published by Ural Federal University
eISSN2411-1414
chimicatechnoacta.ru

ARTICLE
2025, vol. 12(1), No. 12106
DOI: 10.15826/chimtech.2025.12.1.06

Rapid voltammetric determination of hemagglutinin in saliva using magnetic nanoparticles modified with triazolotriazine in a portable cell design

Yulia Sabitova [a], Tatiana Svalova [a]*, Margarita Medvedeva [a], Natalia Malysheva [a], Vladimir Rusinov [ab], Anatoly Matern [a], Alisa Kozitsina [a]

a: Institute of Chemical Engineering, Ural Federal University, Ekaterinburg 620009, Russia
b: I.Ya. Postovsky Insititute of Organic Synthesis of the Ural Branch of the Russian Academy of Sciences, Ekaterinburg 620137, Russia
* Corresponding author: t.s.svalova@urfu.ru

Abstract

Express determination of the viral nature of the diseases is one of the most important tasks of modern medicine. Portable electrochemicalanalytical devices are used to complement standard laboratory analytical methods and implement analysis at the patient's bedside. A rapid approach of voltammetric determination of the viral marker protein hemagglutinin (HA) in saliva using portable electrochemical microcell based on the three-electrode planar system (TPS) and magnetite nanoparticles modified with 3-nitro-4-hydroxy-1,4-dihydro-7-propargylthio-1,2,4-triazolo[5,1-c][1,2,4]triazine (MNPs-Aza) was developed. MNPs-Aza were synthesized by carbodiimide cross-linking and characterized by infrared spectroscopy and square wave voltammetry. Modified MNPs were used simultaneously as carriers of the HA receptor and as agents for HA-Aza complexes concentrating on the surface of the working electrode. The normalized difference between the peak current values of the first stage of electroreduction of Aza before and after selective interaction with the HA in the analyzed sample (I^*) was used as the analytical signal. Under the selected operating conditions of the developed portable microcell, linear calibration I^* = (12.81±0.26) lg c_{HA} + (136.75±1.73) was obtained in the range of 10^{-3}–10^{-9} M. The limit of detection (LOD) calculated by the 3σ criteria was $8.4 \cdot 10^{-10}$ M. The developed approach was successfully tested on model solutions and real saliva samples.

Key findings

- The approach of voltammetric determination of hemagglutinin using magnetic nanoparticles in a portable microcell was developed.
- An algorithm of magnetic nanoparticle modification with 3-nitro-4-hydroxy-1,4-dihydro-7-propargylthio-1,2,4-triazolo[5,1-c][1,2,4]triazine was developed.
- Determination of hemagglutinin-containing viruses such as H1N1 can be realized.
- The developed approach is highly sensitive and selective.

Accompanying information

Article history
Received: 25.11.24
Revised: 03.12.24
Accepted: 04.12.24
Available online: 09.12.24

Keywords
Hemagglutinin; 3-nitro-4-hydroxy-1,4-dihydro-7-propargylthio-1,2,4-triazolo[5,1-c][1,2,4]triazine; portable electrochemical microcell; magnetite nanoparticles; voltammetric rapid analysis; three-electrode planar system

Funding
The study was supported by the Russian Science Foundation, project no. 20-13-00142 (https://rscf.ru/project/20-13-00142/).

Supplementary information
Supplementary materials: READ
Transparent peer review: READ

Sustainable Development Goals

1. Introduction

Infectious diseases of viral etiology have become a serious threat to human life safety due to their high contagiousness, severe disease course and frequent complications [1]. Quick and accurate determination of the viral nature of the pathogen “point-of-care” facilitates the timely prescription of adequate therapy, reducing the risks of possible complications and the number of contact patients [2]. Express, sensitive and portable (bio)sensors and test systems today successfully complement traditional laboratory methods of analysis as means of primary rapid diagnostics. As a rule, the patient’s blood, saliva and samples taken from the nasopharynx or oropharynx are used as research objects, and the pathogen is identified both directly by the content of viral particles and signaling proteins (C-reactive

protein, hemagglutinin, serum amyloid A, etc.), and by antibodies to them at later stages of the disease [3, 4]. At the same time, non-invasive express diagnostic tools have an absolute advantage. The detection method and the nature and structure of the bio/chemical receptor layer play a key role in ensuring the required analytical performance of portable devices.

The authors of [5] developed immunochromatographic test strips for determining the hepatitis B virus. The test strip consists of a sample injection zone, a conjugation zone coated with gold nanoparticles with immobilized mouse monoclonal antibodies to the hepatitis B virus, and a control line. Blood is used as the analyzed sample. After injection, the sample moves along the strip to the test zone under the influence of capillary effect. The assay reliably detects hepatitis B surface antigens at levels of 50 IU/mL for all genotypes and at lower concentrations for some genotypes. The authors of [6] developed an improved method of immunochromatographic analysis for the determination of influenza A and B viruses, which uses antibodies conjugated to fluorescent beads. The sensitivity and specificity of this test are 100%. The disadvantages of the mentioned approaches include the high cost and low stability of the immunoreagents as well as qualitative or semi-quantitative analysis results with insufficient sensitivity of optical detection methods. The work [7] describes immunochromatographic test strips for determining the influenza A H1N1 virus. The test strip consists of a zone for the introduction of saliva, a conjugation zone coated with monoclonal antibodies which recognize the nucleoproteins of the influenza A virus, and a control line. The test kit provides a rapid (10–20 min), non-invasive and cost-effective diagnosis of the virus. The sensitivity of the analysis does not exceed 60%, and the detection limit is 10^3 units.

The sensitivity and accuracy of differential rapid determination of infectious agents can be improved by using electrochemical portable test devices and sensors based on nanomaterials [8, 9]. An electrochemical immunosensor for the determination of Sars-CoV-2 in saliva was developed in [10]. The determination algorithm consists of incubating magnetic nanoparticles coated with monoclonal antibodies, polyclonal antibodies against Sars-CoV-2, as well as polyclonal antibodies conjugated with alkaline phosphatase used as a label, 1-naphthylphosphate and the analyzed saliva sample. After 30 min of incubation, the resulting suspension is applied to a three-electrode planar screen-printed system. Using magnetic separation, the immunocomplex is concentrated in the working area of the electrode, after which the differential pulse voltammetric response is recorded. The analytical performance of the electrochemical immunoassay was assessed in a standard solution of spike (S) and nucleocapsid (N) proteins in buffer solution and in untreated saliva. A detection limits for proteins S and N in untreated saliva were 19 ng/ml and 8 ng/ml, respectively. A diamond biosensor [11] allows the specific detection of influenza A virus. The surface of the diamond electrode was modified with 4-aminobenzoic acid, bovine serum albumin, and polyclonal antibodies against the influenza antigen. The determination algorithm included taking a swab from the nasopharynx or pharynx, placing it in a buffer solution containing 0.5% nonionic surfactant Triton X-100 ($C_{14}H_{22}O$ $(C_2H_4O)_n$), incubation and recording the electrochemical response using electrochemical impedance spectroscopy. The detection limit was 0.7 fg, the linear range – 1–100 fg.

According to the literature, primarily due to the simplicity of the design, the most common portable devices for detecting viruses are immunochromatographic systems. The receptors in such devices are antibodies to the virus being detected. At the same time, the transition to electrochemical devices is promising, since they allow quantitative assessment of the virus in the sample, which increases the sensitivity of the analysis and tracks the dynamics of the disease.

A laboratory prototype of an electrochemical test platform based on magnetite nanoparticles, used simultaneously as a signal-forming label, a carrier of the immunoreceptor layer, and concentration agents for the rapid analysis of *S. thyphimurium* antigens, was developed [12]. Antibodies are immobilized on the surface of magnetic nanoparticles through carbodiimide cross-linking. Unlike most known approaches and devices, in this design the formation of the immunocomplex occurred in the sample volume placed in the chamber of the flow cell, after which the immunocomplexes were concentrated on the surface of the three-electrode planar system under the influence of a magnetic field. Input and output of reaction components and cell washing were carried out using a stationary laboratory micropump. The analytical signal was the difference in the electroreduction currents of MNPs before and after incubation in the analyzed medium. Such design ensured the stability of the quantitative determination characteristics over 10 cycles of measurements on one electrode in the flow-injection mode.

The formation of a receptor layer of electrochemical sensors using small heterocyclic molecules as elements of independent (bio)molecular recognition (due to the ability to selectively bind to the compound being determined) is especially relevant today and can significantly expand the analytical capabilities and prospects for the practical application of such devices. Triazolotriazine compounds are a group of condensed azaheterocyclic compounds that have a wide range of biological activity [13]. Triazolotriazines occupy an important place among synthetic heterocyclic compounds with antiviral activity. On the one hand, they are structurally similar to natural purine bases, which determines their activity as antimetabolites or agonists of various receptors. On the other hand, they have a high potential for designing various structures using various synthetic approaches [14]. Electrochemical activity and selective interaction with the envelope protein of many RNA viruses (hemagglutinin) make them useful as cross-linkers for affinity immobilization and non-biological agents for biomolecular

 DOI: 10.15826/chimtech.2025.12.1.06

recognition. This makes it possible to use them for quantitative rapid determination of antibodies to measles virus and quality control of influenza vaccines [15–17]. 3-nitro-4-hydroxy-1,4-dihydro-7-propargylthio-1,2,4-triazolo[5,1-c][1,2,4]triazine shows the best characteristics of selective affine interaction with hemagglutinin ($K_a = 4.35 \cdot 10^7$ M^{-1}) [17]. The long incubation time of the modified electrode, as well as the need to wash unreacted components are the disadvantages of the existing approaches.

This work deals with the development of the voltammetric determination of hemagglutinin in model solutions of saliva using magnetic nanoparticles modified with 3-nitro-4-hydroxy-1,4-dihydro-7-propargylthio-1,2,4-triazolo[5,1-c][1,2,4]triazine (Aza), in the design of an original portable electrochemical cell.

2. Experimental

2.1. Reagents and solutions

Ferrous chloride tetrahydrate, ferric chloride hexahydrate, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), aminoethanethiol, dimethyl sulfoxide (DMSO), azobisisobutyronitrile (AIBN), hemagglutinin, diethylene glycol 99% were purchased from Sigma-Aldrich, USA. Glycine 99% were purchased from PanReac AppliChem, Spain. Universal Britton-Robinson buffer (BRB, (pH 5.0)) was prepared according to standard method [18]. Deionized water was obtained using a combined membrane installation DVS-M/1NA(18)-N series (Mediana-Filter, Russia).

Aza (Figure 1) was synthesized according to the known method [19].

2.2. Equipment and measuring instruments

The synthesis of magnetic nanoparticles was carried out using a stirrer IKA EUROSTAR digital 200 (IKA, Germany). pH values were measured using an Expert-pH ion meter (Econix-Expert, Russia).

FT-IR spectra were recorded using a LUMOS IR Fourier spectrometer (Bruker, Germany).

Electrochemical studies were carried out using an Autolab PGSTAT 204 potentiostat/galvanostat (Metrohm Autolab, The Netherlands) connected to both a stationary laboratory electrochemical cell and a developed portable microcell. The 3D model for microcell was created using the online service http://tinkercad.com. The microcell was manufactured using stereolithography method on a Form-Labs Form 3B 3D printer (Formlabs, USA) from Clear Resin V4 (Form 3) (Formlabs, USA). The measurements were performed using a screen-printed three-electrode planar system (TPS, Rusens LTD, Russia), consisting of a carbon-based working and counter electrodes and an Ag/AgCl pseudo-reference electrode.

2.3. Synthesis of magnetic nanoparticles (MNPs)

The Fe_3O_4 MNPs were synthesized by the controlled precipitation method (Equations 1) using ferrous chloride tetrahydrate, ferric chloride hexahydrate and ammonium hydroxide as precursors [20]:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- = Fe_3O_4 + 4H_2O. \quad (1)$$

0.0428 g of ferrous chloride tetrahydrate and 0.1163 g of ferric chloride hexahydrate were dissolved in 50 mL of water, and 0.5 mL of 25% NH_4OH was added drop by drop into the solution while stirring. The solution was stirred for 30 min at a speed of 2200 rpm. The magnetic suspension was washed with deionized water until pH 7.0–8.0 was achieved.

2.4. Modification of MNPs with 3-nitro-4-hydroxy-1,4-dihydro-7-propargylthio-1,2,4-triazolo[5,1-c][1,2,4]triazine

Carboxylation of MNPs was performed with glycine. Amination of Aza was carried out by thiol-yne reaction with aminoethanethiol. Final carboxylated MNPs and aminated Aza coupling was conducted with carbodiimide cross-linking. The whole algorithm is presented in Figure 2.

1 mL of MNPs suspension (0.1 mg/mL) was washed with a diethylene glycol once; then MNPs were transferred to a round-bottom flask, to which 30 mL of a diethylene glycol and 0.45 g of glycine were added. The resulting suspension was kept for 4 h at 175 °C under N_2 atmosphere. After cooling, the MNPs were washed with ethanol and distilled water.

Thiol-yne reaction was carried out in another one-neck flask using solution of 0.0109 g of Aza in 2 mL of DMSO, 0.0026 g of aminoethanethiol, 0.0035 g AIBN and the 18 ml of t-BuOH. The mixture boiled for 3 h at 85 °C.

After cooling, 1 mL of the resulting mixture, 0.0206 g of EDC, 0.0124 g of NHS and 5 ml of BRB (pH 5.0) were added to the flask with MNPs. This reaction was carried out in a flask isolated from light to minimize the formation of by-products. The flask was kept under stirring for 1 h. The resulting modified magnetic nanoparticles (MNPs-Aza) then were washed with a BRB solution (pH 5.0) and finally dispersed in BRB solution (pH 5.0) to form 0.1 mg/mL suspension.

2.5. Fourier-transform infrared spectroscopy (FT-IR) research

FT-IR spectroscopy was carried out after each stage of magnetic nanoparticles modification.

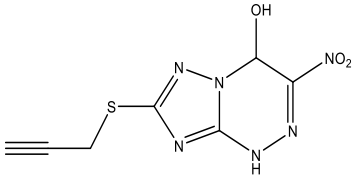

Figure 1 Structure of Aza.

 DOI: 10.15826/chimtech.2025.12.1.06

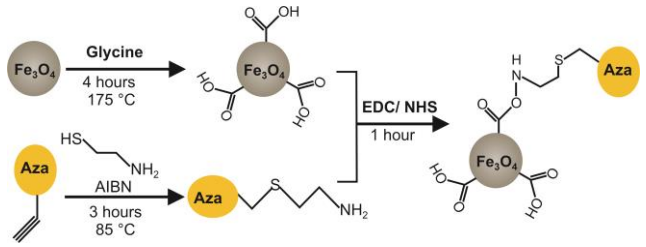

Figure 2 Scheme of immobilization of Aza on the surface of magnetic nanoparticles.

The nanocomposite was mixed with KBr powder and placed in a mold to form a tablet. FT-IR spectra of the modified magnetic nanoparticles are given in Supplementary materials.

2.6. Electrochemical research

The electrochemical response from Aza was recorded using square wave voltammetry under operating conditions given in Table 1 [16].

2.7. Algorithm for quantitative rapid determination of hemagglutinin in saliva in the portable electrochemical microcell

An algorithm for the quantitative determination of HA in saliva is given in Figure 3a.

- At the first stage, a saliva sample is taken using a sterile cotton swab.
- At the second stage, this swab is placed in a test tube containing 400 μL of 0.1 mg/mL MNPs-Aza suspension in a BRB solution (pH 5.0), shook for 1 min and then removed. Further incubation of MNPs-Aza and the sample from the swab lasts 15 min.
- At the third stage, the 50 μL aliquot of the liquid from the test tube is introduced into a sample hole of a microcell on the working zone of pre-installed TPS. The scheme and photo of the microcell are shown in Figure 3b and 3c, respectively. The nanoconjugates MNPs-Aza-HA are magnetized onto the surface of the work electrode of TPS.
- At the fourth stage, a square wave voltammogram is recorded.

The relative difference in the reduction current of triazolotriazine immobilized on the surface of magnetic nanoparticles before and after incubation in the sample was used as an analytical signal and calculated by Equation 2.

$$I^* = \frac{I_{\text{MNP–Aza}} - I_{\text{MNP–Aza–HA}}}{I_{\text{MNP–Aza}}} \cdot 100\% \qquad (2)$$

3. Results and Discussion

3.1. Modification of magnetic nanoparticles with 3-nitro-4-hydroxy-1,4-dihydro-7-propargylthio-1,2,4-triazolo[5,1-c][1,2,4]triazine

A approach for magnetic nanoparticles modification was developed to determine the viral nature of diseases. The approach includes a carbodimide cross-linking reaction between carboxylated MNPs and triazolotriazine linked with aminoethanethiol.

MNPs-Aza was characterized by FT-IR spectroscopy and square wave voltammetry based on the cathodic peak current of the nitro group of triazolotriazine immobilized on the MNPs surface. FT-IR spectra (Figure S1) of carboxylated MNPs show the presence of characteristic absorption bands in the range of 750–1250 cm^{-1}, confirming the presence of amino acids and Fe_3O_4 in the sample.

Table 1 SWV-s registration parameters.

Parameter	Value
Start potential	−0.2 V
Stop potential	−1 V
Step potential	−0.005 V
Modulation amplitude	0.1 V
Frequency	20 Hz
Interval time	0.05 s

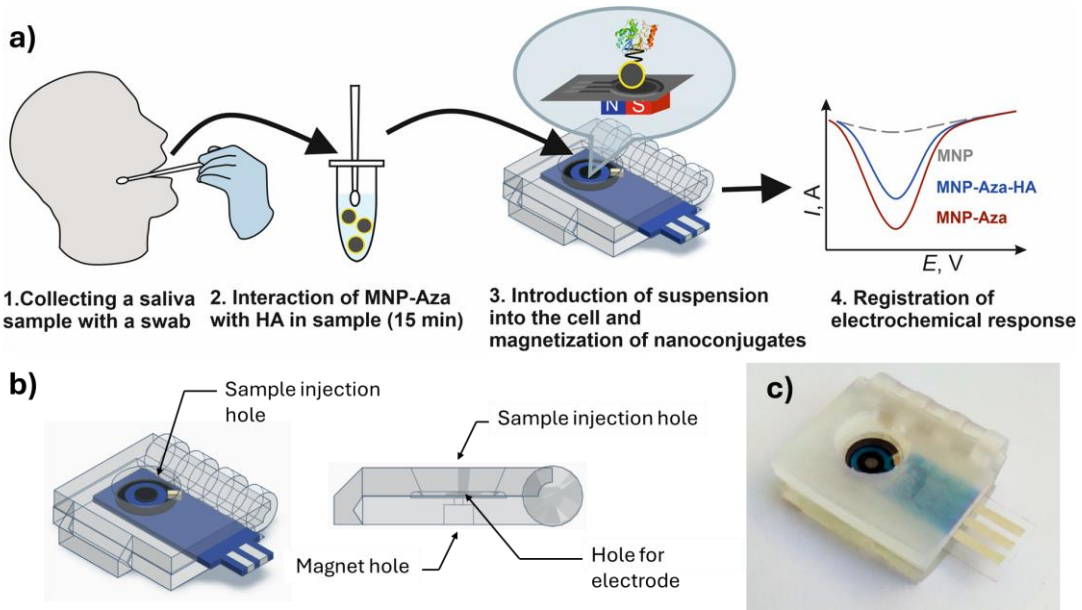

Figure 3 Design of the portable electrochemical cell: (a) Algorithm for voltammetric determination of HA in the design of the portable electrochemical cell, (b) scheme of the portable cell (c) photo of the cell.

 DOI: 10.15826/chimtech.2025.12.1.06

The characteristic bands in the range of 1300–1500 cm^{-1} and 1550–1650 cm^{-1} apparently corresponding to vibrations of –OH,–NH, $–NO_2$ groups were observed in the spectra of MNPs-Aza.

The peak at ~–0.62 V, corresponding to the first stage of electroreduction of the $–NO_2$ group of azoloazine [16], is observed in the square-wave voltammograms (SWV) (Figure 4a). The results obtained indicate the successful immobilization of Aza on the surface of MNPs using the developed approach.

The influence of the time of the thiol-yne reaction on the nanoparticles electrochemical response was investigated. As the reaction time between Aza and aminoethanethiol reached 180 min, an increase of the cathodic peak current was observed. Therefore, MNPs-Aza obtained from thiol-yne cycloaddition for 180 min was used in further studies (Figure 4b). MNPs are the carriers of the receptor molecule (Aza), and concentrating Aza MNPs after reaction with HA on the TPS surface is possible. The electroreduction peak current depends linearly on the concentration of MNPs-Aza in the range of 0.5–10 mg/mL and can, therefore, be used as an analytical signal (Figure 4c).

The investigation of the MNPs-Aza and HA incubation time showed that I^* reaches saturation after 15 min. Therefore, 15 min of MNPs-Aza incubating with HA was applied in further studies (Figure 4d). A 2-fold decrease in the analysis time was achieved compared to the approach developed in our previous studies [16].

3.2. Voltammetric determination of hemagglutinin using MNPs-Aza in model solutions and real saliva samples

Under selected operating conditions, using the developed algorithm and 2 type of cells (stationary laboratory and portable microcell), linear calibrations I^* (%) = f(lg c_{HA}) were obtained, and analytical characteristics were calculated (Figure 5, Table 2).The results indicate similar values of the analytical characteristics of HA determination in both laboratory and developed portable electrochemical microcell. The detection limits calculated using 3σ-criterion are more than 100 times higher than the values obtained in our previous work [16]. This is probably due to the more efficient formation of MNPs-Aza-HA conjugates in the case of the biorecognition reaction in the volume of the analyzed sample with subsequent concentration conjugates on the surface of the working electrode.

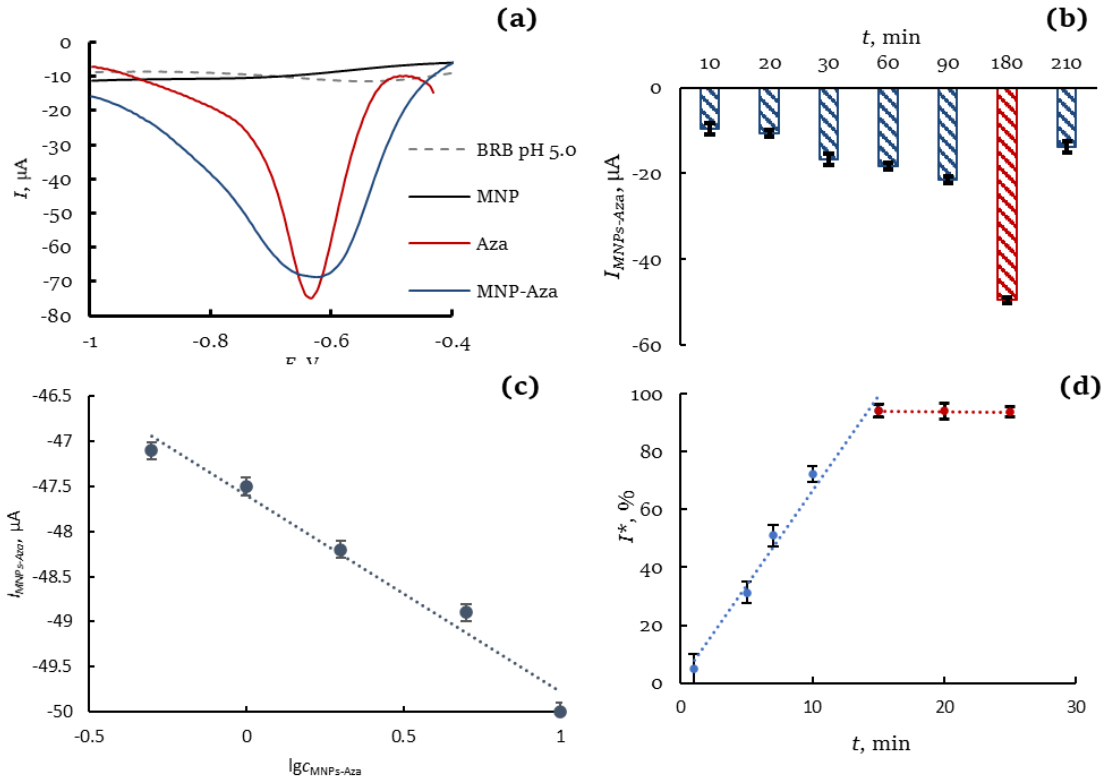

Figure 4 SWV-s of unmodified and modified MNPs (laboratory cell with three-electrode screen-printed planar system, background electrolyte: BRB (pH 5.0) (a); effect of thiol-yne reaction time on the MNPs-Aza electrochemical response (b); effect of MNPs-Aza concentration (mg/mL) on electrochemical response I(μA) = (2.0±0.3)·lg $c_{MNPs-Aza}$+(47.6±0.9), R^2 = 0.96 (c); effect of incubation time MNPs-Aza with HA (c_{HA} = 10^{-4} M) on the analytical signal (I^*)(d).

Table 2 Analytical characteristics of the voltammetric determination of HA using TPS-MNPs-Aza using stationary laboratory and portable microcells.

Cell type	I^* vs lg c_{HA}	R^2	LOD (M)	Linear range (M)
Laboratory cell	I^* = (16.7±0.3) lg c_{HA} + (163±4)	0.98	$6.7·10^{-10}$	10^{-3}–10^{-9}
Portable microcell	I^* = (12.8±0.3) lg c_{HA} + (137±2)	0.97	$8.4·10^{-10}$	10^{-3}–10^{-9}

 DOI: 10.15826/chimtech.2025.12.1.06

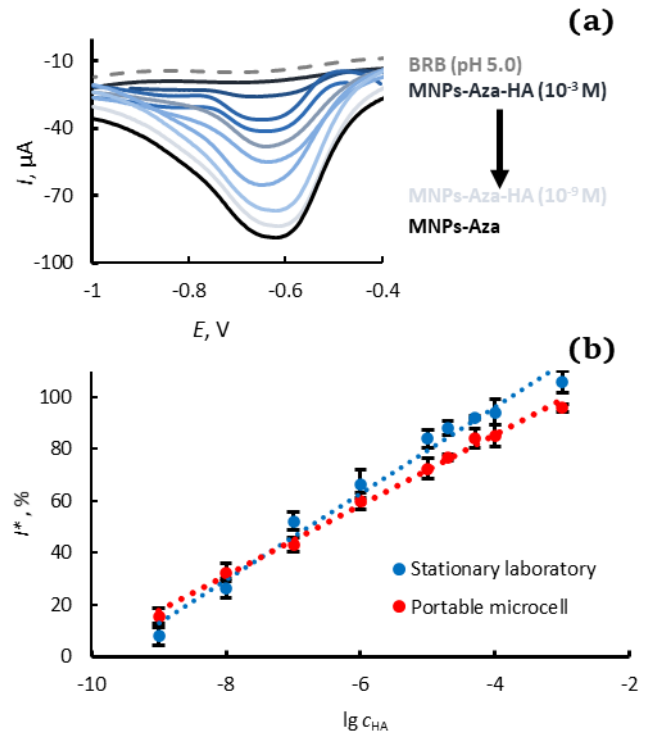

Figure 5 SWV of standard HA solutions (portable microcell with three-electrode screen-printed planar system, scan rate 100 mV/s) (a), I^*(%) vs (lg c_{HA}) (n = 5, P = 0.95) in a stationary laboratory and portable microcell (b).

The approach proposed in this study is comparable in terms of analytical characteristics and analysis time with the literature data presented in Table 3, which makes it promising for further development of a portable device.

Figure 6 shows the effect of introducing various interfering components on the electrochemical response of the MNP- AZA-HA. The list of components and their contents were selected according to the literature data [21]; the model solutions consisted of α-amylase ($c_{\alpha\text{-amilase}}$ = 450 IU/L·10³), urea(c_{Urea}=0.198 g/L), KCl (c_{KCl} = 0.202 g/L), NH_4NO_3 ($c_{NH_4NO_3}$ = 0.328 g/L), KH_2PO_4 ($c_{KH_2PO_4}$ = 0.636 g/L), NaCl (c_{NaCl} = 1.594 g/L), BSA (c_{BSA} = 1 g/L), respectively. The model saliva consisted of all these components in one solution.

The figure shows that interfering model mixtures do not have a significant effect on the results of the analysis, which confirms selectivity of the interaction of azoloazine derivatives with HA and indicates the possibility of its quantitative determination in real samples of saliva using the developed approach.

Table 4 shows the results of the quantitative determination of HA in non-spiked and spiked saliva samples from 5 volunteers. The calculated values of the Student's t-test do not exceed the critical value, which indicates the accuracy of the developed approach for the quantitative determination of HA in saliva samples.

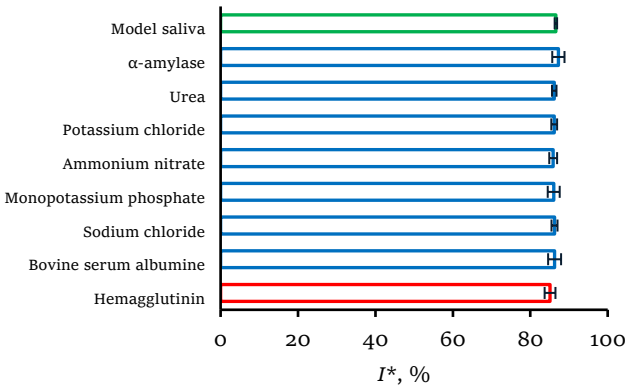

Figure 6 Diagram for the determination of HA (c = 10⁻⁴ M) in model solutions ($c_{\alpha\text{-amilase}}$ = 450 IU/L·10³, c_{Urea}=0.198 g/L, c_{KCl} = 0.202 g/L, $c_{NH_4NO_3}$ = 0.328 g/L, $c_{KH_2PO_4}$ = 0.636 g/L, c_{NaCl} = 1.594 g/L, c_{BSA} = 1 g/L) and model saliva.

Table 4 HA determination in saliva samples using the developed approach in the portable electrochemical cell t_{crit} = 2.570 (P = 0.95, n = 5).

Sample	Spiked, c_{HA}, M	Found, c_{HA}, M	*t-value*
1	0	0.00±0.02	0
2	10^{-3}	10^{-3}·(0.82±0.03)	2.367
3	10^{-4}	10^{-4}·(0.81±0.04)	2.004
4	10^{-5}	10^{-5}·(0.84±0.04)	1.990
5	10^{-7}	10^{-7}·(0.79±0.04)	2.007
6	10^{-9}	10^{-9}·(0.82±0.03)	2.410

Table 3 Portable devices for different virus determination.

Method of analysis	Analyte	Receptor	Object of analysis	LOD	Time, min	Ref.
Immunochromatographic	Hepatitis B	Gold nanoparticles with immobilized mouse monoclonal antibodies	Blood	50 IU/ mL	20	5
Immunochromatographic	Influenza A	Monoclonal antibodies	–	–	10-20	6
Immunochromatographic	Influenza A and B	Monoclonal antibodies	Nasal swab samples	10^3 units	–	7
Voltammetry	Sars-CoV-2	Nanoparticles coated with monoclonal antibodies	Untreated saliva	8 ng/mL	30–60	10
Impedance spectroscopy	Influenza A	Polyclonal antibodies	Saliva	0.7 fg	5	11
Voltammetry	Hemahhlutinin	MNP-Aza	Saliva	$8.4\cdot10^{-10}$ M (0.92 ng/mL)	20	This work

 DOI: 10.15826/chimtech.2025.12.1.06

4. Limitation

The main limitations of the developed approach are related to the instrument base not being adapted for use by the consumer outside the laboratory. Also, when scaling the magnetite modification approach, additional clarification of the operating conditions for the implementation of individual stages may be required. Further developments will be aimed at adapting the developed approach for conducting measurements on a micro-potentiostat and developing software for automatic processing of measurements when using the device. It is also necessary to develop a protocol for cleaning the cell for its repeated use.

5. Conclusions

A portable electrochemical microcell was designed for the quantitative voltammetric assessment of HA in saliva samples. The approach includes collecting a saliva sample, incubating it with modified MNPs in a test tube, and transferring them into the electrochemical microcell equipped with TPS for square-wave voltametric detection. The MNPs acted both as carriers for the receptor (Aza), which can selectively interact with HA, and as agents for concentrating Aza-HA complexes on the working electrode surface. The peak current during the initial stage of electroreduction of the triazolotriazine derivative served as the analytical signal. The operating parameters for preparing MNPs-Aza conjugates and the duration of their incubation with HA in the analyzed medium were optimized. The approach yielded linear calibration curves given by $I^* = (12.81\pm0.26)\ lg\ c_{HA} + (136.75\pm1.73)$. The approach's analytical characteristics (LOD $8.4\cdot10^{-10}$ M, linear range 10^{-3}–10^{-9} M) are comparable to those reported in literature. The components of model saliva do not affect the accuracy of hemagglutinin determination. Overall, the straightforward design and accessibility of the developed portable electrochemical microcell alongside the impressive analytical features of the proposed voltammetric determination for HA suggest promising applications for quick diagnostics of the viral nature of infectious diseases.

Supplementary materials

This manuscript contains supplementary materials, which are available on the corresponding online page.

Figure S1: IR spectra of modified magnetic nanoparticles.

Data availability statement

We collected and shared the data in the article.

Acknowledgments

None.

Author contributions

Conceptualization: S.T.S.
Data curation: S.T.S.
Formal Analysis: M.N.N., M.M.V.
Funding acquisition: K.A.N., R.V.L.
Investigation: S.Yu.E., M.M.V.
Methodology: S.T.S., M.M.V
Project administration: S.T.S.
Resources: K.A.N., R.V.L.
Software: M.M.V.
Supervision: M.A.I.
Validation: K.A.N.
Visualization: S.Yu.E., M.M.V.
Writing – original draft: S.Yu.E., S.T.S.
Writing – review & editing: M.N.N.

Conflict of interest

The authors declare no conflict of interest.

Additional information

Author IDs:

Tatiana Svalova, Scopus ID 56539980000;
Margarita Medvedeva, Scopus ID 57211444404;
Natalia Malysheva, Scopus ID 57190867923;
Vladimir Rusinov, Scopus ID 7006493788;
Anatoly Matern, Scopus ID 12142454900;
Alisa Kozitsina, Scopus ID 16432620500.

Websites:

Ural Federal University, https://urfu.ru/en/;
I.Ya. Postovsky Insititute of Organic Synthesis of the Ural Branch of the Russian Academy of Sciences, https://iosuran.ru/.

References

1. Kiselev D, Matsvay A, Abramov I, Dedkov V, Shipulin G, Khafizov K. Current Trends in Diagnostics of Viral Infections of Unknown Etiology. Viruses. 2020;12(2):211. doi:10.3390/v12020211
2. Elaheh K.Goharshadi EK, Goharshadi K, Moghayedi M. The use of nanotechnology in the fight against viruses: A critical review. Coordinat Chem Rev. 2022;464:214559. doi:10.1016/j.ccr.2022.214559
3. Ozer T, Geiss BJ, Henry ChS. Review—Chemical and Biological Sensors for Viral Detection. Electrochem Soc. 2020;167(3):037523. doi:10.1149/2.0232003JES
4. Nirbhaya V, Chaudhary C, Chandra R, Kumar S. Biofunctionalized carbonaceous nanoflakes based efficient electrochemical biosensor for SAA biomarker detection. Appl Surf Sci Adv. 2023;13:100368. doi:10.1016/j.apsadv.2023.100368
5. Gish RG, Gutierrez JA, Navarro-Cazarez N, Giang K, Adler D, Tran B, Locarnini S, Hammond R, Bowden S. A simple and inexpensive point-of-care test for hepatitis B surface antigen detection: serological and molecular evaluation. J Viral Hepatitis. 2014;21(12):905–8. doi:10.1111/jvh.12257
6. Miyoshi-Akiyama T, Narahara K, Mori S, Kitajima H, Kase T, Morikawa S, Kirikae T. Development of an Immunochromatographic Assay Specifically Detecting Pandemic H1N1 Influenza Virus. J Clinical Microbiol. 2009;48(3):703–8. doi:10.1128/jcm.02262-09
7. Sakurai A, Takayama K, Nomura N, Kajiwara N, Okamatsu M, Yamamoto N, Tamura T, Yamada J. Fluorescent Immunochromatography for Rapid and Sensitive Typing of Seasonal Influenza Viruses. PLoS ONE. 2015;10(2):e0116715. doi:10.1371/journal.pone.0116715
8. Chen A, Chatterjee S. Nanomaterials based electrochemical sensors for biomedical applications. ChemSocRev. 2013;12:5425–38. doi:10.1039/C3CS35518G
9. Zhu Ch, Yang G, Li H, Du D, Lin Yu. Electrochemical Sensors and Biosensors Based on Nanomaterials and Nanostructures. Anal Chem. 2015;87(1):230–249. doi:10.1021/ac5039863
10. Fablani L, Saroglia M, Fillo S, Luca V, Faggoni G, D'Amore N, Regalbuto E, Salvatori P. Magnetic based combined with carbon black-based screen-printed electrodes for COVID-19: A reliable and miniaturized electrochemical immunosensor for

SARS-CoV-2 detection in salvia. Biosensors Bioelectronics. 2021;171:112686. doi:10.1016/j.bios.2020.112686
11. Nidzworski D, Siuzdak K, Niedziałkowski P, Bogdanowicz R, Sobaszek M, Ryl J, Weiher P, Sawczak M, Wnuk E, Goddard III WA, Jaramillo-Botero A, Ossowski T. A rapid-response ultrasensitive biosensor for influenza virus detection using antibody modified boron-doped diamond. Sci Rep. 2017;7(1):15707. doi:10.1038/s41598-017-15806-7
12. Svalova TS, Malysheva NN, Zaidullina RA, Medvedeva MV, Mazur AV, Morshchinin IV, Kozitsina AN. Novel electrochemical immunosensing platform based on magnetite-antibody conjugate as a direct signal label: design and application for Salmonella typhimurium. Anal Lett. 2023;56(16):2572–85. doi:10.1080/00032719.2023.2180015
13. Singla Pr, Luxami V, Paul K. Triazine as a promising scaffold for its versatile biological behavior. Eur J Med Chem. 2015;102:39–57. doi:10.1016/j.ejmech.2015.07.037
14. Rusinov VL, Charushin VN, Chupakhin ON. Biologically active azolo-1,2,4-triazines and azolopyrimidines. Russ Chem Bull. 2018;67(4):573–599. doi:10.1007/s11172-018-2113-8
15. Ivoilova A, Mikhalchenko LV, Tsmokalyuk A, Leonova M, Lalov A, Mozharovskaia P, Kozitsina AN, Ivanova AV, Rusinov VL, Redox Conversions of 5-Methyl-6-nitro-7-oxo-4,7-dihydro-1,2,4triazolo[1,5-a]pyrimidinide L-Arginine Monohydrate as a Promising Antiviral Drug. Molecules. 2021;26(16):5087. doi:10.3390/molecules26165087
16. Svalova TS, Medvedeva MV, Mazur AV, Drokin RA, Butorin II, Tsmokalyuk AN, Malysheva NN, Rusinov VL, Kozitsina AN. Voltammetric determination of hemagglutinin using triazolotriazine derivatives as agents for the biomolecule recognition. Electrochimica Acta. 2024;481:143954. doi:10.1016/j.electacta.2024.143954
17. Medvedeva MV, Mazur AV, Svalova TS, Balin IA, Rusinov VL, Matern AI, Kozitsina AN. Voltammetric determination of measles virus antibodies using a glassy carbon electrode modifed with 2-propargyltio-6-notro-7-hydroxy-4H-1,2,4-triazolo-4,7-dihydro[5,1-c]-1,2,4-triazine. J Anal Chem. 2024;78:1694–1700. doi:10.1134/S1061934823120109
18. Sucha L, Betteridge D, Kotrly S. Solution Equilibria in Analytical Chemistry London:Van Nostrand Reinhold Company: 1972. 371 p.
19. Drokin RA, Fesenko EA, Mozharovskaia PN, Medvedeva MV, Svalova TS, Kozitsina AN, EsaulkovaYaL, Volobueva AS, Zarubaev VV, Rusinov VV. 4-Hydroxy-3-nitro-1,4-dihydrotriazolo[5,1-c][1,2,4]triazines:synthesis, antiviral activity, and electrochemical characteristics. Russ Chem Bull. 2022;71(11):2460–6. doi:10.1007/s11172-022-3674-0
20. Liu ZL, Liu YJ, Yao KL, Ding ZH, Tao J, Wang X. Synthesis and Magnetic properties of Fe_3O_4. J Mater Synthesis Processing. 2002;10:83–87. doi:10.1023/A:1021231527095
21. Sarkar A, Xu F, Lee S. Human saliva and model saliva at bulk to adsorbed phases - similarities and differences. Adv Colloid Interface Sci. 2019;273:102034. doi:10.1016/j.cis.2019.102034

 DOI: 10.15826/chimtech.2025.12.1.06