www.elsevier.com/locate/talanta

Novel electrochemical sensing platform based on a nanocomposite of PVA / PVP / RGO applied to IgG anti-*Toxoplasma gondii* antibodies quantitation

Eduardo A. Takara, Sirley V. Pereira, María L. Scala-Benuzzi, Martín A. Fernández-Baldo, Julio Raba, Germán A. Messina

PII: S0039-9140(18)31222-0
DOI: https://doi.org/10.1016/j.talanta.2018.11.070
Reference: TAL19303

To appear in: *Talanta*

Received date: 22 August 2018
Revised date: 16 November 2018
Accepted date: 21 November 2018

Cite this article as: Eduardo A. Takara, Sirley V. Pereira, María L. Scala-Benuzzi, Martín A. Fernández-Baldo, Julio Raba and Germán A. Messina, Novel electrochemical sensing platform based on a nanocomposite of PVA / PVP / RGO applied to IgG anti-*Toxoplasma gondii* antibodies quantitation, *Talanta,* https://doi.org/10.1016/j.talanta.2018.11.070

Novel electrochemical sensing platform based on a nanocomposite of PVA / PVP / RGO applied to IgG anti- *Toxoplasma gondii* antibodies quantitation

Eduardo A. Takara, Sirley V. Pereira, María L. Scala-Benuzzi, Martín A. Fernández-Baldo, Julio Raba, Germán A. Messina*

INQUISAL, Departamento de Química, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco 917, D5700BWS. San Luis, Argentina.

*Author to whom correspondence should be addressed: INQUISAL, Departamento de Química. Universidad Nacional de San Luis, CONICET. Chacabuco 917. D5700BWS. San Luis, Argentina. (e-mail) messina@unsl.edu.ar. (Tel.) +54-266-4425385; (Fax) +54-266-443-0224.

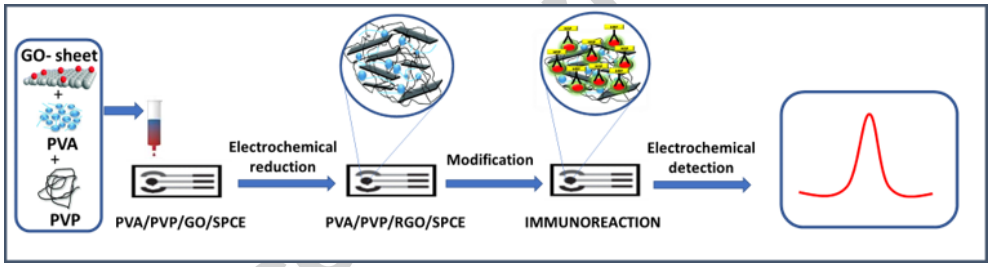
Abstract

This article describes the development of a new electrochemical platform composed by a polymer mixture and graphene oxide (GO). The working electrode of a screen-printed carbon electrode (SPCE) was modified with nanocomposite constituted by poly-vinyl alcohol (PVA), poly-vinylpyrrolidone (PVP) and GO, which was electrochemically reduced to obtain PVA/PVP/RGO/SPCE. The interactions and morphology of the PVA/PVP/GO nanocomposite were investigated by SEM, FTIR and UV-Vis. SEM images indicated an excellent dispersion of the GO sheets in the polymer matrix. Besides, FTIR and visible UV studies revealed strong interactions between

polymer mixture and GO sheets. According to electrochemical studies, the new platform increased the electroactive surface area by a factor of 20.46 compared to the unmodified SPCE. Also, the PVA/PVP/RGO/SPCE had a fast electron kinetics transfer process with a value of $k_s = 9.6\ s^{-1}$.

The modified electrode was applied to the determination of IgG anti-*T. gondii* antibodies for the serological diagnosis of toxoplasmosis. The IgG anti-*T. gondii* antibodies quantification showed a detection limit of 0.012 U mL^{-1}, and the coefficients of variation intra-day and inter-day assays were lower than 4.5 % and 6.2 %, respectively. The electrochemical platform proved to be a sensitive and easily applicable tool applied to the serological diagnosis of toxoplasmosis. Therefore, the developed nanocomposite represents an excellent alternative for the electrochemical biosensor fabrication.

Graphical abstract



Keywords: Electrochemical immunosensors; Toxoplasmosis; Nanocomposite; Graphene oxide; poly-vinyl alcohol; Poly-vinylpyrrolidone.

1. Introduction

In the last years, electrochemical immunosensors have demonstrated to be interesting alternatives for clinical diagnosis due to their sensitivity and selectivity compared to conventional methodologies *[1]*. The electrochemical immunosensors-based on SPCEs have been considered as potential point-of-care testing devices because they are portable, easy to use, inexpensive, require small volume of reagents, and provides rapid analysis results *[2]*. Moreover, the SPCEs surface allows to incorporate different kind of materials. Particularly, the incorporation of graphene represents an attractive strategy due to its intrinsic properties. Reduced graphene oxide (RGO) is a carbon based nanomaterial which exhibit important properties for sensor applications, such as excellent electric conductivity, elevated electron mobility, high surface area, high sensitivity and low cost *[3]*. Nevertheless, RGO is a hydrophobic material which tends to agglomerate irreversibly in an aqueous medium *[4]*.

Polymer blending is a simple and practical method to generate novel materials. The GO incorporation to these materials is an interesting option to overcome the mentioned disadvantage *[5]*. The mixture of polymers and RGO allows the nanomaterial dispersion and the possibility of covalent biomolecules immobilization. Among commonly used polymeric matrices, PVP is an amphiphilic polymer with good electrical properties*[6]* while PVA is a semicrystalline polymer which presents OH groups and poor electrical properties.

According to our scientific bibliography search, there is few reported articles related to the combination of these three materials PVA/PVP/GO, furthermore there are no works developed in the field of biosensors *[7, 8]*. However, some related articles have been reported, for example, the polymer mixtures PVA/PVP were widely used in the field of membranes; these have shown a great compatibility, good conductivity and excellent stability in physiological conditions *[9-11]*. Some articles describe that

PVA/GO nanocomposite is a homogeneous mixture that exhibits an excellent biocompatible surface to immobilize biological elements *[12]*. While, the PVP/GO nanocomposite shows higher conductivity than PVA/GO and the polymer presents an excellent compatibility with GO and RGO *[13]*. Then, PVA/PVP/GO nanocomposite could offer an easily functionalization surface, improvement of the conductivity and increased surface area. For these reasons, the SPCE modification with the PVP/PVA/GO nanocomposite and its subsequent electrochemical reduction represents an attractive strategy for the development of electrochemical immunosensors.

In this work, the PVP/PVA/RGO/SPCE as sensing platform was applied for the design of an immunosensor for IgG-anti *T. gondii* antibodies determination. Toxoplasmosis is a zoonotic disease caused by the intracellular parasite *Toxoplasma gondii [14]*. The infection by *T. gondii* has an acute and chronic phase. In immunocompetent people, the acute phase is often asymptomatic *[14]*. For this reason, the diagnosis of toxoplasmosis is difficult and of great importance because clinical features are not sufficient to discriminate between toxoplasmosis and other illnesses *[15]*. Serological tests are the most widely used tools for the diagnosis of toxoplasmosis *[15, 16]*. However, these methods have poor efficiency, require highly qualified personnel, consume a lot of time and need sophisticated instrumentation *[17]*. Therefore, the development of a new method with high sensitivity and specificity for the direct determination of specific IgG anti-*T. gondii* antibodies is highly desirable.

The goal of this work was the development and characterization of a nanocomposite applied to the electrochemical immunosensor fabrication. The analytical performance of the immunosensor was tested by the IgG anti-*T. gondii* antibodies quantification in serum samples. SPCE was modified with a nanocomposite of PVA, PVP and GO which was electrochemically reduced. The nanocomposite was

characterized morphologically, chemically and electrochemically. The excellent qualities of the designed immunosensor clearly indicate that PVA/PVP/RGO/SPCE represents a promising analytical tool for the clinical diagnosis of toxoplasmosis.

2. Experimental

2.1 Reagents and solutions

Glutaraldehyde (25% aqueous solution) was purchased from Merck, Darmstadt. 4-Tert-Butylcatechol (4-TBC), PVA Mw 89,000-98,000, PVP Mw 84,000 and graphite were purchased from Sigma aldrich. All reagents used were of analytical grade without prior purifications. The commercial ELISA kit (ab108776) for the quantitative determination of IgG anti-*T. gondii* biomarker was purchased from abcam (Tecnolab S.A) and it was used according to the manufacturer's instructions. Aqueous solutions were prepared with water Milli-Q.

2.2 Instrumentation

The electrochemical characterizations were performed by a BAS 100 B/W (electrochemical analyzer Bioanalytical System, West Lafayette, IN), while amperometric detection was carried out using a BAS LC-4C potentiostat. SPCE with CE (carbon) + RE (Silver) + WE (carbon) was purchased from Ital Sens IS C (Palmsens, Italy). According to the manufacturer the geometric area and Ø of WE are 7.07 mm^2 and 3 mm, respectively. UV-Vis characterization studies were performed using a UV–vis spectrophotometer model UV-1650 (Shimadzu, USA). The infrared spectroscopy evaluations were carried out by a Varian 640 Spectrometer. SEM images were obtained by LEO 1450VP.

2.3 Synthesis of nanocomposite

Graphite oxide (GO) was synthesized by a modified Hummer's method *[18]*. GO was suspended in distilled water obtaining a brown dispersion. After, it was exposed to an exfoliation process by ultrasonication for 10 h. Unexfoliated graphite oxide present in the solution was removed by centrifugation at 3700 rpm for 30 min. Finally, graphene oxide solution with a final concentration of 7 mg. mL^{-1} was obtained.

In order to obtain the PVA/PVP/GO nanocomposite, the GO was dispersed in PVA/PVP polymer solution. PVA solution was prepared by dissolving 20 mg of the polymer in distilled water (1 mL) with continuous stirring for 2 h at 90 ° C. While 20 mg of PVP were dissolved in water (1 mL) under continuous stirring for 1 h at room temperature to obtain the PVP solution. The optimal concentrations of GO, PVA and PVP were adequately studied by cyclic voltammetry.

2.4 Electrode modification

The electrode surface was pretreated to oxidize graphite impurities and obtain a more hydrophilic surface *[19]*. Briefly, a 0.25 M acetate buffer (pH 4.4) solution containing 0.1 mM KCl was added to SPCE and anodic potentials of 1.6 V for 2 min and 1.8 V for 1 min were applied. Then, the electrode was washed several times using 0.01 M phosphate buffered saline (PBS, pH 7.2) and preserved until its use in the same buffer at 4 °C.

After electrode preconditioning, 5 μL of PVA/PVP/GO solution (0.7 mg mL^{-1} oxidation graphene, 0.01% PVP, 0.0066% PVA) were placed onto the working electrode area and dried at 50 °C for 1 hour. Then, the PVA/PVP/GO/SPCE was

electrochemically reduced for 1200 s at -1.2 V (0.5 M $NaNO_3$, pH 4.0), obtaining the PVA/PVP/RGO/SPCE (Fig. 1).

2.5 Characterization of modified electrode

The modified electrode surface was characterized by SEM. The interactions of the PVA/PVP/GO nanocomposite were investigated by UV-Vis and FTIR. FTIR spectra were obtained with resolution of 4 cm^{-1} and consisted of approximately 3500 points. Integration times were of 60 s (1 s per scan) and the number of scans for each sample of 64.

The electrochemical performance of the PVA/PVP/RGO/SPCE sensor was studied by cyclic voltammetry (scan rate of 100 mVs^{-1} at 25 °C). In order to know the electroactive surface area, the Randles–Sevcik equation was used *[20]*. Besides, the electron transfer rate constant (k_s) for the modified electrode was calculated by the Laviron equation *[21]*. The theoretical models used are present in supplementary data (SM. 1).

2.6 Functionalization of PVA/PVP/RGO/SPCE

In order to applied the PVA/PVP/RGO/SPCE for immunosensor development, its surface was functionalized by the addition of glutaraldehyde solution (5 % w/w, pH = 2 HCl) and it was left to react for 2 h at room temperature. In this step, the aldehyde groups reacted with free -OH groups present in the nanocomposite. Then, the electrode was washed and immersed in the *T. gondii* antigen solution (Ag) (100 µg mL^{-1}) for 2 h at 37 °C (*T. gondii* antigen obtainment is described in supplementary material, SM. 1). The free aldehydes on the electrode surface covalently reacted with the -NH_2 groups of

antigens, thus, the functionalized surface of the modified electrode was obtained (Ag/PVA/PVP/RGO/SPCE).

2.7. IgG anti-*T. gondii* antibodies detection

Before the IgG anti-*T. gondii* antibodies detection, the unspecific bindings were blocked by immersing the electrode in a 1 % BSA solution in 0.01 mol L^{-1} PBS (phosphate buffered saline pH 7) for 5 min at room temperature. After each step, the excess of reagents was eliminated by three consecutive washes with PBS. Later, the electrode was immersed in a serum sample (diluted 100-fold with 0.01 mol L^{-1} PBS pH 7) for 10 min at 37 °C, where anti-*T. gondii* antibodies specifically recognized the immobilized antigen. Then, it was exposed to anti-human IgG antibodies conjugated with HRP (horseradish peroxidase) (anti-IgG–HRP) for 10 min at 37 °C (anti-IgG–HRP, dilution of 1/1000 in 0.01 mol L^{-1} PBS pH 7). Subsequently, a substrate solution (1 mmol L^{-1} H_2O_2 and 1 mmol L^{-1}, 4-TBC in 0.01 mol L^{-1} phosphate–citrate buffer pH 5) was added on the electrode surface at 25 °C. HRP, in the presence of H_2O_2, catalyzes the oxidation of 4-TBC (H_2Q) to o-benzoquinone (Q). When a potential of −0.15 V is applied, Q is electrochemically reduced to H_2Q. The obtained current peak from the product of the enzymatic reaction was proportional to the IgG anti-*T. gondii* concentration (Fig. 1).

3. Results and discussion

3.1 Characterization of nanocomposite

The interactions and morphology of the PVA/PVP/GO nanocomposite were investigated by SEM, FTIR and UV-Vis.

The SEM images of the modified electrode show a homogeneous surface, indicating an excellent dispersion of graphene oxide in the polymeric solution (Fig. 2-A and B).

UV–vis measurements were carried out for GO and polymers-GO aqueous dispersions in a region from 190 to 400 nm using pure water as reference. Fig. 2-C shows the absorption UV-Vis spectra of pure GO, PVA and PVP. The GO spectrum exhibited two characteristic bands, a major absorption peak at ~233 nm, attributed to π / π * transition and a shoulder peak at ~300 nm originated from n / π *, due to the presence of different oxygen functional groups in GO *[22]*. For PVA, the absorption peak at ~209 nm could be attributed to the n → σ* transition which is very sensitive to hydrogen bonding *[23]*. While PVP presented a band at 218 nm assigned to n / π*. Another absorption shoulder was observed at about 225–235 nm, it could be attributed to unsaturated bonds *[14]*. The PVA/PVP/GO nanocomposite showed adsorption bands very similar to GO, attributed to the superposition of bands corresponding to each component microstructure. However, it was possible to visualize a decrease of the shoulder signal at 300 nm. This could be related to the interaction of different oxygen functional groups in the GO structure with the polymers matrices.

Infrared spectra were obtained by a Fourier transform infrared spectrometer in a region from 4000 to 900 cm^{-1}. FTIR patterns of PVA, PVP, GO, PVA/GO, PVP /GO and PVA/PVP/GO are shown in Fig. 3. The characteristic bands of the GO *[24]*, PVP *[25]*, and PVA *[26]* structures are detailed in Table 1.

The spectrum of PVP/GO indicated the decrease of the bands at 1726 and 1258 cm^{-1} (corresponding to GO) and a shifted of the C=O signal from 1637 to 1643 cm^{-1} (corresponding to PVP), compared with PVP spectrum (Fig. 3-B). Also, the decrease of the OH groups signal at about 3325 cm^{-1} was observed. These mentioned behaviors

could be due to the hydrogen bond between C=O of PVP and hydroxyl groups on the surface of GO (C=O/-OH) *[27]*. The combination of GO with PVA showed that the band of OH groups was shifted to smaller wavenumbers, from 3310 to 3290 cm^{-1} compared with PVA spectrum (Fig. 3-B), which could be attributed to the dissociation of the hydrogen bond among the PVA hydroxyl groups *[28]*. Chenlu Bao et al showed that the decrease of hydrogen bonds between PVA chains is probably due to the incorporation of GO nano-sheets, which interferes with the hydrogen bonds. In addition, they demonstrated that new hydrogen bonds are generated between GO nano-sheets and the PVA matrix *[29]*.

The PVA/PVP/GO spectrum (Fig. 3-A) indicated a greater decrease and widening of the OH band at about 3345 cm^{-1} and a reduction of C=O signal at 1726 cm^{-1}, compared with PVA/GO and PVP/GO. Also, it was observed that the C–N single bond strength increased, which caused the slight increase of the wavenumber from 1280 to 1284 cm^{-1} of the C-N group. These phenomena indicated that the mixture PVA/PVP/GO increased the H-bonds due to interactions among -OH and C=O of GO, C=O of PVP and -OH of PVA, mainly *[30, 31]*. The IR results confirm that the polymers successfully wrap the graphene surface, generating a great stability of the suspension and an excellent GO layers dispersion. Moreover, it is important to highlight the presence of free -OH groups in the nanocomposite which provide reaction sites for biomolecule covalent immobilization.

3.2 Characterization of the PVA/PVP/RGO/SPCE electrode surface

The obtained nanocomposite film thickness on the SPCE surface depends of the PVA, PVP and GO concentrations, and this affect the electrochemical response. This

effect was electrochemically studied using a 1 mmol L^{-1} $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ in 0.1 mol L^{-1} KCl solution. The GO concentration effect on the oxidation current peaks was studied varying the concentration of GO on the electrode surface and its subsequently electroreduction (RGO). As shown in Fig. 4-A, a RGO concentration of 0.7 mg/mL generated the major increase of the current peak. This increase of the current could be attributed to the excellent RGO electrical conductivity and the increase of the electroactive surface area. Fig. 4-B shows the different polymeric concentrations studied at a fixed concentration of GO (PVA or PVP/RGO/SPCE). The Fig. 4-B shows the reduction of the electrode conduction at different PVA or PVP concentrations, compared with RGO/SPCE. This is attributed to the insulating character of PVA and the interruption of the electron transfer (PVA and PVP polymer matrix) causing an increase of the VC base line *[32]*. However, the PVP/PVA mixture allowed us to obtain a homogeneous nanocomposite with high adhesion to SPCE surface and chemical groups for covalent immobilization of biomolecules. Experimental results indicated that 0.7 mg mL^{-1} for GO, 1 mL 0.01% PVP and 1 mL 0.006% PVA was the optimum relation.

PVA/PVP/RGO/SPCE provided a considerable increase in the anodic and cathodic peak currents (4.9 times) compared to the unmodified SPCE (Fig. 5-A). Furthermore, the voltammograms for SPCE and PVA/PVP/RGO/SPCE at 100 mVs^{-1} exhibited a peak separation (ΔE_p) of 178 and 109 mV, respectively. These peak separation values suggest a more favorable electrochemical interaction at the electrode surface for the modified electrode (smaller ΔE_p) *[33]*. Fig. 5-B shows cyclic voltammograms of the PVA/PVP/RGO/SPCE at various scan rates. When the scan rate increased, the redox potentials (E_{pa} and E_{pc}) of electrode changed lightly. The redox peak current increased linearly with the scan rate (Fig. 5-C): $I_{pa}(\mu A) = 94\ v^{1/2}\ (V.s^{-1})$–

0.16, r = 0.973; $I_{pc}(\mu A) = -89\ v^{1/2}\ (V.s^{-1}) - 1.3$, r = 0.969. An I_{pa}/I_{pc} ratio close to unity was also observed. These behaviors indicated a diffusion-controlled electrochemical process *[34, 35]*. The dependence on the semi-infinite diffusion model established by Randles–Sevcik *[36]* was analyzed graphically (Ep vs log v, Fig. 5-D). The gradient of 0.54 (r =0.975) was obtained for PVA/PVP/RGO/SCPE, indicating that the electrochemical reaction was not trapped within the network of the film (no thin-layer effects) and representing a purely diffusional response *[37]*.

The calculated effective surface area of modified SPCE was 0.134 ± 0.011 cm^2. This result indicated that the modification procedure increased the electroactive surface area by a factor of 20.46 ± 0.3, in accordance with the increase of the current.

The electron-transfer-rate constant (k_s) of PVA/PVP/RGO/SPCE was estimated using the Laviron's model at high scan rates. The obtained plots of E_{pa} and E_{pc} vs. the logarithm of the scan rates were linearly fitting at scan rates from 100 mVs^{-1} to 300 mVs^{-1} (Fig. 5-D), obtaining $E_{pa}(V) = 0.127 \log v\ (V\ s^{-1}) + 0.346$ (r = 0.965) and $E_{pc}(V) = -0.049 \log v\ (V\ s^{-1}) + 0.046$ (r = 0.977), respectively. Then, according to these slopes, an α value of 0.53 ± 0.06 and consequently a k_s of about 9.6 ± 0.31 s^{-1} were obtained. These results indicated that there is a fast electron transfer kinetics process which could be related to the interactions present in the PVA/PVP/RGO nanocomposite.

3.3 Immunosensor parameters Optimization

The immunodetection procedure required the optimization of several parameters: antigen concentration, blocking solution concentration, sample dilution, conjugated dilution, incubation time, H_2O_2 concentration, 4-TBC concentration; and pH

range. The optimization procedures were described in supplementary data (SM. 2). The optimal experimental conditions were summarized in Table 2.

3.4 Analytical performance of immunosensor

The performance of the PVA/PVP/RGO/SPCE functionalized with *T. gondii* antigens was evaluated in 18 human serum samples and 2 control samples using the detection procedure and experimental conditions above described.

An anti-*T. gondii* IgG antibodies calibration plot was obtained. A linear relation, $I\ (nA) = 4.87 + 14.98\ [C]_{IgG\ antibodies}$ ($r = 0.998$), was observed in the range of 0 and 200 U mL^{-1} (see supplementary material SM. 3). The coefficient of variation (CV) for the determination of 100 U mL^{-1} anti-*T. gondii* specific antibodies was below 4.4 % (five replicates). The detection limit (LOD) of the electrochemical method was 0.012 U mL^{-1} and it was calculated based on the signal-to-noise ratio (S/N) of 3.3.

The precision of our system was studied. The anti-*T. gondii* determination showed good precision; the CV within-assay values were below 4.4 % and the between-assay values were below 6.2 % for three consecutive days (Table SM. 4).

The ELISA procedure, which is currently used in clinical diagnosis, was also carried out. Absorbance changes were plotted against the anti-*T. gondii* IgG antibody concentration obtaining the corresponding calibration curve. The linear regression equation was $A = 0.006 + 0.004\ [C]_{IgG\ antibodies}$ ($r = 0.994$). The CV for the determination of 100 U mL^{-1} of anti-*T. gondii* was 5.9 % (five replicate). For ELISA procedure, the LOD was 2.58 U mL^{-1}.

The selectivity of the system was investigated against serum samples from patients who suffer Hidatidosis (*E. granulosus*), Toxocariosis (*T. canis*), Chagas (*T. cruzi*) and hypergammaglobulinemia (HGG) disease. The serum samples from patients who suffer HGG and Hidatidosis exhibited cross-reaction with an increase in the analytical signal of 5.6% and 3.2%, respectively. The other agents displayed negligible signals. (see Fig. SM. 5 supplementary data). The results indicated an excellent selectivity for anti-*T. gondii*.

The 9 positive, 9 negative serum samples and 2 control samples confirmed with ELISA test Kit were analyzed with our proposed immunosensor and showed excellent concordance. The cut off value used to identify positive and negative samples according to Toxoplasmosis disease prevalence was 30 U mL^{-1}. A correlation of both methods is presented in the supplementary material (Fig., SM. 6).

Regarding to the stability, the modified electrode was lyophilized and kept it in storage conditions at 4 °C for 2 months. After this time, the currents values obtained decreases at 98% of its initial value. This result indicates the excellent stability of the PVA/PVP/RGO/SPCE functionalized with *T. gondii*.

Currently, there are few reported articles that employ the mixture PVA/PVP /GO for electrode modification [38]. So, it is important to highlight that the combination presented in this article (PVA/PVP/RGO/SPCE) is the first reported for the development of biosensors. Considering the excellent electrochemical performance obtained with PVA/PVP/RGO/SPCE, our modified electrode showed to be an attractive tool for the development of electrochemical biosensors. Moreover, in relation to our bibliographic search in scientific literature, some immunosensors have been reported for the serological quantitation of anti-*T. gondii* IgG antibodies. H Wang et al. developed piezoelectric biosensors incorporating Au and silica nanoparticles *[39, 40]* . These

methods generate semicuatitative results expressed in dilution. This reason makes impossible the comparison with the results obtained by using our device. While S. Jiang et al. *[41]* studied an amperometric biosensor based on the use of goldmag nanoparticles and graphene sheets on a glass carbon bare electrode. This quantitative method presented a lower linear range and a higher detection limit than the reported in our work. Medawar et al. *[42]* reported the design and construction of a microfluidic device incorporating ZnO-NPs covered by chitosan as bioaffinity support for anti-*T. gondii* IgG antibodies determination, which has a lower LOD than the present work but using laser-induced fluorescence as detection system. All mentioned immunosensors are summarized in the Table SM 7. To conclude, our immunosensor is based in a novel nanocomposite composed by PVA, PVP and RGO which is easy to fabricate, inexpensive, provides rapid analysis results, and shows an appropriate LOD for the quantification of anti-*T. gondii* IgG antibodies.

Conclusions

A novel nanocomposite based on PVP, PVA and graphene was successfully obtained and applied to the electrochemical quantitation of anti-*T. gondii* IgG antibodies in serum samples. The nanocomposite showed an excellent compatibility between the polymers and GO sheets due to the hydrogen bridge interactions of the PVA, PVP and GO. The SPCE modification with the nanocomposite and the subsequent electroreduction (PVA/PVP/RGO/SPCE), generated a significant increase of the electroactive area and a high electron transfer. Besides, the presence of free -OH groups in the nanocomposite provide reaction sites for the covalent immobilization of different biomolecules. All these features revealed the great potential of this platform to be applied in the biosensor development. The experimental results obtained for anti-*T.*

gondii IgG antibodies indicated that the proposed immunosensor has high stability, excellent selectivity and adequate reliability. The LOD obtained for our designed immunosensor was 20 times lower than the conventional ELISA method. To conclude, the incorporation of PVA/PVP/RGO nanocomposite in the electrode surface represents a promising alternative to the development of highly sensitive electrochemical biosensors.

Acknowledgments

The authors wish to thank the financial support from the Universidad Nacional de San Luis (PROICO-2-1816-22/Q632), the Agencia Nacional de Promoción Científica y Tecnológica and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PICT-2015-2246, PICT 2015-3526, PICT-2015-1575, PICT-2014-1184, PICT-2013-3092).

References

[1] E.B. Aydın, M.K. Sezgintürk, A sensitive and disposable electrochemical immunosensor for detection of SOX2, a biomarker of cancer, Talanta 172 (2017) 162-170.
[2] M.A. Alonso-Lomillo, O. Domínguez-Renedo, A. Saldaña-Botín, M.J. Arcos-Martínez, Determination of ascorbic acid in serum samples by screen-printed carbon electrodes modified with gold nanoparticles, Talanta 174 (2017) 733-737.
[3] S. Li, Q. Zhang, Y. Lu, D. Ji, D. Zhang, J. Wu, X. Chen, Q. Liu, One step electrochemical deposition and reduction of graphene oxide on screen printed electrodes for impedance detection of glucose, Sens. Actuators B 244 (2017) 290-298.
[4] S. Stankovich, R.D. Piner, X. Chen, N. Wu, S.T. Nguyen, R.S. Ruoff, Stable aqueous dispersions of graphitic nanoplatelets via the reduction of exfoliated graphite oxide in the presence of poly (sodium 4-styrenesulfonate), J. Mater. Chem. 16(2) (2006) 155-158.
[5] S. Mallakpour, V. Behranvand, Green hybrid nanocomposites from metal oxides, poly (vinyl alcohol) and poly (vinyl pyrrolidone): Structure and chemistry, Hybrid Polymer Composite Materials, Volume 1, Elsevier 2017, pp. 263-289.
[6] D.R. Kumar, S. Kesavan, T.T. Nguyen, J. Hwang, C. Lamiel, J.-J. Shim, Polydopamine@ electrochemically reduced graphene oxide-modified electrode for electrochemical detection of free-chlorine, Sens. Actuators B 240 (2017) 818-828.

[7] S. Basha, K.V. Kumar, G.S. Sundari, M. Rao, Structural and Electrical Properties of Graphene Oxide-Doped PVA/PVP Blend Nanocomposite Polymer Films, Adv. Mater. Sci. Eng. 2018 (2018).
[8] Z.Q. Zhu, J.F. Dai, C.H. Ma, H.X. Sun, W.D. Liang, A. Li, Facile Preparation of Porous Graphene Oxide-Based Nanocomposite Xerogel for Selective Absorption, Chem. Select 2(27) (2017) 8493-8499.
[9] A.S. Wajid, S. Das, F. Irin, H.T. Ahmed, J.L. Shelburne, D. Parviz, R.J. Fullerton, A.F. Jankowski, R.C. Hedden, M.J. Green, Polymer-stabilized graphene dispersions at high concentrations in organic solvents for composite production, Carbon 50(2) (2012) 526-534.
[10] E. Abdelrazek, I. Elashmawi, A. El-Khodary, A. Yassin, Structural, optical, thermal and electrical studies on PVA/PVP blends filled with lithium bromide, Curr. Appl. Phys. 10(2) (2010) 607-613.
[11] D. Vanitha, S.A. Bahadur, N. Nallamuthu, A. Shunmuganarayanan, A. Manikandan, Studies on Conducting Polymer Blends: Synthesis and Characterizations of PVA/PVP Doped with CaCl2, J. Nanosci. Nanotechnol. 18(3) (2018) 1723-1729.
[12] S.M. Naghib, E. Parnian, H. Keshvari, E. Omidinia, M. Eshghan-Malek, Synthesis, Characterization and Electrochemical Evaluation of Polyvinylalchol/Graphene Oxide/Silver Nanocomposites for Glucose Biosensing Application, Int. J. Electrochem. Sci. 13 (2018) 1013-1026.
[13] J. Wang, T. Zhou, H. Deng, F. Chen, K. Wang, Q. Zhang, Q. Fu, An environmentally friendly and fast approach to prepare reduced graphite oxide with water and organic solvents solubility, Colloids Surf. B: Biointerfaces 101 (2013) 171-176.
[14] E. Abdelrazek, I. Elashmawi, S. Labeeb, Chitosan filler effects on the experimental characterization, spectroscopic investigation and thermal studies of PVA/PVP blend films, Phys. B: Condens. Matter 405(8) (2010) 2021-2027.
[15] M. Shieh, M. Didehdar, R. Hajihossein, F. Ahmadi, Z. Eslamirad, Toxoplasmosis: Seroprevalence in pregnant women, and serological and molecular screening in neonatal umbilical cord blood, Acta Tropica (2017).
[16] C. Dard, H. Fricker-Hidalgo, M.-P. Brenier-Pinchart, H. Pelloux, Relevance of and new developments in serology for toxoplasmosis, Trends in parasitology 32(6) (2016) 492-506.
[17] I. Aly, E.E. Taher, E. Gehan, H.E. Sayed, F.A. Mohammed, R.S. Hamad, E.M. Bayoumy, Advantages of bioconjugated silica-coated nanoparticles as an innovative diagnosis for human toxoplasmosis, Acta tropica 177 (2018) 19-24.
[18] W. Humers, R. Offeman, Preparation of graphitic oxide [J], J. Am. Chem. Soc. 80(6) (1958) 1339.
[19] J. Wang, X. Cai, C. Jonsson, M. Balakrishnan, Adsorptive stripping potentiometry of DNA at electrochemically pretreated carbon paste electrodes, Electroanal. 8(1) (1996) 20-24.
[20] A. J. Bard, Electrochemical methods, Fundamentals and applications 96 (Second. ed.), Wiley, New York (2001).
[21] E. Laviron, General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems, J. Electroanal. Chem. Interfacial Electrochem. 101(1) (1979) 19-28.
[22] S. Saxena, T.A. Tyson, S. Shukla, E. Negusse, H. Chen, J. Bai, Investigation of structural and electronic properties of graphene oxide, Appl. Phys. Lett. 99(1) (2011) 013104.
[23] G. Hirankumar, S. Selvasekarapandian, N. Kuwata, J. Kawamura, T. Hattori, Thermal, electrical and optical studies on the poly (vinyl alcohol) based polymer electrolytes, J. Power Sources 144(1) (2005) 262-267.
[24] P. Manivel, M. Dhakshnamoorthy, A. Balamurugan, N. Ponpandian, D. Mangalaraj, C. Viswanathan, Conducting polyaniline-graphene oxide fibrous nanocomposites: preparation, characterization and simultaneous electrochemical detection of ascorbic acid, dopamine and uric acid, Rsc. Adv. 3(34) (2013) 14428-14437.

[25] A. Biswas, J. Willet, S.H. Gordon, V. Finkenstadt, H. Cheng, Complexation and blending of starch, poly (acrylic acid), and poly (N-vinyl pyrrolidone), Carbohydr. polym. 65(4) (2006) 397-403.
[26] R. Ma, D. Xiong, F. Miao, J. Zhang, Y. Peng, Novel PVP/PVA hydrogels for articular cartilage replacement, Mater. Sci. Eng.: C 29(6) (2009) 1979-1983.
[27] X. Zhang, J. Wang, H. Jia, B. Yin, L. Ding, Z. Xu, Q. Ji, Polyvinyl pyrrolidone modified graphene oxide for improving the mechanical, thermal conductivity and solvent resistance properties of natural rubber, RSC Advan. 6(60) (2016) 54668-54678.
[28] L. Jiang, X.P. Shen, J.L. Wu, K.C. Shen, Preparation and characterization of graphene/poly (vinyl alcohol) nanocomposites, J. Appl. Polym. Sci. 118(1) (2010) 275-279.
[29] C. Bao, Y. Guo, L. Song, Y. Hu, Poly (vinyl alcohol) nanocomposites based on graphene and graphite oxide: a comparative investigation of property and mechanism, J. Mater. Chem. 21(36) (2011) 13942-13950.
[30] Q. Wei, Y. Zhang, Y. Wang, W. Chai, M. Yang, Measurement and modeling of the effect of composition ratios on the properties of poly (vinyl alcohol)/poly (vinyl pyrrolidone) membranes, Mater. Design 103 (2016) 249-258.
[31] D. Mondal, M.M.R. Mollick, B. Bhowmick, D. Maity, M.K. Bain, D. Rana, A. Mukhopadhyay, K. Dana, D. Chattopadhyay, Effect of poly (vinyl pyrrolidone) on the morphology and physical properties of poly (vinyl alcohol)/sodium montmorillonite nanocomposite films, Progress Nat. Sci.: Mater. Int. 23(6) (2013) 579-587.
[32] Q. Liu, X. Zhu, Z. Huo, X. He, Y. Liang, M. Xu, Electrochemical detection of dopamine in the presence of ascorbic acid using PVP/graphene modified electrodes, Talanta 97 (2012) 557-562.
[33] D.A. Brownson, D.K. Kampouris, C.E. Banks, Graphene electrochemistry: fundamental concepts through to prominent applications, Chem. Soc. Rev. 41(21) (2012) 6944-6976.
[34] S. J. Li, D. H. Deng, Q. Shi, S.-R. Liu, Electrochemical synthesis of a graphene sheet and gold nanoparticle-based nanocomposite, and its application to amperometric sensing of dopamine, Microchim. Acta 177(3-4) (2012) 325-331.
[35] B. Silwana, C. van der Horst, E. Iwuoha, V. Somerset, Synthesis, characterisation and electrochemical evaluation of reduced graphene oxide modified antimony nanoparticles, Thin Solid Films 592 (2015) 124-134.
[36] S. Konopka, B. McDuffie, Diffusion coefficients of ferri-and ferrocyanide ions in aqueous media, using twin-electrode thin-layer electrochemistry, Analytical Chemistry 42(14) (1970) 1741-1746.
[37] F.C. Vicentini, A.E. Ravanini, L.C. Figueiredo-Filho, J. Iniesta, C.E. Banks, O. Fatibello-Filho, Imparting improvements in electrochemical sensors: evaluation of different carbon blacks that give rise to significant improvement in the performance of electroanalytical sensing platforms, Electrochim. Acta 157 (2015) 125-133.
[38] M. Rao, Cylicvoltammetric Studies on PVA/PVP: GO Based Polymers. Chem. Sci. Rev. Lett., 6 (2017), 1069-1074.
[39] H. Wang, J. Li, Y. Ding, C. Lei, G. Shen, R. Yu, Novel immunoassay for Toxoplasma gondii-specific immunoglobulin G using a silica nanoparticle-based biomolecular immobilization method, Anal. chim. acta 501(1) (2004) 37-43.
[40] H. Wang, C. Lei, J. Li, Z. Wu, G. Shen, R. Yu, A piezoelectric immunoagglutination assay for Toxoplasma gondii antibodies using gold nanoparticles, Biosens. Bioelectron. 19(7) (2004) 701-709.
[41] S. Jiang, E. Hua, M. Liang, B. Liu, G. Xie, A novel immunosensor for detecting toxoplasma gondii-specific IgM based on goldmag nanoparticles and graphene sheets, Colloids Surf. B: Biointerfaces 101 (2013) 481-486.
[42] V. Medawar-Aguilar, C.F. Jofré, M.A. Fernández-Baldo, A. Alonso, S. Angel, J. Raba, S.V. Pereira, G.A. Messina, Serological diagnosis of Toxoplasmosis disease using a fluorescent immunosensor with chitosan-ZnO-nanoparticles, Anal. biochem. 564–565 (2019) 116-122.

Figure Captions

Figure 1: Schematic representation of the SCPE modification and electrochemical quantitation of IgG anti-*T. gondii* antibodies

Figure 2: SEM images of the surface of the modified electrode (A) 20000 x and (B) 60000x and (C) UV–vis spectra of GO, PVP, PVA and PVA/PVP/GO aqueous dispersions.

Figure 3: (A) Infrared spectra of GO, PVP/GO, PVA/GO and PVA/PVP/GO, (B) PVP and PVP/GO and (C) PVA and GO/PVA powers.

Figure 4: Influence the concentration of A) RGO and B) and PVA/PVP on the peak oxidation current, recorded in 1 mmol L^{-1} $[Fe(CN)6]^{3-}$/$[Fe(CN)6]^{4-}$ in 0.1 mol L^{-1} KCl (Scan rate100 mV s^{-1})

Figure 5: A) Cyclic voltammogram for: (a) blank solution, (b) unmodified SPCE and (c) PVA/PVP/RGO, recorded in 1 mmol L^{-1} $[Fe(CN)6]^{3-}$/$[Fe(CN)6]^{4-}$ in 0.1 mol L^{-1} KCl (Scan rate100 mV s^{-1}), B) Cyclic voltammogram at different scan rates, (C) Current of redox peak vs square roots from 20 to 300 mV s^{-1} and D) relationship of the peak potential (Ep) vs. the logarithm of scan rate (log v).

Table 1 ATR–FT-IR spectra peak assignments of GO, PVP, and PVA.

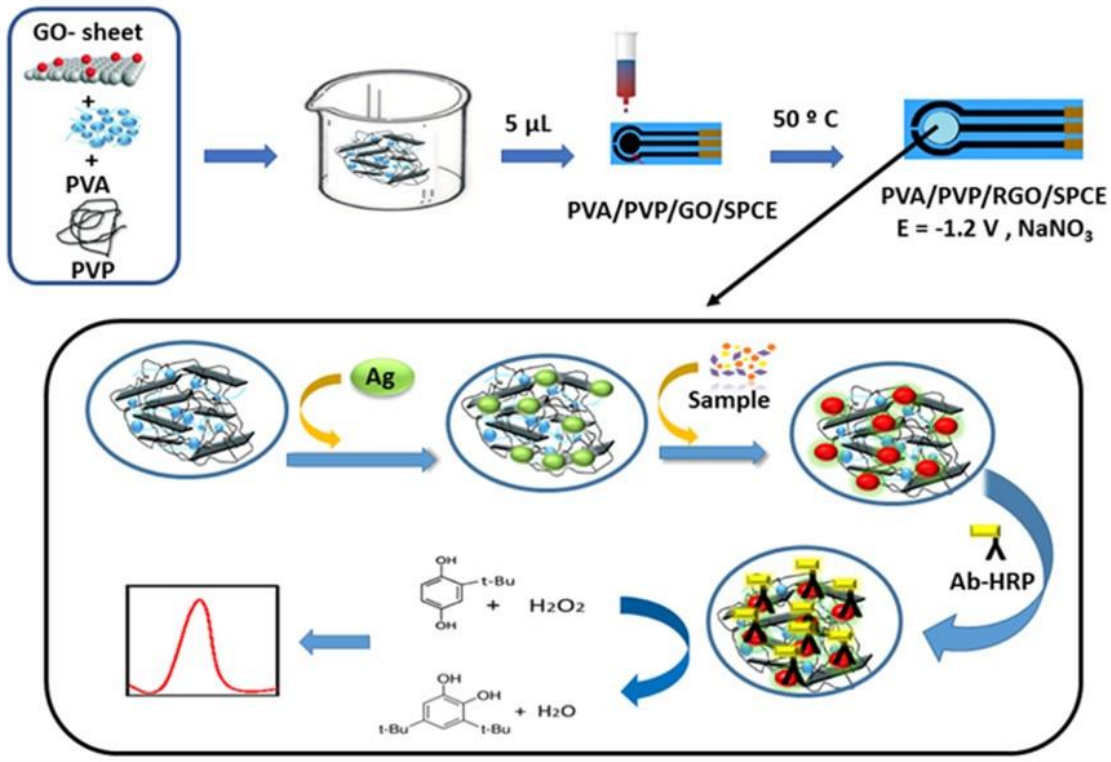
GO ν (cm^{-1})	**PVP** ν (cm^{-1})	**PVA** ν (cm^{-1})	**Description**
3327	3325 *	3310	OH stretching
2923 -2870		2942	asymmetric and symmetrical stretching vibrations of CH_2
1726			carboxyl groups C=O
		1654	CO stretching
1634			C=C stretching

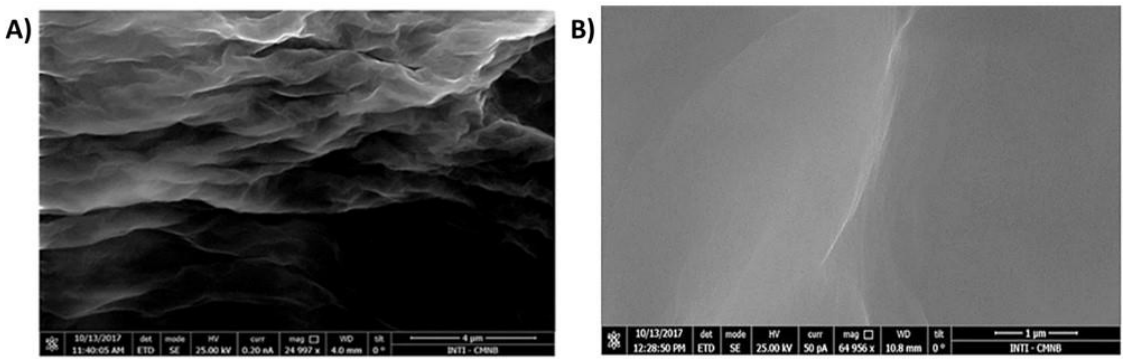
	1539	CN (pyridine ring)
1396		deformation O-H
1258		C-OH stretching
1020		C–O–C epoxide group

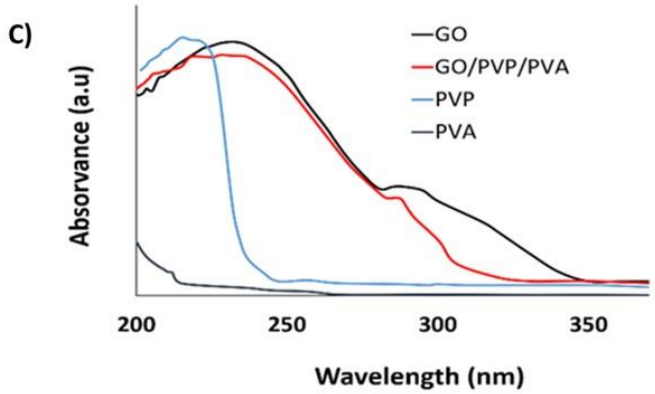
*Hydrophilic nature of PVP - ν: wave number

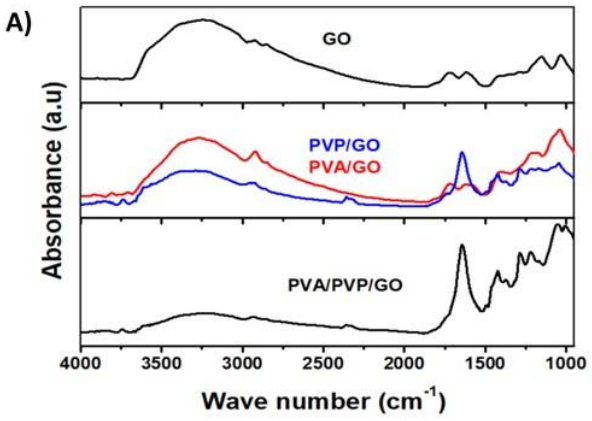
Table 2 Optimal parameters for immunodetection.

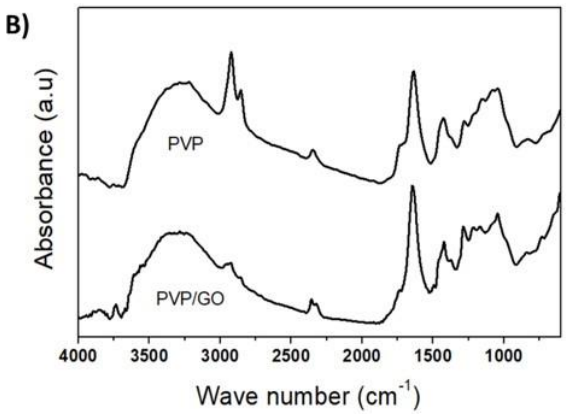
Optimized parameter	***Evaluated range***	***Optimum value***
T. gondii antigen concentration	1-200 ug mL^{-1}	100 ug mL^{-1}
Blocking solution concentration	0.1 – 3 %	1%
Sample dilution	1/10 – 1/200	1/100
Conjugated dilution	1/500 – 1/4000	1/1000
Incubation time	2 – 30 min	10 min
pH of substrate solution	4 - 7	5.03
H_2O_2 concentration	0.5 – 5 mM	1 mM
4-TBC concentration	0.1 – 5 mM	1 mM

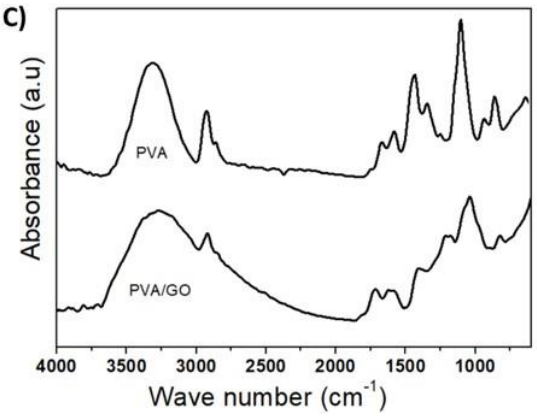


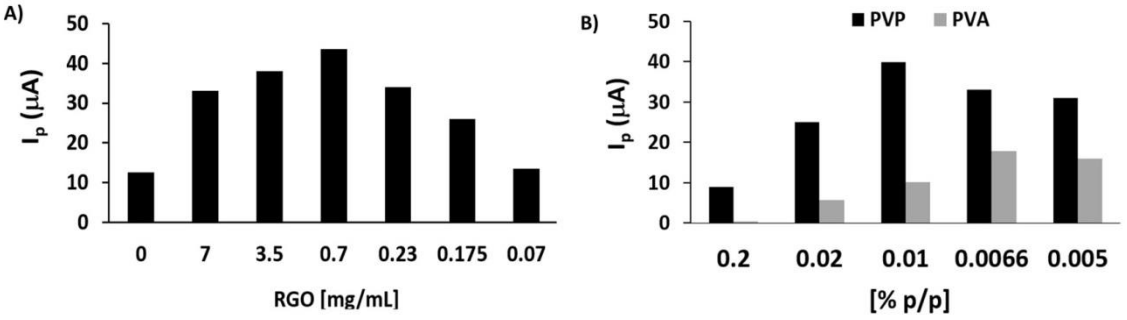


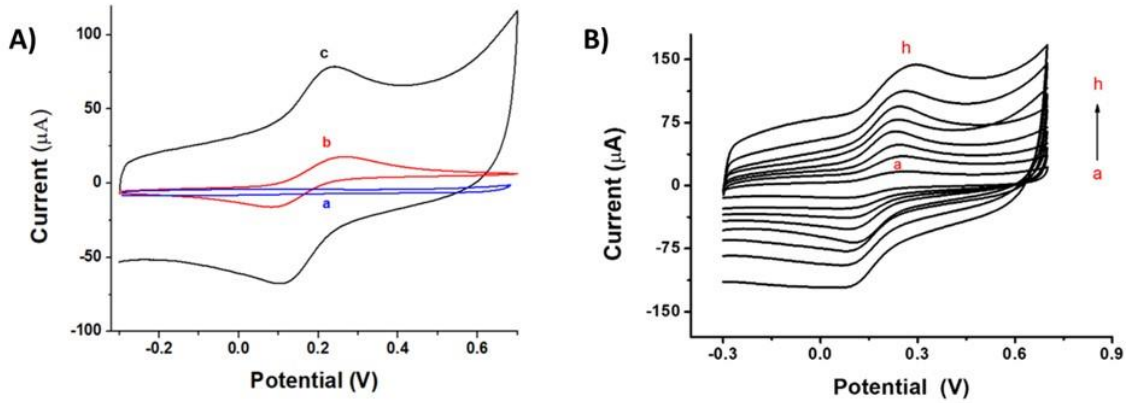


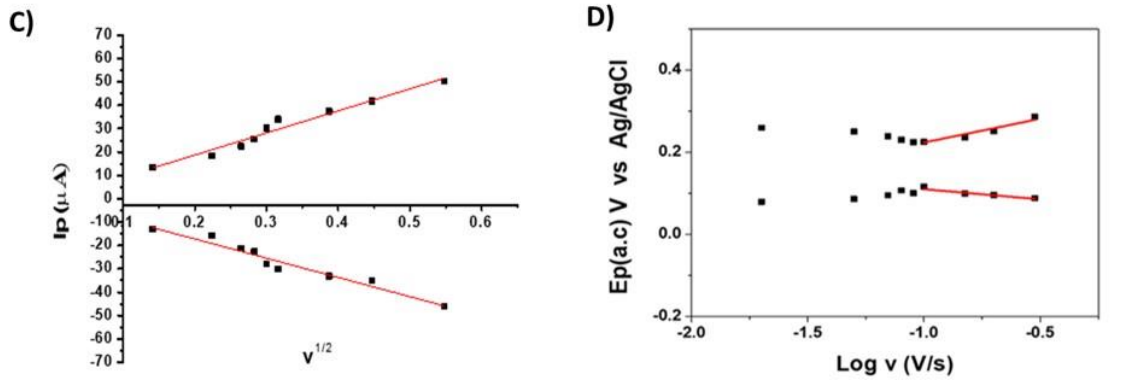












Highlights

- Novel electrochemical platform based on the use a nanocomposite of PVA/PVP/RGO.
- The PVA/PVP/RGO increased the electroactive area and fast electron transfer kinetics.
- The PVA/PVP/RGO components showed excellent dispersion and strong interactions.
- Sensitive serological determination of IgG anti- *T. gondii* antibodies.
- This device represents a novel strategy for the immunosensor development.