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Design of electrochemical aptameric biosensor for the specific and selective detection of chemotherapeutic drugs: Application for paclitaxel and leucovorin

Shamsa Alanazi[1], Amina Rhouati[1], Amani Chrouda[2], Dana Cialla-May[3,4], Jürgen Popp[3,4] & Mohammed Zourob[1]✉

Chemotherapy continues to be a fundamental approach in treating various cancers, with combination therapies frequently improving effectiveness while minimizing toxicity. Recently, the therapeutic potential of combining Paclitaxel and Leucovorin drugs has been highlighted in cancer treatment. However, both agents pose challenges in terms of toxicity and necessitate careful dosing and monitoring. To address this, we report the selection of two ssDNA aptamers and their use in the electrochemical biosensing of Paclitaxel and Leucovorin. Using the SELEX process, five sequences were generated for paclitaxel, labeled P1 to P5, while four aptamers for leucovorin were identified (L1 to L4). Based on affinity studies, the aptamers P3 and L1 exhibiting the lowest dissociation constants, were selected for the design of Paclitaxel and Leucovorin biosensors, respectively. The developed aptasensors were applicable within the ranges of 10–1000 pg/mL and 3–500 pg/mL with the low detection limits of 0.02 and 0.0077 pg/mL for Paclitaxel and Leucovorin, respectively. A good selectivity was also attained against different drugs, including chemotherapeutic compounds. Finally, real samples analysis was also carried out showing good recovery rates ranging from 91.3% to 109% with RSDs lower than 5%. In comparison to traditional techniques, this platform shows high performance and is user-friendly, presenting a novel and efficient approach for monitoring paclitaxel and leucovorin in chemotherapy regimens.

Keywords Chemotherapeutic drug, Paclitaxel, Leucovorin, Aptamer, SELEX, Electrochemical, Biosensor

The World Health Organization (WHO) reported that cancer is one of the leading causes of death globally, with 20 million new cases and 9.7 million cancer-related deaths recorded in 2022[1,2]. Current cancer therapies include surgery, radiotherapy, and chemotherapy, as well as emerging methods such as immunotherapy, targeted drug therapy, and hormone therapy[3–5]. Among these treatments, chemotherapy remains a key method for addressing various types of cancer, though it is often associated with significant side effects that require careful monitoring of drug use[6–8]. Paclitaxel and leucovorin are widely used as chemotherapeutic agents approved by the Food and Drug Administration (FDA) for the treatment of various cancers[9]. Paclitaxel is a complex compound originally isolated from the bark of the Pacific yew tree. It is used to treat a range of cancers, including ovarian, breast, lung, and cervical cancer[10,11]. This molecule stabilizes microtubules and inhibits cell division, resulting in the death of cancer cells. However, its administration may lead to adverse effects such as peripheral neuropathy and

[1]Department of Chemistry, Alfaisal University, Al Zahrawi Street, Al Maather, Al Takhassusi Rd, 11355 Riyadh, Saudi Arabia. [2]Department of Chemistry, College of Science Al Zulfi, Majmaah University, Zulfi, Saudi Arabia. [3]Institute of Physical Chemistry (IPC) and Abbe Center of Photonics (ACP), Friedrich Schiller University Jena, Member of the Leibniz Centre for Photonics in Infection Research (LPI), Helmholtzweg 4, 07743 Jena, Germany. [4]Leibniz Institute of Photonic Technology, Member of Leibniz Health Technologies, Member of the Leibniz Centre for Photonics in Infection Research (LPI), Albert-Einstein-Straße 9, 07745 Jena, Germany. ✉email: MZOUROB@ALFAISAL.EDU

hypersensitivity, particularly at high doses[12,13]. Leucovorin, a reduced folate (tetrahydrofolate), is often used in combination with chemotherapeutic drugs to reduce their toxicity. It is derived from folic acid and plays a crucial role in DNA repair and replication[14]. Leucovorin mitigates the side effects of chemotherapy by protecting healthy cells from damage while allowing the anti-cancer drug to target malignant cells[15].

While these drugs are highly effective in treating cancer, the variability in patient response, drug resistance, and potential toxicity necessitate precise monitoring of their concentrations in biological samples. Currently, high-performance liquid chromatography (HPLC)[16,17], and liquid chromatography-tandem mass spectrometry (LC–MS/MS)[18] are the standard methods for drug monitoring in clinical settings. However, these techniques have significant drawbacks, including high operational costs, time-consuming sample preparation, and the need for skilled personnel. Additionally, they often lack the sensitivity required to detect low concentrations of chemotherapeutic drugs in complex biological matrices[19]. To overcome these limitations, there is increasing interest in developing alternative analytical tools that are more cost-effective, sensitive, and user-friendly. Biosensors, particularly, those utilizing aptamers, offer a promising alternative for the conventional chromatographic tools[20]. Aptamers are short single-stranded oligonucleotides that can fold into specific three-dimensional structures, allowing them to bind selectively to their targeted molecules with high affinity[21]. They are generated in vitro through SELEX (Systematic Evolution of Ligands by Exponential Enrichment) process[22]. Compared to traditional antibodies used in immunoassays, aptamers have several advantages, including lower production costs, ease of synthesis, high stability, and the ability to target a wide range of molecules, from small organic compounds to whole cells[23,24].

Various transducers have been employed in aptasensor design, where electrochemical strategies achieved significant advancements. Electrochemical aptasensors leverage the high specificity of aptamers and the sensitivity of electrochemical transduction, enabling rapid, real-time analysis of drug concentrations with high accuracy. This combination offers a versatile and scalable approach to the development of biosensors tailored for specific applications, such as chemotherapeutic drug detection[25–27]. In this study, we describe the selection of two aptamers via SELEX process, and the design of electrochemical biosensors for the specific and selective detection of two key chemotherapeutic drugs: Paclitaxel and Leucovorin. The developed aptasensors demonstrated high sensitivity, specificity, with a great potential for clinical applications. This approach offers a novel, efficient, and cost-effective solution for real-time monitoring of Paclitaxel and Leucovorin concentrations. It has the potential to enhance patient outcomes by facilitating personalized chemotherapy dosing and reducing treatment-related side effects.

Experimental section

Chemicals and instrumentation

The list of chemicals and the instruments used in this study are mentioned in Supporting Information.

In vitro Selection of Paclitaxel and leucovorin aptamers

First, the DNA library was heated for five minutes at 90 °C, then cooled at 4 °C for 10 min. In parallel, Paclitaxel and Leucovorin were conjugated, separately, to n-hydroxysuccinimide (NHS)-activated Sepharose, according to the manufacturer's protocol. In brief, 1 mg/mL of Paclitaxel and Leucovorin solutions were incubated with 100 µL of the washed beads and put on a rotator overnight, at 4 °C. Then, the mixture was centrifuged for 5 min at 300 rpm and the supernatant was discarded. Afterwards, the beads were washed three times with alternate pH buffers: 0.1 M Tris (pH 8.0) and 0.1 M Acetate NaCl (pH 4.0). Then, 300 nmol of the library were incubated with 100 µL of chemotherapeutic drug-conjugated beads, prepared in binding buffer (BB), under rotation in a centrifuge filter tube for 2 h. Following that, a washing step was carried out by using 400 µL of binding buffer five times. 300 µL of heated elution buffer (90°C) were then added to elute the DNA bound to the chemotherapeutic drugs-coupled beads. After 10 min of incubation, the eluted DNA was collected in a 3 kDa cutoff membrane filter. Finally, ssDNA was amplified by PCR by using a FAM-labelled forward primer. The amplified dsDNA was run on 2% agarose gel electrophoresis for 40 min. The dsDNA PCR band was precipitated by adding 250 µL of 3 M sodium acetate and 750 µL of cold ethanol and incubated at 80 °C for two hours. After 30 min of centrifugation at 4 °C, the collected DNA was re-suspended in a mixture of 1 to 1 BB and loading dye, for 5 min. The fluorescent dsDNA was run on 10% denaturing PAGE at 300 V for 2 h. The obtained band was subsequently cut from the gel, added to 400 µL of TE (Tris–EDTA) buffer and incubated in an incubator overnight at 37°C. Finally, the eluted ssDNA was ultra-filtrated by using a 3 kDa cutoff membrane filter and used as a pool for the next round. After the seventh cycle of SELEX, the ssDNA was conjugated with blank beads. The aim of this counter selection is to eliminate the non-specific sequence by using the same protocol. The collected DNA was concentrated by the cut-off filter then eluted and used to start the next cycle of SELEX.

Cloning, sequencing, and alignment

By the end of the eleventh cycle of SELEX, the ssDNA was cloned to select the best sequences. We performed fresh PCR from the last cycle of SELEX and ligated with a cloning vector, then it was added into competent *E. coli* which was transferred to broth with Catabolite repression (SOC) medium and incubated for one hour at 37°C. Next, two Luria broth (LB) agar plates were used for each analyte after spreading them with 40 mg/mL X-Gal and 50 µg/mL ampicillin. Then, the SOC media that has growing competent cells containing a cloning vector was spread onto the LB agar plates and incubated overnight at 37°C. Then, the good candidate colonies were selected and amplified by PCR by using M13 primers. Finally, we proceeded to the sequencing and PRALINE alignment of the resulting DNA.

Determination of the dissociation constants (K_d)

After selection, the obtained sequences were labelled with carboxyfluorescein to perform the affinity studies. First, aptamer solutions were heated at 90 °C for 10 min, then cooled at 4 °C for 10 min and at 25 °C for 5 min. A fluorescence binding assay was used by incubating different concentrations of the FAM-labeled aptamers, prepared in BB (10, 50, 100, 200 and 300 nM) with Paclitaxel/Leucovorin-conjugated, on a rotator for 1 h at room temperature. After washing with BB, the bound DNA was eluted from the beads by adding the elution buffer at 90 °C for 10 min and centrifuging at 13,000 rpm for 1 min. Then, the DNA amount eluted from each sample was determined by fluorescence measurement with the excitation and emission wavelengths of 470 ± 10 nm and 515nm, respectively. The fluorescence intensities obtained for each aptamer sequence were plotted, and the dissociation constants (K_d) were determined by nonlinear regression analysis of the curves using GraphPad Prism 10.0 software.

Electrochemical aptasensing of Paclitaxel and leucovorin

To develop the electrochemical aptasensors, we selected the aptamers exhibiting the lowest K_d value for each chemotherapeutic drug. Each aptamer was labelled with a thiol group to enable its covalent grafting to the screen-printed gold electrodes (SPGEs). The aptamer sequences were synthesized and labelled by International AG (Planegg, Germany). The aptasensors were fabricated by incubating 10 µL of each aptamer solution (1 µM) on the gold surface, in a water-saturated atmosphere, overnight at 4 °C. Then, the gold electrodes were rinsed with 0.1 M PBS, pH 7.4 and incubated with 1 mM of mercapto-1-hexanol prepared in PBS, for 30 min at room temperature. After that, the functionalized electrodes were washed with PBS and stored at 4 °C for further use. For the chemotherapeutic drugs determination, the fabricated aptasensors were incubated with 10 µL of different concentrations of Paclitaxel and leucovorin for 30 min at room temperature. Finally, the electrochemical response was recorded by square wave voltammetry (SWV) in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution prepared in 0.1 M PBS buffer, pH 7.4, before and after incubation with each concentration.

Results and discussion

Selection of ssDNA aptamers against Paclitaxel and Leucovorin

The conjugation between Paclitaxel/Leucovorin drugs and the solid matrix is a crucial step in SELEX[28]. Paclitaxel was immobilized on agarose beads activated with diamino-dipropylamine, through its terminal carboxyl groups. The COOH groups on the drug molecules were activated and then used to react with the terminal NH_2 groups on the beads through amide bond formation. Leucovorin was covalently immobilized on NHS-activated Sepharose beads via its terminal amino group. To block unbound groups from the beads, 0.1 M Tris was used. This step would minimize the non-specific electrostatic interactions between the DNA and Sepharose/agarose beads. The Paclitaxel/Leucovorin-conjugated beads were used for each round of SELEX.

Figure 1.**a** and **b** illustrate the ssDNA recovery across all SELEX cycles for Paclitaxel and Leucovorin, respectively. As shown in the histogram, fluorescence intensity, corresponding to the amount of recovered ssDNA, steadily increased with each cycle. This increase reflects the enrichment of aptamers with high affinity for the target molecules[29]. However, after the counter-selection step, a notable decrease in fluorescence intensity was observed at rounds 9 and 10, signifying the effective removal of non-specifically bound DNA sequences. The fluorescence intensity then increased again in cycles 10 and 11, indicating the continuous enrichment of specific aptamers.

The SELEX process was concluded after 11 cycles, where the enriched ssDNA pool was sufficient for the target drugs. The recovered DNA was then cloned into E. coli competent cells for amplification. Selected colonies were picked for PCR and sequencing, and the resulting sequences were analyzed using a multiple sequence alignment using PRALINE program (https://www.ibi.vu.nl/programs/pralinewww/). Based on their similarities, the sequences were classified into five and four distinct groups, for Paclitaxel and Leucovorin, respectively. As it is shown in Fig. 1.**c** and **d**, we note that each group displayed significant consensus regions. Therefore, one sequence from each group was selected for binding affinity studies.

Binding affinity studies of the selected aptamers towards Paclitaxel and Leucovorin.

The aptamer sequences selected from the cloning went through a binding affinity study, to identify the most specific aptamers to our targeted analytes. A fluorescent binding assay was employed by incubating 50 µL of Paclitaxel/Leucovorin-conjugated beads with different concentrations (10, 50, 100, 200, 300, and 400 nM) of each sequence, individually. Then, a washing step was performed to eliminate the nonconjugated sequences. In parallel, the bound aptamers were eluted, and their fluorescence intensities were measured at an emission wavelength of 515 nm. The fluorescence intensities were then plotted against the concentrations of each aptamer. Finally, the resulting binding curves were analyzed by a non-linear regression to determine the dissociation constants of each sequence. Table 1 shows the K_d values obtained for each aptamer as well as its sequence.

From the table, we can note that P3 aptamer exhibited the highest affinity for Paclitaxel with a K_d as low as 38.02 nM, while the best affinity for Leucovorin was obtained with the sequence L1 (K_d of 125.7 nM).

Electrochemical biosensing of Paclitaxel/Leucovorin drug using the selected aptamers

Electrochemical characterization of the biosensor fabrication steps

The selected aptamers were conjugated with a thiol group in 5' end and covalently immobilized onto the SPGEs. Thiol chemistry was chosen for its simplicity and efficiency; the spontaneous adsorption of sulfur groups onto metal surfaces enables the formation of uniform layers of well-oriented bioreceptors[30]. Electrochemical characterization was performed to confirm the aptamer immobilization onto the gold surface. First, cyclic

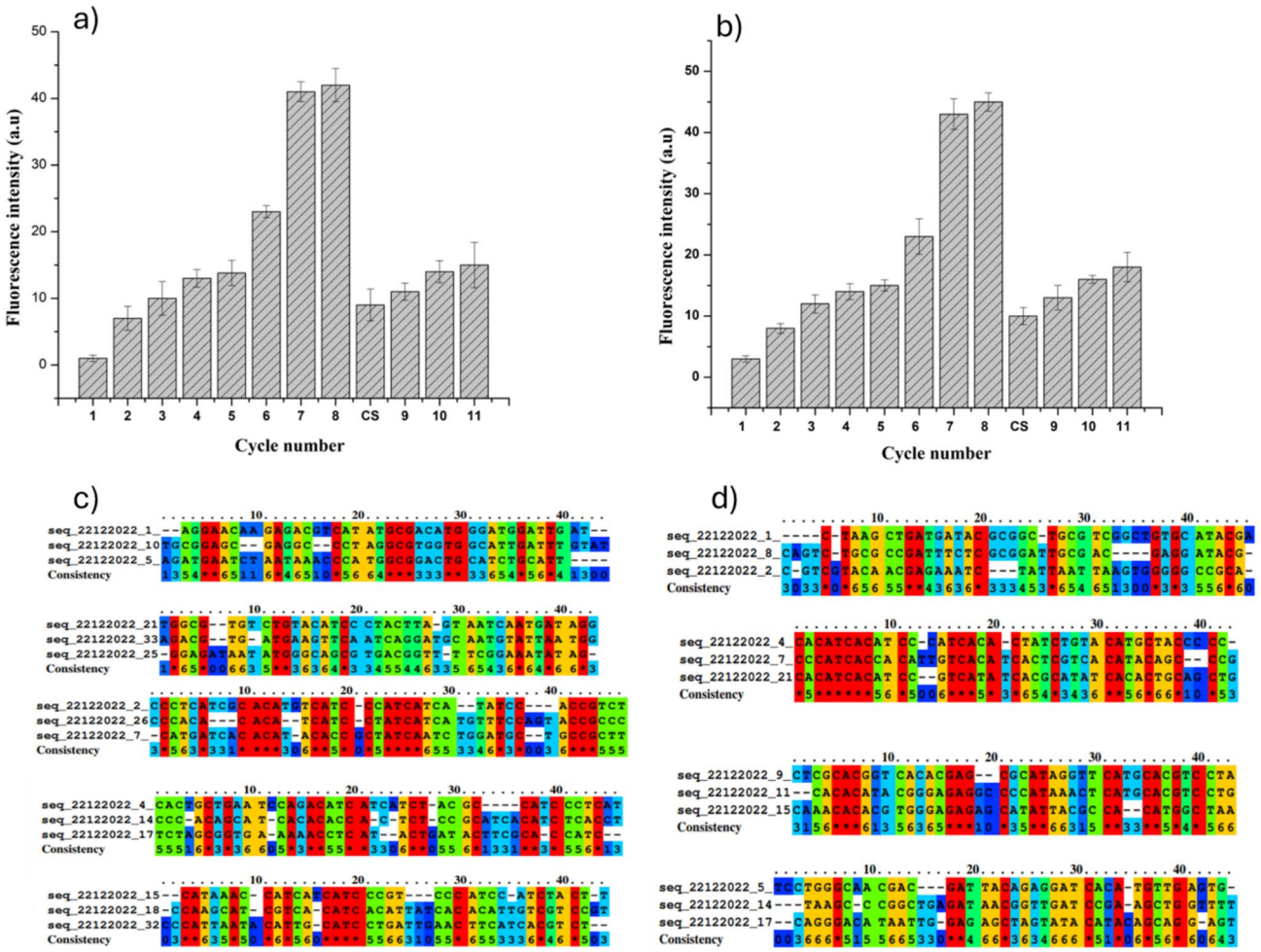


Fig. 1. The fluorescence intensity of the recovered amount of DNA measured after each round of SELEX for Paclitaxel (**a**) and Leucovorin (**b**). Analysis of the selected sequences by multiple sequence alignment using PRALINE software for Paclitaxel (**c**) and Leucovorin (**d**).

Aptamer	Sequence	K_d (nM)
Paclitaxel		
P1	TGC GGA GCG AGG CCC TAG GCG TGG TGG CAT TGA TTT GTA T	139.2
P2	AGA CGT GAT GAA GTT CAA TCA GGA TGC AAT GTA TTA ATG G	484.2
P3	CCA AGC ATC GTC ACA TCA CAT TAT CAC ACA TTG TCG TCC GT	38.02
P4	TGT GCA TTC GTG TGA ATT AAC TCG TCC ACC AGA GCC CGT G	208.1
P5	ACA TGT GTA GGC GGT TGG ATG CAT TAT GAG ATG GGA CAT G	54.07
Leucovorin		
L1	CAG TCT GCG CCG ATT TCT CGC GGA TTG CGA CGA GGA TAC G	125.7
L2	CCC ATC ACC ACA TTG TCA CAT CAC TCG TCA CAT ACA GCC CG	284.2
L3	CAC ACA TAC GGG AGA GGC CCC ATA AAC TCA TGC ACG TCC TG	225.7
L4	TAA GCC CGG CTG AGA TAA CGG TTG ATC CGA AGC TGG TTT T	285.7

Table 1. Dissociation constants (K_d) calculated for the paclitaxel and Leucovorin selected aptamers.

voltammetry (CV) was conducted in a redox solution of 5 mM ferri/ferrocyanide in PBS buffer (pH 7.4), with a potential range of −0.6 V to 0.6 V and a scan rate of 100 mV/s.

Figures 2.**a** and **b**, show the cyclic voltammograms obtained for Paclitaxel and Leucovorin aptasensors, respectively at the different steps of fabrication. By comparing the black line corresponding to the bare SPGE with the red line corresponding to the Aptamer-modified SPGE, we noted that the chemisorption of thiol groups onto the gold surface induced a significant decrease in the oxidation/reduction peaks. This behavior could be

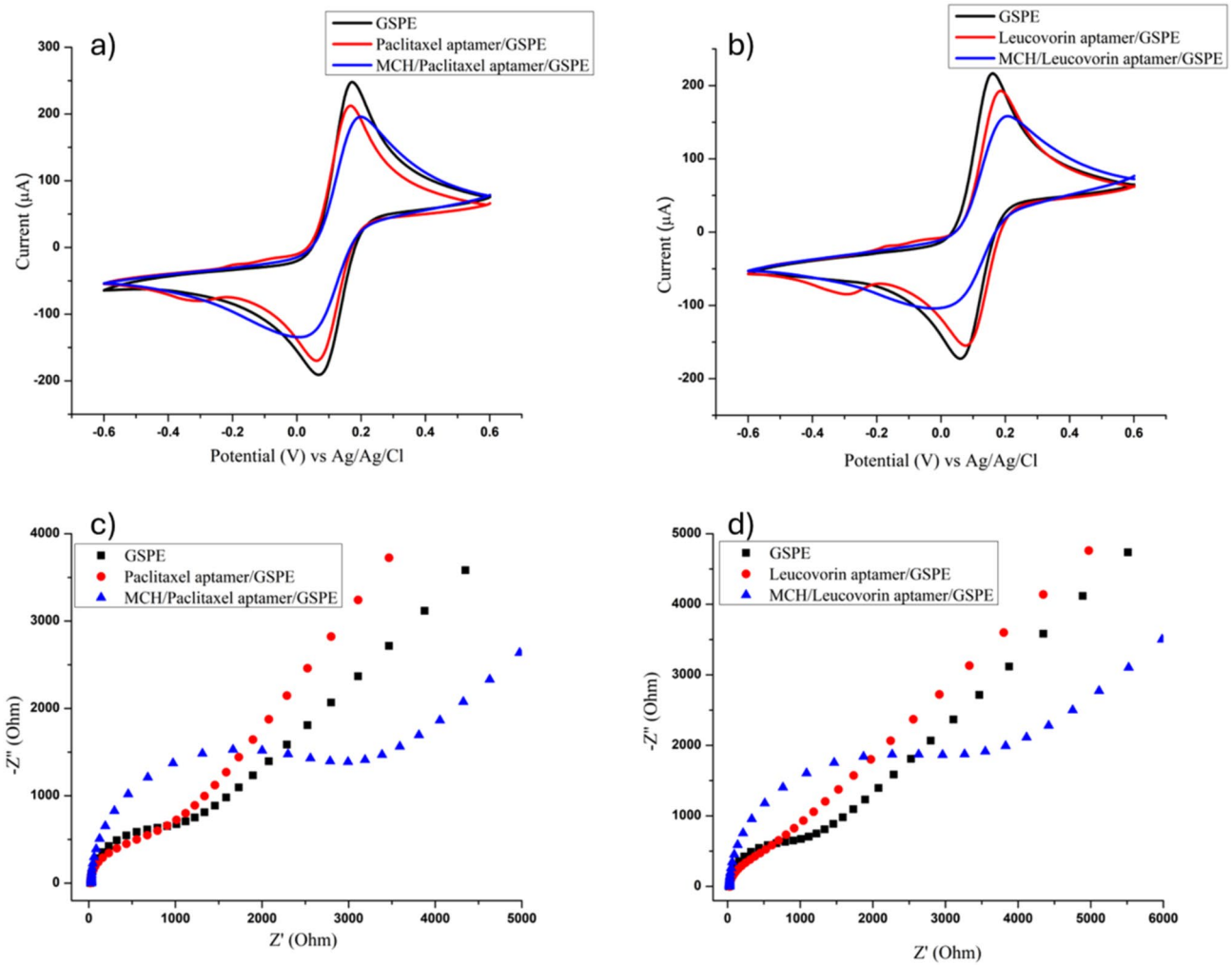


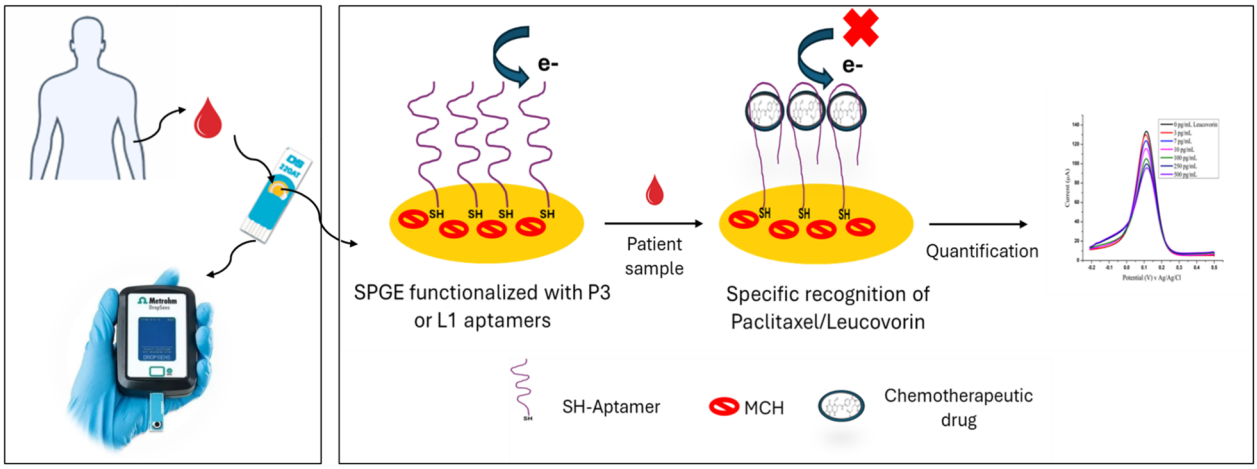
Fig. 2. Cyclic voltammograms and EIS of the paclitaxel/Leucovorin aptasensor fabrication steps; bare SPGE (black), Pacli/Leuco aptamer/SPGE (red) and MCH/aptamer/SPGE (blue). CV was performed in 5mM ferri/ferrocyanide solution prepared in PBS buffer pH 7.4, potential range of (−0.6 to 0.6 V) and a scan rate of 100 mV/s. EIS was performed in the same electrolyte, over the frequency range from 10^5 to 1 Hz, a potential of + 0.4 V and an amplitude of 10 mV.

attributed to the formation of a densely and well-organized self-assembled monolayer (SAM) of aptamers on the gold surface, which hinders the transfer of the redox couple to the electrode surface[31]. Finally, after addition of MCH (blue line), the peak currents decreased thus confirming the blocking of the non-specific sites remaining on the surface.

In parallel, electrochemical impedance spectroscopy (EIS) can provide complementary insights into the surface modifications. Figure 2.**c and d** show the impedance spectra for the two functionalized electrodes prepared for Paclitaxel and Leucovorin, respectively. EIS results are consistent with the CV data where a remarkable increase in the resistance was observed following the aptamer addition to the SPGEs. This increase is mainly due to the negatively charged aptamer layer, which impedes electron transfer between the electrode and the redox probe. After addition of MCH to the aptamer/SPGE surface, an increase in the resistance was also observed. The MCH blocking enhanced electrode coverage by promoting SAM reorganization and reorienting aptamer molecules that adsorbed on the surface via electrostatic interactions[31].

Paclitaxel/Leucovorin aptasensing via square wave voltammetry

To confirm the feasibility of the fabricated aptasensors for Paclitaxel and Leucovorin determination. Each aptasensor was incubated with increasing concentrations of its respective targeted drug. Scheme 1 shows the mechanism of detection of Paclitaxel and Leucovorin. Paclitaxel and Leucovorin concentrations ranging from 3 to 1000 pg/mL were prepared in binding buffer. Then, each concentration was individually incubated with the aptamer-functionalized SPGE for 30 min at room temperature. A washing step was subsequently performed with PBS to remove non-specifically bound elements. Electrochemical measurements were carried out then by SWV in the redox solution of 5mM ferri/ferrocyanide prepared in PBS buffer pH 7.4, potential range of (−0.2 to 0.5 V), frequency 25 Hz, interval time 0.04 s, step potential − 5 mV, scan rate 125 mV/s and amplitude 20



Scheme 1. Schematic illustration of the mechanism of detection of Paclitaxel and Leucovorin using the designed aptasensors.

mV. Figure 3a and b show the square wave voltammograms recorded before and after incubation of the two aptasensors with the different concentrations of Paclitaxel and Leucovorin solutions. As it can be seen from the figures, the peak currents decrease by increasing the drug concentration. This decrease could be explained by the formation of the complex aptamer-drug thus hindering the electron transfer to the aptasensing surface.

To better visualize this variation, the analytical response was calculated as $((i^0\text{-}i)/i^0\%)$, Where i^0 is the peak current of the control and i is the peak current obtained after incubating the aptasensors with the targeted drugs. Then, the calibration curve was obtained by plotting the calculated response versus the different concentrations of the chemotherapeutic drugs (Figs. 3.**c** and **d**). Based on the calibration curves plotted in logarithmic variation, a good linearity was obtained within the ranges of (10–1000 pg/mL) and (3–500 pg/mL) for Paclitaxel and Leucovorin, respectively. Good coefficients of determination were achieved for both aptasensors with the R^2 of 0.99 and 0.96 with the regression equations: $((i^0\text{-}i)/i^0\%) = 0.327 + 8.406$ log [Paclitaxel] (pg/mL) and $((i^0\text{-}i)/i^0\%) = 1.858 + 12.888$ log [Leucovorin] (pg/mL). R^2 values of. The respective detection limits of 0.02 and 0.0077 pg/mL, were then calculated as 3.3 σ/b, where σ is the standard deviation of the blank and b is the slope.

To highlight the originality of the present work, a bibliographic investigation was conducted to compare the performance of various sensors developed for the detection of Leucovorin and Paclitaxel. Different electrochemical sensors were developed for the detection of Leucovorin and Paclitaxel (Table 2). For Leucovorin, the reported approaches rely mainly on monitoring the direct electrochemical response of the molecule on unmodified gold electrodes. The limits of detection in these studies are typically in the micromolar range, significantly higher than the LODs achieved in the present work. Similarly, for Paclitaxel, a variety of surface modifications, such as β-CD/CNT/GCE, have been explored, with detection limits as low as 43 nM. However, the aptamer-based sensor developed in this study outperforms these approaches, achieving an exceptional LOD in the femtomolar range. This could be attributed to the use of aptamers as biorecognition element providing a high affinity for the targeted analytes. The developed aptasensors provide enhanced detection capabilities for both Leucovorin and Paclitaxel when compared to the reported electrochemical methods.

Selectivity studies and real sample applications

To confirm the specificity of the aptasensors for their respective targeted analytes, the as prepared aptamer-functionalized SPCEs were subjected to a variety of potential interfering drugs. The selectivity study was carried out by incubating the aptasensors with a fixed concentration of the different drugs (10 pg/mL), separately. Different chemotherapeutics and antibiotics were tested; Cabazitaxel, 5-Fluorouracil, Penicillin G and Amoxicillin. The analytical procedure was performed in the same experimental conditions and according to the protocol previously described for detection. Figure 4.**a** and **b** show a comparison of the aptasensors responses towards the specific drugs with that obtained for the interfering compounds. We note that the calculated response $((i^0\text{-}i/i^0)\%)$ for the specific drugs is much higher than that obtained for the interfering drugs. These results confirm the selectivity of the selected aptamers for Paclitaxel and Leucovorin.

To demonstrate that the designed aptamer-based strategy could be applied in blood patients' samples without matrix effects, the aptasensors were tested with serum samples. The developed aptasensors were incubated with serum samples diluted in BB (1:50) and spiked with two concentrations of Paclitaxel/Leucovorin drugs (10 and 100 pg/mL). Then, square wave voltammograms were recorded by using the same experimental conditions described above. Table 3 shows the calculated concentrations, the recovery percentage as well as the RSDs (Relative standard deviation) obtained for each aptasensor.

The recovery percentage was calculated by comparing the analytical response of the aptasensor obtained in the binding buffer with that obtained in serum samples by using the equation:

R% = measured concentration/added concentration.

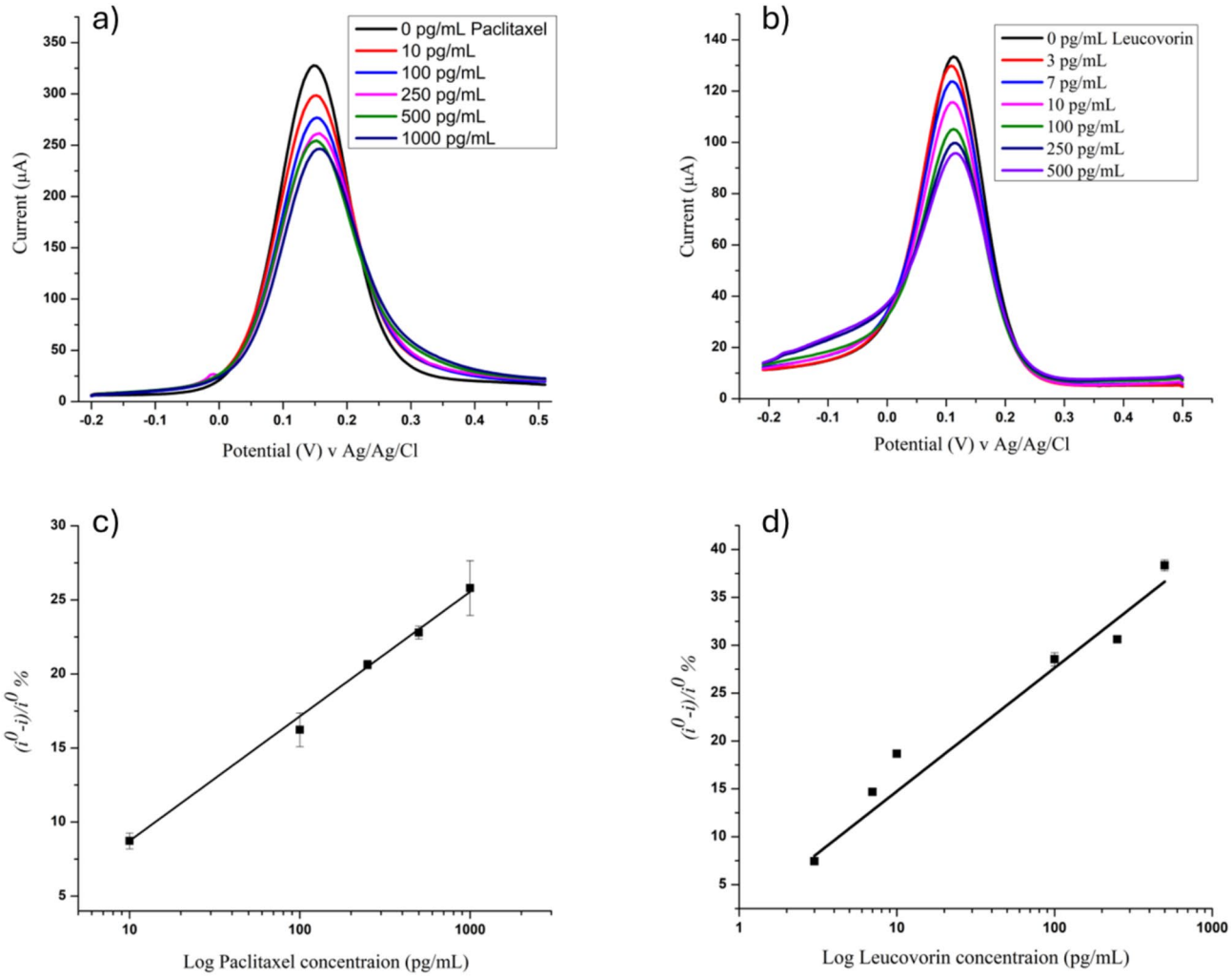


Fig. 3. Square wave voltammograms obtained for increasing concentrations of (**a**) Paclitaxel; and (**b**) Leucovorin. c) Plot of the electrochemical response of the two aptasensors *((i^0-i/i^0) %)* versus the logarithm of (**c**) Paclitaxel and (**d**) Leucovorin concentration (pg/mL). SWV was performed in 5 mM ferri/ferrocyanide, prepared in PBS 7.4 in the range of −0.2 to 0.5 V, at an interval time of 0.04 s, frequency 25 Hz, a scan rate of 125 mV s^{-1}, amplitude: 20 mV and step potential of 5 mV.

	Transducer	Surface modification	Analytical range	LOD	Ref
Leucovorin	Electrochemical	SPCEs	0.5 to 30 µM	2.63 µM	[32]
	Electrochemical	SPCE	0 to 10 µM	0.509 µM	[33]
	Electrochemiluminescence	SPE	6.25 to 100 µM	6.25 µM	[34]
	Electrochemical	Aptamer-functionalized SPGE	6.34 pM to 1.05 µM	0.016 pM	The present work
Paclitaxel	Luminescence	MIP-QDs	0.1 to 2.0 µM	0.043 µM	[35]
	Electrochemical	β-CD/CNT/GCE	5 to 1060 µM	1.7 nM	[36]
	Electrochemical	Aptamer-functionalized SPGE	11.72 pM to 1.72 µM	0.023 pM	The present work

Table 2. Comparison of the analytical performance of the developed aptasensors with other biosensors reported in the literature for Paclitaxel and Leucovorin. MIP-QDs: Molecularly imprinted polymers-Quantum dots, β-CD/CNT/GCE: β-cyclodextrin/carbon nanotubes/glassy carbon electrode.

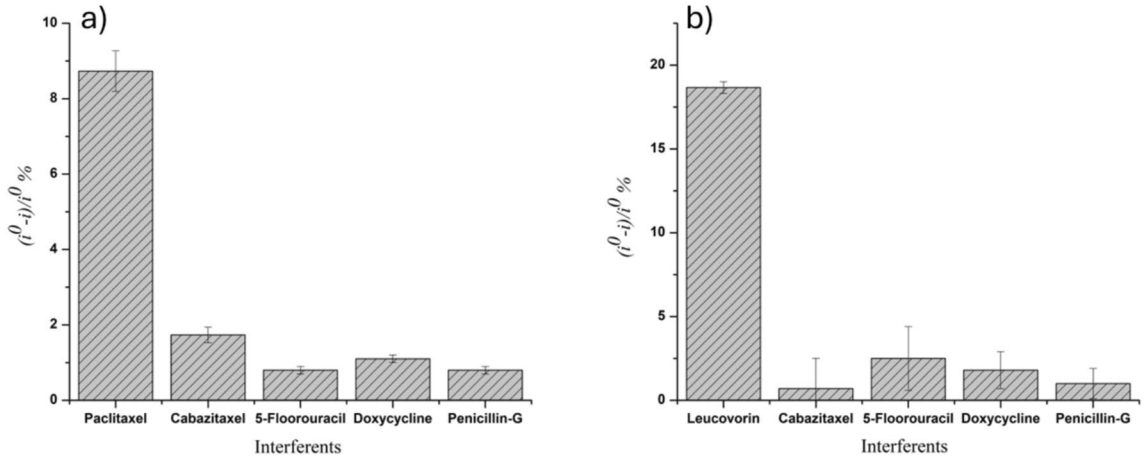


Fig. 4. Cross-reactivity study of the electrochemical aptasensors for: (**a**) Paclitaxel and (**b**) Leucovorin.

	Added concentration (pg/mL)	Found concentration (pg/mL)	Recovery percentage (%)	R.S.D (%)
Paclitaxel	10	9.5	95	1.5
	100	91.3	91.3	3.2
Leucovorin	10	10.9	109	3.46
	100	96.4	96.4	0.86

Table 3. Applicability of the proposed aptasensor for Paclitaxel/Leucovorin detection in spiked serum samples.

Excellent percentages were obtained showing that the developed Paclitaxel/Leucovorin aptasensors could be successfully applied in biological fluids without interferences or matrix effects.

Conclusion

In summary, we successfully selected and characterized single-stranded DNA aptamers for the chemotherapeutic drugs Paclitaxel and Leucovorin. Affinity studies demonstrated that the selected aptamers, P3 and L1, exhibited the lowest K_d values for Paclitaxel and Leucovorin, respectively. The practical application of these aptasensors was validated by integrating them in the design of two electrochemical aptasensors demonstrating excellent analytical performance, with both high specificity and sensitivity. The aptamers P3 and L1 displayed a good applicability in serum samples spiked with varying concentrations of Paclitaxel and Leucovorin, confirming their reliability in complex biological matrices. The aptamer-based sensors offer several key advantages, including low cost, high sensitivity, stability, and the ability to detect small volumes of samples. These properties make them highly promising for clinical applications, particularly in monitoring chemotherapy drugs in cancer patients. By providing accurate drug concentration measurements, these sensors could help minimize the adverse effects of chemotherapy, potentially improving patient outcomes and optimizing treatment regimens.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

S. A, A. R. and A. C. did most of the experimental work and wrote first draft of the manuscript. D.C-M., J.P., and M. Z. supervised the work and proof read the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to M.Z.