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Combined CRISPR/Cas12 system and glassy carbon electrode enable detection and analysis of dopamine solution

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Abstract: *Dopamine (DA) is one of the key neurotransmitters (NTs) in nature and plays a crucial role in the mammalian central nervous system (CNS). Its selective determination in biological fluids is a fundamental need in the field of biomedical research. In this study, a novel electrochemical biosensor was developed, taking advantage of the high selectivity and specificity of the CRISPR/Cas12 system. The aptamer sensor was constructed and characterized using cyclic voltammetry with a glassy carbon electrode (GCE), and its conductivity was demonstrated by electrochemical impedance spectroscopy (EIS). The sensor exhibited a good linear response in the range of 1–10 μM, with a detection limit of 4.98 μmol/L. Compared to conventional methods, the electrochemical sensor developed in this study shows high sensitivity and excellent selectivity. The developed sensor was applied to the determination in analogous samples, yielding satisfactory recovery results.*

Keywords: *CRISPR/Cas12 system, glassy carbon electrode, dopamine assay, electrochemical biosensor*

1. Introduction

Dopamine (DA) is a multifaceted, biologically active molecule that coexists and plays crucial roles within the human body. As a key member of the catecholamine family, DA serves as a vital neurotransmitter, facilitating communication between neurons and regulating a wide range of physiological processes. Its influence extends to cognitive functions such as memory, attention, and motivation, making it essential for maintaining mental acuity and emotional well-being. In the central nervous system (CNS), DA is intricately involved in the regulation of movement and reward mechanisms. In the peripheral nervous system, it modulates cardiovascular and renal functions. Given its widespread presence and critical roles, DA has garnered significant attention for its potential as a biomarker in clinical diagnostics, particularly in the assessment of neurodegenerative diseases like Parkinson's disease and psychiatric conditions such as schizophrenia. Understanding the dynamics of DA levels and its metabolic pathways is crucial for advancing our knowledge of human health and developing targeted therapeutic interventions [1–3]. DA also plays a key role in the functioning of the CNS by regulating hormone secretion, cardiovascular and renal function, vascular tone, gastrointestinal motility, and catecholamine release [4]. It is crucial in cases of drug misuse and a biological precursor of norepinephrine (NP), the primary neurotransmitter in the reward circuit. Different parts of the brain as well as the body's periphery contain DA receptors. A variety of illnesses, including depression, drug addiction, Parkinson's disease, Alzheimer's disease, schizophrenia, and Huntington's disease, may be linked to changes in the ideal concentration of DA as well as any variations in its levels [5,6]. Furthermore, DA regulates a variety of physiological processes, including reward, motivation, control over information flow in the brain, and random movements [7,8].

Numerous techniques for DA detection have been published in recent years. For instance, the determination of DA was done using electrochemical techniques [9,10], molecular functional magnetic resonance imaging (FMRI)[11], and colorimetric detection [12], spectroscopy [13] and high performance liquid chromatography-mass spectrometry (HPLC-MS) [14] . Researchers have focused a lot of attention on electrochemical approaches in particular because of its benefits, which include low cost, minimal time

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consumption, ease of operation, and very inexpensive apparatus [15]. Low cost, high conductivity, electrochemical inertness across a broad potential range, high hardness, strong chemical stability, low permeability, and simple surface modification are all benefits of glassy carbon electrodes (GCEs). Clusters of regularly spaced short palindromic repeat sequences (CRISPR)-related systems (Cas) are adaptive immune systems found in bacteria and archaea as a natural defense against invading nucleic acids [16]. Targeting and cleaving invasive DNA sequences is the main way in which these technologies work. The CRISPR/Cas system has blossomed in biotechnology since its initial use for gene editing in mammalian genomes, and it is now a crucial tool for applications including transcriptional regulation and genome editing [17,18]. Because of their special ability to provide signaling readout when cleaving both target and single-stranded non-target nucleic acids, CRISPR/Cas effectors like Cas13 and Cas12 have recently been used for nucleic acid detection[19,20]. As a result, they have great potential for creating novel biosensors for nucleic acid detection[21,22].

In this study, using a particular aptamer to label the methylene blue moiety (MB), we will use the CRISPR/Cas12a system to detect dopamine solution. Then, we will load the methylene blue (MB)-labeled dopamine Apt single-chain into a glassy carbon electrode and finish building an electrochemical sensor using bovine serum albumin (BSA) as a filler for the confinement agent. Cyclic voltammetry (CV) was used in the sensor fabrication process. Electrochemical impedance spectroscopy (EIS) was used to measure the conductivity, and the CV method was used to characterize and detect the sensor fabrication process. The electrochemical biosensor built using the CRISPR/Cas12 system and glassy carbon electrode shown good DA detection sensitivity, selectivity, and stability, according to the results. As a result, we anticipate that it will be effectively utilized in real-world applications.

2. Experimental design and methods

2.1. Materials and equipment

The nucleic acid sequence was purchased from Shanghai Sangon Biotech. DA, $MgCl_2$, KCl, NaOH, NaCl, and potassium ferricyanide were purchased from Shanghai Aladin Tris-HCl was purchased from Amresco. 0.3 μm, 0.05 μm Al_2O_3 was purchased from Shanghai Chenhua Instrument Co. The working rod glassy carbon electrode (GCE), auxiliary platinum wire electrode (Pt), and reference electrode were Ag/AgCl, and the cyclic voltammetry (CV) curves were carried out by an electrochemical workstation (Koester Cs300M) in 0.5 mm $[Fe(CN)_6]^{3-/4-}$. 0.5 mm $[Fe(CN)_6]^{3-/4-}$ was configured with PBS solution. Ultrasonic shaker (JYD-250), water-bath constant temperature oscillator (SHA-C). All the reagents involved in the experiment are analytically pure.

Name	Squences
DNA1	5’-GTCTCTGTGTGCGCCAGAGAACACTGGGGCAGATATGGGCCA GCACAGAATGAGGCCC-3’
DNA2	5’-GGGCTCATTCTGTGCTGGCCCATATCTGCCCCAGTGTTCTCTGGCTCACACAGACAC-3’
DNA3	5’-GGGTCGTGTCTTACTCGGGTTTTCCCAGCACAGAATGAGCCC-3’
crRNA	5’- UAA UUU CUA CUA AGU GUA GAU GGGTCGTGTCTTACTCGGG-3’
reDNA	5’-MB-TTTATTTATTTATTTATTT-SH-3’

2.2. Preparation of glassy carbon electrode

The glassy carbon electrodes' surface was painstakingly smoothed to attain the ideal level of homogeneity and smoothness. The electrode surface was first treated with 0.3 μm and 0.05 μm aluminum oxide powder (AlO_3). For almost half an hour, the meticulous polishing process was carried out to make sure the surface was appropriately smooth and devoid of any imperfections. In order to improve the electrode's performance in later applications, this process was essential.

After polishing, an ultrasonic cleaning method was used to carefully remove any remaining Al_2O_3 powder. This extensive cleaning process included five-minute washes with ethanol, deionized water, and deionized water in succession. In order to prevent any interference with following reactions or measurements, the series of washes was created to guarantee the total removal of all pollutants and impurities from the electrode surface. To make sure that any last contaminants were removed, the cleaned glassy carbon electrode was thoroughly washed again using deionized water once the cleaning process was finished. A stream of nitrogen gas was then used to properly dry the electrode, eliminating any remaining moisture and guaranteeing that the surface would not become contaminated while drying.

Lastly, the electrode spent 30 minutes submerged in a cDNA solution. In order to immobilize the

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cDNA molecules onto the electrode surface and provide a stable and useful interface for ensuing bioanalytical applications, this process was essential. To get rid of any unbound cDNA molecules, the electrode was carefully rinsed with ultrapure water once the deposition procedure was finished. The cDNA-modified glassy carbon electrode (cDNA/GCE), which was successfully fabricated in this last phase, was now prepared for usage in a variety of electrochemical sensing and analytical applications.

2.3. Preparation of DA solution

Dilute 10×PBS 10 times with deionized water to obtain 1×PBS, which was used as a buffer. 0.038 g of DA was added to 100 mL of 1×PBS to prepare a 20 μM DA solution, and the 20 μM DA solution was diluted with 1×PBS in an appropriate ratio to obtain a lower concentration of DA solution.

2.4. Electrochemical determination of DA

Glassy carbon electrode was used as the working electrode. Ag/AgCl and Pt electrodes were used as reference and auxiliary electrodes, respectively. The electrochemical behavior of different concentrations of DA at the modified electrode was analyzed using CV, EIS and SWV. The CV curves were recorded for 30 cycles using an electrochemical workstation (CHI660e) scanned at a scan rate of 100 mV s^{-1} over a voltage range of - 0.2 ~ 0.4 V. The CV curves were recorded for 30 cycles, the SWV curves were recorded for 1 cycle.

3. Results and discussions

3.1. Principles of DA electrochemical biosensors

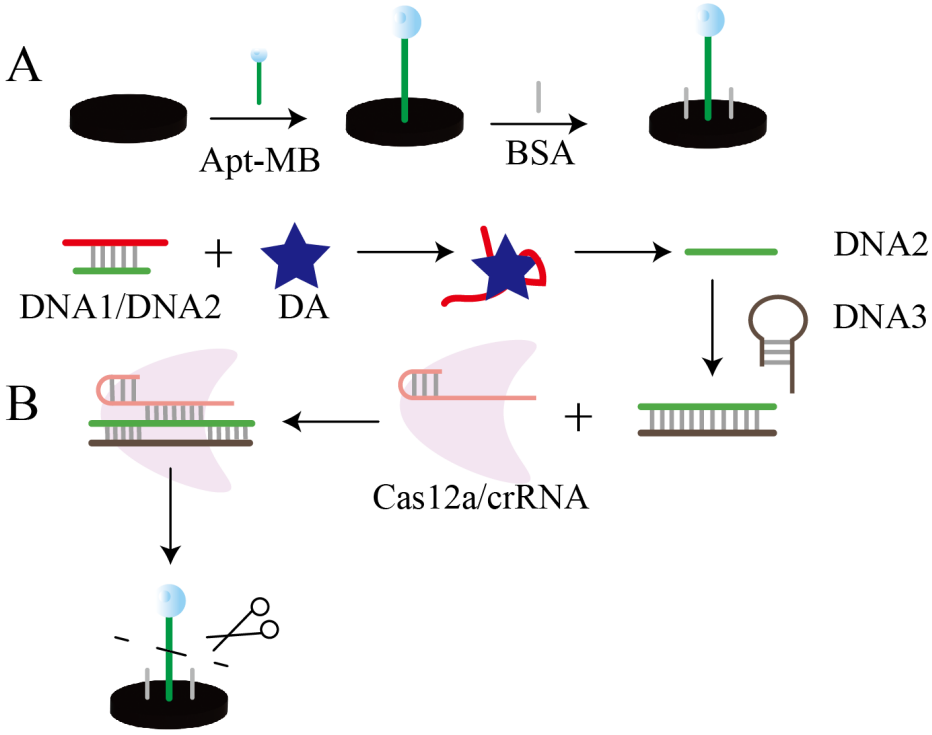

Figure 1: Flowchart of the construction of the sensor and its DA detection

Methylene blue (MB)-labeled reDNA strands are initially deposited onto the surface of a bare electrode in the electrode construction procedure, as shown in Figure 1A. This first stage is essential because it guarantees that the reDNA strands will be electrostatically adsorbently bound on the electrode surface. The reDNA strands are firmly anchored by this adsorption, which offers a solid basis for the following stages of the electrode assembly procedure. After the reDNA strands have been immobilized, any vacant or unoccupied areas on the electrode surface must be addressed. Bovine serum albumin (BSA) is used as a blocking agent to accomplish this. Because of its capacity to attach to surfaces nonspecifically,

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BSA is a protein that is frequently utilized in surface modification to fill in any gaps or areas that could otherwise be open to undesirable interactions. The electrode surface is made more stable and homogeneous by applying a layer of BSA, which completes the functional electrode's creation.

A detectable alteration in the electrochemical signal is the result of a sequence of certain interactions that are started when dopamine is added to the test solution. In example, dopamine can create a stable complex by binding selectively to a specific DNA sequence known as DNA1. Because it sets off a series of molecular interactions, this binding event is a crucial stage in the detection process. The neighboring DNA2 strand is pushed from the DNA1/DNA2 complex on the electrode surface by the mechanical force that dopamine applies when it binds to DNA1. Because it enables interaction between DNA2 and DNA3, which is originally in a hairpin form, this displacement is crucial. A DNA2/DNA3 double-stranded DNA complex is created when DNA2 is released from the electrode surface and is able to unfurl the hairpin shape of DNA3. A crucial step in the detection process is the creation of the DNA2/DNA3 complex. The Cas12a/crRNA complex is a particular enzyme complex that this newly formed complex can bind to. The trans-cleavage activity of Cas12a is activated when the DNA2/DNA3 complex binds to Cas12a/crRNA. The immobilized reDNA strands on the electrode surface can be cleaved by Cas12a in this activated state.

There are fewer MB signaling molecules on the electrode surface as a result of Cas12a cleaving the reDNA strands. Since MB is in charge of producing the electrochemical signal, the total electrochemical signal is significantly reduced when reDNA is cleaved. A quantitative indicator of dopamine concentration, this reduction is exactly proportional to the amount of dopamine in the test solution.

3.2. Electrochemical characterization of DA biosensors

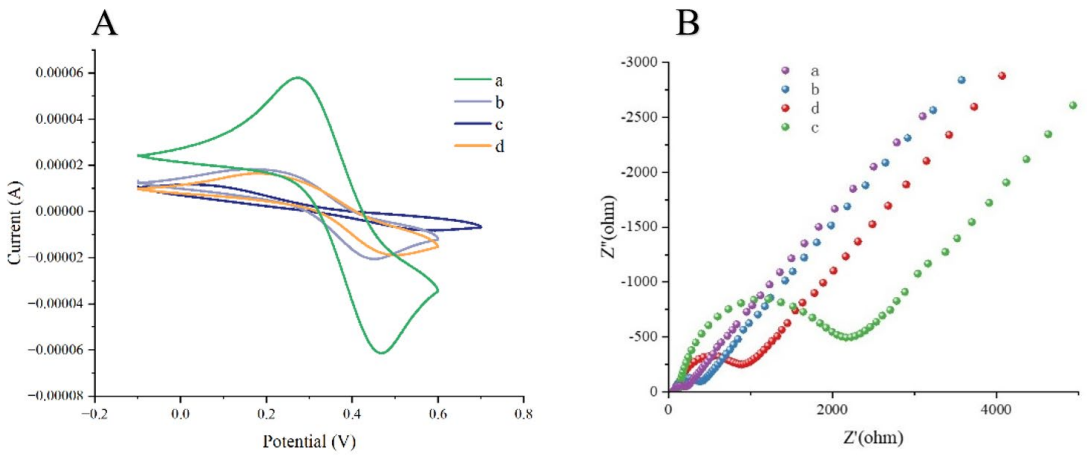

Figure 2: Characterization of biosensor construction using cyclic voltammetry (A) and electrochemical impedance spectroscopy (B).

To describe the process of biosensor fabrication alterations, we employed CV and EIS curves. The bare glassy carbon electrode has the largest redox peak (curve a), as shown in Fig. 2A. The electrode transfer of electrons was blocked when Apt-MB (curve b) and BSA (curve c) were sequentially modified, and the frontal value of the redox peak gradually decreased. The electron transfer rate was increased and the front potential slightly increased (curve d) when CRISPR/Cas12a activity was activated to specifically cleave the DNA, releasing some of the MB-labeled DNA sequences away from the electrode surface.

The electrical impedance of the bare glassy carbon electrode is represented by curve an in Figure 2B. When Apt-MB (curve b) and BSA (curve c) are applied to the bare glassy carbon electrode in succession, the electrochemical impedance value gradually rises and the semicircle gradually widens. Following the activation of CRISPR/Cas12a activity to specifically cut DNA1, the semicircle (curve d) shrunk and the impedance value dropped as a portion of the DNA sequence tagged by MB was freed from the electrode surface. The successful construction of the electrochemical biosensor was demonstrated by the EIS plot curve's change rule matching that of the CV plot curve.

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3.3. Optimization of experimental conditions

To achieve the highest sensitivity and accuracy of the electrochemical sensor, we meticulously optimized the selection of experimental conditions. Two crucial criteria were the main focus of this optimization process: the ideal DA solution concentration and the ideal reaction time. The performance of the sensor is mostly dependent on the DA solution's concentration when coupled with the aptamer (Apt) chain as well as its reaction time. In order to methodically examine the connection between concentration and sensor response, we created a range of distinct DA concentration gradients. In order to determine the appropriate ideal reaction time for every DA concentration, we also investigated the amount of time needed for the reaction to attain a steady state at each concentration level. We were able to optimize the sensor's overall performance by carefully examining these variables and adjusting the testing setup.

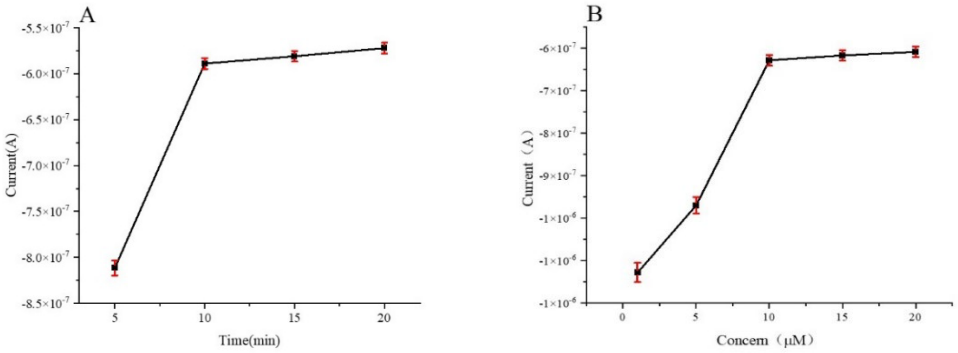

Figure 3: Optimization of experimental conditions. (A) Currents for reaction times of DA solutions. (B) Currents for reactions with different DA concentrations

As the reaction time increases, the electrochemical sensor's current response shows a progressive stabilizing trend, as seen in Figure 3A. In particular, it is noted that as the reaction time reaches roughly ten minutes, the current starts to stabilize. Subsequent observation, however, shows that the current basically stabilizes around 15 minutes. These observations led us to conclude that 15 minutes is the ideal reaction time needed for the solution to achieve a steady state. This duration guarantees that the electrochemical reaction is thorough and repeatable, yielding dependable and consistent outcomes. The concentrations of the DA solutions that we set up were 1 μM, 5 μM, 10 μM, 15 μM, and 20 μM in order to further improve the experimental settings. At the ideal reaction time of 15 minutes, these solutions were made in order to methodically examine the connection between DA concentration and electrochemical signal response. In Figure 3B, the relevant electrochemical signals were plotted after being measured.

Each concentration's electrochemical signal response values were recorded in ascending order: 1 μM, 5 μM, 10 μM, 15 μM, and 20 μM, as illustrated in Figure 3B. It was clear from the data analysis that the 10 μM DA solution had the highest electrochemical signal response value. This result implies that at this specific concentration, the sensor shows the highest level of sensitivity and signal intensity. Thus, with our electrochemical sensor system, we determined that the ideal reaction concentration for DA was 10 μM.

In conclusion, we found that an ideal reaction time of 15 minutes and an ideal DA concentration of 10 μM are critical factors for attaining the greatest performance of our electrochemical sensor after closely examining the reaction kinetics and concentration-dependent signal response. The sensor's maximum sensitivity and dependability for DA detection are guaranteed by these ideal conditions, which is crucial for its possible use in a range of analytical and diagnostic contexts.

3.4. DA electrochemical biosensor platform performance

A variety of DA solution concentrations were measured using the biosensor platform, and the electrochemical signal values that corresponded to each concentration were carefully examined. This thorough investigation was carried out in order to thoroughly assess the established electrochemical biosensing platform's sensitivity in identifying these different DA concentrations. The platform's prospective uses in environmental monitoring and biomedical diagnostics depend heavily on its capacity to detect and quantify DA with accuracy and dependability.

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A detailed illustration of how varying DA solution concentrations affect the electrochemical signals' responsiveness can be found in Figures 5A and 5B. The platform's capacity to distinguish between different DA levels with great precision is demonstrated by these graphs, which clearly show the link between DA concentration and the resulting signal intensity. While Figure 5B further clarifies the relationship between the signal amplitude and the concentration gradient, Figure 5A displays the data showing the electrochemical signals' linear or non-linear response as the DA concentration rises. We can evaluate the biosensor platform's dynamic range, limit of detection, and overall sensitivity thanks to this thorough analysis, which validates that it is capable of detecting DA throughout a wide concentration spectrum.

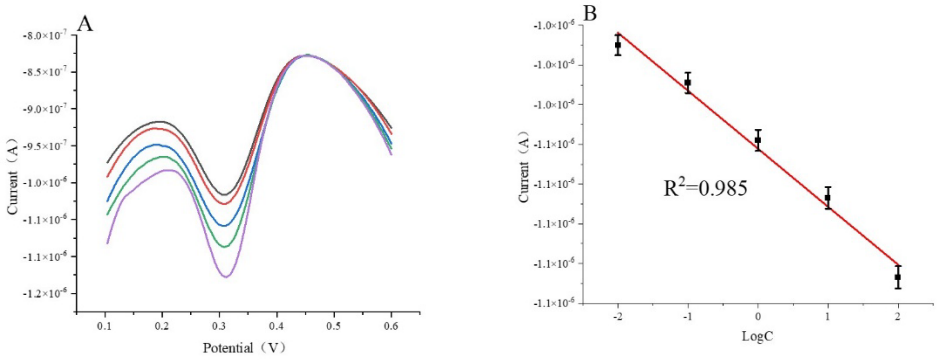

Figure 4: Electrochemical signals of different concentrations of DA solutions treated by the sensor

A linear regression equation was developed in this study, which demonstrated a linear relationship between the level of the electrochemical signal and the concentration of the DA solution. The initial DA concentration in this study was 1 μM /L, and as the DA solution concentration increased, the electrochemical signal was linearly related to the logarithm of the DA solution concentration (lgC, where C represents the DA solution concentration), with $Y = -2.80 \times 10^{-8}LgC - 1.06 \times 10^{-6}$, $R^2 = 0.985$, and the limit of detection was determined to be 4.98 μmol/L according to the general equation $LOD = 3\sigma/k$, where k is the slope of the linear regression equation and σ is the standard deviation. This indicates that our electrochemical biosensor can be utilized to detect DA in the environment because it has a high sensitivity for target detection.

3.5. Specificity of DA electrochemical biosensors

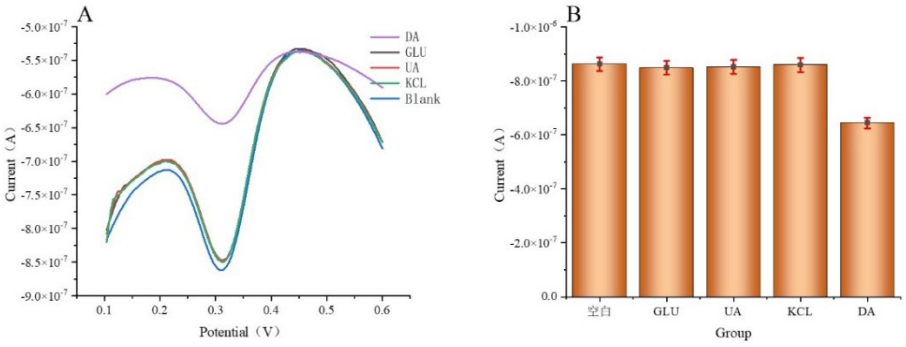

Figure 5: Specificity of DA electrochemical biosensors

In order to verify that the biosensor is specific for the detection of DA, under optimal experimental conditions, we selected different solutions for the detection of sensor specificity. This study was divided into five groups: dopamine solution (DA), potassium chloride (KCL), uric acid solution (UA), 5% glucose solution (GLU), and blank control group (Blank). Figure 5 illustrates that while the sensor responds somewhat to the electrochemical signals of the Glu, UA, Blank control group, and other groups, there is essentially no difference, indicating that the sensor lacks specificity for any of these groups. Furthermore, the DA group's electrochemical signals are significantly weaker than those of the other groups, suggesting that the experimental approach has strong selectivity for DA solution. This alone demonstrates the clear selectivity of the electrochemical sensor technology we created for DA solution

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detection.

3.6. Determination of the recovery of DA

To thoroughly evaluate the practicality of our method, we simulated real-world sample conditions by preparing a serum-like environment. In this environment, glucose (GLU), uric acid (UA), and potassium chloride (KCL) were diluted using phosphate-buffered saline (PBS) solution. In order to evaluate the recovery rates of the DA detection using our electrochemical biosensor technology, we then added different amounts of dopamine (DA) to these solutions. The recovery rates of DA detection with our biosensor varied from 83.93% to 96.26%, as indicated in Table 1. This outcome shows that our electrochemical biosensor is useful and accurate in identifying DA in complicated sample matrices, which is important for its possible use in practical situations. This degree of recovery shows how well the biosensor can distinguish DA from other interfering compounds like GLU, UA, and KCL that are frequently present in biological samples.

These results imply that our biosensor platform has a great deal of promise for real-world DA detection applications, especially in clinical and environmental scenarios where precise and trustworthy detection is crucial.

Table 1: Recovery of DA samples

DA solution concentration (μM /L)	Average (μM/L)	Recovery
1	0.8567	85.67%
2	1.7963	89.81%
5	4.6167	92.33%
10	9.2750	92.75%

4. Conclusions

In this work, we successfully created a new biosensor that uses a glassy carbon electrode in conjunction with the CRISPR/Cas system to detect DA solutions with excellent sensitivity and specificity. To completely maximize the detection performance, we used a variety of electrochemical detection methods, such as CV, EIS, and SWV.

The combination of the CRISPR platform and the glassy carbon electrode forms the basis of this biosensor. The CRISPR/Cas system is well-known for its potent particular DNA recognition capabilities, while glassy carbon electrodes are frequently employed in biosensing because of their superior electrochemical qualities. In addition to achieving highly specific target recognition, we were able to convert this recognition activity into detectable electrochemical signals by combining the specific DNA recognition capability of Cas12a with electrochemical detection technology. This greatly increased the sensor's sensitivity.

In our experiments, we examined simulated serum samples, and the results showed that the electrochemical sensor had good recoveries, indicating its high accuracy and reliability in complex biological samples. This functionality is anticipated to improve patient outcomes and quality of life by drastically reducing the identification time of DA and giving patients prompt and accurate drug treatment recommendations. It is feasible to detect dopamine with high sensitivity by tracking changes in the electrochemical signal. This technique uses the enzymatic activity of Cas12a and the unique interactions between dopamine and the DNA sequences to offer a sensitive and dependable detection platform. This technique is interesting for use in biosensing and diagnostics because it combines these molecular interactions with the electrochemical readout to provide a potent tool for dopamine detection with high accuracy and sensitivity.

In the future, we'll keep working to increase this sensor's sensitivity and investigate its possible use in clinical sample identification. We think that our biosensor, which is based on a glassy carbon electrode and the CRISPR/Cas system, will be able to assist precision medicine and play a significant role in clinical diagnosis and disease monitoring with continued optimization and refinement..

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