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Label-free aptasensor for p24-HIV protein detection based on graphene quantum dots as an electrochemical signal amplifier

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Abstract

Human immunodeficiency virus (HIV) is still considered a pandemic, and the detection of p24-HIV protein has an important role in the early diagnosis of HIV in adults and newborns. The accessibility of these trials depends on the price and execution difficulty of the method, which can be reduced using electrochemical methods by using enzymeless approaches, disposable and accurate devices. In this work, graphene quantum dots were acquired by a simple synthesis and employed as an electrochemical signal amplifier and support for the aptamer immobilization through a feasible and stable modification of disposable screen-printed electrodes. The device has been easily assembled and used to detect p24-HIV protein without the interference of similar proteins or sample matrix. Using the best set of experimental conditions, a linear correlation between analytical signal and log of p24-HIV concentration from 0.93 ng mL⁻¹ to 93 pg mL⁻¹ and a limit of detection of 51.7 pg mL⁻¹ were observed. The developed device was applied to p24 determination in spiked human serum and provided distinct levels of signal for positive and negative samples, successfully identifying real samples with the target protein. This sensor is a step towards the development of point-of-care devices and the popularization of electrochemical methods for trials and diagnostics of relevant diseases.

Introduction

The p24 is a structural protein that makes the Human immunodeficiency virus (HIV) capsid and is present in the blood serum of recently infected individuals due to the primary immune response. Even though the p24 detection is not recommended to be the only test for HIV diagnosis [1], this determination plays an important role in the early diagnosis since that p24 protein can be detected several days before specific antibodies [2]. Also, the higher proportion of infected people presenting for voluntary testing is in the acute stage, when the p24 protein reaches higher concentrations [3]. Another issue covered by the p24 detection is the diagnosis of the infant whose mothers are HIV positive, as the antibodies tests cannot be adequately interpreted until clearance of the maternal antibodies passed prenatally to the baby, which is around 18 months [2]. The long delay in obtaining the results of traditional tests used for the early diagnosis of HIV, and thus the delay in starting antiretroviral treatment can cause losses and consequences for infected patients, especially in situations of limited resources. Various methods for the determination of p24 have been explored, with aptamers showing great potential for application in diagnostics and therapeutics involving HIV [4,5].

Aptamers are single-stranded oligonucleotides (DNA or RNA) generated by the Systematic Evolution of Ligands by Exponential enrichment (SELEX) process, which selects those that bind more efficiently to the target molecule. They can functionally substitute antibodies in diagnostics designs, providing a highly selective characteristic to the method, being easily incorporated into the development of valuable diagnostics [4]. These aspects contribute especially to the reliability of electrochemical affinity sensors in complex samples. Despite their great advantages, especially related to selectivity, the improvement of the limits of detection is still a challenge in the development of electrochemical immunosensors [6]. Therefore, efforts have been driven to the use of electrochemical signal amplifiers, with relevant contributions from nanomaterials like carbon nanotubes [7,8], graphene [9], and other nanoparticles. In addition to providing a high surface area as a stable platform for the immobilization of biomolecules, they enhance the electrochemical signal of the base sensor, improving the limit of detection [6].

In this work, we describe the evaluation of screen-printed electrodes (SPE) modified with Graphene Quantum Dots (GQD) as disposable platforms for the construction of a label-free aptasensor for quantification of p24-HIV protein. The optical and electrochemical advantages provided by GQD have been explored in many ways to develop biomedical sensors [10]. GQD display the qualities of the traditional quantum dots (quantum confinement and edge effect) with the benefits of 2D graphene and graphene oxide sheets (e.g., high surface area, oxygenated groups, excellent

electrochemical properties due to the π - π bonds), resulting in a material that improves the interaction with several analytes [[11], [12], [13]]. They are chemically stable, water-soluble, robust, inert, showed electrocatalytic properties, enhancing detection limits, and sensitivity when used as an electrode modifier [14,15]. Furthermore, GQD with exciting properties has been achieved through quite simple synthesis steps and used as an electrode modifier [16], which can deeply contribute to the development of feasible POC tests, especially for resource-limited settings.

Experimental

Reagents and solutions

All chemicals were analytical or high-purity grade. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimidehydrochloride (EDC), N-Hydroxysulfosuccinimide sodium salt (NHS), Citric Acid, Bovine serum albumin (BSA), and Human serum were purchased from Sigma Aldrich. Potassium hexacyanoferrate (III) was purchased from Merck (Brazil). 0.1 mol L⁻¹ phosphate-buffered saline (PBS) pH 7.4 was used as the supporting electrolyte. The p24 ssDNA aptamer with an amine group in the end, p24-HIV, and p24-HTLV proteins were provided by the Paraná Institute of Molecular Biology (IBMP).

Synthesis and characterization of graphene quantum dots (GQD)

The graphene quantum dots were synthesized as described by Dong et al. [17]. The method is based on the citric acid pyrolysis at 200 °C, ending with the solid liquefaction and color change to orange. NaOH is added to finish the formation of the GQD. The solution is neutralized and stored under refrigeration in amber glass to protect it from light.

GQDs size distribution was determined using transmission electron microscopy (TEM) images obtained using a JEOL JEM 1200 operated at 120 kV. Scanning Electron Microscopy (SEM) images were obtained from a Quanta 450 ESEM FEG scanning electron microscope. A BOMEN spectrophotometer was used for Fourier-transform infrared spectroscopy (FTIR) measurements (64 scans of 4000 cm⁻¹ to 500 cm⁻¹).

Electrochemical measurements

Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) measurements were performed on a Potentiostat/Galvanostat PGSTAT204 containing the FRA32 M module. Experiments were conducted in PBS 0.1 mol L⁻¹ with 5.0 mmol L⁻¹ of potassium ferricyanide K₃[Fe(CN)₆] as the standard electrochemical probe. The

redox probe peak current suppression was monitored during the construction stages and for the determination of p24 due to the non-conductive nature of the molecules. EIS measurements were executed from 100 kHz to 10 mHz with an amplitude of 10 mV in open circuit potential conditions.

Screen-printed electrode modification and aptasensor construction

The SPEs were obtained from Professor Craig E. Banks laboratory at Manchester Metropolitan University, in which the working and counter electrodes are made of graphite conductive ink, an Ag|AgCl conductive ink was used for the pseudo-reference electrode construction, and the final set is supported on a polyester substrate with the electrodes and electrical contacts areas delimited by an insulating ink [[18], [19], [20], [21]].

For modification, an aliquot of GQD dispersion was added directly at the SPE surface, followed by electrodeposition step, which promotes the reduction of oxygenated groups present at the material, decreasing its solubility and depositing the GQD at working electrode [22,23]. The electrodeposition step was carried out by cyclic voltammetry in the potential range of 0.0 and -1.4V (vs. Ag/AgCl) at a scan rate of 50 mVs^{-1} .

The aptamer was immobilized onto the electrode surface by EDC/NHS reaction (4 and 6 mmol L^{-1} , respectively, diluted in ultrapure water), forming a covalent bond between the carboxylic groups in the material and the amino group present in the aptamer [24,25]. This was achieved by a two-step method, where the surface was activated with the EDC/NHS solution for 1 h, followed by incubation with the aptamer to create the selective surface. A solution of 1.0 mg mL^{-1} of bovine serum albumin (BSA) was used to block unspecific sites, completing the aptasensor assembly.

The final p24-HIV detection was measured by the decrease in the electrochemical probe peak current caused by the specific interaction of the protein with the surface. A schematic representation of aptasensor construction and the expected electrochemical response for the p24-HIV detection is shown in Fig. 1.

2.5. Stability, selectivity, and analytical curve

The stability of the voltammetric response was evaluated by storing the aptasensor immersed in PBS with a reductive agent (Sodium Azide 0,01%) and under refrigeration (4 °C) for four weeks.

Selectivity was tested by comparing the interaction between the aptasensor and the p24 protein of the HTLV virus, which has similarities with the target protein of HIV; therefore, it acts as a control indicating any cross-reaction that the aptasensor may present.

The response of the aptasensor was evaluated for p24-HIV concentrations varying between 0.93 ng mL⁻¹ and 93 µg mL⁻¹, with four different devices built for each tested concentration. Limits of detection (LOD) and quantification (LOQ) were calculated using 3 and 10 times the standard deviation from blank measurements divided by the slope of the analytical curve, respectively [26].

Trial in spiked real sample

The developed aptasensor was applied in a commercial spiked human serum sample with HIV-p24. The serum was used with a dilution rate of 1:100 in PBS, and the spiked p24 reached a final concentration of 9.3 ng mL⁻¹. The measurement and detection procedures were conducted as previously described.

Results

Characterization of GQD as affinity-based bioplatfrom platform

The GQD has been used for the development of an affinity-based bioplatfrom for p24-HIV. The immobilization of the aptamer on the electrode surface was carried out by exploring the carboxylic groups characteristic of the GQD. These oxygenated groups are often present on these nanomaterials [27], and can be confirmed by simple FTIR analyzes (Fig. S1A). SPE modification with GQD has been performed under cyclic voltammetry conditions. This electrochemical approach promotes a progressive reduction of oxygenated groups present at the GQD surface by formation of sp² sites, which can improve surface adhesion, but it cannot be used for the construction of the aptasensor [22,23]. So, the GQD deposition is a crucial step for the aptasensor preparation, not only for being the support for the immobilization of further species but also enhance the electrochemical characteristics of the proposed device. The results obtained for the GQD electrodeposition by cyclic voltammetry are shown in Fig. 2A.

As previously reported [16,28], oxygenated groups decrease significantly by the electrochemical reduction occurring near $-0.8V$ forming less hydrophilic species on the modified surface electrode. So, under CV conditions, the number of carboxylic groups available for the aptamer immobilization with EDC/NHS can decrease with rising voltammetric cycles in the electrodeposition step, directly impacting the sensitivity of the sensor. To optimize the number of electrodeposition cycles, the aptamer was immobilized in SPEs with GQD deposited using different voltammetric conditions. CV experiments were performed in a solution with the electrochemical probe before and after aptamer immobilization. The variation on signal (Fig. 2B) caused by the aptamer immobilization (non-conductive material) was used indirectly to verify the GQD modification. The use of GQD as a platform for the aptasensor construction is justified since an increase in the amount of aptamer immobilized at the modified surface was observed. Also, the deposition of GQD with three voltammetric cycles led to a higher decline in the probe signal, related to a higher quantity of available functional groups after the GQD electrodeposition, thus, an optimal condition for the aptamer immobilization.

Additionally, the CV and EIS measurements that corroborate the electrochemical advantages of using the GQD for SPE modification are presented in Fig S1B/C. From EIS spectra is possible to observe the semicircle diameter for the unmodified SPE is larger than that of modified GQD since the electron transfer processes between the electrode and the redox probe are facilitated by the presence of the modifier, reducing the electron transfer resistance from 38.4 k Ω to 2.43 k Ω . Besides better electron-

transfer, there is an improvement in mass transport since GQD is a nano-size material with an average diameter of 7.0 nm (Fig. S1D), which also promotes an effective interaction with the bioreceptor.

Aptasensor construction and optimization

After the SPE modification with GQD, a layer of carboxylic groups is present at the electrode surface, allowing the immobilization of the aptamer via the EDC/NHS reaction. The parameters involving the activation of the surface and the incubation with the aptamer were thoroughly optimized, seeking a selective surface with a higher sensitivity. The time of reaction was studied between 30 min and 2 h. After the EDC/NHS reaction, the electrode was incubated by 1 h in presence of 1.0 ng mL^{-1} of aptamer. The efficiency of the activation step was evaluated by CV measurements recording the suppression in the electrochemical probe signal. The decrease observed is directly correlated to the amount of material immobilized on the SPE surface. The variation in the voltammetric signal for each time of activation studied is shown in Fig. S2A. As can be seen in Fig. S2A, there is an increase in signal variation from 30 min to 1 h, and higher times do not provide any significant difference to the aptamer immobilization that justifies the rise in time. From this, 1 h of the reaction was considered an optimal time to activate the surface, while 30 min is not enough to provide adequate surface activation.

After optimizing the experimental conditions for EDC/NHS reaction, aptamer (APT) can be immobilized at the electrode surface to form the selective layer. This step was studied using APT concentration of 1.0 nmol mL^{-1} and varying the incubation time between 1 and 4 h. Like the activation step, CV measurements were carried out in presence of the electrochemical probe and the decrease in voltammetric signal was compared. The results obtained for different incubation time are presented in Fig. S2B. It is possible to observe, from Fig. S2B, that 2 h is a period enough to incubate the APT on the electrode surface, generating a very satisfactory response signal. Higher incubation times present a significant signal loss and lower reproducibility, which can be attributed to the blocking or saturation of the surface caused by the long period of the interaction of the species with the electrode surface.

Selectivity and stability

Selectivity plays a major role in diagnostics and clinical trials because any potential biological sample has elevated complexity and might offer some level of cross-reactivity or unspecific adsorption. To evaluate the selectivity of the proposed aptasensor, the p24 protein of the Human T-lymphotropic virus (HTLV) was used to contrast, as it is

similar to the HIV-p24 structure. The shift in the signal after incubation with 100.0 ng mL^{-1} of both p24 proteins is presented in Fig. 3.

As can be seen in Fig. 3A, there was no signal suppression caused by the p24-HTLV immobilization, instead, a slight increase in the signal that could be caused by a mild desorption of some immobilized species during the incubation. The p24-HIV incubation caused a suppression of $4.99 \mu\text{A}$ in the peak current due to the specific interaction with the aptamer immobilized in the electrode (Fig. 3C). These results suggest high selectivity from the aptamer, showing an excellent ability to interact specifically with the target protein and confirming the possibility to use it to develop a selective trial.

The stability of the aptasensor was evaluated within four weeks, recording the base signal of the built sensor before and after storage, and a detectability test with 100.0 ng mL^{-1} HIV-p24. The results indicated that the electrodes showed a current maximum variation of 8% at the end of 4 weeks when compared to the initial measurements, demonstrating no significant desorption during storage period. In contrast, the detectability of the aptasensor was slightly altered providing 54% of the expected signal variation at the end of the test. Two weeks of storage maintained a stable signal and detectability, as the p24-HIV resulted in 91% of the expected decrease in peak current.

Analytical application

Using the best set of experimental conditions, the proposed aptasensor was submitted to different concentrations of p24-HIV to evaluate the linear response range. Representative CV measurements recorded in $\text{K}_3[\text{Fe}(\text{CN})_6]$ (5.0 mmol L^{-1}) solution before and after incubation of different p24-HIV concentrations, varying between 0.93 ng mL^{-1} and $93 \mu\text{g mL}^{-1}$ ($n = 4$), and the analytical curve obtained are shown in Fig. 4A e 4B, respectively.

A linear relationship was obtained between anodic peak and log of p24-HIV concentration for all ranges investigated with a sensitivity of $1.293 \mu\text{A dec}^{-1}$ and R^2 0.996 (Fig. 4B). The calculated limits of detection (LOD) and quantification (LOQ) of 51.7 and 930.0 pg mL^{-1} were obtained, respectively. For each evaluated concentration, the standard deviation between the signals was close to 5% of the medium response, except for the lower concentration that showed a variation over 10%. The proposed sensor provides a broad linear range reaching pg mL^{-1} concentrations for p24-HIV detection with a simple strategy, showing great reproducibility of the fabrication process, especially inside the linear range. The linear dynamic range and limit of detection

achieved using the proposed sensor were compared to other p24-HIV sensors described in the literature in Table 1.

As can be seen, the developed sensor has a compatible limit of detection compared to other sensors. The method developed by Ma et al. reached a lower LOD with a MWCNT based MIP on glassy carbon electrodes, which require higher overnight preparation of the platform using higher-grade materials than the proposed method. With a simple approach from the GQD synthesis and SPE platform, the present work is a proof of concept that compatible limits can be achieved through this simpler and less time-consuming method, which can be easily escalated, converted, and explored for other affinity molecules as well.

Trial of real spiked samples

The p24-HIV concentration in blood during early infections spans between 0.1 and 103 pg mL⁻¹ [2], with upper concentration inside the limits of detection achieved for this sensor. To evaluate how the device behaves in a complex biological sample, commercial human serum was diluted and fortified with p24-HIV to a final concentration of 9.3 ng mL⁻¹ (inside the linear range of the proposed sensor) and tested in pair with the serum without the target protein. This study is a rough approximation as biological samples go through some stages of sample preparation, like precipitation and purification that often concentrates the material in a smaller volume, but can provide valuable information about unspecific adsorption and reactivity with a biological medium. The results obtained for the sample trial can be seen in Fig. 5.

Fig. 5A and C shows the differentiation between the serum with and without p24-HIV, respectively. The signal obtained for the fortified serum with 9.3 ng mL⁻¹ of p24-HIV (Fig. 5B – red bar) was slightly higher than the signal achieved for the same concentration in the analytical curve but is expected, and justified within-sample conditions. Instead, the serum without the target protein (Fig. 5B – green bar) provided almost no interference to the test, with a medium signal of 0.275 μA. The conditions used to differentiate the real samples can diverge from the ones used to the spiked samples in this work, and require another set of studies to evaluate the best conditions to perform the test, which may involve different degrees of dilution or sample preparation. However, with the great selectivity and low interference of the biological matrix, the proposed device has the potential to differentiate real samples with lower levels of the biomarker.

Conclusion

In summary, we have shown that GQD can be easily synthesized and employed as a screen-printed electrode modifier. The nanomaterial has a simple synthesis and can be quickly immobilized in the SPE, boosting the sensor sensitivity and providing functional groups for further modifications. The aptamer used provided a high selectivity to p24-HIV to the electrochemical device, showing almost no interference from similar protein or sample matrix. Through the quantification of a biomarker in concentrations near ELISA kits often achieve, the device provides valuable information with a simple strategy and quick results. So, the GQD modified SPE as support for the label-free aptasensor applied in the quantification of p24-HIV is a step towards the popularization of electrochemical detection and the development of reliable POC trials. Despite the common design, the proposed sensor showed essential features that allow it to be applied in early HIV infection with a broad work linear range, good selectivity, and low interference from the sample matrix.

CRedit authorship contribution statement

Jeferson L. Gogola: Data curation, Investigation, Methodology, Writing – original draft. Gustavo Martins: Data curation, Investigation, Methodology. Ava Gevaerd: Data curation, Investigation, Methodology, Writing – original draft. Lucas Blanes: Resources, Writing – review & editing. Josiane Cardoso: Resources, Visualization. Fabricio Klerynton Marchini: Resources, Visualization. Craig E. Banks: Resources, Visualization. Márcio F. Bergamini: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision. Luiz H. Marcolino-Junior: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision.

Declaration of competing 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.

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References

- [1] WHO, First WHO Model List of Essential IVDs (2018)
- [2] E.R. Gray, R. Bain, O. Varsaneux, R.W. Peeling, M.M. Stevens, R.A. McKendry
P24 revisited: a landscape review of antigen detection for early HIV diagnosis
Aids, 32 (2018), pp. 2089-2102,
- [3] W.S. Tan, E.P.F. Chow, C.K. Fairley, M.Y. Chen, C.S. Bradshaw, T.R.H. Read
Sensitivity of HIV rapid tests compared with fourth-generation enzyme immunoassays or HIV RNA tests; *Aids*, 30 (2016), pp. 1951-1960
- [4] J. Bala, S. Chinnapaiyan, R.K. Dutta, H. Unwalla, Aptamers in HIV research diagnosis and therapy, *RNA Biol.*, 15 (2018), pp. 327-337,
- [5] Q. Zhu, G. Liu, M. Kai, A.O.A. Miller, DNA aptamers in the diagnosis and treatment of human diseases *Molecules*, 20 (2015), pp. 20979-90997
- [6] I.H. Cho, J. Lee, J. Kim, M.S. Kang, J.K. Paik, S. Ku, H.M. Cho, J. Irudayaraj, D.H. Kim, Current technologies of electrochemical immunosensors: perspective on signal amplification, *Sensors (Switzerland)*, 18 (2018), pp. 1-18,
- [7] E. Sánchez-Tirado, A. González-Cortés, P. Yáñez-Sedeño, J.M. Pingarrón, Carbon nanotubes functionalized by click chemistry as scaffolds for the preparation of electrochemical immunosensors. Application to the determination of TGF-beta 1 cytokine, *Analyst*, 141 (2016), pp. 5730-5737
- [8] C. Tlili, L.N. Cella, N.V. Myung, V. Shetty, A. Mulchandani, Single-walled carbon nanotube chemoresistive label-free immunosensor for salivary stress biomarkers *Analyst*, 135 (2010), pp. 2637-2642,
- [9] T. Kuila, S. Bose, P. Khanra, A.K. Mishra, N.H. Kim, J.H. Lee, Recent advances in graphene-based biosensors, *Biosens. Bioelectron.*, 26 (2011), pp. 4637-4648,
- [10] B.D. Mansuriya, Z. Altintas, Applications of graphene quantum dots in biomedical sensors, *Sensors (Switzerland)*, 20 (2020),
- [11] S. Dong, Q. Bi, C. Qiao, Y. Sun, X. Zhang, X. Lu, L. Zhao, Electrochemical sensor for discrimination tyrosine enantiomers using graphene quantum dots and β -cyclodextrins composites, *Talanta*, 173 (2017), pp. 94-100,
- [12] A. Gevaerd, C.E. Banks, M.F. Bergamini, L.H. Marcolino-Junior, Nanomodified Screen-Printed Electrode for direct determination of Aflatoxin B1 in malted barley samples, *Sensor. Actuator. B Chem.*, 307 (2020), pp. 1-7
- [13] H. Rao, X. Zhao, X. Liu, J. Zhong, Z. Zhang, P. Zou, Y. Jiang, X. Wang, Y. Wang
A novel molecularly imprinted electrochemical sensor based on graphene quantum dots coated on hollow nickel nanospheres with high sensitivity and selectivity for the rapid determination of bisphenol S; *Biosens. Bioelectron.*, 100 (2018), pp. 341-347,
- [14] M. Bacon, S.J. Bradley, T. Nann, Graphene quantum dots, *Part. Part. Syst. Char.*, 31 (2014), pp. 415-428,
- [15] S.H. Choi, Unique properties of graphene quantum dots and their applications in photonic/electronic devices, *J. Phys. D. Appl. Phys.*, 50 (2017)
- [16] A. Gevaerd, C.E. Banks, M.F. Bergamini, L.H. Marcolino-Junior; Graphene

quantum dots modified screen-printed electrodes as electroanalytical sensing platform for diethylstilbestrol; *Electroanalysis*, 31 (2019), pp. 838-843,

[17] Y. Dong, J. Shao, C. Chen, H. Li, R. Wang, Y. Chi, X. Lin, G. Chen, Blue luminescent graphene quantum dots and graphene oxide prepared by tuning the carbonization degree of citric acid; *Carbon N. Y.*, 50 (2012), pp. 4738-4743,

[18] D. Agustini, M.F. Bergamini, L.H. Marcolino-Junior, Tear glucose detection combining microfluidic thread based device, amperometric biosensor and microflow injection analysis, *Biosens. Bioelectron.*, 98 (2017), pp. 161-167,

[19] F.R. Caetano, E.A. Carneiro, D. Agustini, L.C.S. Figueiredo-Filho, C.E. Banks, M.F. Bergamini, L.H. Marcolino-Junior, Combination of electrochemical biosensor and textile threads: a microfluidic device for phenol determination in tap water; *Biosens. Bioelectron.*, 99 (2018), pp. 382-388,

[20] A. Gevaerd, E.Y. Watanabe, K. Fernandes, M.A.P. Papi, C.E. Banks, M.F. Bergamini, L.H. Marcolino-Junior, Electrochemically reduced graphene oxide as screen-printed electrode modifier for fenamiphos determination, *Electroanalysis* (2020), pp. 1689-1695,

[21] M.C. Oliveira, E.Y. Watanabe, D. Agustini, C.E. Banks, L.H. Marcolino-Júnior, M.F. Bergamini; Nonenzymatic sensor for determination of glucose in blood plasma based on nickel oxyhydroxide in a microfluidic system of cotton thread; *J. Electroanal. Chem.*, 840 (2019), pp. 153-159,

[22] N. Hashemzadeh, M. Hasanzadeh, N. Shadjou, J. Eivazi-Ziaei, M. Khoubnasabjafari, A. Jouyban, Graphene quantum dot modified glassy carbon electrode for the determination of doxorubicin hydrochloride in human plasma, *J. Pharm. Anal.*, 6 (2016), pp. 235-241,

[23] Y. Yu, W. Liu, J. Ma, Y. Tao, Y. Qin, Y. Kong; An efficient chiral sensing platform based on graphene quantum dot-tartaric acid hybrids; *RSC Adv.*, 6 (2016), pp. 84127-84132,

[24] J.L. Gogola, G. Martins, F.R. Caetano, T. Ricciardi-Jorge, C.N. Duarte dos Santos, L.H. Marcolino-Junior, M.F. Bergamini; Label-free electrochemical immunosensor for quick detection of anti-hantavirus antibody; *J. Electroanal. Chem.*, 842 (2019), pp. 140-145,

[25] R. Pauliukaite, M. Ghica, Comparative study of different cross-linking agents for the immobilization of functionalized carbon nanotubes within a chitosan film supported on a Graphite–Epoxy composite electrode; *Anal. Chem.*, 81 (2009), pp. 5364-5372,

[26] J.C. Miller, J.N. Miller, *Statistics and Chemometrics for Analytical Chemistry* (fourth ed.), Pearson Education (2000),

[27] S. Campuzano, P. Yáñez-Sedeño, J.M. Pingarrón, Carbon dots and graphene quantum dots in electrochemical biosensing, *Nanomaterials*, 9 (2019), pp. 1-18, 10.3390/nano9040634

[28] S.K. Tuteja, R. Chen, M. Kukkar, C.K. Song, R. Mutreja, S. Singh, A.K. Paul, H. Lee, K.H. Kim, A. Deep, C.R. Suri; A label-free electrochemical immunosensor for the detection of cardiac marker using graphene quantum dots (GQDs); *Biosens. Bioelectron.*, 86 (2016), pp. 548-556,

[29] F. Kheiri, R.E. Sabzi, E. Jannatdoust, E. Shojaeefar, H. Sedghi; A novel

amperometric immunosensor based on acetone-extracted propolis for the detection of the HIV-1 p24 antigen; *Biosens. Bioelectron.*, 26 (2011), pp. 4457-4463,

[30] F. Li, H. Li, Z. Wang, J. Wu, W. Wang, L. Zhou, Q. Xiao, Q. Pu, Mobile phone mediated point-of-care testing of HIV p24 antigen through plastic micro-pit array chips *Sensor. Actuator. B Chem.*, 271 (2018), pp. 189-194,

[31] Y. Ma, X.L. Shen, Q. Zeng, H.S. Wang, L.S. Wang, A multi-walled carbon nanotubes based molecularly imprinted polymers electrochemical sensor for the sensitive determination of HIV-p24, *Talanta*, 164 (2017), pp. 121-127,

[32] Y.S. Fang, X.J. Huang, L.S. Wang, J.F. Wang; An enhanced sensitive electrochemical immunosensor based on efficient encapsulation of enzyme in silica matrix for the detection of human immunodeficiency virus p24; *Biosens. Bioelectron.*, 64 (2015), pp. 324-332

[33] N. Gan, T.H. Li, J.P. Lei, L.Y. Wang, X. Yang, Electrochemical immunosensor for human immunodeficiency virus p24 antigen based on Mercapto succinic acid hydrazide copper monolayer modified gold electrode, *Fenxi Huaxue/Chinese J. Anal. Chem.*, 36 (2008), pp. 1167-1171

[34] M. Du, N. Li, G. Mao, Yucheng Liu, X. Wang, S. Tian, Q. Hu, X. Ji, Yalan Liu, Z. He, Self-assembled fluorescent Ce(III) coordination polymer as ratiometric probe for HIV antigen detection; *Anal. Chim. Acta*, 1084 (2019), pp. 116-122

Fig. 1. Schematic representation of the aptasensor construction (steps numbered) and expected response for the final detection of p24-HIV by electrochemical probe signal suppression.

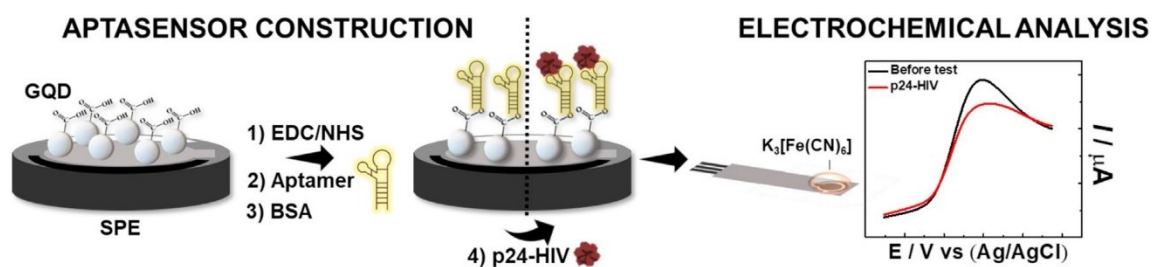


Fig. 2. A) Voltammetric profile of the GQD electrodeposition step. Cycles: 1 to 10. Scan rate: 50 mV s^{-1} . B) Decrease in the voltammetric probe signal after aptamer (1.0 nmol mL^{-1} / 2 h) immobilization on SPE and GQD-SPE (varying voltammetry cycles for electrodeposition of GQD). Scan rate of 100 mV s^{-1} and $5.0 \text{ mmol L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]$ in PBS pH 7.4.

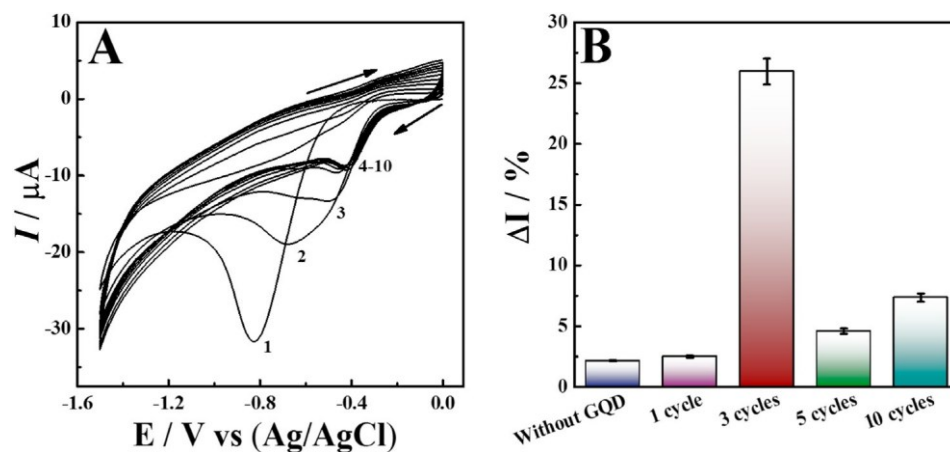


Fig. 3. A) Voltammetric probe profile before and after incubation with control protein p24-HTLV (100.0 ng mL⁻¹). B) Comparison between the decrease in signal for target protein p24-HIV (right) and control protein p24-HTLV (left); C) Voltammetric probe profile before and after incubation with control protein p24-HIV (100.0 ng mL⁻¹). 5.0 mmol L⁻¹ K₃[Fe(CN)₆] in PBS pH 7.4. Scan rate: 100 mV s⁻¹.

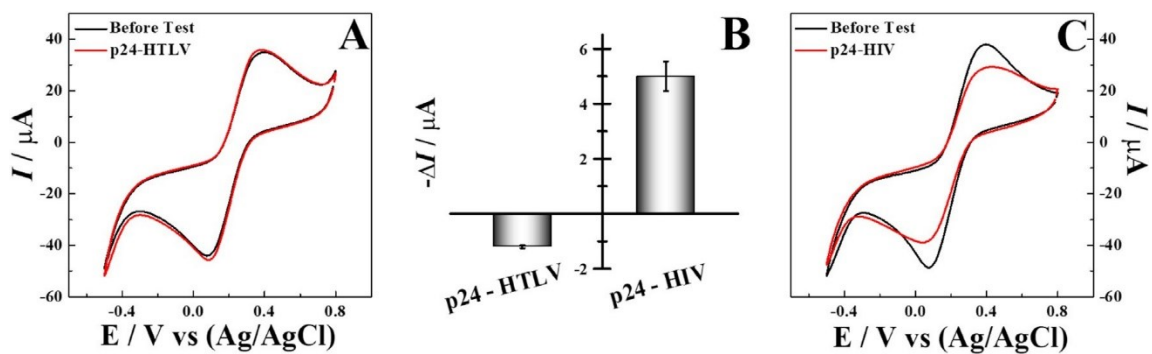


Fig. 4. A) Anodic peak section of the cyclic voltammetric response before and after incubation with 0.93 ng mL^{-1} (red), 93 ng mL^{-1} (blue), and 93 mg mL^{-1} (green) of p24-HIV; B) The analytical curve obtained by the proposed device. $5 \text{ mmol L}^{-1} \text{ K}_3\text{Fe}(\text{CN})_6$ in PBS pH 7.4. Scan rate: 100 mV s^{-1} $n = 4$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

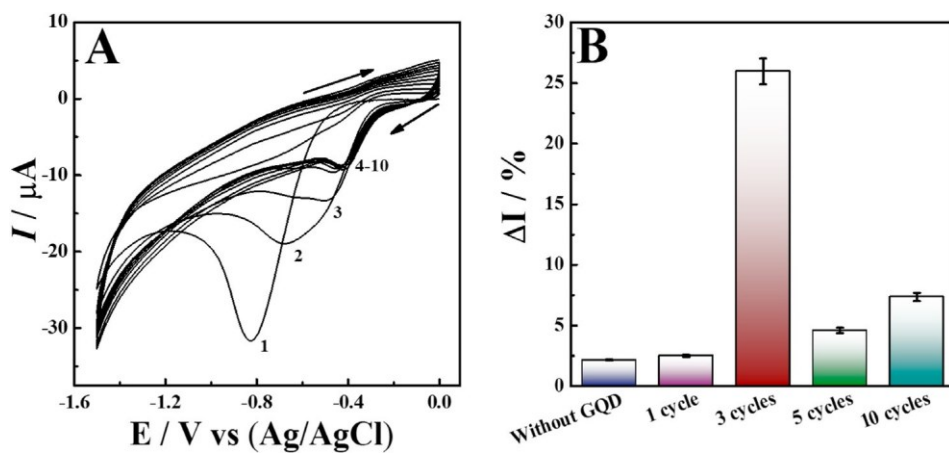


Table 1
 Linear detection range and limit of detection of p24-HIV sensors in literature.

Electrode	Method	LDR	LOD/ $\mu\text{g mL}^{-1}$	Reference
GQD-SPE/Aptamer	Electrochemical (Label-free)	0.93 ng mL^{-1} – 93 $\mu\text{g mL}^{-1}$	51.7	This work
GNP/MWCNTs/AEP	Electrochemical (HRP)	0.01–60 ng mL^{-1}	6.4	[29]
μPACs	Colorimetric (HRP)	0.1 ng mL^{-1} – 1 $\mu\text{g mL}^{-1}$	190.0	[30]
MWCNTs based MIP	Electrochemical (Label-free)	0.1 pg mL^{-1} –2 ng mL^{-1}	0.083	[31]
Graphene oxide-Carbon nanotubes-silica electrode	Electrochemical (HRP)	0.5 pg mL^{-1} – 8.5 ng mL^{-1}	0.15	[32]
Au/CuL	Electrochemical (HRP)	0.5–200 ng mL^{-1}	200	[33]
Ce(III) coordination polymer	Fluorescence	4–28 pg mL^{-1}	1.1	[34]

LDR = Linear detection range, LOD = Detection limit, HRP = Horseradish peroxidase, GNP = Gold nanoparticles, AEP = Acetone-extracted propolis MWCNT = Multiwalled Carbon Nanotubes, MIP = Molecularly Imprinted Polymer, μPACs = Plastic micro-chips with micro-pit array, Au/CuL = Mercapto succinic acid hydrazide copper (II) (CuL) monolayer modified gold electrode.

Fig. 5. A) Cyclic voltammety response for the positive serum spiked with p24-HIV (9.3 ng mL⁻¹). B) The signal shift obtained for positive serum (p24-HIV e red bar) and negative serum (green bar). C) Cyclic voltammety response for the negative sample, without the target protein. 5 mmol L⁻¹ K₃[Fe(CN)₆] in PBS pH 7.4; n = 4. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

