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Cyclic Voltammetric Investigation of Interactions between Bisnitroaromatic Compounds and ds.DNA

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ABSTRACT. Herein, the cyclic voltammetric (CV) investigations of structurally similar bisnitrocompounds (N3, N4, N5, N6, having different $-CH_2-$ spacer length) is presented. CV study offered interesting interactional possibilities of bisnitrocompounds with chicken blood ds.DNA at physiological pH 4.7 and human body temperature, 310 K. The results indicated strong interaction by these symmetric molecules with ds.DNA and strength of binding is found to depend on length of CH_2 spacer group in their molecular structure. Thermodynamics derived from electrochemical binding parameters also favored the irreversible interactions. Moreover, threading intercalation mode of binding is suggested based on thermodynamic and kinetic binding parameters extracted from CV studies.

Key words: Bisnitrocompounds, ds.DNA binding, Cyclic voltammetry, Threading intercalation

INTRODUCTION

Deoxyribonucleic acid (DNA) is a double helical biomacromolecule which controls all the life processes. DNA can be damaged under oxidative conditions, especially by interaction with molecular radicals and this damage may lead to various pathological diseases in living organisms Under oxidative circumstances, the repeating unit of (double stranded) ds.DNA may act as free radical and undergo excessive replications, thus causing cancerous abnormalities.[1,2] That is why researchers have great interest in exploring the binding of small molecular functionalities targeted to DNA for the rational drug design and in understanding their therapeutic efficiency.[3–6]

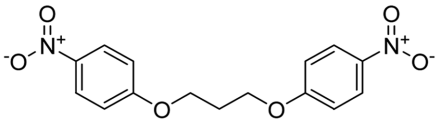
A number of research reports are available which address the toxicity, kinetics of the electroreduction, structure-reactivity relation of drugs containing nitro-group.[7–12] The use of nitrocompounds as antibiotics, antibacterial, antiprotozoal and anticancer agent is also focus of many reports.[13,14] The pharmaceutical properties of clinically used nitroaromatics is of interest because of their selective potentiality in cancer therapy.[15–18]

Although the binding modes of small nitrocompounds with DNA have been studied by using various techniques,[19–22] the binding of structurally symmetric bisnitroaromatics has not been explored and needs more elaboration. Electrochemical methods especially cyclic voltammetric (CV) is constantly used to detect the binding of small molecules with ds.DNA for the rapid, reproducible results. Recently, Munos et al.[23] have demonstrated the formation and stability of radical and biradical anionic structures in dinitrobenzene (DNB) using CV technique. These voltammetric analyses can be used to address the redox pathways in the nitroaromatic-molecular models because the reduction products are thought to actually take part in the DNA binding.[24]

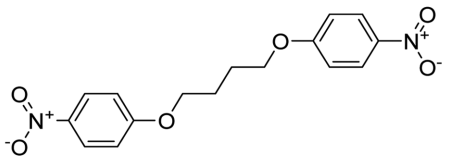
Herein, we report the interaction of a series of bisnitrocompounds (N3, N4, N5 and N6) with ds.DNA studied in 0.1 M acetate buffer (pH = 4.7) by using cyclic voltammetry. The structures are given in *Scheme* 1 which shows threading nature due to structural symmetry.[25] The electrokinetics of pure bisnitrocompounds is found to be temperature dependent and diffusion controlled. It yielded information about the dependence of redox phenomenon upon the diffusion process and size of molecule. Increasing binding tendency with ds.DNA was indicated with the molecular chain length. "Nitromics" term is suggested for such binding study of these compounds with ds.DNA based on the parameters retrieved from CV data.

EXPERIMENTAL

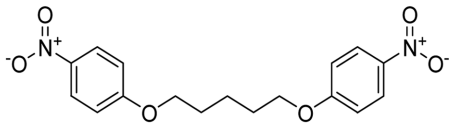
Bisnitrocompounds were synthesized following reported method.[26,27]Acetate buffer with pH 4.7 in water-ethanol (3:7 v/v) was used as the solvent for all measurements which served as supporting electrolyte, as well. Fresh chicken blood ds.DNA was extracted in the laboratory by commonly used Falcon method.



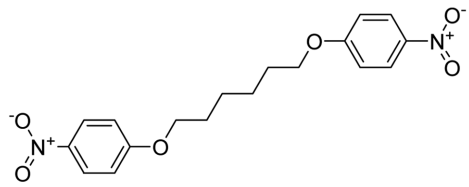
1-Nitro-4-(3-(4-nitrophenoxy)propoxy)benzene, N3.



1-Nitro-4-(4-(4-nitrophenoxy)butoxy)benzene, N4.



1-Nitro-4-(5-(4-nitrophenoxy)pentyloxy)benzene, N5.



1-Nitro-4-(6-(4-nitrophenoxy)hexyloxy)benzene, N6.

***Scheme* 1.** The structures of series of bisnitrocompounds.

Auto-Lab equipped with GPES 4.9 software, Eco-Chemie, was used for cyclic voltammetric studies at glassy carbon electrode (GCE) with an active area of 0.07 cm^2 as working electrode against saturated calomel electrode, SCE (3 M KCl) as reference electrode with Pt wire as counter electrode. Background CV for solvent system (blank) was run in the potential window of selected bisnitrocompounds which ensured that observed CV is only for electrochemical (EC) system and not the solvent. Argon gas was purged during measurements. Three electrode assembly was setup so as to maintain the vicinity of the working and reference electrodes for compensation of IR drop. Before each experiment, GCE was polished with fine alumina slurry followed by thorough rinsing with distilled water and finally with the working solvent.

The electrokinetics (including heterogeneous rate constant, diffusion coefficient) of pure bisnitrocompounds were investigated at various temperatures, scan rates and their interaction with ds.DNA could be studied at various scan rates, and the body temperature, 310 K, all in pH 4.7 solvent which also acted as the supporting electrolyte.

RESULTS AND DISCUSSION

CV Profile of Pure Bisnitrocompounds

Cyclic voltammetric investigations were carried out to observe scan rate effects, diffusional behaviour with respect to structural variation, the critical scan rate, heterogeneous rate constants, and effect of temperature on the electrokinetics of pure nitrocompounds. Voltammetric profile could be clearly connected with their structural subtleties. A single oxidation peak (E_p^a I) and two reduction peaks (E_p^c II and E_p^c III) are observable in the CV responses of all the studied nitrocompounds as depicted in *Fig.* 1 for 1 mM N3 in −1.5 to 1.0 V potential window. Background CV for acetate buffer (as solvent system or blank) was run and showed no electroactivity in the potential window of N3 bisnitrocompounds EC system. The CV profiles matched with the reported correlation of the electrochemical responses of nitrocompounds containing the positional nitro-group(s) in their structures.[28,29] The electrochemical process for reduction peak at −1.097 V is attributable to nitro-group reduction product.[4] N3-N6 electrochemical (EC) systems were found to be irreversible from E_p–$E_{p/2}$ values and a negative shift in the cathodic peak position with increasing scan rate from 0.02 to 1 Vs^{-1}.[24] The irreversibility could also be envisioned from the correlation coefficient value of 1 for a plot (not shown here) between E_p^c (V) and logυ.[7]

Cyclic voltammetric behaviour of 1 mM of all other bisnitrocompounds (N4-N6) offered the same electrochemistry under similar conditions of solvent and scan rate with single anodic peak and two cathodic peaks, *Table* 1. These electroactive systems were all irreversible as inferred from the criteria used for N3 EC system.[6] One can apprehend from

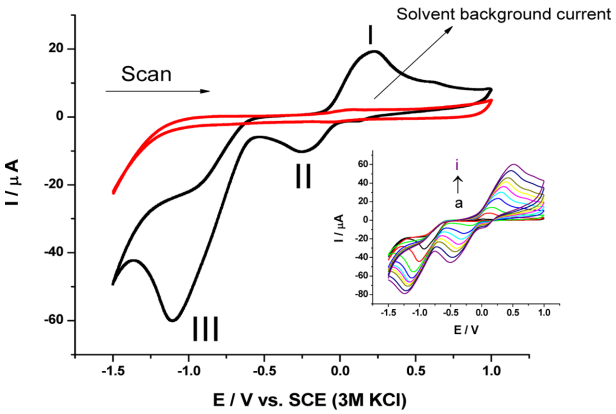


***Figure* 1.** Cyclic voltammograms of N3 (1mM) in acetate buffer pH 4.7 and at 100 mVs^{-1}. Inset A: Effect of scan rate on I-E response; a) 20, b) 50, c) 100, d) 200, e) 300, f) 400, g) 500, h) 800, i) 1000 mVs^{-1}.

***Table* 1.** Cyclic voltammetric parameters for all bisnitrocompounds

Compounds	Anodic peak, I $(E_p^a - E_{p/2}^a)$/V	Cathodic peaks $(E_p^c - E_{p/2}^c)$/V		αn*
		II	III	
N3	0.159	0.095	0.145	0.33
N4	0.187	0.099	0.153	0.31
N5	0.205	0.110	0.158	0.30
N6	0.211	0.114	0.163	0.29

*For irreversible cathodic peak (III) only.

the CV data that the reduction products once formed in the cathodic step become electrochemically inactive thus rendering the system as irreversible.[7]

The irreversibility in CV responses of investigated pure bisnitrocompounds was attributable to the peak shift (positively in oxidation and negatively in reduction scan direction) with increasing scan rate and also the absence of the reverse peak for the III reduction peak, see *Fig.* 1.[30] Importantly, the reduction product of the second step is rendered electro-inactive which may be due to the usual formation of the amino-hydroxyl analogue.[9] Thus the voltammetric responses of the studied bisnitrocompounds may be correlated with two-step EC mechanism as taking place in an aqueous medium:[12,31]

$$R\text{-}NO_2 \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} R\text{-}NO_2^- + 3e^- + 4H^+ \rightarrow R\text{-}NHOH + H_2O \quad (1)$$

Here, the first step is a type of reversible redox production of anion radical which in aqueous medium, accepts one more electron along with four protons to form the reduction derivative, hydroxylamine in the subsequent irreversible electrochemical (EC) step. The electrochemical (EC) parameters for all bisnitrocompounds along with αn values for the prominent cathodic peak only, calculated using equation: $(E_p^c - E_{p/2}^c) = 47.7/\alpha n$,[32] are given in *Table* 1. [Here, n can be considered as 3 according to equation 1. For detailed mechanism, please see *Scheme* 1 in reference 7. Authors in reference 8 have discussed more details where n=1 gives best results, see *Table* 1].

Therefore, the present and reported nitro-typical CV responses have been termed as "nitromics" for the voltammetric studies on nitroaromatic compounds.

Gileadi's heterogeneous rate constant ($k_{s,h}$)

The reactivity and kinetics of electro-reduction and subsequent binding of the reduction products could be assessed with heterogeneous rate constant, $k_{s,h}$ as determined by method,[33] by using critical rate constant, the υ_c value. Accordingly, a plot of Ep values versus the logarithm of scan rates

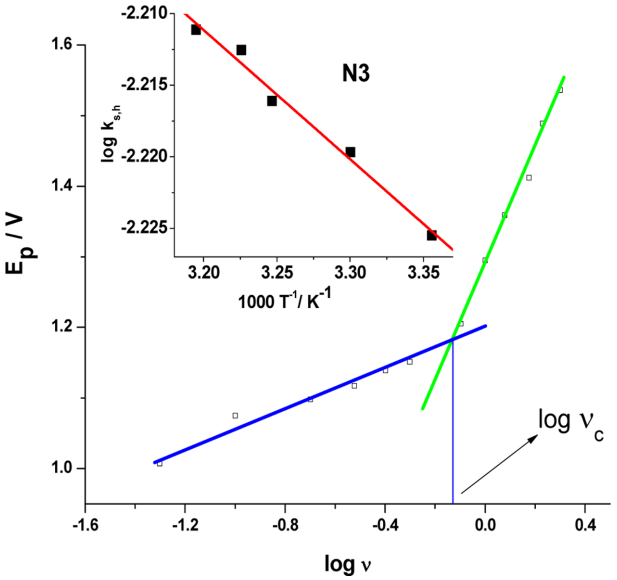


***Figure* 2.** Functional plot of E versus logυ for the calculation of critical scan rate at 310 K and pH 4.7. Inset: Effect of temperature on electrokinetics (as $k_{s,h}$) of N3.

gives a linear trend with relatively less slope at low scan rates and a second ascending line with greater slope at higher scan rates which is used to get υ_c value. Extrapolation of both curves intersects at a point known as "toe" which corresponds to the log ν_c. *Fig.* 2 is plotted between log υ and Ep values for N3 to graphically know the υ_c value. Hence, critical scan rates were used in calculating the heterogeneous rate constants, $k_{s,h}$ as given in *Table* 2 using the following equation:

$$\log k_{s,h} = -0.48\alpha + 0.52 + \log\left[\frac{nF\alpha\nu_c D_o}{2.303RT}\right]^{1/2} \quad (2)$$

where α is the transfer coefficient and D_o is the diffusion coefficient of the electrophore (nitro-group in this case) with the other usual parameters.

Th heterogeneous rate constant increases linearly with increasing temperature as shown in inset of *Fig.* 2. This facilitated molecular mobility towards electrode with temperature leading to substantial acceleration in electron

***Table* 2.** Heterogeneous rate constants of bisnitrocompounds at various temperatures

Hetrogenous rate constant, $k_{s,h}$ (cm s^{-1}) × 10^3 for all bisnitrocompounds					
	298 K	303 K	308 K	310 K	313 K
N3	9.23	9.37	9.46	9.51	9.64
N4	7.82	7.95	8.09	8.14	8.21
N5	7.12	7.24	7.33	7.40	7.44
N6	5.95	6.03	6.08	6.13	6.15

transfer processes renders the EC process as diffusion controlled.[32]

Besides the temperature dependent electrokinetics, the structural subtleties of the studied bisnitrocompounds were interesting. The heterogeneous rate constant values are apparently affected by the molecular size effect and are observed to decrease as the $-CH_2-$ spacer group in their structure increased, thus rendering EC process to be diffusion limited.[19]

CV Profiles of Bisnitrocompound-DNA Interactions

For fixed concentration (1 mM) of all bisnitrocompounds (N3 through N6) cyclic voltammograms were recorded in the potential window range of −1.5–0 V while adding 1 μM to 5 μM DNA in acetate buffered solution (pH 4.7), 310 K and at 0.1 V s^{-1} (*Fig.* 3). E_p and $E_p-E_{p/2}$ values were shifted positively by 0.058 V and 0.019 V, when 5 μM DNA was added to 1 mM of N3 at 100 mV s^{-1}. The pronounced decrease in peak current and positive shift in the reductive peak potential is attributable to irreversible behavior[7,34] resulting from the intercalation of the planar part of interacting compound into the stacked base-pair domain of DNA double helix.[35]

Based on the shift of peak potential and decrease in peak height, the interaction with chicken blood ds.DNA could be inferred. Upon titration with ds.DNA, shift in peak position and decrease in peak height is observed up to ~5 μM DNA addition in N3 system. This saturation point occurred in all interacting systems but to a different extent as depicted by the % decrease in the peak currents thus clueing to their structural influence upon binding with DNA. The percent decrease in peak current or hypoelectric effect, %I was calculated using (Eq. 3):

$$\%\Delta I = \frac{(Ip - Ip_o)}{Ip_o} \times 100 \quad (3)$$

where Ip_o and Ip is the peak current of the reduction peak III without and with DNA. Firstly, %I is observed to increase with added ds.DNA. This trend then becomes independent after a certain limiting DNA concentration which was nearly 5 μM in the case of N3 compound. The substantial diminution in peak current is attributable to the decrease in free concentration of electrophore due to the formation of heavy, slowly diffusing, N3-DNA adduct as a result of strong interactions.

For 5 μM added ds.DNA into 1 mM bisnitrocompound, the %I trends are in the order: N3 (16%) < N4 (23%) < N5 (24%) < N6 (44%) and are shown for N3 and N6 in *Fig.* 4 for comparison. This hypoelectric effect (decrease in peak current due to intercalation) observed upon voltammetric titration of all the investigated nitrocompounds with DNA suggested sufficient interaction but a much pronounced effect by N6.[24]

The maximum %I was observed for N6 nitrocompound bound to ds.DNA i.e., ~44%. This trend may be attributable to mixed mode of binding that is threading intercalation which includes both intercalation and groove binding interactions. As an outcome of this study, threading intercalation is being proposed which is strongly dependent upon the relative increase in the molecular size and spacer chain of

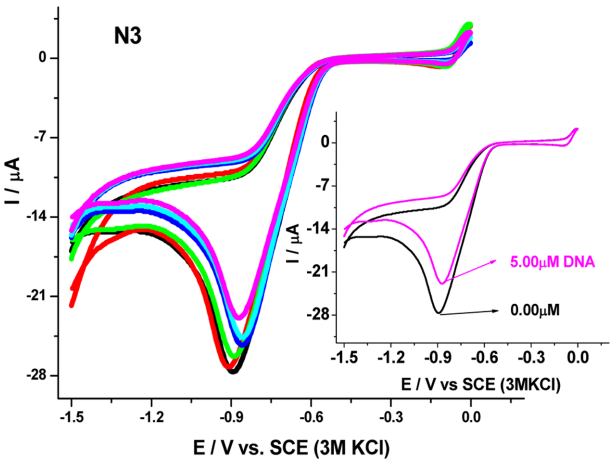


***Figure* 3.** CV responses for N3 (1 mM) without and with different concentration of DNA at 100 mVs^{-1}. Concentration of DNA (μM) added are: a) 0.00, b) 1.00, c) 2.00, d) 3.00, e) 4.00, f) 5.00. Inset shows the maximum decrease in current with addition of 5.00 μM DNA.

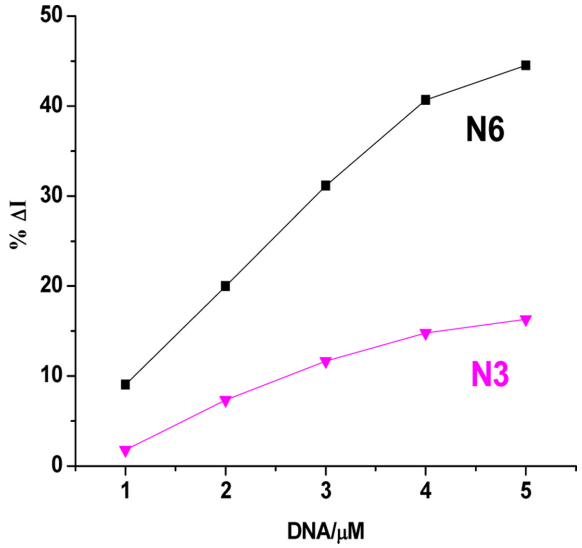


***Figure* 4.** Comparative trends of %decrease in current with increasing [DNA] for N3 and N6.

bisnitrocompounds. This phenomenon can be comprehended by comparing the peak currents of N3 and N6 where 5 μM DNA addition causes about three times decrease in peak current of N6 electrochemical system compared to N3, *Fig*. 4. N6 has more number of methylene spacer groups and its threading intercalation capacity is highest of all studied nitrocompounds and hence it binds more strongly with ds.DNA as compared to N3 thus decreasing the electrophore concentration and its peak current.[36]

Diffusion Coefficient Calculations

The diffusion coefficients, D_o (cm^2s^{-1}) of all the studied electroactive bisnitrocompounds in the absence and presence of DNA were obtained from CV profiles at scan rate of 100 mVs^{-1} and are collected in *Table* 3 and shown in *Fig*. 5. These were calculated by using Randles-Sevcik equation for an irreversible EC process:[37,38]

$$Ip = 2.99\times10^5\ n\ (\alpha n_\alpha)^{1/2}\ A\ C_o^*\ D_o^{1/2}\ \nu^{1/2} \qquad (4)$$

where Ip is peak current in amperes (A), n is the charge transfer number being one,[8] n_α is the number of electrons transferred up to and including the rate determining step, α being the transfer coefficient, C_o is the bulk concentration of the diffusion species in mol cm^{-3}, ν is potential scan rate in Vs^{-1}. In logarithmic form, slope of this equation (log Ip versus log υ) should be 0.5 which is also a criterion of diffusion process. The diffusion coefficient is observed to decrease linearly with the addition of ds.DNA (*Table* 3).

While 1 mM each of N3, N4, N5, and N6 was titrated against DNA (up to 5 μM), the D_o values decreased gradually. This trend is according to the expected structural features i.e., more addition of DNA into a fixed concentration of bisnitrocompounds enhances the intercalative interactions between them thus increasing DNA-bound nitrocompound and decreasing amount of free electroactive species. Comparing D_o values, the structure and size of the molecules matter. Here, D_o value of N6 molecule is nearly half order of magnitude compared to 3.67×10^{-6} mol cm^{-3} for N3. N6 itself is slowly diffusing specie with small diffusion coefficient value which is apparently due to increased hydrophobic alkyl chain that may help in extended intercalative binding with helical structure of ds.DNA.

***Table* 3.** Diffusion coefficients of nitrocompounds in the absence and presence of ds.DNA

[DNA]/μM	Diffusion coefficient, $D_o/cm^2s^{-1}\times10^{-6}$			
	N3	N4	N5	N6
0.00	3.67	3.37	3.44	1.84
1.00	3.44	3.09	3.19	1.52
2.00	3.19	2.93	2.79	1.23
3.00	3.05	2.77	2.50	0.986
4.00	2.88	2.61	2.27	0.709
5.00	2.43	2.41	2.16	0.469

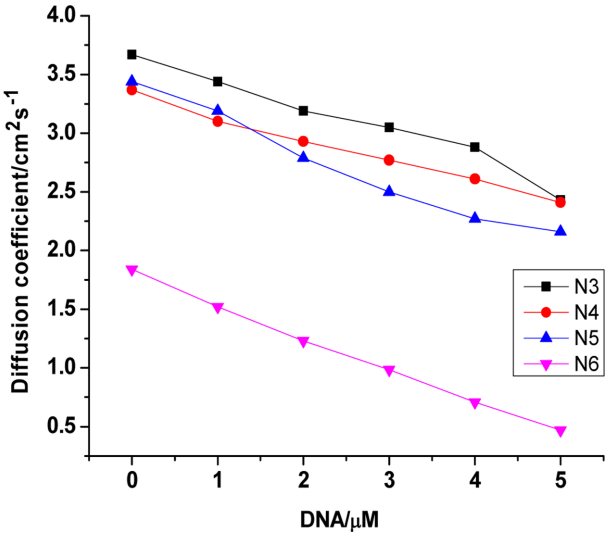


***Figure* 5.** Trends in diffusion coefficient of bisnitrocompounds with respect to added ds.DNA.

The present study indicates a clear trend of decreasing D_o values of small symmetric molecules with their structure and size. These results correspond to a recent report where diffusion coefficients of electroreduction products for various substituted nitrobenzenes varied linearly with their molecular structure.[39]

Formation Constants of Bisnitrocompound–DNA Complexes

The interaction of small molecules with a host (macromolecule) results in a bigger complex and the thermodynamic of this complex can yield the information about binding or formation constant K_f (M^{-1}). K_f values of bisnitrocompound–DNA complexes were determined using the peak current values and following equation.[40]

$$Ip^2 = \frac{1}{K_f[\mathrm{DNA}]}(Ip_o^2 - Ip^2) + Ip_o^2 - [\mathrm{DNA}] \qquad (5)$$

Under excess concentration of N3-N6 (mM) and negligible concentration of ds.DNA (μM), a plot of Ip^2 versus $(Ip_o^2 - Ip^2)$ / [DNA] gives a straight line with a slope equal to the reciprocal of formation constant. The order of formation or binding constants of the bisnitrocompounds-DNA complexes obtained by cyclic voltammetric data in M^{-1} as follows; 3.3×10^4 (N3), 5.1×10^4 (N4), 7.3×10^4 (N5) and

1.0×10^5 (N6). Enhanced binding is also apparent from the highest value of K_f for N6 which reveals that N6-DNA complex is the most stable one, may be due to possibility of its extended interaction with ds.DNA.

The formation constant data and Gibbs equation were used to evaluate the change in Gibbs free energy, G;

$$\Delta G = -RT \ln K_f \text{ (kJ mol}^{-1}\text{)} \quad (6)$$

The calculated G values for nitrocompounds-DNA complexes are –26.81, –27.88, –28.86, and –29.67 kJ mol^{-1}, for N3, N4, N5, N6, respectively. The order of magnitude in G values increase with the respective structure of each bisnitrocompounds. The thermodynamics corresponds to the length of CH_2-spacer in their structures and points to stronger binding of N6 in comparison to N3. The negative values of G indicate spontaneity of binding of nitrocompounds with DNA, as well.[35]

Threading intercalation is inferred from the observed data as well as the corresponding symmetric structures of the bisnitrocompounds under study, which consist of both the hydrophobic and the hydrophilic parts necessary for threading intercalation which is actually wrapping of ds.DNA by combined effect of intercalation and groove binding favored by planar and non-planar features in small chained molecular configurations.[41]

Binding Site Size Calculations

The binding site size (s) per base pairsof DNA could be evaluated by using the following equations:[31,40]

$$C_b/C_f = K_f \{[\text{DNA}] / 2s\} \quad (7)$$

$$C_b/C_f = (I - I_{DNA}) / I_{DNA} \quad (8)$$

where C_b/C_f is the concentration ratio of bound and free bisnitrocompounds. Binding site-sizes are numbers of free base pairs (bp) in ds.DNA interacting with the guest, bisnitrocompound. The number of site-sizes of ds.DNA bound with bisnitrocompounds gradually increased with the size of the molecules in terms of CH_2-spacer group.[39,40] Binding site (s) is in the increasing order: 0.94 (N3) < 1.14 (N4) < 1.2 (N5) < 1.43 (N6) which again points to the enhanced threading intercalation by N6 compound.

CONCLUSIONS

The electrokinetics including heterogeneous rate constant of pure bisnitrocompounds was studied using Gileadi's method at various temperatures and it showed temperature-facilitated electron transfer process. All the EC systems are diffusion controlled. Nitrocompounds interacted with ds.DNA via threading intercalation,[36] as indicated by the shift of peak potentials where intercalated complex is supposed to form between ds.DNA and the reduction products. Hypoelectric effect observed upon addition of DNA in the voltammetric profile of the investigated bisnitrocompounds suggested sufficient interaction.[24,41]

The diffusion coefficients of free and bound nitrocompound, percent decrease in current, free energy change (ΔG) of nitrocompound-DNA complex were also obtained, which ascertained the greater affinity of N6 with DNA as compared to other studied bisnitrocompounds apparently due to longer alkyl chain with enhanced hydrophobicity. Keeping in mind the structures of investigated nitrocompounds, the CV responses, binding constants and resulting thermodynamaics in the presence of ds.DNA, threading intercalation was suggested to be dominent mode of interaction. The structurally favoured interactions of such nitrocompounds with ds.DNA could be efficiently addressed using CV technique and is termed as "Nitromics".

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