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Development and validation of an electroanalytical method for the quantification of antiviral molnupiravir and its application to the pharmaceutical sample

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ABSTRACT: In this study, a sensitive, fast, economical, and practical voltammetric method was developed for analysing molnupiravir (MLP), an antiviral drug extensively used during the COVID-19 pandemic, without the need for modifying the glassy carbon (GC) electrode surface. During the electroanalysis, an oxidation peak for MLP was detected at 0.72555 V on the GC electrode. The method exhibited linearity across a concentration range of 1 - 250 µM for MLP with 0.33 µM of detection limit. Furthermore, with a 99.81% recovery from its pharmaceutical form, the method's effectiveness was validated. These results strongly indicate that this method is suitable for routine quality control of molnupiravir and may serve as a model for analysing other antiviral drugs.

KEYWORDS: Antiviral agent; molnupiravir; electroanalytical chemistry: differential pulse voltammetry.

1. INTRODUCTION

Viral diseases, caused by virus-called particles lacking independent cellular machinery and relying on nucleic acid for genetic material, pose a significant challenge in modern medicine. They manifest in various forms, including acute infections, chronic conditions, and virus-related cancers [1-3]. Viral diseases have been known since ancient times and have significantly affected humankind throughout history. In the past two decades, we have encountered numerous viral epidemics caused by various virus families, including flaviviruses, noroviruses, filoviruses, myxoviruses, alphaviruses, and hepaniviruses, among others [4, 5]. Finally, a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced viral disease (COVID-19), which started in China and then affected the whole world, forced all humanity into a very strict isolation, brought daily life to a standstill, caused serious economic crises on a global scale, and was responsible for the deaths of millions of people, was declared a pandemic by the World Health Organization (WHO) [6-9]. Viral diseases have given rise to antiviral therapy over time. Although the COVID-19 pandemic caught humanity by surprise, effective and safe vaccines were rapidly developed, helping to lower mortality rates and control the spread of the disease. However, widespread scepticism about these vaccines has led to an increased emphasis on monoclonal antibody therapy and antiviral treatment [7, 8, 10]. Molnupiravir (MLP), an antiviral agent, molecular structure shown in Figure 1, is a synthetic nucleoside derivates which affects by inhibiting the replication of RNA viruses, was approved in the United Kingdom in November 2021 for use in adult COVID-19 patients and is reported to reduce hospitalization and mortality by around 50% [11-14].

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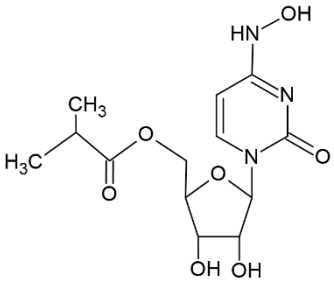

Figure 1. Molecular structure of MLP

To achieve the desired therapeutic effect and avoid toxic side effects, precise dose adjustment is essential in drug treatment. Therefore, quality control during production must be conducted meticulously. Various analytical methods are being developed to ensure these controls are effectively implemented. Electroanalytical methods are distinguished among analytical techniques for their rapid response, portability, cost-effectiveness, straightforward procedures, high sensitivity, and minimal interference from drug excipients. Consequently, they are becoming an essential approach in pharmaceutical analysis [15-20]. In addition, Electroanalytical methods offer several advantages over traditional analytical techniques, including the ability to perform in-situ and real-time analysis without the need for extensive sample preparation. This not only speeds up the analytical process but also reduces the potential for sample contamination and loss [21-23]. In recent years, several electroanalytical studies on MLP analysis have been reported, but they all involve material synthesis and characterization, as well as sensor modification and characterization [24-27]. These steps are both costly and time-consuming. In contrast, glassy carbon (GC) electrodes are particularly advantageous due to their wide potential window, chemical inertness, low background current, and excellent conductivity. These properties allow GC electrodes to detect small changes in current with high precision, making them highly effective for the electrochemical detection of various drugs. Furthermore, GC electrodes are robust and reusable, which contributes to the overall cost-effectiveness of the method [28, 29]. In this study, a selective, cost-effective, rapid, and easily applicable electroanalytical method for MLP analysis was developed, which does not require material synthesis or surface modification processes. This method was successfully applied to analyse MLP directly from pharmaceutical sample.

2. RESULTS AND DISCUSSION

2.1. Electrochemical behaviour of MLP

The electrochemical behaviour of 100 µM MLP on the GC electrode surface was examined in a Britton–Robinson buffer solution (BR) at pH 2 with the differential pulse voltammetry (DPV). As illustrated in Figure 2, a distinct oxidation peak appeared at 0.72555 V with a peak current of 1.16 µA.

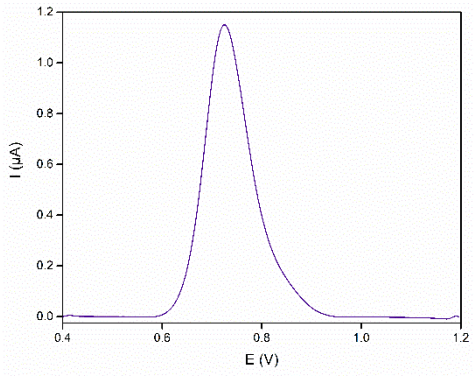

Figure 2. Baseline corrected DPV of 100 µM MLP in pH 2 BR.

2.2. Evaluation pH effect

As is well known, the acidity or alkalinity of the electrolyte solution is a key factor in electrochemical studies, significantly influencing analytical results. Therefore, pH optimization is essential to achieve the

best experimental conditions. The electrochemical responses of 100 µM MLP were compared across different pH values, ranging from 2 to 9. As shown in Figure 3, the electroanalytical response of MLP decreased as the pH shifted from acidic to neutral, and nearly disappeared in alkaline conditions. Consequently, pH 2 was selected as the optimal value for subsequent experiments.

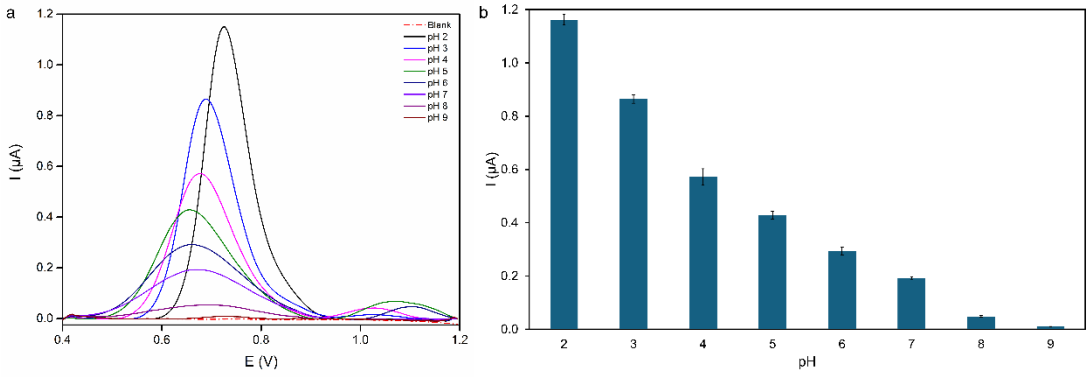

Figure 3. Baseline corrected DPVs of 100 µM MLP at different pH values of BR (a), average (N = 3) peak current value of 100 µM MLP at different pH values of BR (b).

2.3. Evaluation of electrochemical reaction

The electrochemical reaction at the electrode surface can be governed by adsorption, diffusion, or a combination of both. To assess the electro-oxidation mechanism of MLP on the GC electrode surface, cyclic voltammetry (CV) was performed on 100 µM MLP across a range of scan rates from 0.01 V·s^{-1} to 0.5 V·s^{-1}. As shown in Figure 4, the logarithm of the scan rate (log v) exhibits a linear relationship with the logarithm of the peak current (log I), described by the regression equation log I (µA) = 0.4402 log v (V·s^{-1}) +0.6216 (R^2 = 0.9973). The slope, being less than 0.5, suggests that the reaction is diffusion-controlled on the electrode surface, with adsorption playing no role in the electro-oxidation of MLP. This finding enables the development of a faster and more practical method, eliminating the need for accumulation potential and accumulation time steps.

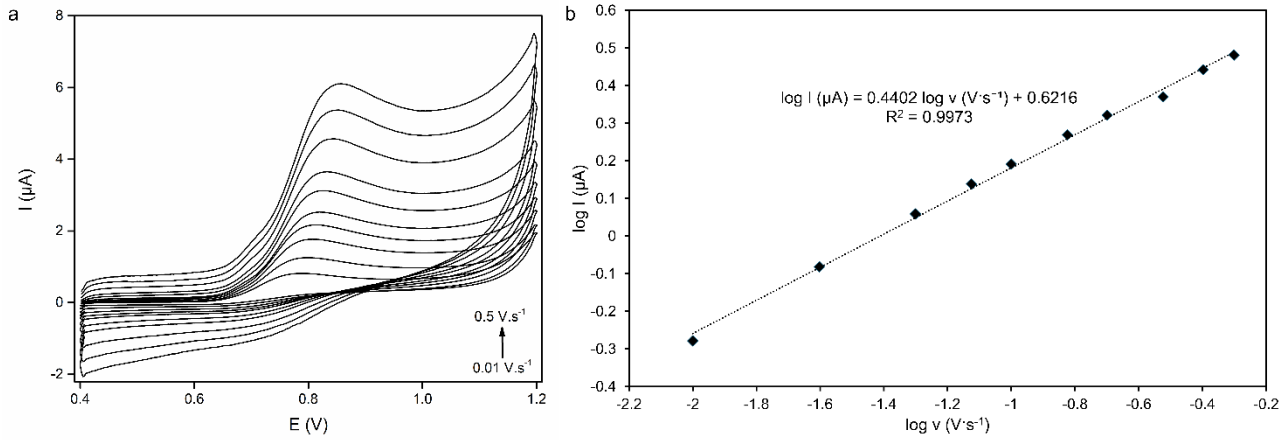

Figure 4. CVs of 100 µM MLP in pH 2 BR at increasing scan rates (a), plot of log v vs. log I (N = 3).

2.4. Analytical performance

Once the optimal pH value and reaction mechanism were established, the next step was to determine the linear working range. Prior to measurements, all factors influencing the method's performance were optimized, as outlined in Table 1. Under these optimal conditions, the analytical response of MLP exhibited linearity across a concentration range of 1 to 250 µM, as illustrated in Figure 5. The corresponding regression equation is as follows:

I (µA) = 0.0082 C_{MLP} (µM) + 0.0785 (R^2=0.9924)

The limit of detection (LOD) was determined using the formula $3SD/m$, where SD represents the standard deviation of the lowest concentration within the linear working range, and m is the slope of the regression equation. The LOD was calculated to be 0.33 µM, confirming the method's sensitivity. All analytical results summarized in Table 2. As mentioned above, a distinct oxidation peak appeared at 0.72555 V. Although there have been some negligible shifts, the peak currents were located at similar potentials during the measurements that made for construction of regression equation.

Table 1. Optimized analytical parameters of DPV for MLP detection.

Parameter	Value
Start potential (V)	0.4
Stop potential (V)	1.2
Step (V)	0.005
Modulation amplitude (V)	0.025
Modulation time (s)	0.05
Interval time (s)	0.1
Scan rate ($V \cdot s^{-1}$)	0.05

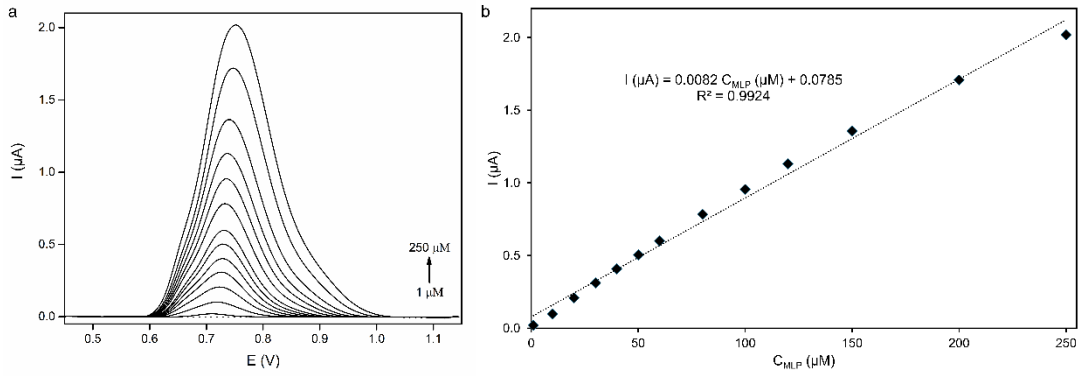

Figure 5. Baseline c orrected DPVs of MLP at increasing concentration in the pH 2 BR. Dotted line is DPV of pH 2 BR off MLP (a), the calibration graph of MLP (b).

Table 2. Analytical parameters obtained from MLP analysis.

Parameter	Value
Linear working range (µM)	1 – 250
Slope	0.0082
Intercept	0.0785
LOD (µM)	0.33

2.5. Repeatability

In analytical studies, it's essential to validate the developed method for repeatability to ensure consistent results across different times or operators. To assess the repeatability of the method, 100 µM MLP was analyzed five times consecutively using DPV with a GC electrode. The relative standard deviation (RSD) was found to be 4.19%, indicating that the method is reliably repeatable and suitable for routine MLP analysis.

Table 3. The repeatability results of MLP at the surface of GC electrode

Parameter	Value
	0.7143
	0.6874
I (µA)	0.6854
	0.6625
	0.6389
Mean	0.6777
SD	0.0284
RSD	4.19%

2.6. Selectivity

The ability to accurately analyze the target analyte within a complex matrix is a key requirement in analytical method development, making selectivity studies crucial. In this regard, 10 µM MLP was tested in the presence of potential interferences at 100 times their concentration, including glucose, urea, Na^+, K^+, Ca^{2+}, Cl^-, NO_3^-, and CO_3^{2-}. The change in the analytical response was only 0.95%, demonstrating that the developed method can effectively analyze MLP without interference from these substances.

2.7. Pharmaceutical sample analysis

To assess the method's applicability, the MLP content in commercially available capsules was analysed using the standard addition method. A slight positive shift of ~0.05 V in the peak potential was observed, likely due to the complex matrix of the capsule contents. The average recovery value, based on three replicates, was calculated to be 99.81%, confirming that the developed method is well-suited for analysing MLP in its pharmaceutical form.

Table 4. Determination of MLP in capsule (N = 3).

Labelled (mg)	Found (mg)	Recovery (%)
200	199.63	99.81

3. CONCLUSION

In this study, a rapid and sensitive voltammetric method for MLP analysis on GC electrode surfaces was developed and validated. Unlike other voltammetric methods reported for MLP, this approach is practical and cost-effective, as it eliminates the need for material synthesis and surface modification. The method demonstrated a broad linear working range and successfully analysed MLP in pharmaceutical samples. Considering all the findings, this electroanalytical method has been introduced to the literature as a reliable tool for routine quality control of MLP and as a potential model for analysing other antiviral drugs.

4. MATERIALS AND METHODS

4.1. Chemicals and reagents

MLP was generously provided as a gift by Abdi İbrahim Pharmaceuticals Industry and Trade Corp. (Istanbul, Turkey). All chemicals used in the experiments were of analytical grade, and ultra-pure water was used as the solvent to prepare the MLP stock solution.

4.2. Apparatus

All electroanalytical measurements were performed using a Metrohm-Autolab potentiostat-galvanostat system (AUT204FRA32M, Netherlands), controlled by NOVA 2.1 software. The conventional three-electrode system consisted of a 3 mm diameter GC electrode as the working electrode, an Ag/AgCl (3 M KCl) electrode as the reference electrode, and a platinum wire as the counter electrode (all from BASi®).

4.3. Electroanalytical measurements

Voltammetry, specifically CV and DPV, was selected as the electroanalytical technique. Before measurements, the surface of the GC electrode was polished with an alumina slurry and then washed with water and ethanol, respectively. BR was used as the electrolyte solution, and all measurements were conducted under ambient conditions.

4.4. Pharmaceutical sample preparation

The contents of a capsule containing 200 mg of MLP were mixed with ultrapure water in a volumetric flask and placed in an ultrasonic bath for 15 minutes to fully dissolve the MLP. Afterward, the insoluble excipients were allowed to settle at the bottom. The solution was then filtered through a 0.45 µm syringe filter. Before use, it was diluted with BR to reach the desired concentration.

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REFERENCES

[1] Qu J-M, Cao B, Chen R-C. Respiratory virus and COVID-19. In: Qu J-M, Cao B, Chen R-C (Eds). COVID-19 The Essentials of Prevention and Treatment. Elsevier, USA, 2021, pp. 1-6.

[2] Kotra LP. Viral Disease. In: Enna SJ, Bylund DB (Eds). xPharm: The Comprehensive Pharmacology Reference. Elsevier, New York, 2007, pp. 1-3.

[3] Dunn EF, Connor JH. HijAkt: The PI3K/Akt Pathway in Virus Replication and Pathogenesis. In: Shenolikar S.(Ed.) Progress in Molecular Biology and Translational Science. Academic Press, USA, 2012, pp. 223-250.

[4] Chappell JD, Dermody TS. Biology of Viruses and Viral Diseases. In: Bennett JE, Dolin R, Blaser MJ (Eds). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Elsevier/Saunders, Philadelphia, 2015, pp. 1681-1693.e1684.

[5] Meganck RM, Baric RS. Developing therapeutic approaches for twenty-first-century emerging infectious viral diseases. Nat Med. 2021; 27(3): 401-410. https://doi.org/10.1038/s41591-021-01282-0.

[6] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395(10229): 1054-1062. https://doi.org/10.1016/S0140-6736(20)30566-3.

[7] Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Pérez Marc G, Moreira ED, Zerbini C. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020; 383(27): 2603-2615. https://doi.org/10.1056/NEJMoa2034577.

[8] Brown AN, Lang Y, Zhou J, Franco EJ, Hanrahan KC, Bulitta JB, Drusano GL. Why molnupiravir fails in hospitalized patients. Mbio 2022; 13(6): e02916-02922. https://doi.org/10.1128/mbio.02916-22.

[9] WHO, World Health Organisation. https://data.who.int/dashboards/covid19/deaths?n=c (accessed 22 August 2024)

[10] Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021; 384(5): 403-416. https://doi.org/10.1056/nejmoa2035389.

[11] Syed YY. Molnupiravir: First approval. Drugs 2022; 82(4): 455-460. https://doi.org/10.1007/s40265-022-01684-5.

[12] Singh AK, Singh A, Singh R, Misra A. Molnupiravir in COVID-19: A systematic review of literature. Diabetes Metab Syndr Clin Res Rev. 2021; 15(6): 102329. https://doi.org/10.1016/j.dsx.2021.102329.

[13] Ashour NA, Abo Elmaaty A, Sarhan AA, Elkaeed EB, Moussa AM, Erfan IA, Al-Karmalawy AA. A systematic review of the global intervention for SARS-CoV-2 combating: from drugs repurposing to molnupiravir approval. Drug Des Devel Ther. 2023; 685-715. https://doi.org/10.2147/DDDT.S354841.

[14] Mahase E, Covid-19: Molnupiravir reduces risk of hospital admission or death by 50% in patients at risk, MSD reports. https://www.bmj.com/content/375/bmj.n2422 (accessed 22 August 2024)

[15] Uslu B, Ozkan SA. Electroanalytical methods for the determination of pharmaceuticals: A review of recent trends and developments. Anal Lett. 2011; 44(16): 2644-2702. https://doi.org/10.1080/00032719.2011.553010.

[16] Munoz R, Almeida E, Angnes L. Sample preparation techniques for the electrochemical determination of metals in environmental and food samples. Refer Mod Chem Mol Sci Eng. 2013; 1-10. https://doi.org/10.1016/B978-0-12-409547-2.05750-4. .

[17] Yadav SK, Chandra P, Goyal RN, Shim Y-B. A review on determination of steroids in biological samples exploiting nanobio-electroanalytical methods. Anal Chim Acta 2013; 762:14-24. https://doi.org/10.1016/j.aca.2012.11.037.

[18] Kulikova T, Porfireva A, Shamagsumova R, Evtugyn G. Voltammetric sensor with replaceable polyaniline-DNA layer for doxorubicin determination. Electroanalysis 2018; 30(10): 2284-2292. https://doi.org/10.1002/elan.201800331.

[19] Liu Y, Wei M, Hu Y, Zhu L, Du J. An electrochemical sensor based on a molecularly imprinted polymer for determination of anticancer drug mitoxantrone. Sens Actuators B Chem. 2018; 255(1): 544-551. https://doi.org/10.1016/j.snb.2017.08.023.

[20] Huang D, Wu H, Zhu Y, Su H, Zhang H, Sheng L, Liu Z, Xu H, Song C. Sensitive determination of anticancer drug methotrexate using graphite oxide-nafion modified glassy carbon electrode. Int J Electrochem Sci. 2019; 14(4): 3792-3804. https://doi.org/10.20964/2019.04.03.

[21] Karadurmus L, Sahin IF, Kurbanoglu S, Ozkan SA. Electrochemical determination of non-steroidal anti-inflammatory drugs. Curr Anal Chem. 2019; 15(4): 485-501. https://doi.org/10.2174/1573411014666180917113920.

[22] Karadurmus L, Kır D, Kurbanoglu S, Ozkan SA. Electrochemical analysis of antipsychotics. Curr Pharm Anal. 2019; 15(5): 413-428. https://doi.org/10.2174/1573412914666180710114458.

[23] Karadurmus L, Kurbanoglu S, Uslu B, A Ozkan S. Electrochemical DNA biosensors in drug analysis. Curr Pharm Anal. 2017; 13(3): 195-207. https://doi.org/10.2174/1573412912666160422152634.

[24] Erk N, Bouali W, Mehmandoust M, Soylak M. An electrochemical sensor for molnupiravir based on a metal-organic framework composited with poly (3, 4-ethylene dioxythiophene): Poly (styrene sulfonate). ChemSelect. 2022; 7(46): e202203325. https://doi.org/10.1002/slct.202203325.

[25] Kablan SE, Reçber T, Tezel G, Timur SS, Karabulut C, Karabulut TC, Eroğlu H, Kır S, Nemutlu E. Voltammetric sensor for COVID-19 drug Molnupiravir on modified glassy carbon electrode with electrochemically reduced graphene oxide. J Electroanal Chem. 2022; 920:116579. https://doi.org/10.1016/j.jelechem.2022.116579.

[26] Nabil A, Hendawy HA, Abdel-Salam R, Ahmed RM, Shawky A, Emara S, Ibrahim N. A green voltammetric determination of molnupiravir using a disposable screen-printed reduced graphene oxide electrode: Application for pharmaceutical dosage and biological fluid forms. Chemosensors 2023; 11(9): 471. https://doi.org/10.3390/chemosensors11090471.

[27] Vural K, Karakaya S, Dilgin DG, Gökçel Hİ, Dilgin Y. Voltammetric determination of Molnupiravir used in treatment of the COVID-19 at magnetite nanoparticle modified carbon paste electrode. Microchem J. 2023; 184: 108195. https://doi.org/10.1016/j.microc.2022.108195.

[28] Ozcelikay G, Karadurmus L, Kaya SI, Bakirhan NK, Ozkan SA. A review: New trends in electrode systems for sensitive drug and biomolecule analysis. Crit Rev Anal Chem. 2020; 50(3): 212-225. https://doi.org/10.1080/10408347.2019.1615406.

[29] Shahzad S, Karadurmus L, Dogan-Topal B, Taskin-Tok T, Shah A, Ozkan SA. Sensitive nucleic acid detection at NH2-MWCNTs modified glassy carbon electrode and its application for monitoring of gemcitabine-DNA interaction. Electroanalysis. 2020; 32(5): 912-922. https://doi.org/10.1002/elan.201900597.