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Electrochemical, spectroscopic and molecular docking studies on the interaction of calcium channel blockers with dsDNA

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ABSTRACT

This study presents evaluation of the possible interaction mechanism between calf thymus dsDNA and three calcium antagonists; nifedipine, lercanidipine and amlodipine. The interactions between Nifedipine-dsDNA and Lercanidipine-dsDNA were investigated by differential pulse voltammetry using two different interaction methods; at the dsDNA-electrochemical biosensor surface and in bulk incubated solution. Amlodipine was used as model drug in bulk incubated solution. The decrease in the peak current of guanine and adenine were used as an indicator for confirmation of the interaction event in acetate buffer of pH 4.70. In bulk incubated solution, after interaction with Nifedipine and Amlodipine the guanine signal was almost disappeared. At the dsDNA modified glassy carbon electrode surface, the peak currents of guanine and adenine were decreased while Nifedipine and Lercanidipine interacts with DNA. The interactions between Nifedipine-dsDNA and Lercanidipine-dsDNA were further studied by UV–Vis absorption spectroscopy which indicates the intermolecular interaction between these drugs and ds-DNA can be mainly through hydrogen bonding and van der Waals forces. Molecular docking calculations shown that the AMP-1-2, NDP and LDP-1-2-ctDNA having groove binding. Beside spectral data, docking studies elicited that AMP-1-2, NDP and LDP-1-2 complexes have different interaction and conformation trends to target (ctDNA).

1. Introduction

The electrochemical techniques provide information about in vivo redox process due to the existing similarity between electrochemical and in vivo reactions [1]. Electrochemical investigation presents the opportunity to discern the effects of drugs in DNA structure and elucidate the mechanism of interaction based on their unique electrochemistry. This has led to the development of DNA biosensors or geno sensors. The electrochemical DNA biosensor is a device that incorporates immobilized DNA as a molecular recognition element on the electrode surface and enables rapid, simple and low-cost detection capabilities for biological binding events. Simulating interactions of nucleobases in DNA with phospholipid membranes and proteins by electrochemical DNA biosensors to mimic in vivo situation as well as potential environmental health hazardous compounds elicits the basic model of interaction of drugs with DNA though formation of a complex. [2–8]

The binding mechanism of some small molecules of interest in pharmaceuticals like drugs with DNA has been a key research topic during the past few decades for rational designing of new more effective targeted drugs. These interactions can either modify or inhibit the normal function of DNA. Binding of peptides, drugs or small organic and inorganic molecules to DNA can interfere in numerous processes, including transcription and replication of DNA. Generally, DNA-acting drugs can be classified as: covalently bound molecules, noncovalent interacting agents though groove binding or by intercalative mode or DNA backbone cleaving reagents. The covalent mode of binding of drug-DNA either through alkylation or crosslinking inter- or intra-strands is irreversible with high binding strengths and conducts directly to cell death. The non-covalent mode of binding is reversible but can change DNA conformation, lead to topoisomerase unmodulated DNA torsional stress, interrupt process of transcription by disrupting protein–DNA interactions, and potentially lead to DNA strand breaks. Non-covalent binding includes electrostatic interaction with the negatively charged nucleic acid sugar-phosphate structure, intercalation of the planar aromatic ring systems between base pairs and minor/major DNA groove binding interaction as well as external binding by

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1567-5394/

electrostatic mode with anionic backbone of DNA [9]. The driving binding interactions can include electrostatic, hydrophobic, allosteric, hydrogen bonding and/or van der Waals interactions [10].

The differences in the electrochemical behaviour of the drugs or electroactive DNA bases after interaction with each other, can be investigated as follows: (1) the change as a significant decrease or increase in the peak current of the drug; (2) the change as a dramatic decrease or increase in the peak current of the electroactive DNA bases; (3) the shift of the formal potential of the redox couple. Carter and Bard have reported different kinds of interaction modes based on the shifting in peak potential of small molecule, in the presence of dsDNA: a positive shift could be expected if intercalation was the dominant interaction mode of [11], while a negative potential shift would be observed if electrostatic binding was present. This was asserted by Dogan-Topal et al., who investigated the peak potential shift of Lapatinib in a positive direction with the addition of calf thymus dsDNA confirming intercalation mode of interaction [12]. Similar studies were conducted by Wang et al., for interaction of daunomycin with dsDNA in solution phase and dsDNA anchored on carbon paste electrode suggesting intercalation binding [13]. In another study, Dogan-Topal and Ozkan have noted that the interaction of Leuprolide in different concentrations with dsDNA immobilized either on the electrode or in solution phase, resulted in a decrease of guanine signal exhibiting interaction of the drug with redox active guanine base [14].

In this paper, the efficacy of possible interaction mechanism between calcium antagonists and dsDNA is presented. Calcium channel blockers, or calcium antagonists, treat a variety of conditions, such as high blood pressure, angina, arrhythmias, and circulatory disorders. Calcium channel blocking agents restrict the amount of calcium entering cardiac and smooth muscle cells by blocking voltage-gated calcium channels. This causes blood vessels to relax and widen (vasodilate), improves oxygen supply to the heart, and lowers blood pressure. Nifedipine (NDP), Lercanidipine (LDP) and Amlodipine (AMP) are calcium channel blockers belonging to the dihyropyridine class (Scheme 1). The binding properties of NDP and LDP with ctDNA were investigated by electrochemical methods, UV–Vis absorption spectroscopy and molecular docking techniques. The interaction between model drug, AMP and dsDNA was studied with differential pulse voltammetry (DPV) (only in bulk incubated solutions) and molecular docking technique. Study of the binding mechanism of target AMP, NDP and LDP with DNA is one of the key steps in their DNA-interaction and designing their new drugs Scheme 1.

2. Experimental

2.1. Apparatus

2.1.1. Electrochemical investigation

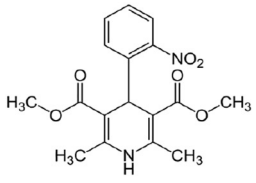
Voltammograms were carried out using an AUTOLAB-PGSTAT 30 electrochemical analysis system with General Purpose Electrochemical Software (GPES) 4.9 software (Eco Chemie, Utrecht, and The Netherlands). A three-electrode cell with an Ag/AgCl (3 M KCl) reference, a Pt wire counter and glassy carbon electrode (GCE) as the working electrodes were used. A pH meter Model 538 (WTW, Austria) was used for adjustment of pH values having a combined glass-reference electrode.

Cyclic voltammetric (CV) measurements were also realized with using BAS 100 W (Bioanalytical System, USA), electrochemical analyser with a standard three-electrode configuration. The three electrode system consisted of a GCE (BAS: $\Phi = 3$ mm, diameter) as working electrode, a platinum wire counter electrode, and an Ag/AgCl saturated KCl reference electrode. GCE was polished manually with aqueous slurry of alumina powder ($\Phi = 0.01$ μm) on a damp smooth polishing cloth (BAS velvet polishing pad) just before each measurement. All measurements were achieved at room temperature. CV was used with conditions given as follows; initial potential: 100 mV, high potential: 1500 mV, scan rate: 100 mV/s, sensitivity: 10 μA/V and quiet time: 2 s.

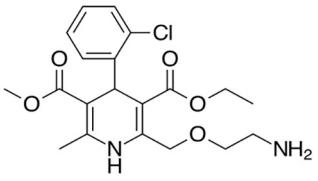
DPV was used with conditions given as follows; step potential: 0.00195 V; modulation amplitude: 0.0505 V; modulation time: 0.07 s; interval time: 0.4 s. The DP voltammograms were used after baseline correction with General Purpose Electrochemical Software (GPES 4.9). Average baseline correction was applied using a 'peak width' of 0.01 V. Prior to each measurement, GCE was polished with alumina slurry ($\phi = 0.01$ μm) on a polishing cloth.

2.1.2. UV–Vis absorption study

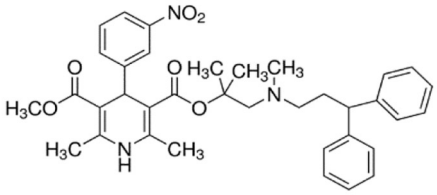
UV–Vis spectra were recorded on an Agilent Cary 60 UV–Vis Spectrophotometer using 1 × 1 cm quartz cell. Temperature were controlled by Single Cell Peltier accessory with a high-precision.



(A) Nifedipine



(B) Amlodipine



(C) Lercanidipine

Scheme 1. The chemical structures of (A) Nifedipine: dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (B) Amlodipine: 3-*O*-ethyl 5-*O*-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate and (C) Lercanidipine: 5-*O*-[1-[3,3-diphenylpropyl(methyl)amino]-2-methylpropan-2-yl] 3-*O*-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

2.2. Chemicals

Calf thymus ct-dsDNA was obtained from Sigma. NDP, LDP and AMP were supplied by Bayer®, Recordati®, Sanovel®, respectively. All other chemicals for the preparation of buffers and supporting electrolytes were reagent grade (Merck or Sigma). The concentrations of ct-dsDNA in ultra-pure water were prepared as 575 μg mL^{-1}. A stock solution of NDP and LDP were prepared in ethanol and AMP was prepared in ultra-pure water. These stock solutions were diluted to the working concentration with the selected supporting electrolytes. All supporting electrolyte solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system.

2.3. Process

2.3.1. For characterization by electrochemical method

For scan rate studies, stock solutions of NDP and LDP (100 ppm) were prepared in ethanol and AMP was prepared in ultra-pure water and kept in the dark at 4 °C. Their working solutions for voltammetric Investigations were prepared by dilution of the stock and contained 20% methanol. For the voltammetric studies, 0.1 M acetate buffer (pH 4.70) were prepared in double-distilled water. All experiments were carried out at room temperature.

The interaction between drug and DNA was investigated in solution and at the electrode surface by electrochemical methods. The surface confined interaction required a smaller volume of sample solution (i.e 50 μL) compared to the interaction performed in solution phase (i.e 1000 μL).

In bulk incubation procedure, 100 μg mL^{-1} ct-dsDNA and 10 μg mL^{-1} NDP in solution form were mixed together in a pH 4.70 (0.1 M acetate buffer), and then incubated at room temperature with different time periods. The same procedure was also used for the following drugs; 1 μg mL^{-1} LDP and 10 μg mL^{-1} AMP as separately. Control solutions of 10 μg mL^{-1} NDP, 1 μg mL^{-1} LDP, 10 μg mL^{-1} AMP, and 100 μg mL^{-1} ct-dsDNA were also prepared separately in pH 4.70 acetate buffer and stored with the same time periods. After each measurement, the GCE surface was polished to a clean finish to avoid blocking of the surface by adsorption of electro-active species.

The multi-layer ct-dsDNA-modified electrode was prepared by depositing three drops of 5 μL each containing 50 μg mL^{-1} ct-dsDNA on the GCE surface. After placing each drop on the electrode surface, the biosensor was allowed to dry at 35 °C. The ct-dsDNA-electrochemical biosensor was immersed in each of the drug solution separately (10 μg mL^{-1} NDP and 0.2 μg mL^{-1} LDP) and incubated at different times. The electrode was then cleaned with blank acetate buffer solution for 5 s for the removal of the unbound ct-dsDNA at the electrode surface. Then the electrode was immersed into cell with the blank acetate buffer solution and DP voltammograms were recorded between the range 0.2 and 1.5 V. A freshly biosensor was prepared for each experiment. Using the signal of multilayer ct-dsDNA, the between-day reproducibility results (RSD %) for guanine and adenine peak currents were found as 1.52% and 2.88%, respectively.

2.3.2. For UV–Vis absorption spectroscopy

The concentration of the stock solution of ct-DNA (1.40×10^{-3} M) was determined by UV absorbance at 260 nm using the molar extinction coefficient = 6600 M^{-1} cm^{-1}. Titration were carried out by adding DNA solution to fixed concentrations of NDP (10 μM) and LDP (30 μM) solutions in acetate buffer (0.1 M; pH 4.70). Spectra were recorded between 250 and 500 nm and 250–450 nm for NDP-DNA titration and LDP-DNA titration respectively. The titrations were performed by keeping temperatures at 283 K, 293 K and 303 K for both LDP and NDP to observe the behaviour of interaction at different temperatures and derive thermodynamic parameters.

2.3.3. Molecular docking

Discovery Studio (DS) 2018 software [15] was used to search the possible orientations and interaction mechanismes of AMP as positive control, NDP and LDP with DNA as target. The crystal structure of DNA (ID:1BNA) [16] were downloaded from protein data bank. AMP and LDP have two possible configuration which are S and R form. The S and R configurations of possible compounds (AMP and LDP) were named 1 and 2, respectively. The geometry and energy optimization of all possible AMP, NDP and LDP configurations as the ligands were done at DFT/B3LYP/6-31G* level by using Gaussian 09 (G09) [17]. The chosen target was also minimized CHARMm force field and the adopted-basis Newton-Raphson (ABNR) method [18] to prepare the target for molecular docking. Analyze Ligand Poses and Calculated Binding Energy subprotocols of the DS 2018 were used to analyze the docking results.

3. Results and discussion

3.1. Scan rate studies

The most popular technique in electrochemical studies is CV for obtaining information about fairly complicated electrode reactions. It is very important technique for studying electron transfer-initiated chemical reactions including catalysis. CV is powerful technique commonly employed to investigate the electrochemical processes of molecular species. The experiments are controlled by the scan rate according to the how fast the applied potential is scanned.

Useful information involving electrochemical mechanism generally can be acquired from the relationship between peak current and scan rate. Therefore, the voltammetric behaviour of the NDP, LDP and AMP was studied using CV at different scan rates for clarifying of the transfer of the compound under diffusion or adsorption controlled (S1).

The scan rate studies varied from 5 to 750 mV/s for 10 μg mL^{-1} NDP, LDP and AMP. The equations are noted below in acetate buffer at pH 4.70:

$$\log ip(A) = 0.660 \log v\left(mVs^{-1}\right) - 1.527, r = 0.995\ (n = 7)\ \text{for NDP} \quad (1)$$

$$\log ip\ (A) = 0.475 \log v\left(mVs^{-1}\right) - 1.352, r = 0.997\ (n = 7)\ \text{for LDP} \quad (2)$$

$$\log ip\ (A) = 0.792 \log v\left(mVs^{-1}\right) - 1.271, r = 0.999\ (n = 5)\ \text{for AMP} \quad (3)$$

A plot of logarithm of *ip* versus logarithm of scan rate gave a straight line with a slope of 0.66 and 0.79 for NDP and LDP, respectively, show the diffusion-adsorption mixed controlled process [19]. The slope of log ip – log v were found as 0.48 for LDP, shows the diffusion controlled process.

The peak potential shifted to more negative potential values with the increase in the scan rates. As could be seen in the equation, Ep shifted to more anodic values with increasing scan rate confirming and supporting the irreversibility. The linear relationship between Ep and log*v* can be expressed by the following equation:

$$Ep(V) = 0.079 \log v(V\ s^{-1}) + 0.957, r = 0.991\ (n = 9)\ \text{for NDP} \quad (4)$$

$$Ep\ (V) = 0.049 \log v\ (V\ s^{-1}) + 1.068, r = 0.998\ (n = 9)\ \text{for LDP} \quad (5)$$

$$Ep\ (V) = 0.070 \log v\ (V\ s^{-1}) + 0.855, r = 0.999\ (n = 6)\ \text{for AMP} \quad (6)$$

3.2. Electrochemical interaction studies

Electrochemical detection of the interaction between ds-DNA and NDP or LDP based on the changes of guanine and adenine signals have

not been studied before on any electrode. The model compound, AMP has been studied by Svitkova et al., to investigate the fish sperm DNA-modified boron doped diamond electrode (BDDE) incubated in the presence of AMP at different incubation times [20]. In this published study, to evaluate the interaction process, the changes were studied as a dramatic decrease of peak current of guanine by DPV and peak current of redox indicator by CV [20]. In our study, the interaction between DNA and calcium antagonists were investigated at dsDNA-electrochemical biosensor surface and in bulk incubated solution based on the changes of guanine and adenine signals.

3.2.1. Bulk incubated dsDNA solution studies

The electrochemical investigation of the NDP-dsDNA interaction was studied in incubated solutions containing 10 μg mL^{-1} NDP and 100 μg mL^{-1} ct-dsDNA, in pH 4.70 (0.1 M) acetate buffer. As seen Fig. 1, the DP voltammograms of the NDP-ct-dsDNA interaction were recorded for GCE after different incubation times (0 min, 10 min, 60 min, 120 min, 180 min, and 240 min). Control solutions of 100 μg mL^{-1} ct-dsDNA and 10 μg mL^{-1} NDP prepared in acetate buffer explained in Section 2.3.2 were analysed after incubation for the same period of time. The electrochemical behaviour of ct-dsDNA was recorded as control to clarify the NDP-ct-dsDNA interaction. The DP voltammograms of ct-dsDNA showed two peaks corresponding to the oxidation of deoxyguanosine (dGuo) at 1.01 V and deoxyadenosine (dAdo) at 1.26 V as shown in Fig. 1. The DP voltammograms of NDP oxidation were shown as one peak; at 0.82 V (Fig. 1). For increased incubation times, the dGuo peak was disappeared, and peak current of dAdo was decreased. Also, the peak potential of NDP was slightly shifted and the peak current decreased.

Similar experiments were performed for LDP and AMP. Although, the chemical structure of NDP is different from LDP and AMP, the dihydropyridine structure is similar. This approach has been used for understanding of preferential dsDNA interactions with dihydropyridine structure which have various functional groups. The interaction of ct-dsDNA and LDP or AMP was studied by DPV with different incubation times as shown in Figs. 2 and 3.

As seen in Fig. 2, the interactions of LDP with ct-dsDNA were studied in solution with different incubation times by DPV. The DP voltammograms of LDP showed two peaks at 0.75 V (first peak) and 0.94 V (second peak). As it can be observed from Fig. 2 the oxidation peak of dGuo and second peak of LDP have overlapped at almost 0.98 V. So, to evaluate the interaction only dAdo signal was remarked. The electrochemical study of the LDP-ct-dsDNA interaction by DPV was carried out incubating 1 μg mL^{-1} LDP with 100 μg mL^{-1} ct-dsDNA in pH 4.70 (0.1 M) acetate buffer with different incubation periods

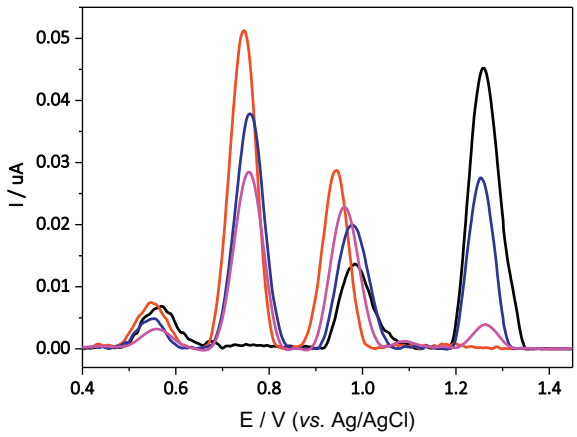


Fig. 2. DP voltammograms of 100 μg mL^{-1} ct-dsDNA (black), 1 μgmL^{-1} LDP (red) and ct-dsDNA incubated with LDP under different incubation time: 0 min (blue), 120 min (pink) in pH 4.7 acetate buffer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(0 min – 240 min). After 120 min incubation time, the peak current of dAdo significantly was decreased. Also, the first peak current of LDP decreased with a slight shift in peak potential.

The interaction between AMP and dsDNA has already been studied by Svitkova et al [20]. That's why, in our study, this interaction was only studied in bulk incubated solution. As observed in Fig. 3, the interactions of AMP with ct-dsDNA were studied in solution by DPV. The DP voltammograms of AMP exhibits one peak at 0.80 V. The electrochemical study of the AMP-ct-dsDNA interaction was carried out by incubating 10 μg mL^{-1} AMP with 100 μg mL^{-1} ct-dsDNA in pH 4.70 (0.1 M) acetate buffer with different incubation periods (0 min - 240 min). After 60 min incubation time, the dGuo peak almost disappeared while peak current of dAdo decreased significantly. This disappearing of dGuo peak was also observed for NDP. Also, the peak potential of AMP was slightly shifted and the peak current decreased.

3.2.2. In situ study using the ct-dsDNA-electrochemical biosensor

For a better understanding the calcium antagonists-ct-dsDNA interaction, a DNA electrochemical biosensor was prepared for NDP and LDP. Throughout the electrochemical investigation of interaction between drug-DNA in incubated solutions, an incomplete network film of co-adsorbed free DNA, free drugs and drug-DNA complex occurred at the electrode surface [21]. The DNA biosensor consists of an electrode with DNA immobilized on the surface and the DNA film completely

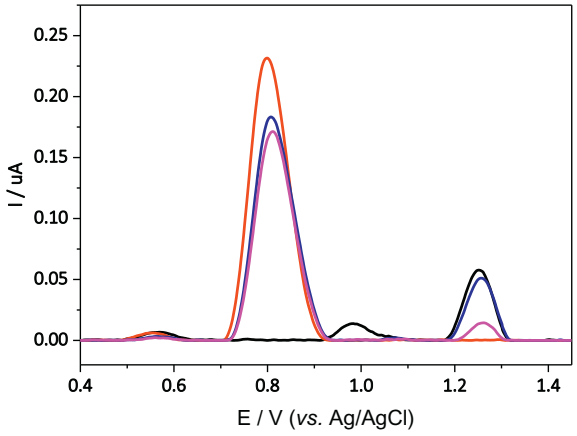


Fig. 1. DP voltammograms of 100 μg mL^{-1} ct-dsDNA (black), 10 μgmL^{-1} NDP (red) and ct-dsDNA incubated with NDP under different incubation time: 60 min (blue), 120 min (pink) in pH 4.7 acetate buffer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

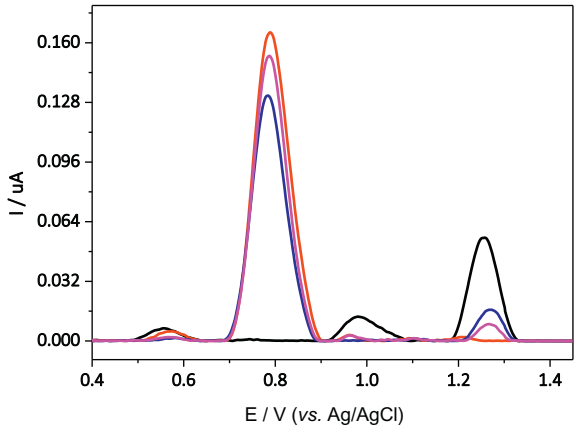


Fig. 3. DP voltammograms of 100 μg mL^{-1} ct-dsDNA (black), 10 μgmL^{-1} AMP (red) and ct-dsDNA incubated with AMP under different incubation time: 30 min (blue), 60 min (pink) in pH 4.7 acetate buffer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

covers the GCE surface. Therefore, DNA biosensor has its own significance to avoid the undesired drug adsorption to the electrode surface.

As seen in Fig. 4, the oxidation peaks of ct-dsDNA biosensor were obtained at 1.0 V (dGuo) and 1.26 V (dAdo), respectively. The oxidation peak of NDP occurred at about 0.8 V using DPV in pH 4.70 acetate buffer solution after free adsorption of a 10 μg mL^{-1} NDP solution on bare GCE. The freshly prepared ct-dsDNA-electrochemical biosensor was then incubated between 1.0 and 10.0 min in 10 μg mL^{-1} NDP, was washed carefully with ultra-pure water to remove unbound NDP molecules and transferred to an acetate buffer solution where a DP voltammogram was recorded. This experiment was always repeated using a freshly prepared biosensor. After interaction of ct-dsDNA with NDP, peak currents of dGuo and dAdo were decreased. These results obtained were consistent with results of bulk incubated solution.

The oxidation peaks of LDP were obtained at 0.76 V and 0.96 V using DPV in pH 4.70 acetate buffer solutions (Fig. 5). The prepared ct-dsDNA-electrochemical biosensor was incubated between 1.0 and 10.0 min in 0.2 μg mL^{-1} LDP and was dipped into an acetate buffer where a DP voltammogram was recorded. After interaction with LDP, peak currents of dGuo and dAdo decreased.

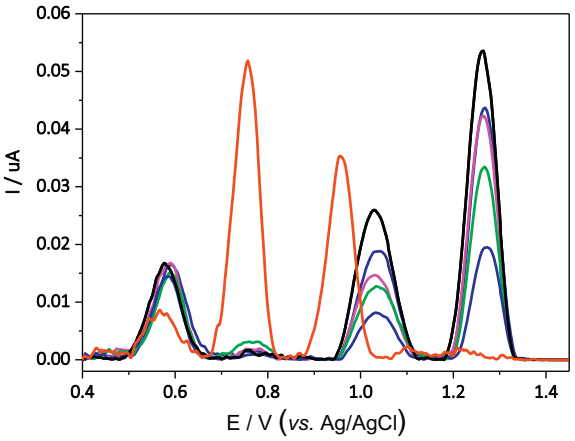


Fig. 5. DP voltammograms of 50 μg mL^{-1} ct-dsDNA biosensor (black), 0.2 μgmL^{-1} LDP (red), ct-dsDNA biosensor interacted with bare acetate buffer (blue) and ct-dsDNA biosensor interacted with LDP under different interaction time: 3 min (pink), 5 min (green), 7 min (navy) in pH 4.7 acetate buffer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Possible electrochemical interaction mechanism

Electrochemical research on DNA is of great relevance to explain many biological mechanisms [21]. The interaction mechanism of some small molecules with DNA has been a key topic during the past few decades. These interactions are usually closely related to toxicological, carcinogenic, and/or pharmacological activity of the assayed substances [22–27]. A change in peak current of the drug by adding DNA could be used for the determination of binding constant and binding site size, while the shift in peak potential could be used to determine the mode of interaction [28].

The published manuscripts concerned with the interaction between calcium antagonist and dsDNA was researched. By Svitkova et al., the DNA-modified BDDEs were developed to investigate the interaction between DNA and AMP using CV and DPV [20]. Svitkova et al., obtained that the peak current of guanine signal was decreased when the biosensor was incubated in AMP solution by DPV. Similarly, in our study, as seen in Fig. 3, it was noticed that the interaction of AMP with ct-dsDNA in bulk solution resulted in disappearance of dGuo peak.

The other published study, interaction between nimodipine, isopropyl 2 - methoxyethyl 1, 4 - dihydro - 2, 6 - dimethyl - 4 - (*m*-nitrophenyl) - 3, 5 – pyridinedicarboxylate, and ct-DNA were investigated by CV and DPV [28]. The peak current of nimodipine decreased with increasing concentration of ct-DNA [29].

In our study, the peak potential of NDP and AMP were obtained almost at same potential in bulk incubated solutions. The monitored interactions between NDP or AMP and dsDNA cause more significant changes in the oxidation current signals of dGuo which almost disappeared after interaction with these drugs in bulk incubated solutions (Figs. 1 and 3). However, the peak currents of dAdo decreased gradually depending on incubation time. At dsDNA biosensor, after interaction with NDP and LDP, the peak currents of dGuo and dAdo were significantly decreased (Figs. 4 and 5). Also, the changes for peak potential and current for dGuo and dAdo are tabulated in Table 1 for both interactions at the electrode surface and in bulk incubated solutions.

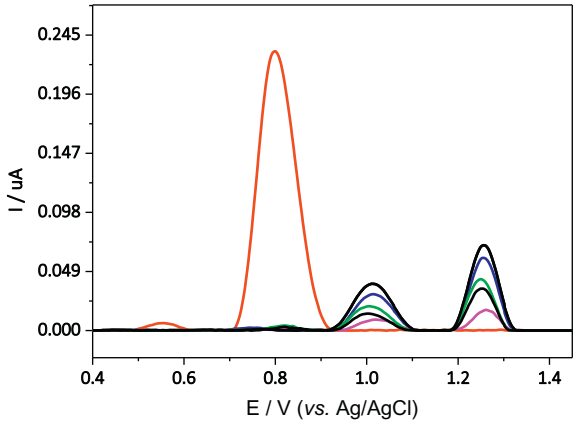


Fig. 4. DP voltammograms of 50 μg mL^{-1} ct-dsDNA biosensor (black), 10 μgmL^{-1} NDP (red), ct-dsDNA biosensor interacted with bare acetate buffer (blue) and ct-dsDNA biosensor interacted with NDP under different interaction time: 3 min (green), 5 min (navy), 7 min (pink) in pH 4.7 acetate buffer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. UV–Vis spectrophotometric study of the interactions

Interactions between NDP – ds-DNA and LDP–ds-DNA have further been studied by using UV–Vis absorbance spectroscopy at three different temperatures (Fig. 6 and Fig. S2). Although according to molecular docking study AMP-2 has remarkable interaction with DNA, racemic mixture of AMP has very low response in UV–Vis titration and it was impossible to attain information about AMP-DNA interaction from UV–Vis titration. The main effect of DNA on the spectrum of studied drugs was

Table 1
Comparison of the peak potential and current of dGuo and dAdo.

Interaction mode	Drug	Time	dGuo		dAdo	
			E_p (mV)	i_p (μA)	E_p (mV)	i_p (μA)
In Incubated solutions	Nifedipine	0 min	983	0.014	1251	0.058
		60 min	–	–	1255	0.051
		120 min	–	–	1261	0.015
	Lercanidipine	0 min	983	0.014	1259	0.045
		10 min	977	0.020	1253	0.028
		120 min	955	0.024	1269	0.004
	Amlodipine	0 min	981	0.013	1257	0.055
		30 min	–	–	1271	0.017
		60 min	–	–	1267	0.009
dsDNA biosensor	Nifedipine	0 min	1011	0.039	1257	0.071
		3 min	1005	0.020	1249	0.043
		5 min	1003	0.014	1253	0.035
		7 min	1017	0.009	1261	0.017
	Lercanidipine	0 min	1029	0.039	1265	0.084
		3 min	1031	0.015	1265	0.042
		5 min	1035	0.013	1267	0.033
		7 min	1033	0.008	1273	0.020

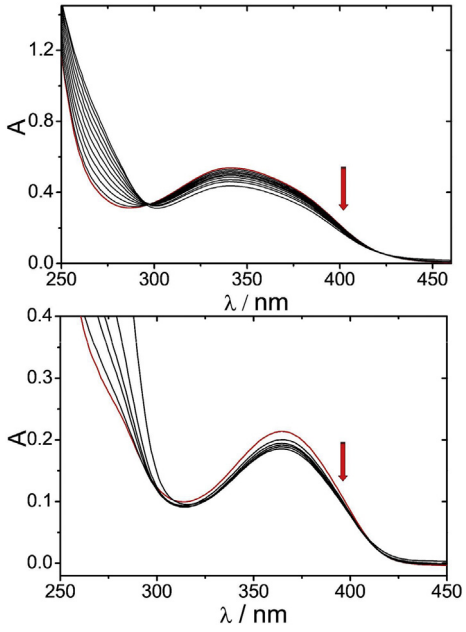


Fig. 6. UV absorption spectra for nifedipine-DNA titrations (top) and lercanidipine-DNA (bottom) titrations at 283 K.

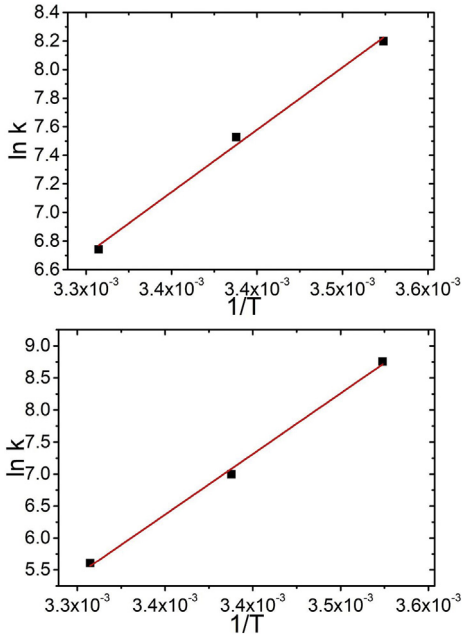


Fig. 7. Van't Hoff plots of nifedipine-DNA (top) and lercanidipine-DNA (bottom) interactions.

followed through the region over 300 nm which is free from DNA absorbance (Fig. S3). The results of interaction on the spectrum of drugs is hypochromism and we used the absorbance decrease at 341 nm for NDP and 366 nm for LDP for calculations.

Binding constants were calculated using the following equation:

$$1/(A_0-A) = 1/A_0 + 1/kA_0[DNA] \quad (7)$$

Where A_0 and A are absorbance of analytes in the absence and in the presence of DNA respectively. [DNA] is the molar concentration of ds-DNA and k is the binding constant between analytes and DNA. Fig. S4 shows $1/A_0$ [DNA] vs $1/(A_0\text{-}A)$ plot for NDP and LDP. Binding constants were derived using these plots and the strength of interactions between both analytes and DNA can be rated as moderate (Table 2). Van't Hoff plots were used for calculation of thermodynamic parameters (Fig. 7). Enthalpy and entropy change of binding can be calculated using of 1/T vs lnK plot since the slope term is ΔH/R and intercept is ΔS/R according to following equations:

$$\ln K = \Delta H/RT + \Delta S/R \quad (8)$$

$$\Delta G = \Delta H - T\Delta S \quad (9)$$

Finally, ΔG were calculated using entropy and enthalpy change at different temperatures. ΔG are negative for both drugs and at three temperatures showing the binding is favourable. The contribution of enthalpy term to Gibbs free energy is very high in both interactions (Table 2). In these spontaneous interactions, analytes bind to DNA exothermically and the binding constants decreases as temperature increases. On the other hand, entropy term is unfavourable with a negative value. These results may indicate that the intermolecular interaction between analytes and ds-DNA can be mainly through hydrogen bonding and van der Waals forces while hydrophobic interaction is poor comparing to other types of interactions.

Table 2
Thermodynamic parameters for NDP–ds-DNA and LDP–ds-DNA.

Temperature	K[a] (M^{-1})	ΔG° (kcal/mol)	ΔS (cal K^{-1} mol^{-1})	ΔH (kcal/mol)
Nifedipine – ds-DNA				
283 K	3.64×10^3	−4.62	−27.4	−12.4
293 K	1.86×10^3	−4.35		
303 K	0.85×10^3	−4.07		
Lercandipine – ds-DNA				
283 K	6.34×10^3	−4.90	−77.5	−26.8
293 K	1.09×10^3	−4.12		
303 K	0.27×10^3	−3.35		

[a] Binding constants.

3.5. Molecular docking results

Docking was exerted to estimate binding site and affinity of AMP-1-2, NDP and LDP-1-2, as ligands in the target model, DNA. In this way, experimentally unexplained situations will be elucidated by docking studies. Therefore, the optimized ligands (AMP-1-2, NDP and LDP-1-2) including detail information at Fig. S6-S10 and Table S1–5 in supplementary part and DNA were docked with help of CDOCKER docking protocol of DS software. The obtained docking results were evaluated based on three-dimensional residue interactions, the calculated binding energy and inhibition constant (Ki) values. The ten possible conformation of each ligand were generated, and the best pose was defined based on the lowest energy values for each complex. Moreover, relative orientations of AMP-1-2, NDP and LDP-1-2, for each complex including lowest binding energy values of AMP-2-DNA, NDP-DNA and LDP-1-DNA were shown in Fig. 8 a, b and c, others were given at Fig. S11- S12 in supplementary part.

3.5.1. Molecular docking calculations of ligands - DNA complex

In this part of this study, 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl) -4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (AMP) as reference ligand was used to get obtain its interaction mechanism with DNA. The docking results show that hydrophilic interactions are more dominant than other interaction types in AMP-2-DNA complex. AMP-2 compound has eleven hydrogen bonds which first two one are conventional hydrogen bonds, residues are carbon hydrogen bonds with A: DC3, A: DG4, A: DA5, A: DA6, B: DG22, B: DC23 and B: DG24 nucleotides of DNA (seen in Fig. 8a). The real aim of this study, molecular docking was implemented to probe the relative orientation and affinity of NDP and LDP against to DNA. Additionally, they were

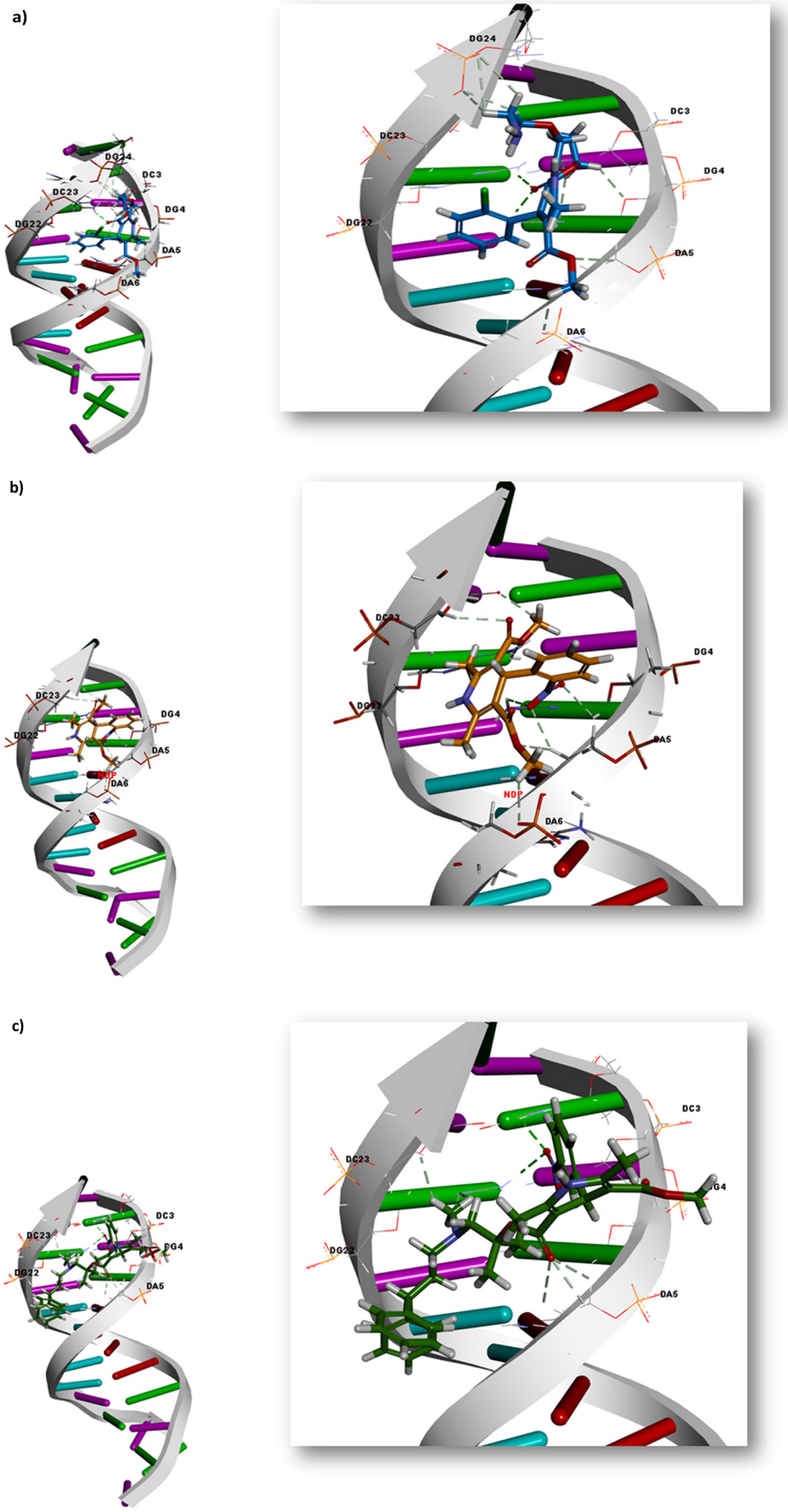


Fig. 8. Global minimum energy binding poses and interactions of AMP-2 (a), NDP (b) and LDP-1(c) in the binding site of DNA.

compared with the docking result of AMP-2-DNA. NDP compound have weak interactions with A:DG4, A:DA6, B: DG22 and B:DC23 nucleotides in active site of DNA, as compared with AMP-DNA complex, (see Fig. 8b). The other compound, LDP-1 form hydrogen bonds with DNA like AMP-2 and NDP, Fig. 8c. Besides these, it also shows one hydrophobic interaction with DNA. The reason is LDP-1 has hydrophobic groups such as phenly, methyl groups in its chemical structure. Fig. 9 show the best pose of the ligands, NDP, LDP-1 and superimposed form with AMP-2 in binding site of DNA. Table S6 can also be seen in supplementary information part including non-binding interaction kinds and distances of the compound, AMP-1-2, NDP and LDP-1-2.

Specifically, for AMP-1-2, NDP and LDP-1-2, as can be seen in Table 3, it was reveals that LDP-1 configuration has the best binding affinity value (BE: −7.61 kcal/mol) with DNA target, according to other conformer LDP-2. Because, LDP-1 has highest surface area in all compounds, so it has more potential than AMP and NDP to good interaction with DNA. Second best binding affinity is AMP-2 with −6.57 kcal/mol and last one, NDP is −5.80 kcal/mol against to DNA.

Table 3
Molecular docking binding energy and inhibition constant (Ki) values of AMP-1-2, NDP and LDP-1-2 with DNA complexes. The lowest binding energy values of ligands are indicated in italic format.

Complex	Binding energy (kcal/mol)	Ki (μM)
AMP-1-DNA	−5.77	58.91
AMP-2-DNA	*−6.57*	*15.33*
LDP-1-DNA	*−7.61*	*2.64*
LDP-2-DNA	−7.21	5.18
NDP-DNA	−5.80	55.90

4. Conclusion and future aspect

The electrochemical techniques supply great advantages such as simplicity, rapidity, relatively low cost, satisfactory sensitivity, and suitability for the development of inexpensive and portable analytical devices for rational designing of drugs 30-33].

In this study, the interaction between the NDP-dsDNA and LDP-dsDNA were investigated in incubated solutions and at a dsDNA electrochemical biosensor surface by DPV. The decrease in the peak current of dGuo and dAdo were used as an indicator for the interaction in pH 4.70 acetate buffer. In incubated solutions, the interaction was also compared with model drug, AMP. After interaction with NDP and AMP in the bulk incubated solution, dGuo signal was almost disappeared. At dsDNA biosensor, after interaction with NDP and LDP, the peak currents of dGuo and dAdo were almost decreased by 100 to 24%.

The interactions were further investigated by UV–Vis spectrophotometry. The binding constants between drugs and dsDNA have been calculated. The binding constants were found as 3.64×10^3 for NDP-dsDNA interaction, 6.34×10^3 for LDP-dsDNA interaction at 283 K. The spectroscopic studies were confirmed that the binding between drugs and dsDNA is favourable. The results may display that the intermolecular interaction can be through hydrogen bonding and van der Waals forces.

Furthermore, the calculation of binding energy (−7.61, −6.57 and − 5.80 kcal/mol) of LDP-1, AMP-2, NDP, respectively and docking interactions with ctDNA from CDOCKER in DS 2018 exhibited that the hydrogen bond were dominant forces in the binding process, which was consistent with the other experiment results. The study may provide a good model to explain binding properties of AMP-1-2, NDP and LDP-1-2 like compounds to DNA and protein.

This interaction studies by electrochemically, spectrophotometrically and molecular docking offer the opportunity to know about the effects of drugs in DNA structure and know about mechanism of interaction and it may be useful to design new drugs. Also, binding

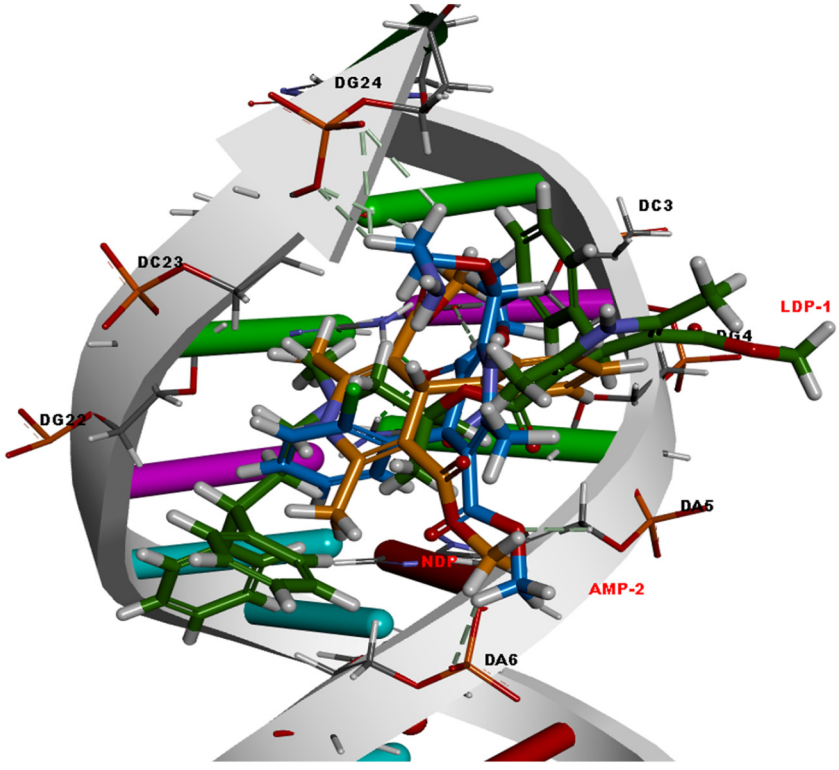


Fig. 9. Global minimum energy binding poses and interactions of AMP-2 (dark blue), NDP (orange) and LDP-1 (dark green) superimposed form in DNA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

constant is a basic experimental parameter in many clinical studies, such as pharmacokinetic drug interaction.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2018.12.007.

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