Brazilian Journal of Analytical Chemistry
2024, Volume 11, Issue 43, pp 15-20
doi: 10.30744/brjac.2179-3425.letter.brazacaLC

BrJAC

LETTER

Electrochemical Biosensors for the Detection of Viruses: Must-Have Products or Just Science for Publication?

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The SARS-CoV-2 pandemic has brought significant light to the urgent need for rapid, precise, and low-cost diagnosis tools. The scientific community has responded as quickly, overflowing the literature with papers describing interesting biosensors for aiding in the diagnosis of COVID-19.[1,2] However, almost none of them, mainly the electrochemical ones have reached the market or never will, with only a few traditional formats used in the daily combat of the virus, including ELISA (enzyme-linked immunosorbent assay), lateral flow assays, and, mainly, PCR (polymerase chain reaction).

The drawbacks of PCR and the need for improved analytical tools

Although PCR-based methods are currently the gold standard for detecting viruses worldwide, these still present various drawbacks. Usually, the commercial detection of viruses (such as SARS-CoV-2) uses the combination of standard PCR (or RT-PCR) and gel electrophoresis due to its sensitivity, reliability, and low price (if compared to other PCR-based methods such as real-time PCR). This approach relies, mainly, on the use of a standard thermal cycler and an electrophoresis tank by a specialized worker. While electrophoresis tanks can be quite affordable, with some of them costing a few hundred dollars,[3] even simple thermal cyclers cost around 5,000 USD[4] – significantly enhancing the investment required for testing. Furthermore, the complete analysis of a sample is slow and can take up to six hours to complete, which prevents an effective sanitary barrier at borders and crowded events, for example. The samples need to be transported to the lab, as no reliable portable PCR and gel electrophoresis equipment are available. The results commonly take from two to five days to be generated – an extremely long delay when considering that these can seriously influence the health of a patient and the spread of the virus. Last, standard PCR does not provide quantitative information – which is vital in some cases to aid in diagnosing the severity of an infection.

Cite: Brazaca, L. C.; Bonacin, J. A.; Muñoz, R. A. A.; Janegitz, B. C.; Carrilho, E. Electrochemical Biosensors for the Detection of Viruses: Must-Have Products or Just Science for Publication? *Braz. J. Anal. Chem.* 2024, *11* (43), pp 15-20. https://doi.org/10.30744/brjac.2179-3425.letter.brazacaLC

Submitted January 19, 2024; Resubmitted February 1, 2024; Accepted February 12, 2024; Available online March 1, 2024.

Techniques derived from PCR (such as qPCR, for example), on the other hand, can provide quantitative and more rapid results, but are also more expensive and still require sample transportation. Equipment for performing qPCR ranges from 15,000 USD to 90,000 USD[4] and the use of specific reaction kits containing fluorescent markers also corresponds to a significant increase in analysis costs.

Other commercially available methods for the detection of viruses, ELISA and lateral flow assays, also present significant drawbacks. While ELISA is time demanding (6 h) and requires specialized professionals and equipment to be adequately performed, some lateral flow assays present results with low precision,[5,6] being useful for massive triages in the case of COVID-19, for example.

Although presenting such limitations, PCR-based techniques are still the gold standard for the detection of viruses. This is probably due to its sensitive and well-established features, being widespread along with many medical and research centers around the globe. Furthermore, the development of PCR-based diagnosis kits in urgent scenarios, such as the one imposed by SARS-CoV-2, is straightforward and allows rapid responses from health organizations and governments. The technique can also provide low limits of detection (LOD), with a gold standard RT-PCR assay for COVID-19 presenting a LOD of ~100 copies of viral RNA per mL of transport media, for example. It is important to mention, however, that the LOD of currently approved assays for COVID-19 varies over 10,000-fold, which will generate immense false-negative rates.[7]

How can biosensors improve the diagnosis of viral diseases?

Biosensors present interesting properties to overcome some of the drawbacks presented by PCR. Although thousands of papers have been published in the last years based on the detection of several diseases, almost all the material published has focused on the formation of human resources and not on the market (Table I). There are few discussions in the electrochemical meetings and a tremendous demand to produce new selling and profitable devices for the environment, food, medical, and forensic analyses. In this context, portable potentiostats are commonly available on the market at prices that range from a few thousand dollars (2,000 – 3,000 USD) for full desktop equipment[8] to a few hundred dollars for equipment devoted to a single analysis. There is also significant research interest in the development of portable, miniaturized, and low-cost potentiostat, as highlighted by some articles published in recent years.[9–11] Colorimetric biosensors, in turn, can rely on responses readable with the naked eye or using widespread smartphones. The use of smartphones can also contribute to compiling results and acquiring additional information such as patient location and data. Therefore, if compared to PCR-based techniques, instrumentation costs are decreased while its portability allows point-of-care analysis, significantly increasing the accessibility to tests in remote areas. Analysis time is also greatly diminished as results can be obtained in only a few minutes. Both of these features are of extreme importance when considering healthcare applications that commonly require quick or real-time responses. Furthermore, immunosensors do not require previous sample preparation even when using complex biological fluids, decreasing analysis costs and making it even more rapid. Last, biosensors can be easy to use, usually requiring lower previous preparation from the operator if compared to traditional techniques (Figure 1).

Table I. Examples of recent (2021-2024) publications of electrochemical biosensors for the detection of viruses and their characteristics

Virus	Description	Detection range	Validation	Reference
Herpes simplex virus type 2 (HSV-2)	Electrodes were modified with human cellular receptor nectin-1 and electrochemical impedance spectroscopy (EIS) was applied for the determinations	1 to 10^5 HSV-2 (PFU/mL)	Tested in biological matrix	14

(continues on the next page)

Table I. Examples of recent (2021-2024) publications of electrochemical biosensors for the detection of viruses and their characteristics (continuation)

Virus	Description	Detection range	Validation	Reference
SARS-CoV-2	Gold electrodes were modified with nanochannels based on polystyrene (PS) containing bioreceptors. The blockage of the nanochannels with viruses hampers the diffusion of a redox probe.	1 to 10^8 particles/mL	Tested in biological matrix	15
Hepatitis C vírus (HCV)	Electrodes were modified with fragments of the cell receptor CD81 to determine HCV E2 envelope protein	0.1 to 5 µg/mL of hepatitis C virus-mimetic particles	Tested in synthetic plasma	16
Enterovirus 71 (EV71)	Determination based on the aggregation of AgNPs promoted by the incorporation of EV71	10^{-4} to 10 EV71 (PFU/mL)	Tested in biological matrix	17
SARS-CoV-2	Inkjet-printed nanostructured gold electrodes promote the multiplexed detection of SARS-CoV-2 ORF1ab and N genes with the use of an also inkjet-printed battery-free near-field communication (NFC) potentiostat	10^{-10} to 10^{-5} mol/L of ORF1ab and N genes	Tested in buffer	18

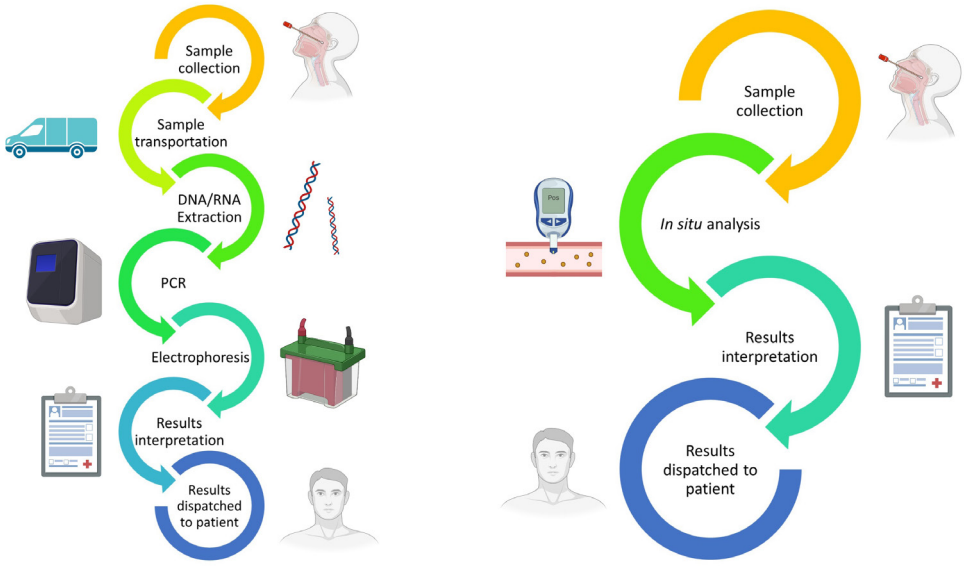

Figure 1. Steps typically involved in a molecular method (e.g., PCR) (left) and the simplicity of a biosensor (right) analysis of biological samples. Created with BioRender.com.

Biosensors can also be readily developed in urgent scenarios, as proven with COVID-19. Numerous examples of electrochemical, colorimetric, and mass-sensitive devices for aiding in the diagnosis of the disease were described in the literature only a few months after the start of the pandemic event.[1,12,13] Devices are commonly validated in biological samples, providing precise results in a rapid, cheap, and simple manner. So, a relevant question is, why are most of these devices still out of the consumers' reach?

Why are biosensors still out of the market?

Despite their advantages, electrochemical biosensors are rarely seen in the market except for particular examples such as the glucometer and a few lateral flow assays. In our opinion, diverse aspects contribute to the existing barrier between publication and commercialization.

1) The biorecognition layer, commonly composed of biomolecules such as antibodies, genetic material, and enzymes, may present stability issues regarding storage, temperature, and chemical conditions. The organization and structural integrity of such elements are essential for the adequate functioning of the devices, which is still a challenge to the field. This aspect mainly influences the shelflife of biosensors, hampering its commerciality.
2) To improve the analytical performance of devices, many of them use complex constructs or high-cost materials, such as nanoparticles, rare elements, or liquid crystals. While the complex constructs might bring a significant challenge for batch manufacturing, increased prices might favor the use of traditional techniques such as immunoassays.
3) For industries to be interested in the fabrication of biosensors, different barriers to market entrance must be transcended and the final product must be profitable. For example, a clear market demand must exist and regulatory agencies must approve the use of the device. Furthermore, the manufacturing must be adequate for low-cost batch production and the adaptation of the machinery or new processes should present cost-efficacy and availability.
4) The validation studies and the development of prototypes should be more discussed for the scientific community and should be a link between the industries and the academy around the world. Among other parameters, the accuracy of the developed tests, for example, needs to be carefully assessed in different scenarios, being compared to well-established, validated techniques to ensure that customers will get precise results.
5) Last, although biosensors present an adequate performance under controlled environments, they commonly present limitations when applied to raw biological samples. The reasons for that are diverse, including the presence of interfering species, biofouling, the formation of complexes, or the nature of the analyte itself. Therefore, the direct application of samples is still a problem.[12]

Recent advancements, however, present great potential to address these challenges. The use of 3-D printed electrodes, for example, might decrease the cost of electrode production while increasing the accessibility of devices, especially in low-resource settings.[19] Using new assembles and labels, in turn, presents the potential to increase the stability, sensitivity, and reliability of biosensors. To improve the biorecognition layer stability, the use of innovative receptors such as biomimetic enzymes, molecularly imprinted membranes, and DNA origami can be of great value,[20-22] while the development of flexible devices can improve the range of their application – including wearables, for example.[23] Last, the combination of artificial intelligence for data analysis and the Internet of Things is crucial for the automation of the procedure and can improve the analytical techniques beyond human potential.[24,25]

It must be clear for analytical chemists that, for achieving these new grounds, innovation and entrepreneurship are essential, stimulating the creation of startups, spin-offs and collaborations with existing companies.[26] Therefore, biosensors are beyond the publication hype and are an inspiration for the future, moving constantly closer to being accessible to the population, being, undoubtedly, not meant to be limited to journal pages.[12]

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

São Paulo Research Foundation (FAPESP) [2018/19750-3; 2013/22127-2; 2014/40867-3]. National Council for Scientific and Technological Development (CNPq) [315838/2021-3; 401256/2020-0;

308835/2019-0; 465389/2014–7]. Coordination for the Improvement of Higher Education Personnel (CAPES) [88887.504861/2020-00; 23038.003012/2020-16].

REFERENCES

(1) Samson, R.; Navale, G. R.; Dharne, M. S. Biosensors: Frontiers in Rapid Detection of COVID-19. *3 Biotech* **2020**, *10* (9), 385. https://doi.org/10.1007/s13205-020-02369-0
(2) Choi, J. R. Development of Point-of-Care Biosensors for COVID-19. *Front. Chem.* **2020**, *8*, article 517. https://doi.org/10.3389/fchem.2020.00517
(3) Wolflabs. *Horizontal Gel Electrophoresis Tank*. Available at: https://www.wolflabs.co.uk/laboratory-products/gel-electrophoresis-tanks-horizontal [Accessed June 2022].
(4) Johnson, M. PCR Machines. *Mater. Methods* **2013**, *3*, 193. https://doi.org/10.13070/mm.en.3.193
(5) Flower, B.; Brown, J. C.; Simmons, B.; Moshe, M.; Frise, R.; Penn, R.; Kugathasan, R.; Petersen, C.; Daunt, A.; Ashby, D.; et al. Clinical and Laboratory Evaluation of SARS-CoV-2 Lateral Flow Assays for Use in a National COVID-19 Seroprevalence Survey. *Thorax* **2020**, *75* (12), 1082–1088. https://doi.org/10.1136/thoraxjnl-2020-215732
(6) Mattioli, I. A.; Hassan, A.; Oliveira, O. N.; Crespilho, F. N. On the Challenges for the Diagnosis of SARS-CoV-2 Based on a Review of Current Methodologies. *ACS Sens* **2020**, *5* (12), 3655–3677. https://doi.org/10.1021/acssensors.0c01382
(7) Arnaout, R.; Lee, R. A.; Lee, G. R.; Callahan, C.; Yen, C. F.; Smith, K. P.; Arora, R.; Kirby, J. E. SARS-CoV-2 Testing: The Limit of Detection Matters. *bioRxiv* Preprint **2020**, Jun 4. https://doi.org/10.1101/2020.06.02.131144
(8) Wuhan Corrtest Instruments Corporation. *Portable Electrochemical Voltammetric Analyzer*. Available at: https://corrtest.en.made-in-china.com/product/MvSmWwIDllRj/China-Portable-Electrochemical-Voltammetric-Analyzer.html [Accessed June 2022].
(9) Bezuidenhout, P.; Smith, S.; Land, K.; Joubert, T.-H. A Low-Cost Potentiostat for Point-of-Need Diagnostics. *2017 IEEE Africon Conference*, pp 83–87. https://doi.org/10.1109/AFRCON.2017.8095460
(10) Kellner, K.; Posnicek, T.; Ettenauer, J.; Zuser, K.; Brandl, M. A New, Low-Cost Potentiostat for Environmental Measurements with an Easy-to-Use PC Interface. *Procedia Eng.* **2015**, *120*, 956–960. https://doi.org/10.1016/j.proeng.2015.08.820
(11) Cruz, A. F. D.; Norena, N.; Kaushik, A.; Bhansali, S. A Low-Cost Miniaturized Potentiostat for Point-of-Care Diagnosis. *Biosens. Bioelectron.* **2014**, *62*, 249–254. https://doi.org/10.1016/j.bios.2014.06.053
(12) Brazaca, L. C.; dos Santos, P. L.; de Oliveira, P. R.; Rocha, D. P.; Stefano, J. S.; Kalinke, C.; Abarza Muñoz, R. A.; Bonacin, J. A.; Janegitz, B. C.; Carrilho, E. Biosensing Strategies for the Electrochemical Detection of Viruses and Viral Diseases – A Review. *Anal. Chim. Acta* **2021**, *1159*, 338384. https://doi.org/10.1016/j.aca.2021.338384
(13) Asif, M.; Ajmal, M.; Ashraf, G.; Muhammad, N.; Aziz, A.; Iftikhar, T.; Wang, J.; Liu, H. The Role of Biosensors in Coronavirus Disease-2019 Outbreak. *Curr. Opin. Electrochem.* **2020**, *23*, 174–184. https://doi.org/10.1016/j.coelec.2020.08.011
(14) de Lima, L. F.; Ferreira, A. L.; Awasthi, S.; Torres, M. D. T.; Friedman, H. M.; Cohen, G. H.; de Araujo, W. R.; de la Fuente-Nunez, C. Rapid and Accurate Detection of Herpes Simplex Virus Type 2 Using a Low-Cost Electrochemical Biosensor. *Cell Rep. Phys. Sci.* **2023**, *4* (9), 101513. https://doi.org/10.1016/j.xcrp.2023.101513
(15) Shiohara, A.; Wojnilowicz, M.; Lyu, Q.; Pei, Y.; Easton, C. D.; Chen, Y.; White, J. F.; McAuley, A.; Prieto-Simon, B.; Thissen, H.; Voelcker, N. H. SARS-CoV-2 Virus Detection Via a Polymeric Nanochannel-Based Electrochemical Biosensor. *Small* **2023**, *19* (51). https://doi.org/10.1002/smll.202205281
(16) Antipchik, M.; Korzhikova-Vlakh, E.; Polyakov, D.; Tarasenko, I.; Reut, J.; Öpik, A.; Syritski, V. An Electrochemical Biosensor for Direct Detection of Hepatitis C Virus. *Anal. Biochem.* **2021**, *624*, 114196. https://doi.org/10.1016/j.ab.2021.114196

(17) Sukjee, W.; Sangma, C.; Lieberzeit, P. A.; Ketsuwan, K.; Thepparit, C.; Chailapakul, O.; Ngamrojanavanich, N. EV71 Virus Induced Silver Nanoparticles Self-Assembly in Polymer Composites with an Application as Virus Biosensor. *Sens. Actuators, B* **2023**, *393*, 134324. https://doi.org/10.1016/j.snb.2023.134324

(18) Rossetti, M.; Srisomwat, C.; Urban, M.; Rosati, G.; Maroli, G.; Akbay, H. G. Y.; Chailapakul, O.; Merkoçi, A. Unleashing Inkjet-Printed Nanostructured Electrodes and Battery-Free Potentiostat for the DNA-Based Multiplexed Detection of SARS-CoV-2 Genes. *Biosens. Bioelectron.* **2024**, *250*, 116079. https://doi.org/10.1016/j.bios.2024.116079

(19) Martins, G.; Gogola, J. L.; Budni, L. H.; Janegitz, B. C.; Marcolino-Junior, L. H.; Bergamini, M. F. 3D-Printed Electrode as a New Platform for Electrochemical Immunosensors for Virus Detection. *Anal. Chim. Acta* **2021**, *1147*, 30–37. https://doi.org/10.1016/J.ACA.2020.12.014

(20) Gui, R.; Jin, H.; Guo, H.; Wang, Z. Recent Advances and Future Prospects in Molecularly Imprinted Polymers-Based Electrochemical Biosensors. *Biosens. Bioelectron.* **2018**, *100*, 56–70. https://doi.org/10.1016/j.bios.2017.08.058

(21) Zhang, K.; Huang, W.; Huang, Y.; Li, H.; Wang, K.; Zhu, X.; Xie, M. DNA Tetrahedron Based Biosensor for Argonaute2 Assay in Single Cells and Human Immunodeficiency Virus Type-1 Related Ribonuclease H Detection in Vitro. *Anal. Chem.* **2019**, *91* (11), 7086–7096. https://doi.org/10.1021/acs.analchem.9b00011

(22) Zhao, Y.; Huo, D.; Bao, J.; Yang, M.; Chen, M.; Hou, J.; Fa, H.; Hou, C. Biosensor Based on 3D Graphene-Supported Fe_3O_4 Quantum Dots as Biomimetic Enzyme for in Situ Detection of H_2O_2 Released from Living Cells. *Sens. Actuators, B* **2017**, *244*, 1037–1044. https://doi.org/10.1016/j.snb.2017.01.029

(23) Shin, M.; Yoon, J.; Yi, C.; Lee, T.; Choi, J.-W. Flexible HIV-1 Biosensor Based on the Au/MoS2 Nanoparticles/Au Nanolayer on the PET Substrate. *Nanomaterials* **2019**, *9* (8), 1076. https://doi.org/10.3390/nano9081076

(24) Cui, F.; Yue, Y.; Zhang, Y.; Zhang, Z.; Zhou, H. S. Advancing Biosensors with Machine Learning. *ACS Sens.* **2020**, *5* (11), 3346–3364. https://doi.org/10.1021/acssensors.0c01424

(25) Cadeado, A.; Machado, C.; Oliveira, G.; e Silva, D.; Muñoz, R.; Silva, S. Internet of Things as a Tool for Sustainable Analytical Chemistry: A Review. *J. Braz. Chem. Soc.* **2022**, *33* (7), 681-692. https://doi.org/10.21577/0103-5053.20220048

(26) Carrilho, E. Analytical and Bioanalytical Chemistry – It Is Time We Innovate. *Braz. J. Anal. Chem.* **2022**, *9* (35), 5-6. https://doi.org/10.30744/brjac.2179-3425.point-of-view.ecarrilho.N35