution containing SARS-CoV-2-S glycoprotein and Ppy layer from polymerization solution non-containing SARS-CoV-2-S glycoprotein on Pt-electrode surface.



**Fig. 2.** Electrochemical deposition of the polypyrrole layers on the Pt electrode: **A** – The profile of potential applied during the sequence of potential pulses; **B** – The profile of current registered during the deposition of Ppy layer from polymerization solution containing SARS-CoV-2-S glycoprotein; **C** – The profile of current registered during the formation of Ppy layer from polymerization solution non-containing SARS-CoV-2-S glycoprotein. **D** – The profile of current registered during one potential pulse. **E** – Changes of current measured instantly after a potential step of +950 mV.

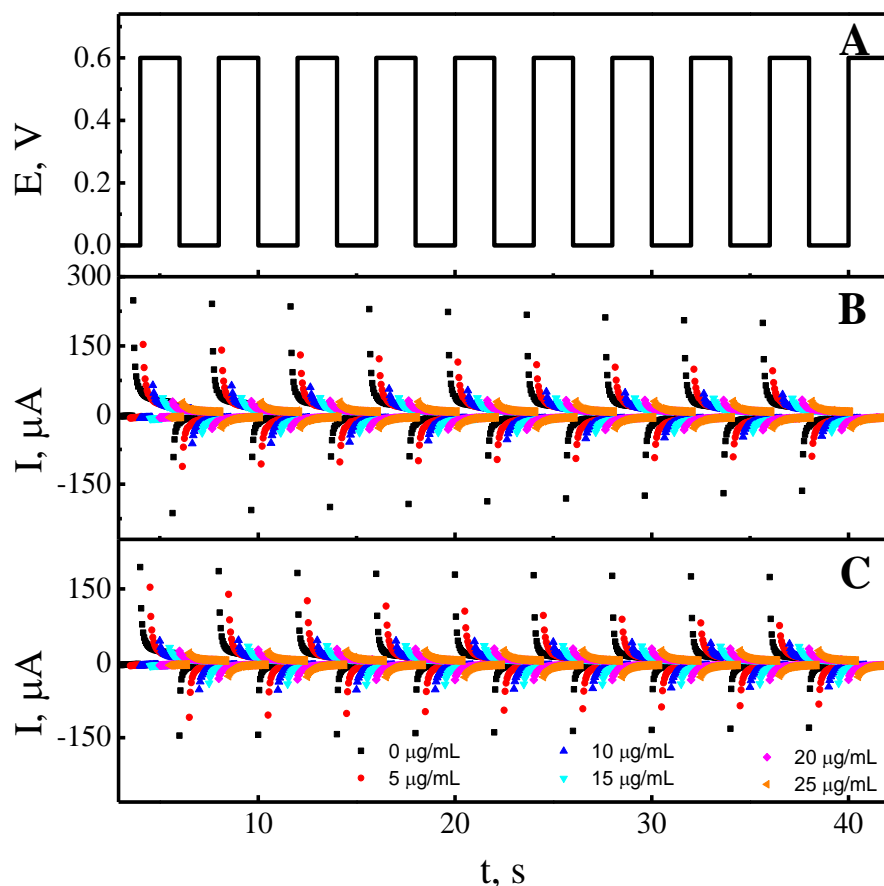
The changes of current at the beginning  $I_1$  and at the end  $I_2$  of pulses of the potential at +950 mV are presented in figure 2E. The current changes were not the object of analysis at the potential of 0 V, because during this potential step the equilibration of monomer and template molecule concentrations in the neighborhood of the working electrode is happening. Previous studies demonstrated that the self-assembly of monomers and template molecules due to the interactions under thermodynamic control prior to polymerization, is significant for the



1 recognition characteristics of the final polymers [57]. Polymerization of Ppy occurs during the  
2 pulses at a potential value of +950 mV. Therefore, only an insignificant Faradaic process was  
3 observed on the electrode at the 0 V potential step. Thus, the current changes during the  
4 potential step when the potential was elevated up to +950 mV were analyzed more in detail.  
5 For the visualization of the current changes during the electrochemical deposition of Ppy layer  
6 from polymerization solution containing SARS-CoV-2-S glycoprotein and Ppy layer from  
7 polymerization solution non-containing SARS-CoV-2-S glycoprotein two current points at the  
8 beginning  $I_1$  and end  $I_2$  of each potential step were taken into account (Fig. 2D). The comparison  
9 of the current changes demonstrated that the current registered during deposition Ppy layer  
10 from polymerization solution non-containing SARS-CoV-2-S glycoprotein is higher than that  
11 registered during deposition of Ppy layer from polymerization solution containing SARS-CoV-  
12 2-S glycoprotein (Fig. 2E). However, the observed difference of current changes is not very  
13 significant in comparison with that registered in our previous researches [27] and in other  
14 researches [27, 58]. The collation of current changes on Pt electrode during the  
15 electrochemical deposition of Ppy/SARS-CoV-2-S and NIP-Ppy layers have illustrated that  
16 current during the deposition of NIP-Ppy increased just by 1.05 times in comparison to that  
17 registered during the deposition of Ppy/SARS-CoV-2-S. From the current changes observed  
18 during the polymerization, it can be presumed that the entrapped protein molecules just  
19 insignificantly affect the conductivity of the formed layers. During the next MIP-Ppy  
20 preparation step, the entrapped SARS-CoV-2-S glycoprotein were removed from the formed  
21 Ppy/SARS-CoV-2-S layer and MIP-Ppy was formed. In the same way as MIP-Ppy, NIP-Ppy was  
22 also exposed to 0.05 M  $H_2SO_4$  to eliminate any differences caused by the extraction procedure  
23 on the formed MIP-Ppy, NIP-Ppy layer properties.

24 In the following part of the research, the formed MIP-Ppy and NIP-Ppy layers were  
25 evaluated using pulsed amperometric detection by a sequence of 10 potential pulses of +600 mV  
26 and 0 V for 2 s each as it was suggested in our previous research [42]. Various aspects of  
27 charging-discharging of conducting polymer polypyrrole was well discussed by Heinze et al.  
28 [59]. Also there was stated that overoxidation of the un-substituted Ppy already occurs at 0.65  
29 V vs Ag/AgCl<sub>(3M KCl)</sub> [60]. Hence, taking into account these findings a potential pulse values of 0  
30 V and +600 mV were selected for the determination of SARS-CoV-2-S glycoproteins.

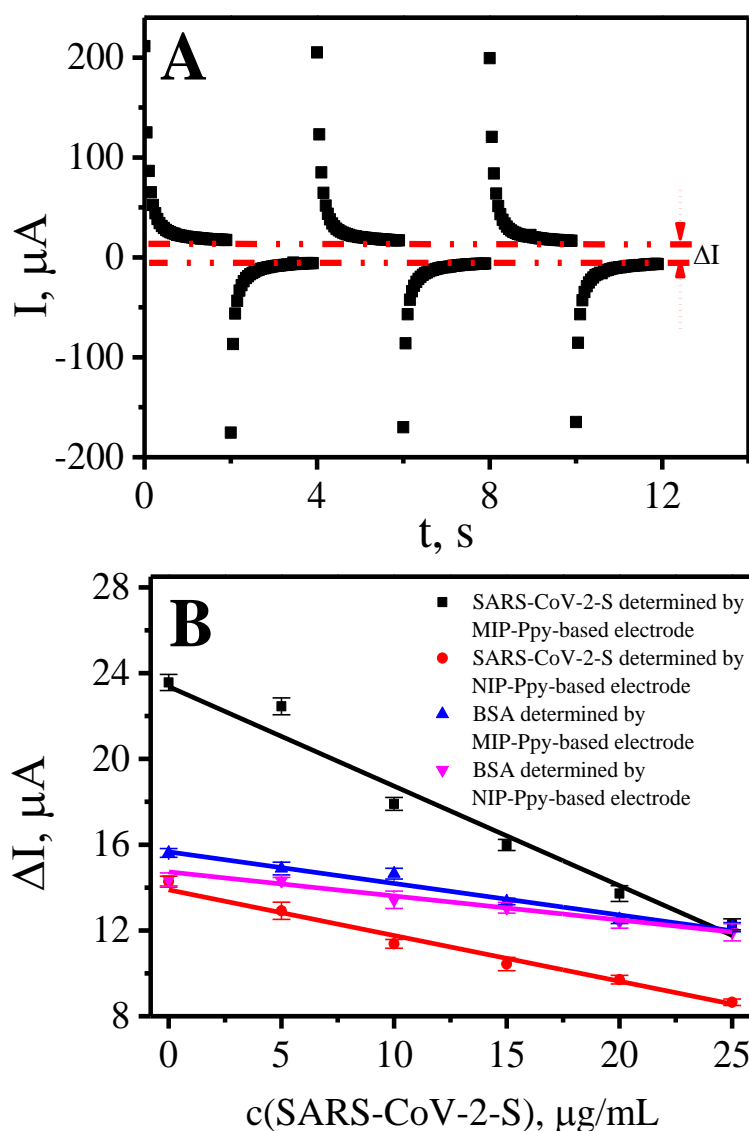
31 The profile of the potential pulse sequence is presented in figure 3A.



**Fig. 3.** Electrochemical evaluation of MIP-Ppy and NIP-Ppy layers was performed by the potential pulse sequence. **A** – potential pulse profile. Typical chronoamperograms (during pulsed amperometric detection) were obtained at: **B** – MIP-Ppy and **C** – NIP-Ppy modified Pt electrode in the absence of SARS-CoV-2-S glycoprotein (▪) and in the presence of SARS-CoV-2-S glycoprotein from 5  $\mu\text{g/mL}$  up to 25  $\mu\text{g/mL}$  in PBS solution, pH 7.4 (offset 0.5).

The concentration of SARS-CoV-2-S glycoprotein was varying in the range from 0  $\mu\text{g/mL}$  to 25  $\mu\text{g/mL}$ . Some other reports described instability of the proteins in presence of salts [61, 62], but during the preparation of required concentrations no signs of instability of the SARS-CoV-2-S glycoprotein solubilized in PBS were observed. Figures 3B and C demonstrate the dependence of the chronoamperometric response (during pulsed amperometric detection) of MIP-Ppy and NIP-Ppy Pt electrodes modified with SARS-CoV-2-S glycoprotein in the PBS solution. The change in the chronoamperometric response is related to the adsorption of less conductive protein molecules on the MIP-Ppy and NIP-Ppy layers. When SARS-CoV-2-S glycoprotein concentration in solution was increased, the registered chronoamperometric response of both MIP-Ppy and NIP-Ppy-modified Pt electrodes decreased. Higher currents were registered before the incubation of electrode in SARS-CoV-2-S glycoprotein containing solution. This effect is determined by the presence of water

1 molecules and electrolyte ions in the places where molecular imprints were formed. After the  
 2 incubation in SARS-CoV-2-S glycoprotein containing solution, the ions of solvent and the  
 3 electrolyte were replaced by the molecules of SARS-CoV-2-S glycoprotein and thus the  
 4 registered current at the potential of +600 mV decreased.



**Fig. 4.** Calibration curves of  $\Delta I$  vs concentration of SARS-CoV-2-S glycoprotein and BSA on MIP-Ppy and NIP-Ppy according to the  $\Delta I$  calculated in respect to: **A** – the principal of  $\Delta I$  measuring;

**B** –  $\Delta I$ . RSD% was in range from 2 to 4.3% of current values of 5 potential pulses for the listed data points.

**Table 1.** Linear regression characteristics of current ( $\Delta I$ ,  $\mu A$ ) vs concentration of SARS-CoV-2-S glycoprotein (c,  $\mu g/mL$ ) on the MIP-Ppy and NIP-Ppy modified Pt electrodes.

	$y = ax+b$	a	b	$R^2$
SARS-CoV-2-S determined by MIP-Ppy-based electrode		$-0.46 \pm 0.04$	$23.4 \pm 0.7$	0.96
SARS-CoV-2-S determined by NIP-Ppy-based electrode		$-0.21 \pm 0.01$	$13.9 \pm 0.3$	0.98
BSA determined by MIP-Ppy-based electrode		$-0.15 \pm 0.01$	$15.7 \pm 0.2$	0.97
BSA determined by NIP-Ppy-based electrode		$-0.1 \pm 0.01$	$14.7 \pm 0.1$	0.97

The magnitude of current differences, which are registered during potential pulses at instants when potentials were stepped from 0 mV up to +600 mV and +600 mV down to 0 mV, has decreased with increasing SARS-CoV-2-S glycoprotein concentration in PBS solution (Fig. 4). Figure 4A represents the current profile, which was registered during potential pulses, and the way in which the analytical signals ( $\Delta I$ ) for the calibration curve was depicted. According to this calibration curve, linearity of analytical signal dependence on analyte concentration was observed at all evaluated SARS-CoV-2-S glycoprotein concentrations in the range from 0  $\mu g/mL$  to 25  $\mu g/mL$ .

The slope derived using the linear regression equation for the changes of current ( $\Delta I$ ,  $\mu A$ ) vs concentration of SARS-CoV-2-S glycoprotein (c,  $\mu g/mL$ ) registered by NIP-Ppy-modified Pt electrode was of  $-0.22 \mu A/(\mu g/mL)$  with  $R^2 = 0.98$  (Table 1). While the slope of linear regression for the Pt electrode modified with SARS-CoV-2-S glycoprotein imprinted MIP-Ppy was  $-0.47 \mu A/(\mu g/mL)$  with  $R^2 = 0.96$  (Table 1). The sensitivity calculated from the calibration curves of the MIP-Ppy modified Pt electrode towards SARS-CoV-2-S glycoprotein in the linear dependence interval according to the  $\Delta I$  measurements was approximately 2.1 times higher than that of NIP-Ppy modified Pt electrode. This difference is significant and therefore can be applied in the design of sensors based on MIP-Ppy modified Pt electrodes.

The same MIP-Ppy and NIP-Ppy modified Pt electrodes were evaluated for the interaction with BSA (Fig. 4B) to evaluate the selectivity of MIP-Ppy layer towards different proteins. The slope values for these measurements were derived using linear regression and they are represented in Table 1. The slope value ( $-0.15 \mu A/(\mu g/mL)$ ) registered by the MIP-Ppy modified Pt electrodes incubated in BSA containing solution was significantly lower.

The comparison of the sensitivity/selectivity results among studies, which are reporting MIPs sensors based on the different polymers is rather complicated, because several factors are playing an important role on the final result: (i) the design of the electrochemical cell, (ii) the electrochemical method used for evaluation of the sensor, (iii) nature of the polymer, etc.

There are published only very few studies concerning the application of molecular imprinting technology for the analysis of SARS-CoV-2 proteins. There was described the application study of o-phenylenediamine deposited on the macroporous gold screen-printed electrode with the receptor-binding domain of SARS-CoV-2-RBD for impedimetric measurements [55]. The described sensor was sensitive to the concentrations of SARS-CoV-2-RBD molecules in the range of pg/mL. In another study m-phenylenediamine (mPD) was imprinted with SARS-CoV-2 nucleoprotein (ncovNP). The sensitivity of the sensor according to the DPV signal was in the range of fM [54]. In purpose to demonstrate the selectivity of the sensor BSA and some more proteins were used in the study. The Ppy was imprinted with gp51 and was applied in the design of electrochemical sensor [42]. The sensitivity of the sensor according to the results of simplified pulsed amperometric detection was in the range of µg/mL. The electrochemical sensors based on Ppy with imprints of prostate-specific antigen (PSA) was reported in 2020 [15]. The square wave voltammetry technique was used to determinate PSA concentration. The described sensor was sensitive to the concentrations of PSA molecules in the range of pg/mL. The electrochemical MIP sensor based on Ppy and aminophenylboronic acid (p-APBA) bilayer was imprinted with lysozyme [46]. The sensitivity of the sensor according to the CV signal was in the range of ppm. Hence, several factors govern the sensitivity of the MIP sensors. The electrochemical method of chronoamperometry (pulsed amperometric detection) by the sequence of potential pulses was only occasionally used in previous studies. In present our study, it was demonstrated that the obtained MIP-Ppy modified Pt electrodes can be applied for the determination of imprinted SARS-CoV-2-S glycoproteins.

## Conclusions

Pt electrode was modified by two types of Ppy layers: (i) MIP-Ppy layer, which was modified by imprints of SARS-CoV-2-S glycoprotein and (ii) NIP-Ppy, which was formed without the imprint of any proteins. The comparison of the current changes on Pt electrode during the electrochemical deposition of MIP-Ppy and NIP-Ppy has demonstrated that the current for NIP-Ppy increased approximately by only 1.05 times more than that registered during the

deposition of MIP-Ppy layer. This means that the SARS-CoV-2-S glycoprotein, which serves as the template molecule for MIP-Ppy layer, does not have a crucial effect on the thickness of the deposited polymer layer and the initial characteristics of the formed MIP-Ppy and NIP-Ppy layers are comparable.

The comparison of calibration curves registered after the incubation of MIP-Ppy and NIP-Ppy modified Pt electrodes revealed that the interaction of SARS-CoV-2-S glycoprotein with MIP-Ppy generates 2.1 times higher change of current for MIP-Ppy modified electrode, in comparison with that registered for NIP-Ppy modified Pt electrode. The selectivity of SARS-CoV-2-S imprinted MIP-Ppy modified Pt electrode was tested in comparison to BSA solution. The obtained slope values during the evaluation of MIP-Ppy modified Pt electrode sensitivity towards BSA were significantly lower when compared with that towards SARS-CoV-2-S glycoprotein. The results of application of MIP-Ppy modified Pt electrodes demonstrated higher current changes in respect can be applied for selective determination of the imprinted SARS-CoV-2-S glycoprotein. Therefore, it can be concluded that the molecular imprinting of the conducting polymer might be applied for the development of the electrochemical sensor for the detection of SARS-CoV-2-S glycoprotein.

#### **Declaration of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### **CRedit authorship contribution statement:**

**Vilma Ratautaite** – Methodology, Investigation, Data analysis, Writing - original draft, Interpretation of data, Data analysis;

**Raimonda Boguzaite** – Methodology, Investigation, Data analysis, Writing - original draft, Interpretation of data, Data analysis;

**Ernestas Brazys** – Methodology, Investigation, Data analysis, Writing - original draft, Interpretation of data, Data analysis;

**Almira Ramanaviciene** – Writing - review & editing, Data analysis, Interpretation of data;

**Evaldas Ciplys** – Production of protein;

**Mindaugas Juozapaitis** – Production of protein;

**Rimantas Slibinskas** – Production of protein;

**Mikhael Bechelany** – Writing - review & editing;

1   **Arunas Ramanavicius** – Interpretation of data, Data analysis, Supervision, Conceptualization,  
2   Writing - review & editing, Funding acquisition.  
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