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DNA sensor based on carbon black/Methylene Blue arylamine analogue composite for doxorubicin detection

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Abstract

In modern analytical chemistry, various methods are being actively developed to simplify and reduce the cost of electrochemical sensor fabrication, thereby increasing the accessibility of these technologies for a wide range of users, including medical institutions, industrial enterprises, and research laboratories. In this study, we present a DNA sensor based on a screen-printed carbon electrode modified with a carbon black suspension in propylene carbonate and diffusion-free 3,7-bis(4-aminophenylamino)phenothiazin-5-ium chloride for doxorubicin detection. The sensor operates via drop-based analysis using a printed graphite electrode, which ensures compactness and enhanced practical usability. The addition of carbon black to the SPCE surface significantly enhances the signal of diffusion-free phenothiazine in buffer solution, thereby improving both sensitivity and the quality of the analytical signal during biospecific analyte–DNA interactions. The specificity of this interaction was confirmed by substituting sodium polystyrene sulfonate for DNA. The sensor demonstrated high sensitivity in detecting doxorubicin both in buffer solutions and in model biological fluid samples containing high levels of interfering components. The detectable doxorubicin concentration ranges from 1 pM to 1 μM, with a limit of detection of 0.8 pM. The developed DNA sensor offers several advantages that render it promising for clinical applications, including minimal reagent requirements, high sensitivity, and ease of use.

Key findings

- DNA sensor based on carbon black/Methylene Blue arylamine analogue composite was developed.
- Carbon black increased the electrode's effective surface area, thereby enhancing the arylamine phenothiazine signal.
- The doxorubicin concentration ranges from 1 pM to 1 μM, with a limit of detection 0.8 pM.

Accompanying information

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DNA sensor; biosensor; doxorubicin; phenothiazine; carbon black; amperometry

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Supplementary information
Supplementary materials: READ
Transparent peer review: READ

Sustainable Development Goals

1. Introduction

Currently, there is growing interest in electrochemical sensors in fields such as environmental monitoring, healthcare, and quality control [1]. According to marketing reports, the global market for electrochemical sensors was valued at USD 12.9 billion in 2025, projected to grow to USD 23.15 billion by 2032. This reflects market demand for sensor technologies, driven by rising demand for customized diagnostic tools and quality control systems [2].

Electrochemical methods are widely used in the development of sensor systems [1], owing to their low cost [3], rapidity [4], and high sensitivity [5]. An important advantage of such analytical devices is their potential for miniaturization, enhancing portability and usability [6]. This

technology holds promise for personalized medicine, enabling early disease diagnosis, monitoring progression, and developing individualized treatment plans [7].

One of the key representatives of electrochemical sensors that combines all these properties is a biosensor [8]. Biosensors are transducer-based devices that convert biochemical interactions with an analyte into a measurable electrical or optical signal. They find widespread applications in diverse fields, including medical diagnostics and environmental monitoring. Biological recognition elements in biosensors include enzymes [9], tissue samples [10], bacterial cells [11], yeasts [12], and DNA [13].

DNA sensors are used to detect low-molecular-weight compounds that interact with nucleic acids [14]. These sensors address several critical analytical challenges, including identification of biological material via nucleotide sequence analysis [15–19], advancement of early diagnostic methods and detection of oncological diseases [20–23], and assessment of anticancer drugs and DNA-damaging agents [24, 25]. One such anticancer drug is doxorubicin, an anthracycline with intercalating properties [26]. The mechanisms of its action on cancer cells are well established, and its adverse effects on the human body have been extensively studied [27].

The sensors currently used to detect doxorubicin are predominantly challenging to manufacture due to the use of numerous reagents, leading to increased production costs [28]. This high cost restricts the accessibility of these technologies for end-users, including medical institutions, enterprises, and research laboratories.

Modern analytical chemistry provides multiple approaches to simplify sensor fabrication. Scientific literature highlights carbon nanomaterials as a promising solution to this challenge [29]. These materials can be used to create multilayer functional coatings incorporating DNA, metal nanoparticles, or organic reagents, thereby enhancing the selectivity and sensitivity of the sensor response [31-32].

Additionally, polymer films containing DNA fragments or other biomolecules, produced via electropolymerization, serve as modifying coatings in doxorubicin-detecting sensors [33-36]. This approach streamlines and automates the sensor fabrication process.

In addition, developing effective and sensitive electrochemical DNA sensors for anti-cancer drug detection requires selecting an electron transfer mediator and capture agents that facilitate analyte accumulation at the electrode surface.

The key challenge lies in meeting stringent requirements for effective sensor operation. Specifically, the mediator and capture agents must exhibit high electrochemical activity for signal amplification, specific interaction with DNA, and stability under physiological conditions.

Previously, we developed a sensitive electrode layer using a phenothiazine dye copolymer as an electron transfer mediator [37–40]. Subsequent investigation of novel phenothiazine derivatives demonstrated enhanced electrochemical responses compared to the parent phenothiazine compound [41, 42].

The challenge of miniaturization for analytical devices remains relevant for practical clinical applications. In this work, we report a compact and cost-effective DNA sensor based on a composite of 3,7-bis(4-aminophenylamino)phenothiazin-5-ium chloride (PhTz-$(NH_2)_2$) and carbon black (CB) suspensions in propylene carbonate, designed for detecting DNA intercalating agents using doxorubicin as a model analyte. The use of diffusionally free PhTz-$(NH_2)_2$ as a recognition element significantly enhanced both the sensitivity and signal quality in response to DNA-mediated biospecific interactions. The sensor demonstrated high sensitivity in detecting doxorubicin in both buffer media and model biological fluid samples with numerous interfering matrix components. Furthermore, the sensor enables microdrop analysis using a screen-printed carbon electrode (SPCE), ensuring compactness and practical usability in real-world settings.

2. Materials and methods

2.1. Reagents

CB is manufactured by IMERYS (France). The oxidation of CB was carried out according to the method previously developed by our research group [43]. The oxidized CB was dispersed in 1.5 mL of propylene carbonate to obtain a CB suspension with a concentration of 0.66 mg/mL.

All other reagents were of chemical-grade purity. The solutions were prepared using deionized water from a Millipore-Q Simplicity® water purification system (Merck Millipore, France).

PhTz-$(NH_2)_2$ was synthesized according to a previously reported method [44, 45].

2.2. Modification of the SPCE surface

The SPCE was fabricated by screen printing using a DEK 248 printer (DEK, UK) on a Lomond PE DS Laser Film polyester substrate (125 μm thick; Lomond Trading Ltd.). The process involved the sequential deposition of four layers: 1 – conductive silver tracks (PSP 2 paste, NPP DELTA PASTE LLC); 2 – Ag/AgCl pseudo electrode layers (PSC P1 paste, NPP DELTA PASTE LLC); 3 – carbon tracks for the working and auxiliary electrodes (C2030519P4 paste, Gwent Electronic Materials, UK); 4 – protective dielectric layer (D2140114D5 paste, Gwent Electronic Materials, UK).

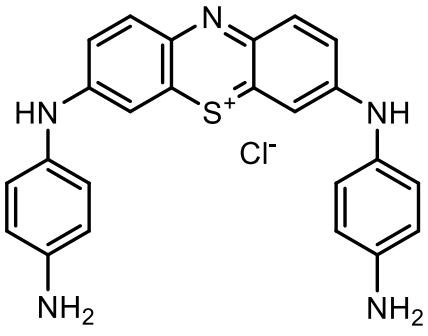

Figure 1 Chemical structure of PhTz-$(NH_2)_2$.

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Each layer was thermally cured in an oven at 80 °C. The geometric area of the electrode's working surface was 3.8 mm².

The SPCE was modified by drop casting 1 μL of CB suspension (0.66 mg/mL in propylene carbonate or dimethylformamide (DMF)), followed by thermal drying in an oven at 100°C. For electrochemical activation, the modified electrode was placed in an electrochemical cell containing phosphate buffer solution and C_2H_5OH (1:1 vol.), pH 7.0. A cyclic voltammogram was recorded over the potential range −0.5 to 0.5 V at a scan rate of 0.1 V/s.

2.3. Development of a DNA sensor

The modified SPCE was washed with water, and the measurements were performed in a phosphate buffer solution and C_2H_5OH (1:1 vol.), pH 7.0. Cyclic voltammograms were recorded in the potential range from −0.7 to 0.7 V at a scanning rate of 0.1 V/s. Differential pulse voltammograms were recorded in the range from −0.8 to 0 V, with a potential step of 0.004 V, an amplitude of 0.05 V, a pulse width of 0.06 s, and a pulse interval of 0.5 s.

For the DNA sensor, the measurements were carried out in a phosphate buffer solution containing 0.1 mM PhTz-$(NH_2)_2$ and 1 mg/mL of native DNA, with the SPCE modified with CB placed in the solution. The voltammograms were recorded using a CHI 660E potentiostat (CH Instruments Inc., USA).

2.4. Determination of doxorubicin

Doxorubicin was added to a 0.01 M phosphate buffer solution (pH 7.0) containing 0.1 M KCl, C_2H_5OH (1:1 vol.), 0.1 mM PhTz-$(NH_2)_2$, and 0.5 mg/mL native DNA, in a concentration range from 1 pM to 1 μM. Subsequently, the SPCE modified with CB was immersed in the solution, and a differential pulse voltammogram was recorded using the following parameters: potential step: 0.004 V; amplitude: 0.05 V; pulse width: 0.06 s; pulse interval: 0.5 s. The analytical signal was defined as the change in the oxidation peak current of PhTz-$(NH_2)_2$.

To assess the matrix effect on doxorubicin determination, artificial plasma was used, containing NaCl: 2 mM; $NaHCO_3$: 0.2 mM; *L*-tryptophan: 0.21 mM; *L*-tyrosine: 0.2 mM; *L*-alanine: 0.2 mM; *L*-aspartic acid: 22 μM; *DL*-lysine: 5 μM; *L*-arginine: 5 μM; *L*-methionine: 4 μM; *L*-phenylalanine: 4 μM; *L*-histidine: 3.5 μM; *L*-glycine: 3.5 μM; *L*-cysteine: 1.3 μM [46].

3. Results and Discussion

Previously, we determined the voltammetric parameters for recording the PhTz-$(NH_2)_2$ signal and investigated its electrochemical properties using cyclic voltammetry on a bare glassy carbon electrode (GCE) [45]. In the present work, we examined the effect of CB deposited on the surface of the SPCE on the signal of diffusionally free PhTz-$(NH_2)_2$ in buffer solution. The diffusion-free property of PhTz-$(NH_2)_2$ facilitates direct DNA interaction, leading to improved signal quality in biospecific analyte-DNA assays.

3.1. Electrochemical behavior of SPCE modified with CB in the presence of diffusionally free PhTz-$(NH_2)_2$

The choice of SPCE as the sensor platform, which integrates a working electrode, auxiliary electrode, and reference electrode into a single miniature device, enables the fabrication of compact sensors. These sensors can be readily integrated into commercially available portable analyzers, making them highly suitable for point-of-care applications.

Figure 2 shows cyclic voltammograms recorded on a bare SPCE and on an SPCE modified with CB suspended in DMF or propylene carbonate in the presence of 0.1 mM PhTz-$(NH_2)_2$.

The morphology of the PhTz-$(NH_2)_2$ voltammograms obtained on SPCE retained its characteristic shape, with broadened peaks resembling those recorded on a GCE [45]. In contrast, the SPCE/CB exhibited a significant increase in peak currents within the potential range from −0.45 to −0.2 V, attributed to phenothiazine, compared to the unmodified electrode.

Moreover, for SPCE/surface, the oxidation peak of PhTz-$(NH_2)_2$ in the potential range from −0.3 to −0.2 V was more pronounced than the reduction peak. In contrast, on bare SPCE, similarly to what was observed on bare GCE [45], an increase in the cathodic peak was more prominent.

The effect of CB on the PhTz-$(NH_2)_2$ signal arises from two factors: CB increases the effective surface area of the working electrode [47], and CB enhances the electrode's negative surface charge, which promotes phenothiazine sorption into the CB layer via electrostatic interaction with its positively charged groups. This, in turn, alters the electron density at the electrode/solution interface.

The use of suspension in propylene carbonate yielded a greater effect compared to CB suspension in DMF. This difference in suspension media arises from the more uniform distribution of CB across the electrode surface when using propylene carbonate, which is attributed to its higher viscosity.

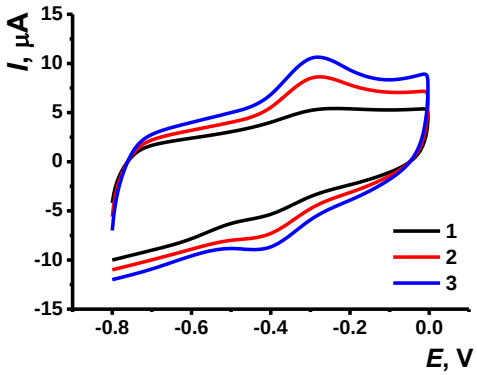

Figure 2 Cyclic voltammetric curves of SPCE in a 0.1 mM PhTz-$(NH_2)_2$ solution. (1) SPCE, (2) SPCE/CB in DMF, (3) SPCE/CB in propylene carbonate. Voltammetric measurements were performed in 0.01 M phosphate buffer solution + 0.1 KCl and C_2H_5OH (1:1 vol.), pH = 7.0. Scan rate 0.1 V/s.

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Although both solvents can disperse CB, propylene carbonate offers a superior combination of optimal viscosity, controlled evaporation rate, and enhanced safety profile. Consequently, propylene carbonate was selected as the preferred medium for CB suspension in subsequent experiments.

When varying the pH of the buffer solution, the redox peak currents of PhTz-$(NH_2)_2$ increase and plateau at pH 7 (Figure 3). This behavior aligns well with the properties of other phenothiazine-series compounds, for which a pH range of 7–9 is optimal [48].

The dependence of the peak oxidation current on the concentration of PhTz-$(NH_2)_2$ in solution (Figure S1) is linear over the concentration range from 1 to 200 μM (I_{pa}, μA = (0.002 ± 0.0001) + (32.099 ± 0.142)·log(*C*, mM), R^2 = 0.9987, *n* = 5). Increasing the PhTz-$(NH_2)_2$ concentration resulted in a corresponding rise in peak currents.

3.2. The effect of DNA on the PhTz-$(NH_2)_2$ signal

When native DNA is added to a PhTz-$(NH_2)_2$ solution, an increase in the PhTz-$(NH_2)_2$ redox peaks is observed over the concentration range of DNA phosphate bases from 0.01 to 3.0 mM (Figure 4). Presumably, the interaction between DNA and PhTz-$(NH_2)_2$ follows this mechanism: the PhTz-$(NH_2)_2$ molecule carries a positive charge on its nitrogen atoms, while the DNA molecule acts as a polyanion, serving as a template for phenothiazine dye accumulation via electrostatic interactions. However, previous spectroscopic studies of the interaction between phenothiazine and DNA in solution [44] have demonstrated the presence of other, more specific interaction mechanisms. Based on this, it was of interest to verify the specificity of this interaction under electrochemical experimental conditions.

The specificity of PhTz-$(NH_2)_2$/DNA interaction in the sensor was confirmed by substituting sodium polystyrene sulfonate (PSS) for DNA. Both PSS and DNA are polyanionic electrolytes capable of forming polyelectrolyte complexes with positively charged species. However, due to its rigid structure, DNA-with charged phosphate groups aligned along the backbone-forms more specific complexes than PSS. The latter has a flexible architecture: its charged anionic groups reside at the ends of bulky side aromatic moieties, resulting in a larger average distance between adjacent charges [49].

Figure 4 displays the dependence of the anodic peak current of 0.1 mM PhTz-$(NH_2)_2$ on the concentration of DNA phosphate bases or PSS sulfonate groups, as recorded on a CB-modified SPCE. When PSS replaced DNA, PhTz-$(NH_2)_2$ redox peaks shifted similarly, though the effect was markedly weaker.

Thus, the DNA– PhTz-$(NH_2)_2$ interaction is not only electrostatic. It likely involves steric complementarity between cationic and anionic center, phenothiazine intercalation into DNA, and groove-binding interactions. These conclusions align with our prior spectroscopic studies of PhTz-$(NH_2)_2$–DNA interactions [44].

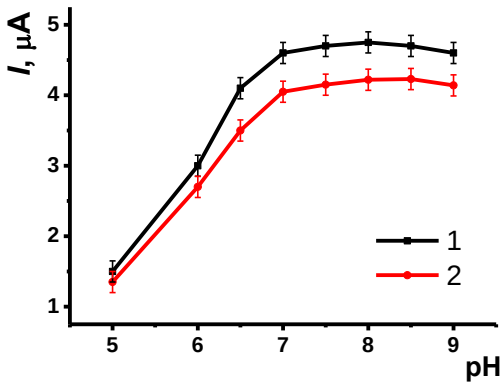

Figure 3 pH-dependence of (1) I_{pa} and (2) I_{pc} of SPCE/CB in propylene carbonate in a 0.1 mM PhTz-$(NH_2)_2$ solution. Voltammetric measurements were performed in 0.01 M phosphate buffer solution + 0.1 KCl and C_2H_5OH (1:1 vol.), pH = 7.0. –0.8÷0.0 V, scan rate 0.1 V/s, *n* = 5.

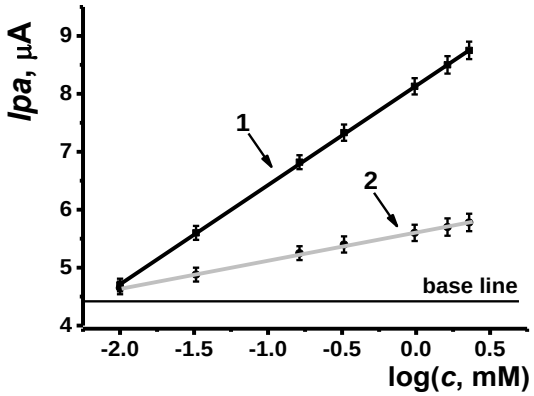

Figure 4 Dependence of the I_{pa} of the 0.1 mM PhTz-$(NH_2)_2$ peak in solution on the concentration of (1) DNA phosphate bases and (2) PSS sulfonate groups on SPCE/CB in propylene carbonate in solution. Voltammetric measurements were performed in 0.01 M phosphate buffer solution + 0.1 KCl and C_2H_5OH (1:1 vol.), pH = 7.0, –0.8÷0.0 V, scan rate 0.1 V/s, n=5.

3.3. Determination of doxorubicin

Based on the established ability of PhTz-$(NH_2)_2$ to bind specifically to DNA, we deemed it advisable to develop an electrochemical sensor for detecting low molecular weight compounds that interact specifically with DNA. The sensor was designed using PhTz-$(NH_2)_2$ and SPCE modified with CB.

Since PhTz-$(NH_2)_2$ has demonstrated the ability to intercalate into the DNA double helix, we selected the cytostatic drug doxorubicin as the target analyte. This anthracycline drug also exhibits intercalating properties, enabling the use of competitive interaction between doxorubicin and phenothiazine to enhance the sensitivity of cytostatic detection.

Although PhTz-$(NH_2)_2$ was previously shown to form electroactive polymer coatings on electrode surfaces [44], in this DNA sensor development (as in earlier work stages) we employed PhTz-$(NH_2)_2$ as a diffusion free mediator. This approach was feasible because the PhTz-$(NH_2)_2$ signal remained stable and reproducible under experimental conditions. Consequently, this strategy both simplified the DNA sensor fabrication procedure and enhanced the efficiency of the competitive interaction between the two intercalators.

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Figure 5 shows cyclic voltammograms recorded on a CB modified SPCE upon addition of native DNA or DNA/doxorubicin complexes to a PhTz-$(NH_2)_2$ solution. Doxorubicin (10 μM) was added to a phosphate buffer solution containing 0.1 mM PhTz-$(NH_2)_2$ and 0.5 mg/mL native DNA, with a CB modified SPCE immersed in the solution.

In the presence of doxorubicin, the redox peak currents of PhTz-$(NH_2)_2$ increased more significantly than when DNA alone was added to the PhTz-$(NH_2)_2$ solution. This enhancement may result from the competitive displacement of PhTz-$(NH_2)_2$ from the DNA double helix, which increases the proportion of free PhTz-$(NH_2)_2$ molecules in solution. The competitive displacement of PhTz-$(NH_2)_2$ from the DNA double helix by doxorubicin process can be a thermodynamically driven competitive intercalation mechanism: doxorubicin exhibits structural advantages, including a larger aromatic surface area, a groove-binding moiety, and stronger non-covalent interactions, that enable it to outcompete PhTz-$(NH_2)_2$ for DNA intercalation sites. Consequently, this leads to higher reactivity in the redox process occurring at the electrode surface.

Differential pulse voltammetry was employed to enhance detection sensitivity. Figure 6 displays differential pulse voltammograms recorded on a CB modified SPCE upon addition of varying concentrations of doxorubicin to a solution containing 0.1 mM PhTz-$(NH_2)_2$ and 0.5 mg/mL native DNA. Figure S2 presents the calibration curve for doxorubicin quantification using the developed biosensor system.

As the doxorubicin concentration increased, the voltammetric peaks associated with PhTz-$(NH_2)_2$ exhibited a significant rise. The detectable doxorubicin concentration range spanned from 1 pM to 1 μM, with a limit of detection (LOD) 0.8 pM calculated based on S/N=3:1 (I_{pa}, μA = (2.9 ± 0.02) + (41.16 ± 0.23)·log(C, M), R^2 = 0.9984, n = 5).

The determination of doxorubicin using the developed biosensor system was also performed in synthetic blood plasma mixed with a working buffer solution (Table 1).

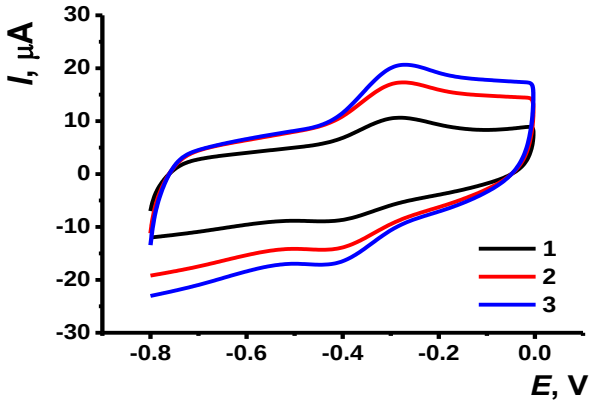

Figure 5 Cyclic voltammograms registered for SPCE/CB in propylene carbonate in the presence of (1) 0.1 mM PhTz-$(NH_2)_2$, (2) 0.1 mM PhTz-$(NH_2)_2$ and 0.5 mg/mL DNA, (3) 0.1 mM PhTz-$(NH_2)_2$, 0.5 mg/ mL of DNA and 10 μM of doxorubicin. Voltammetric measurements were performed in 0.01 M phosphate buffer solution + 0.1 KCl and C_2H_5OH (1:1 vol.), pH = 7.0. –0.8÷0.0 V, scan rate 0.1 V/s.

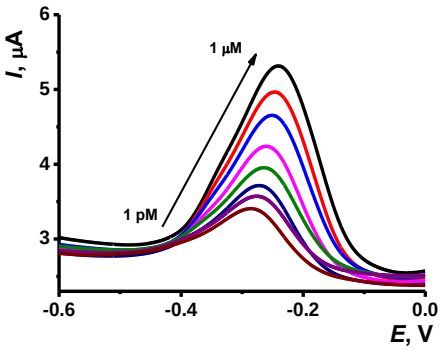

Figure 6 Differential pulse voltammograms obtained for SPCE/CB in propylene carbonate in a solution containing 0.1 mM PhTz-$(NH_2)_2$, 0.5 mg/mL of DNA in the presence of different concentrations of doxorubicin. Voltammetric measurements were performed in 0.01 M phosphate buffer solution + 0.5 mg/mL DNA + 0.1 KCl and C_2H_5OH (1:1 vol.), pH = 7.0. –0.8÷0.0 V, scan rate 0.1 V/s.

Table 1 Accuracy of doxorubicin quantification in synthetic blood plasma (n = 5).

Dilution of doxorubicin	Doxorubicin, μM		Recovery, %
	Added	Found	
1:100	0.1	0.134 ± 0.003	134 ± 3
1:500		0.115 ± 0.003	115 ± 3
1:1000		0.102 ± 0.002	102 ± 2

Given that therapeutic doses of doxorubicin are substantially higher than the obtained LOD [50], the developed sensor demonstrates suitability for detecting cytostatics in biological fluid samples.

Table 2 compares the analytical performance of the developed DNA sensor, based on CB-modified SPCE and diffusion-free PhTz-$(NH_2)_2$, with the previously reported sensors. The proposed sensor exhibits comparable or superior sensitivity for doxorubicin detection. Furthermore, the DNA sensor retained its activity toward doxorubicin when stored dry at 4 °C for at least 45 days.

Table 2 Comparison of the electrochemical characteristics of the sensors developed with other electrochemical sensors for doxorubicin determination described in literature.

	Concentration range, M	LOD, M	Ref.
Indium tin oxide electrode/vertically-ordered mesoporous silica films and N-doped graphene quantum dots	$5.0\cdot10^{-9}$ – $1.0\cdot10^{-6}$	$5.0\cdot10^{-10}$	[51]
GCE/DNA/GCE/DNA/ single-walled carbon nanotubes	$1.0\cdot10^{-9}$ – $2.0\cdot10^{-5}$	$6.0\cdot10^{-10}$	[52]
SPCE/multi-walled carbon nanotubes/Poly-L-lysine	$2.5\cdot10^{-9}$ – $2.5\cdot10^{-7}$	$1.0\cdot10^{-9}$	[53]
GCE/Nitrogen-doped carbon nanoonions	$2.0\cdot10^{-10}$ – $1\cdot10^{-5}$	$6.0\cdot10^{-11}$	[54]
GCE/Acetylene Black	$1.0\cdot10^{-8}$ – $2.5\cdot10^{-6}$	$3.0\cdot10^{-9}$	[55]
SPCE/CB in propylene carbonate, PhTz-$(NH_2)_2$ and DNA in solution	**$1.0\cdot10^{-12}$ – $1.0\cdot10^{-6}$**	**$0.8\cdot10^{-13}$**	**This work**

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When synthetic blood plasma was diluted 100-fold, the recovery reached 134%, which can be attributed to the interfering effects of plasma components on the sensor signal for doxorubicin. This interference was minimized by diluting the synthetic blood plasma 1000-fold — in this case, the recovery decreased to 102%.

4. Limitations

The study has several limitations: analytical specificity was assessed only in model biological fluids, not in real clinical samples containing complex interferents; sensor performance was optimized at pH 7 but not evaluated under physiologically relevant acidic or alkaline conditions; the approach was validated solely for doxorubicin, not for other DNA-intercalating drugs; no in vivo or clinical sample validation was performed. These gaps highlight the need for future work on real-sample testing, stability optimization, and broader analyte validation.

Conclusions

Thus, the developed sensor based on SPCE modified with carbon black and diffusion free PhTz-$(NH_2)_2$, demonstrated high sensitivity for doxorubicin detection both in buffer media and in model biological fluid samples containing numerous interfering matrix components. This suggests potential future applications in medicine and pharmacology.

Additionally, we investigated the electrochemical properties of the CB based modifying coating in the presence of PhTz-$(NH_2)_2$ CB increased the electrode's effective surface area, thereby enhancing the PhTz-$(NH_2)_2$ signal.

The resulting biosensor outperforms existing analogues in key analytical parameters: the detectable doxorubicin concentration ranges from 1 pM to 1 μM, with a limit of detection of 0.8 pM (based on S/N ratio of 3:1). These features render the DNA sensor highly promising for cost effective monitoring of doxorubicin in patients' biological fluids.

Moreover, the system can be readily adapted to detect other DNA intercalators similar to doxorubicin and identify low molecular weight compounds that interact specifically with DNA via alternative mechanisms.

The DNA sensor also exhibits high stability, retaining activity against doxorubicin when stored dry at 4 °C for at least 45 days. The combination of minimal reagent requirements, high sensitivity, and the inherent convenience of SPCE platforms makes this DNA sensor particularly promising for clinical applications.

Supplementary materials

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

Data availability statement

The raw/processed data required to reproduce the above findings cannot be shared at this time as the data also forms part of an ongoing study.

Acknowledgments

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Author contributions

Conceptualization: D.S, I.S.
Data curation: P.P.
Formal Analysis: D.S, K.K., D.K.
Funding acquisition: D.S.
Investigation: D.S., K.K, P.P.
Methodology: D.S., P.P.
Project administration: D.S., I.S.
Resources: I.S., P.P.
Software: K.K., D.S.
Supervision: I.S.
Validation: D.K., K.K.
Visualization: D.S.
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Writing – review & editing: D.S., P.P., I.S.

Conflict of interest

The authors declare no conflict of interest.

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Kazan (Volga region) Federal University, https://kpfu.ru/Eng/.

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