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# Electrochemical Biosensor for the Detection of Viruses and Possibly Covid-19

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## Abstract

The bio electrochemical behavior of West Nile virus at mixed carbon-phosphate paste electrode modified with Peroxidase-Secondary Antibody Conjugate (PAS) is reported. A Cyclic Voltammetry (VC), Square Wave Voltammetry and Impedance Spectroscopic methods were investigated. The influence of variables such as the accumulation time, pH solution and PAS loading was tested by different electrochemical methods. The detection limit is 1.8.10-3 and the quantification limit is 7.2.10-2. The bio electrode developed has shown high efficiency for the detection of antibodies to the viruses tested but also shows promising signs for the detection of covid-19 antibodies

**Keywords:** Biosensor; Modified electrode; Cyclic voltammetry; Covid-19; Detection of viruses; ELISA; West Nile virus

## Introduction

In recent years, a great deal of attention has been paid to virology, with the objective to develop appropriate and affordable methods for the detection of whole viruses or their fragments. Conventional methods commonly used for the detection of antibodies against viruses are related to Enzyme Linked Immunosorbent Assays (ELISA) [1], Hemoagglutination Inhibition (HI) [2,3] and Western Blot assay (WB) [4]. However, they are often laborious and time-consuming or require expensive instruments and are only available in experienced laboratories. Furthermore, emergency cases require a rapid portable detection system.

Consequently, an important need still exists to create simple, sensitive and inexpensive diagnostic methods for the detection of antibodies against different types of viruses. Immunosensors incorporating specific antigen are promising alternative systems for the direct detection of biomolecules such as immunospecies. Low sample consumption, reasonable instrument cost and good possibility for miniaturization and minimization of analysis time in immunoassay are the main reasons for the extensive development of electrochemical immunosensors for immunoassay.

Several different types of immunosensors have been successfully developed for the detection of various types of antibodies [5], his-tagged proteins [6] or antigens.

In this paper, we present a sensitive and selective immunosensor for the detection of antibodies against West Nile virus which could also be used to detect the presence of corvid-19 antibodies. The basis is the species-induced electrical changes in the biological species, in particular the specific interaction between the peroxidase-secondary antibody conjugate (HRP-conjugate) and West Nile virus-specific antibodies (antibody/secondary antibody binding) evaluated mainly by Cyclic Voltametry (CV) and Square Wave Voltametry (SQW) and electrochemical impedance spectroscopy in the presence of NaCl as electrolyte media. Finally, the immunosensor was used for the detection of West Nile virus-specific antibody response in quail serum samples and the results were compared with those obtained with ELISA [6-9].

## Materials and Methods

### Apparatus and software

**Electrochemical techniques:** Cyclic Voltammeter (VC) and Impedance Spectroscopy (IES) were carried out using a voltalab potentiostat (model PGSTAT 100, Eco Chemie BV, Utrecht, The Netherlands) controlled by Voltalab Master 4 software. A conventional three-electrode system was used; the prepared electrodes are used as the working electrode. The platinum plate (1 cm × cm) is a counter electrode and the Ag-AgCl electrode is used as the reference electrode.

**Electrodes:** The carbon paste electrode is shaped by the following method: by mixing the graphite carbon powder with Natural Phosphate and ethanol in a petri dish to obtain the desired paste shape, then a few drops of paraffin oil, it is a binder that ensures the intermolecular cohesion of the products, after the dough is introduced into the port of the prepared electrode, the electrical conduction is provided by a carbon rod. The modified electrodes were immersed in a Peroxidase-Secondary Antibody conjugate (PAS) solution. The effect of time of cumulation and concentration of solution were studied. Then the modified electrodes (CPE-NP modified with PAS) were immersed in a cell 20 ml of distilled water, 0.1M NaCl and the antibodie suspensions and then characterized as a function of time by cyclic voltammetry and by electrochemical impedance spectroscopy.

Figure 1 represents the cyclic voltammograms recorded at the CPE-NP electrode modified with PAS, with a scanning speed equal to 100 mV/s in the NaCl solution (0.1 mol/l) containing or not antibodie in a range of potential between -2V and 2V. It can be seen that the presence of antibodie is manifested by the appearance of a reduction peak at around 0.2V.

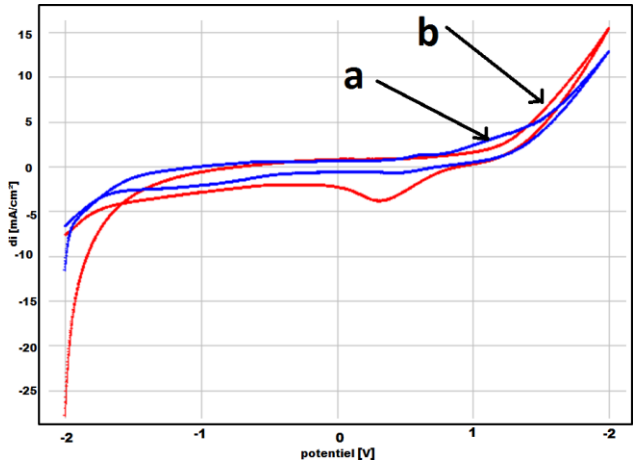


**Figure 1:** Overlay of cyclic voltammograms of CPE-NP/PAS (a) without antibodies and CPE-NP/PAS with antibodies (b), in NaCl at 0.1 M; v=100 mV/s; pH=7.

The morphology of the electrode surface of CPE-NP modified with PAS, before incubation in antibodies (curve a) and after 20 min incubation in antibodies (curve b), was observed by optical scanning microscopy (Figure 2). It can be seen that the presence of the antibody is manifested by the appearance of three-dimensional reliefs, deposited over the entire surface of the electrode. The antibodies have attached to the electrode surface probably *via* redox properties.

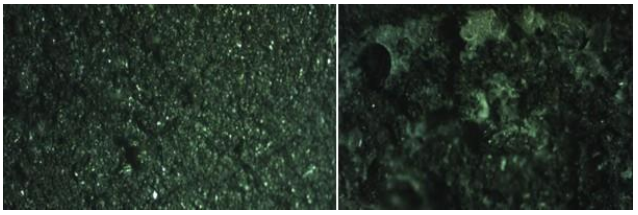

**Figure 2:** Micrograph of the CPE-NP/PAS electrode before incubation in antibodies (a) and after 20 min incubation in antibodies (b).

Figure 3 illustre the overall scenario reflecting the proposed mechanism from the development of the PAS-modified CPE-NP electrode to the adsorption of the antibody using the redox properties of the PAS and the biological analyte (Antibody).

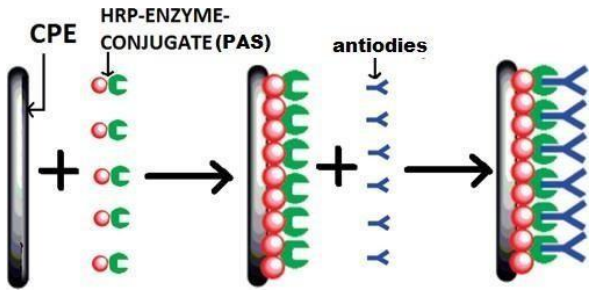


**Figure 3:** Schematic representation of the preparation of an immuno-detection layer.

## Effect of antibody incubation time

The CPE-NP/PAS immunological sensor was incubated with a solution containing antibodies at different times. The results are shown in Figure 4, by cyclic voltammetry. During the first 20 min of antibody contact with the CPE-NP electrode modified with PAS, the cathode peak current density increases rapidly with the incubation time, which indicates a good electroactivity on the electrode surface due to the reaction between the antibodies and the secondary peroxidase-antibody, more precisely to the formation of the bond between the antibodies and the secondary antibodies, After 20 min, it becomes almost constant and the tendency to saturate, due to the probable saturation of the antibody-secondary antibody binding sites conjugated to peroxidase-conjugated peroxidase.

Thus, 20 min was selected as the optimal incubation time to maximize the signal and minimize the assay time.

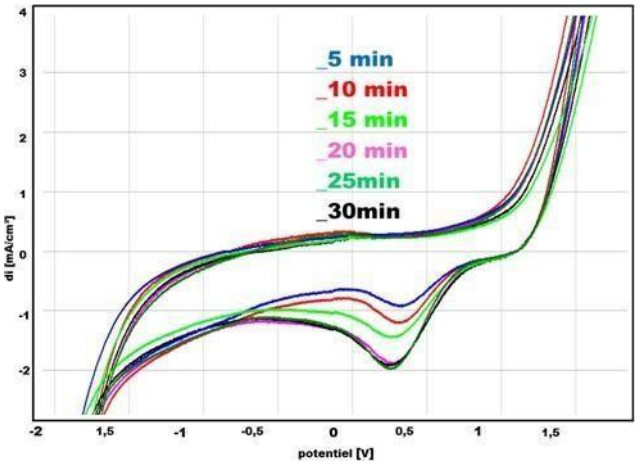


**Figure 4:** Superimposition of cyclic voltammograms of CPE-NP/PAS at different deposition times, in NaCl at 0.1 M; v=100 mV/s, from -2V to 2V; pH=7.

After each incubation time of the PAS-modified CPE-NP electrode, samples of the solution containing the antibodies were taken to measure the optical density of the sample after the Elisa test. The results are shown in Table 1 and in Figure 5.

| Time (m) | 5 | 10 | 15 | 20 | 30 | 40 | 50 |
|---|---|---|---|---|---|---|---|
| DO | 0.235 | 0.265 | 0.272 | 0.289 | 0.3 | 0.298 | 0.3 |
| S/N | 0.144 | 0.162 | 0.166 | 0.173 | 0.184 | 0.182 | 0.184 |

**Table 1:** Optical Density as a function of contact time of CPE-NP/PAS with antibodies.

S/N is the percentage given by the following equation:

$$S/N = \frac{DOechantillon}{DOcn} \times 100$$

- Samples with S/N less than or equal to 50% are considered negative.

- Samples with an S/N greater than 50% are considered positive.

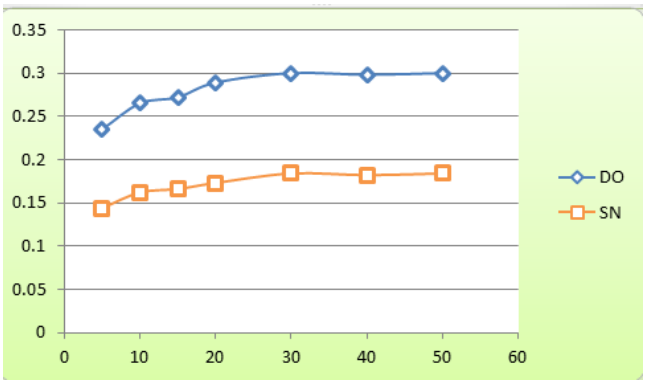


**Figure 5:** Optical density as a function of contact time of CPE-NP/PAS With the antibodies.

## Effect of antibody dilution factor (the concentration effect)

The immunosensor was incubated in different media containing increasing concentrations of antibodies for 20 min. An increase in current density was recorded as the concentration of antibodies increased. As shown in Figure 6. The detection power of the electrode therefore increases with increasing antibody loading up to C2 and then the tendency to saturate

At low concentrations of adsorbed antibodies, the signal is very low but not zero. Antibodies immobilized on the surface do not ensure complete coverage of all active sites.

At higher concentrations, it is evident that the signal increases, revealing the specific adsorption of antibodies to the adsorbed conjugate. A slight tendency to saturation is shown at the highest concentration (>C2); this tendency is attributed to saturation of the surface binding sites.

This electrochemical behavior of the electrode is confirmed by electrochemical impedance spectroscopy (Figure 7), EIS curves have the shape of a half-loop, the diameter of which corresponds to the electron transfer resistance.

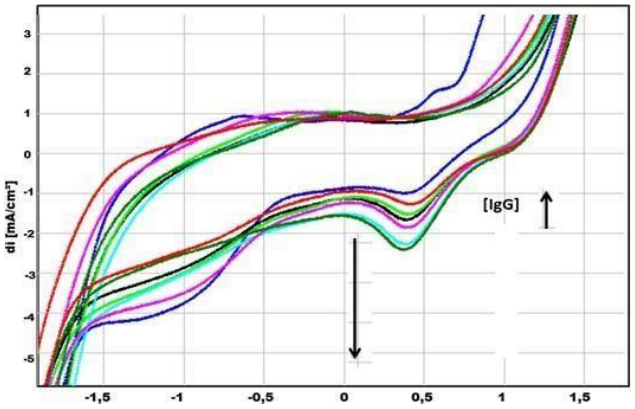


**Figure 6:** Superimposition of cyclic voltammograms of CPE-NP/PAS at different antibody concentrations in NaCl at 0.1 M; v=100 mV/s, from -2V to 2V; pH=7.

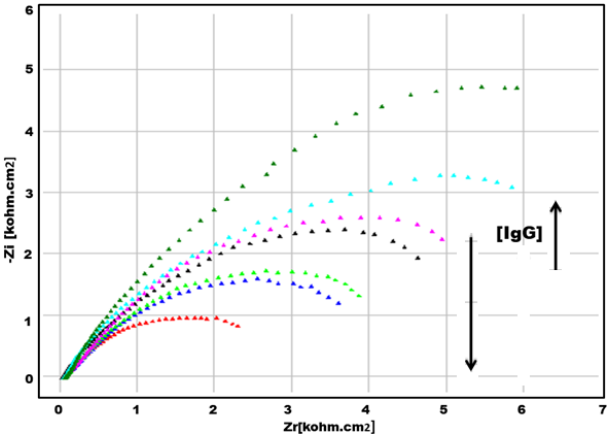


**Figure 7:** Superimposition of electrochemical impedance spectra of CPE-NP/PAS at different antibody concentrations in NaCl at 0.1 M; 100 mHz at pH=7.

## Study of the effect of Optical Density (OD) of antibodies on CPE-NP/PAS

The calibration curve was plotted under the optimised conditions, previously determined, using cyclic voltammetry. The current intensity of the cathodic peak on CPE-NP/PAS is proportional to the Optical Density (OD) of the antibodies obtained after the classical ELISA method, in the range of C6 to C1 (Table 2). This linearity is expressed by the following relationship: Di=11.79, DO=5.292, $R^2$=0.982.

| Sample | C1 | C2 | C3 | C4 | C5 | C6 |
|---|---|---|---|---|---|---|
| Current Density | -0.75 | -0.92 | -1.28 | -1.53 | -1.86 | -2.28 |
| DO | 0.3898 | 0.3689 | 0.3413 | 0.3112 | 0.2852 | 0.2651 |

**Table 2:** Influence of antibody concentration on the intensity of reduction peaks Obtained by VC on the surface of CPE-NP/PAS.

## Determination of the limit of detection and quantification of CPE-NP/PAS

According to Miller and Miller [2], the Standard Deviation of the measured mean current (SD) can be modeled by the equation:

$$SD = \frac{1}{(n-2)} \sum_{j=1}^{n} (i_j - I_j)^2$$

Where ij is the experimental value of the current identified at manipulation j and Ij is the corresponding value calculated at the same optical density using the calibration equation. n is the number of measurements performed. The calculated SD (Standard Deviation) value is used to determine the Detection Limit (LD) and Quantitation Limit (LQ).

LD= 3 × SD/slope

LQ= 10 × SD/slope

For the PAS-modified CPE-NP electrode, the detection limit is 1.8.10-3 and the quantification limit is 7.2.10-2.

### Analytical application

The applicability of the immunosensor for the detection of antibodies in quail serum was demonstrated. 5 quail serum samples with different degrees of infection were tested. The results are given in Table 3, which demonstrate that the immunosensor meets the requirements for clinical analysis to assess the degree of West Nile infection in quail serum.

| Sample quailserum (2 ml) | Volume added positive serum(µl) | Current density (mA.cm-2) | OD found electrochemically | OD according to Elisa test | S/N found electrochemically | | S/N according to Elisa test | |
|---|---|---|---|---|---|---|---|---|
| 1 | 100 | -1,23 | 0,3445 | 0,35989 | 33% | positive | 34% | positive |
| 2 | 150 | -1,61 | 0,3122 | 0,32132 | 30,3% | positive | 30,8 % | positive |
| 3 | 200 | -1,92 | 0,2860 | 0,29124 | 27,5% | positive | 28% | positive |
| 4 | 250 | -2,47 | 0,2393 | 0,25523 | 23% | positive | 24,3 % | positive |
| 5 | 300 | -3,78 | 0,1282 | 0 ,1412 | 12,32% | positive | 13,35 | positive |

**Table 3:** Analytical application results.

## Conclusion

This work demonstrated that the mixed carbon-phosphate paste electrode modified with PAS is a feasible alternative for the bio analytical determination of various viruses. Analytical results show that the proposed is comparable to the ELISA test, but much faster and much less expensive, with good sensitivity and reproducibility.

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