Sodium 1, 4-dihydroxy-9, 10-anthraquinone-2-sulphonate interacts with calf thymus DNA in a way that mimics anthracycline antibiotics: An electrochemical and spectroscopic study

ARTICLE *in* JOURNAL OF PHYSICAL ORGANIC CHEMISTRY · JUNE 2009

Impact Factor: 1.38 · DOI: 10.1002/poc.1624

3 AUTHORS, INCLUDING:

Saurabh Das
Jadavpur University
35 PUBLICATIONS **363** CITATIONS

SEE PROFILE

Research Article

Journal of Physical Organic Chemistry

Received: 6 August 2009, Accepted: 22 August 2009, Published online in Wiley InterScience: 24 November 2009

(www.interscience.wiley.com) DOI 10.1002/poc.1624

Sodium 1, 4-dihydroxy-9, 10-anthraquinone-2-sulphonate interacts with calf thymus DNA in a way that mimics anthracycline antibiotics: an electrochemical and spectroscopic study

Partha Sarathi Guin[a,b†], Saurabh Das[b*] and P. C. Mandal[a]

The anthracycline drugs, adriamycin and daunorubicin, efficient in the treatment of various human cancers, form strong intercalation complexes with DNA. The therapeutic efficiency and toxic properties of the drugs are associated with electron transfer processes, which correlate well with the redox behaviour of the compounds. Sodium 1,4-dihydroxy 9,10-anthraquinone-2-sulphonate (sodium quinizarin-2-sulphonate, $NaLH_2$) (Na-Qz-2S) is a molecule that resembles anthracycline drugs and has a simpler structure in comparison to these drugs. Two electrons in the course of chemical action reduce this molecule like the anthracyclines. Electrochemical methods were used to identify this process. UV-Vis and fluorescence spectroscopy were used to analyse binding of the compound to calf thymus DNA. The binding constant and site size were evaluated for Na-Qz-2S and the same compared to that of the anthracyclines. Such comparisons are essential in order to understand whether the simpler hydroxy-anthraquinones can be a substitute for anthracycline drugs in cancer chemotherapy. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: binding constant; binding site size; calf thymus DNA; chronocoulometry; fluorescence; sodium 1,4-dihydroxy-9,10-anthraquinone-2-sulphonate; square wave voltammetry; UV-Vis

INTRODUCTION

The anthracycline drugs adriamycin and daunorubicin are widely used as anticancer agents.[1] They are effective against acute leukemia, malignant lymphomas and active in solid tumours, particularly in the case of breast cancer.[1] Such drugs are important ingredients for the successful treatment of ovarian, bladder, Hodgkin's and non Hodgkin's lymphomas, Wilmi's tumour and neuroblastoma. The drugs are beneficial in a wide range of sarcomas, including osteogenic, Ewing's and soft tissue sarcomas, including carcinoma of the endometrium, testes, prostrate, cervix, head and neck.[1] Reports indicate that various analogs and derivatives of these drugs are prepared and tried clinically.[2–4] Studies on such compounds have concentrated on interaction with DNA.[5–9] The compounds form strong intercalation complexes with DNA that inhibit DNA replication and RNA transcription.[5] Infectivity and replication of the human immunodeficiency virus (HIV) *in vitro* has been hindered by anthracyclines, which indicates an application of the compounds as antiviral agents in the treatment of acquired immunodeficiency syndrome (AIDS).[10]

However, the major limitations of the use of these drugs include their acute and chronic toxicities, of which cardiotoxicity is an aspect that requires most attention.[11,12] Aspects of cardiotoxicity[11,12] are associated with several electron transfer processes involving the respiratory chain generating $H_2O_2/O_2^{\bullet-}$/$OH^{\bullet}$, which in turn is also required for oxidative phosphorylation, complexation of phospholipids, and initiation of peroxidation of lipids, thus making studies on electron transfer with these molecules very relevant. It was shown that anthracyclines enhance the flow of electrons from nicotinamide adenine dinucleotide (NADH) to molecular oxygen forming the superoxide radical through the enzyme NADH dehydrogenase. Formation of metal complexes of anthracyclines is seen to reduce formation of superoxide radical anion.[13] Similar behaviour was seen for 1,2-dihydroxy-9,10-anthraquinone where the free quinone is capable of generating $O_2^{\bullet-}$ but its metal complexes are able to reduce formation of $O_2^{\bullet-}$ to a considerable extent. The most significant is that of the Cu(II) complex.[14] Therefore, a very close comparison between anthracycline drugs,

* *Correspondence to: S. Das, Department of Chemistry, Jadavpur University, Raja S. C. Mullick Road, Kolkata-700032, India.*

a *P. S. Guin, P. C. Mandal*
Chemical Sciences Division, Saha Institute of Nuclear Physics, 1/AF-Bidhannagar, Kolkata-700064, India

b *P. S. Guin, S. Das*
Department of Chemistry, Jadavpur University, Raja S. C. Mullick Road, Kolkata-700032, India

† *Present address: Department of Chemistry, Shibpur Dinobundhoo Institution (College), Howrah, India.*

Abbreviations: *$NaLH_2$, Sodium 1,4-dihydroxy 9,10-anthraquinone-2-sulphonate.*

their metal complexes and those of the anthraquinones and their metal complexes is imperative. This study is a continuation of our earlier observations[14] and if a comparison can be drawn, it remains to be seen whether anthraquinones and their metal complexes can work with similar efficiency to that of the anthracyclines in anticancer activity. Any difference in reactivity and binding with DNA if detected through experiments would also be indicative of the role of sugar units present in the case of anthracyclines thus enabling one to identify the utility of having sugar units in such drugs. It is known that sugar units allow a drug to recognize cancer cells for which its presence is essential.[15] However, since most of the drug chemistry is centred around the quinone our task is to establish whether a formulation lacking the sugar unit of anthracycline is also effective. The absence of a sugar unit on one hand would reduce cost of such drugs substantially, but it remains to be seen whether it would easily bind DNA, as drug binding is a pre-requisite for most of the chemical actions.

In order to arrive at such a conclusion, two essential aspects need to be checked; firstly, whether anthraquinones mimic the chemistry of anthracyclines, i.e. whether electron transfer processes are similar or different, and secondly, the types of interactions anthracyclines have with DNA – whether these can be compared with those found for the anthraquinones. This is important, as aspects of therapeutic efficiency are associated with electron transfer processes and these have a good correlation with the redox behaviour of the compounds.[16–19]

Therefore, this study of an aqueous solution of sodium 1,4-dihydroxy-9,10-anthraquinone-2-sulphonate (sodium quinizarin-2-sulphonate, $NaLH_2$) attempts to show that the compound is reduced by two electrons, as is known for the anthracycline drugs.[20–25] Chronocoulometry was used to determine the number of electrons involved in electrochemical reduction. Square wave voltammetry, UV-Vis and fluorescence spectroscopy were used to analyse binding of the compound to calf thymus DNA. The binding constant and site size have been evaluated.

Today, investigation of drug-DNA interaction is important to understand molecular mechanisms of drug action and to the design of specific DNA-targeted drugs. Keeping in mind all of the above facts, it may be said that the interaction of molecules with DNA and the mode of electrochemical reduction are two key factors determining their biochemical activities. Sodium 1,4-dihydroxy- 9,10-anthraquinone-2-sulphonate was chosen as it possesses similar binding sites to those of adriamycin and daunorubicin and being highly soluble in water can be used under physiological conditions for which it may be considered an important model for anthracyclines for understanding how small molecules interact with DNA.

MATERIALS AND METHODS

Sodium 1,4-dihydroxy-9,10-anthraquinone-2-sulphonate (Sodium quinizarin-2-sulphonate) (Na-Qz-2S) was prepared by sulphonation of 1,4-dihydroxy-9,10-anthraquinone (Quinizarin) (Sigma-Aldrich) with aqueous sodium sulphite in the presence of cupric oxide.[26] Na-Qz-2S was recrystalized from ethanol water mixture. For characterization purposes ^{1}H NMR was done (Fig. 1) and the responses for the phenolic-OH protons were obtained at the characteristic region of 4.76 to 4.57 ppm. The aromatic protons were obtained at 7.46, 7.67 and 7.88 ppm. Elemental analysis of the compound was carried out on a 2400 Series II CHN Analyser, Perkin Elmer. Elemental analysis showed contents of C and H as 49.20 and 2.01 wt% respectively (calculated C: 49.12% and H: 2.05%).

The quinone moiety being sensitive to light, solutions were prepared just before the experiment and kept in the dark. Highly polymerized CT DNA was purchased from SRL (Sisco Research Laboratory), India and after dissolution in buffer, purity was checked from the absorbance ratio A_{260}/A_{280}. For all the solutions the absorption ratio was in the range $1.8 < A_{260}/A_{280} < 1.9$.

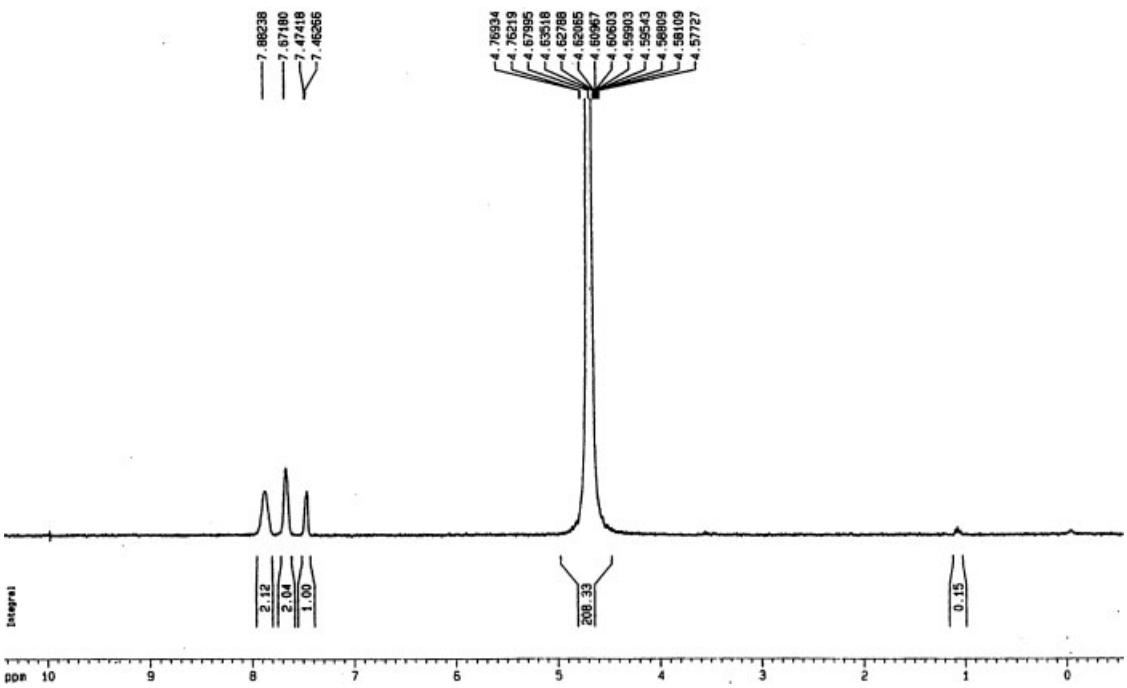

Figure 1. ^{1}H NMR spectra of sodium 1,4-dihydroxy-9,10-anthraquinone-2-sulphonate (Na-Qz-2S)

Therefore, no further deproteinization of DNA was required. Concentration of DNA in terms of nucleotide was determined taking $\varepsilon_{260} = 6600\,M^{-1}\,cm^{-1}$ per base for CT DNA. In all the experiments, DNA concentration has been expressed in terms of base. Analytical grade Hepes buffer (N-2-hydroxyethylpiperazine-N-2-ethane-sulphonic acid, 10 mM) (Spectrochem Pvt. Ltd., India) was used to maintain pH and in all the experiments physiological pH 7.4 was maintained. 500 mM sodium chloride (AR grade) obtained from Merck, was used to maintain ionic strength. For electrochemical experiments sodium chloride also served the purpose of a supporting electrolyte. All solutions were prepared in triple distilled water. Square Wave voltammetry experiments were carried out using the conventional three-electrode system at 25 °C. A glassy carbon electrode served as the working electrode, a platinum wire acted as the counter electrode, while Ag/AgCl was the reference electrode. Experiments were done using a EG & G Potentiostat Model 263A. Voltammetric experiments of Na-Qz-2S were done in pure aqueous buffer in the presence and absence of CT DNA. All experimental solutions were degassed for 30 min with high-purity argon gas before any voltammetry experiment was done. Microprocessor pH/ION meter (pMX 3000) was used for recording pH-values of different solutions. UV-Vis spectroscopic studies were carried out using a spectrophotometer (model UNICAM UV 500). A pair of 10×10 mm path length quartz cuvette was used for absorption experiments. Fluorescence spectroscopic measurements were carried out using a fluorimeter (model Jobin Yvon Fluoro Max 3). A 10×10 mm fluorescence cuvette was used for fluorescence measurements. In all the experiments the temperature was maintained at 25 °C.

RESULTS AND DISCUSSION

Chronocoulometric determination of the number of electrons involved in the reduction of Na-Qz-2S

Controlled-potential coulometry is a useful method for determining the number of electrons involved in electrochemical reduction. In controlled-potential coulometry the total number of coulombs consumed in electrolysis is used to determine the amount of substance electrolyzed. In aqueous buffer, the formal reduction potential of Na-Qz-2S varies with pH[27] with values of −420 mV, −470 mV and −485 mV at pH 5.0, 7.0 and 9.0, respectively. At all these three pH, i.e. acidic, neutral and alkaline, 10^{-6}(M) Na-Qz-2S solutions were electrolyzed against constant potential of −400 mV for more than 90 min. The total number of coulombs consumed in electrolysis (Q) in such electrolysis was monitored against time (seconds). The experimental data was plotted according to Eqn (1).

$$Q = Q^0\,(1 - e^{-pt}) \qquad (1)$$

where Q and Q^0 are the number of coulombs at time t and infinite time, respectively, and p is a constant.[28]

$$Q^0 \text{ can be given by : } \quad Q^0 = n\,F\,N_0 = n\,F\,V\,C_0^* \,(0)$$

where N_0 represents the total number of moles of reactant, n is the number of electrons involved in the reduction, F is Faraday, V is the volume of Na-Qz-2S taken and $C_0^*(0)$ is its initial concentration. From the chronocoulometric studies, Q^0 values were obtained and it provides a value of $n = 2$ for all three pH.

Therefore, whatever may be the pH of the solution, two electrons reduce Na-Qz-2S. From the literature[19–25] it is found that two electrons are required to reduce anthracycline drugs and some of their analogs. A similarity in reduction, therefore, hints at a possible similarity in chemical behaviour when such molecules would interact at the cellular level.

Interaction of Na-Qz-2S with CT DNA determined by square wave voltammetry

From cyclic voltammetry studies we found that two electrons at −465 mV[27] can reduce sodium 1,4-dihydroxy-9,10-anthraquinone-2-sulphonate taken in Hepes buffer, at pH 7.4, in presence of NaCl reversibly. The square wave voltammetry of the compound also showed a reduction peak at −465 mV. In this experiment, the compound solution was titrated with CT DNA. It was found that as the amount of CT DNA increased the reduction peak current gradually decreased indicating a possible interaction of the compound with DNA (Fig. 2). It was observed from Fig. 2 that in presence of DNA there is no other reduction peak. This observation also indicates that following an interaction of the compound with DNA the newly generated compound-DNA species is not electroactive.

Spectrophotometric studies on interaction of Na-Qz-2S with CT DNA

The binding constant of Na-Qz-2S with calf thymus DNA was evaluated by spectrophotometry using different models. Keeping the concentration of Na-Qz-2S constant at 10 μM, the solution was titrated with increasing amounts of calf thymus DNA. Figure 3 shows the absorption spectrum of Na-Qz-2S in the absence and presence of calf thymus DNA, with a peak for the compound at 465 nm. When calf thymus DNA was added to a solution of the compound at physiological pH, the intensity of the peak at 465 nm decreased with a slight red shift of about 6 nm. This hypochromic effect is due to the interaction between the electronic states of the anthraquinone chromophore and those of the DNA bases.[29–32] It is seen that the strength of this electronic interaction is expected to decrease as the cube of the distance of separation of the compound chromophore and DNA. The hypochromism observed in this study indicates a close proximity of the compound to DNA bases.[31] In addition to a hypochromic shift, slight bathochromic shifts are observed. These spectral features are indicative of the intercalation of the compound into the DNA helix,[32] which results in an ordered stacking of the compound in between aromatic heterocyclic base pairs and the intercalating surface is sandwiched tightly between the base pairs and stabilized electronically in the helix by π–π stacking and dipole–dipole interactions.[30]

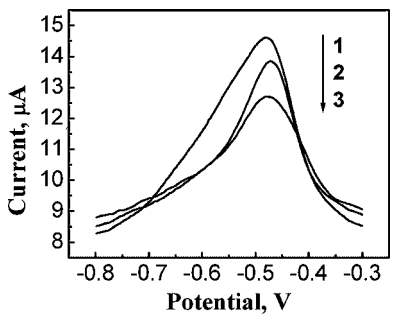

Figure 2. Square wave voltammogram of 10 μM Na-Qz-2S in absence (1) and presence of different CT DNA concentrations: 5.0 μM (2), 10.0 μM (3)

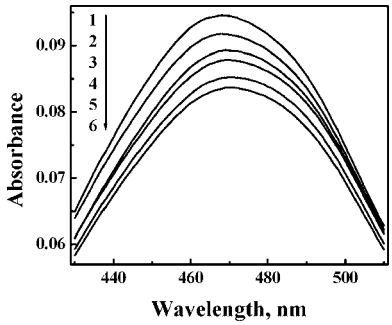

Figure 3. Absorbance spectrum of 10 μM Na-Qz-2S in absence (1) and presence of different CT DNA concentrations: 113.52 μM (2), 283.85 μM (3), 567.7 μM (4), 738.01 μM (5), 908.32 μM (6); [NaCl] = 500 mM, pH = 7.4, $T = 25\ ^{\circ}C$

Based on the variations in the absorption spectra of Na-Qz-2S upon binding to DNA, the binding constant, K was calculated according to Eqn (2)[33]:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \frac{1}{K[DNA]} \quad (2)$$

where A_0 and A are the absorbances of the compound in the absence and presence of DNA, and ε_G and $\varepsilon_{H\text{-}G}$ are the absorption coefficients of Na-Qz-2S and its complex with DNA, respectively. Plot of $A_0/(A-A_0)$ *versus* 1/[DNA] (Fig. 4) is linear, generating a binding constant $K = 2.02 \times 10^3\ M^{-1}$.

The binding constant may be determined from the double reciprocal plot of the changes of apparent extinction coefficient of Na-Qz-2S *versus* DNA concentration. Eqn (3) represents such a relationship[34,35]

$$\frac{1}{\Delta\varepsilon_{ap}} = \frac{1}{\Delta\varepsilon K[DNA]} + \frac{1}{\Delta\varepsilon} \quad (3)$$

where $\Delta\varepsilon_{ap} = |\varepsilon_a - \varepsilon_f|$ and $\Delta\varepsilon = |\varepsilon_b - \varepsilon_f|$. The apparent extinction coefficient, ε_a, has been calculated from A_{obs}/[compound] while ε_f and ε_b correspond to the extinction coefficient for the free compound and the extinction coefficient for the compound fully bound to DNA, respectively. Multiplying both sides of Eqn (3) by [DNA], the equation changes to a half reciprocal form, Eqn (4):

$$\frac{[DNA]}{\Delta\varepsilon_{ap}} = \frac{1}{\Delta\varepsilon}[DNA] + \frac{1}{\Delta\varepsilon K} \quad (4)$$

Using Eqn (4), K is obtained as the ratio of the slope ($1/\Delta\varepsilon$) to the intercept ($1/\Delta\varepsilon K$) (Fig. 5). Since a double reciprocal plot gives excessive weight to data points at low DNA concentration, the half reciprocal plot was, therefore, tried as it should generally be more accurate. The binding constant obtained from this plot was

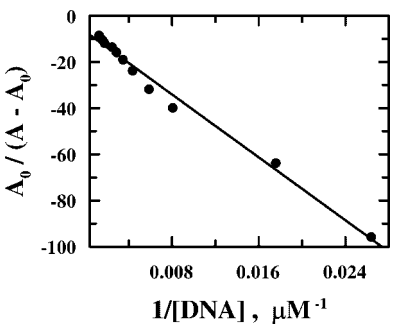

Figure 4. The double reciprocal plot, $A_0/(A-A_0)$ *versus* 1/[DNA], $[NaLH_2] = 10\ \mu M$, [NaCl] = 500 mM, pH = 7.4, $T = 25\ ^{\circ}C$

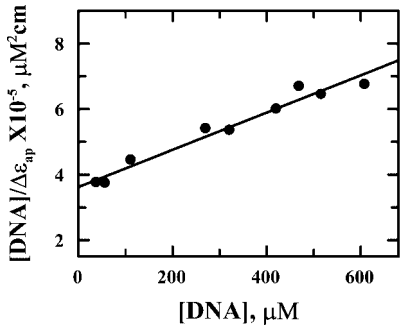

Figure 5. The half reciprocal plot of [DNA]/Δε as a function of DNA concentration as determined from the absorption spectral data; $[NaLH_2] = 10\ \mu M$, [NaCl] = 500 mM, pH = 7.4, $T = 25\ ^{\circ}C$

$1.57 \times 10^3\ M^{-1}$, which agrees reasonably well with that obtained from the double reciprocal plot, as discussed above.

Fluorescence spectroscopic studies: Determination of binding parameters for Na-Qz-2S binding with CT DNA

The interaction of Na-Qz-2S with DNA was studied using fluorescence spectroscopy. The compound has emission maxima at 590 nm. The fluorescence emission spectra of the compound in the absence and presence of micromolar concentration of CT DNA are shown in Fig. 6. Separate solutions were made containing a constant concentration of the compound and different concentrations of CT DNA. The fluorescence spectrum of each such solution was recorded and the change in fluorescence emission intensity at 590 nm was used to construct the binding isotherms. The fluorescence emission intensity decreased gradually with increasing amount of DNA, showing that the fluorescence of Na-Qz-2S was quenched upon binding to DNA. Quenching of fluorescence of the compound was also observed using millimolar concentration of CT DNA (Fig. 7) to show that quenching of fluorescence is due to electron transfer phenomenon and not simply due to dilution.

The Stern–Volmer quenching plot from the fluorescence titration data is shown in Fig. 8. The fluorescence quenching constant (K_{SV}) was evaluated using the Stern–Volmer Eqn (5)[34]:

$$\frac{F_0}{F} = 1 + K_{SV}C_D \quad (5)$$

where F_0 and F are the fluorescence intensities in the absence and presence of CT DNA, respectively. K_{SV} is the Stern–Volmer

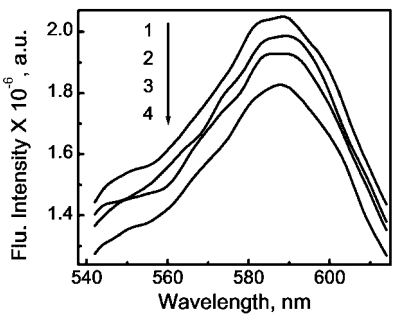

Figure 6. Fluorescence spectrum of 10 μM Na-Qz-2S in absence (1) and presence of different CT DNA concentrations: 70.84 μM (2), 141.68 μM (3), 354.2 μM (4); [NaCl] = 500 mM, pH = 7.4, $T = 25\ ^{\circ}C$

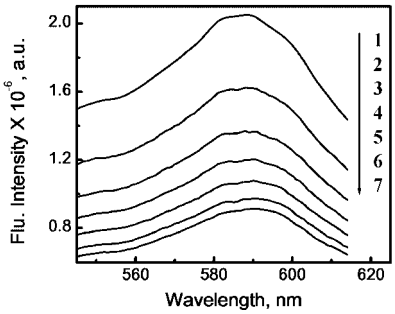

Figure 7. Fluorescence spectrum of 10 μM Na-Qz-2S in absence (1) and presence of different CT DNA concentrations: 0.5161 mM (2), 0.9461 mM (3), 1.3101 mM (4), 1.6220 mM (5), 1.8923 mM (6), 2.1289 mM (7); [NaCl] = 500 mM, pH = 7.4, T = 25 °C

quenching constant which is a measure of the efficiency of quenching by CT DNA. From the titration data, F_0/F values were determined and plotted against CT DNA concentration, C_D. K_{SV} was obtained from the slope of the linear line and was found to be $3.50 \times 10^2\ M^{-1}$. The Stern–Volmer plot is linear, indicating that only one type of quenching process occurs, either static, or dynamic.[36,37]

The binding isotherms were analysed using non-linear curve fitting. To do so, the following ligand–DNA equilibrium was considered[38]

$$L + D \rightleftharpoons LD \tag{6}$$

$$K_d = \frac{[L][D]}{[LD]}$$

L represents Na-Qz-2S and D represents CT DNA. Apparent dissociation constant ($K_d = 1/K_{app}$) was determined using non-linear curve fitting analysis (Eqns (7) and (8)). All experimental points for binding isotherms were fitted by least-square analysis.

This approach is based on the assumption that fluorescence is linearly proportional to the concentration of the compound. Here, the concentration of Na-Qz-2S was 10 μM and CT DNA concentration was kept around 35- to 40-fold greater than that of the compound.

$$K_d = \frac{\left[C_0 - \left(\frac{\Delta F}{\Delta F_{max}}\right)C_0\right]\left[C_D - \left(\frac{\Delta F}{\Delta F_{max}}\right)C_0\right]}{\left(\frac{\Delta F}{\Delta F_{max}}\right)C_0} \tag{7}$$

$$C_0\left(\frac{\Delta F}{\Delta F_{max}}\right)^2 - (C_0 + C_D + K_d)\left(\frac{\Delta F}{\Delta F_{max}}\right) + C_D = 0 \tag{8}$$

ΔF is the change in fluorescence emission intensity of Na-Qz-2S at 590 nm (λ_{ex} = 480 nm) for each point of the titration curve. ΔF_{max} is the same parameter when the compound is totally bound to CT DNA, C_D is the concentration of CT DNA and C_0 is the initial concentration of Na-Qz-2S (compound). Double reciprocal plot (Fig. 9) was used for determination of ΔF_{max} and K_d using Eqn (9):

$$\frac{1}{\Delta F} = \frac{1}{\Delta F_{max}} + \frac{K_d}{\Delta F_{max}(C_D - C_0)} \tag{9}$$

Binding stoichiometry or the binding site size was determined from the point of intersection of two straight lines obtained from the least square fit plot of normalized increase in $\Delta F/\Delta F_{max}$ against the ratio of concentration of CT DNA (in base) to that of the compound. Figure 10 shows the binding isotherms of Na-Qz-2S with CT DNA using which the apparent binding constant was calculated and found to be $2.15 \times 10^3\ M^{-1}$. The apparent binding constant obtained from the double reciprocal plot being $2.59 \times 10^3\ M^{-1}$ indicates that the value obtained from two different techniques are similar. Figure 11 shows the plot of normalized increase of $\Delta F/\Delta F_{max}$ as a function of mole-ratio of DNA to Na-Qz-2S. The point of intersection of two straight lines drawn using points before and after saturation gives the stoichiometry or the binding site-size (n). For Na-Qz-2S the value 'n' of binding to CT DNA is 16 bases, i.e. 8 base pairs. Knowing n, the intrinsic binding constant K, i.e. ($K_{app} \times n$) is obtained as $3.44 \times 10^4\ M^{-1}$.

It is worth mentioning here that the binding constant obtained for the compound, Na-Qz-2S is only an order less than that known for the anthracyclines; there are two possible reasons for this. Firstly, the compound has a negative charge on it and DNA being a negative polymer there is definitely some repulsion that the compound faces in its effort to interact with DNA. Secondly, the sugar moieties present in anthracyclines probably play a role in the binding process that is manifested in a higher binding constant value in the case of established drugs.[15] At the same time what has to be kept in mind is that since binding constant values are close to anthracyclines, an anthraquinone-based drug, if created, would be much less costly. This would definitely be an improvement over the known anthracycline drug molecules belonging to this class. Whatever difference exists in the binding constant values may be improved by seeing if metal complexes of anthraquinones bind to DNA more effectively as the presence of a metal ion is known to increase binding of compounds to DNA.

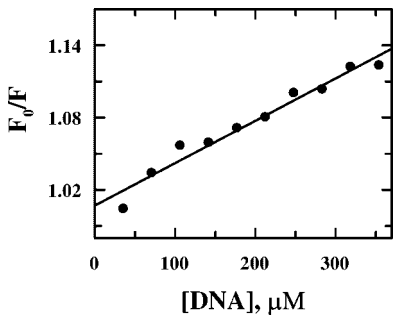

Figure 8. Stern–Volmer quenching plot of Na-Qz-2S with CT DNA; [$NaLH_2$] = 10 μM, [NaCl] = 500 mM, pH = 7.4, T = 25 °C

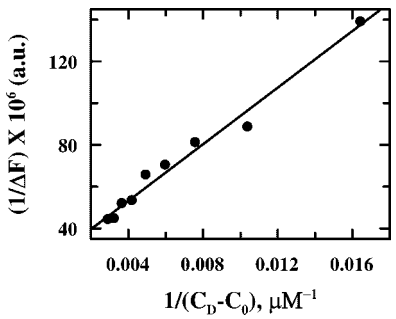

Figure 9. Double reciprocal plot of Na-Qz-2S-CT DNA interaction; [$NaLH_2$] = 10 μM, [NaCl] = 500 mM, pH = 7.4, T = 25 °C

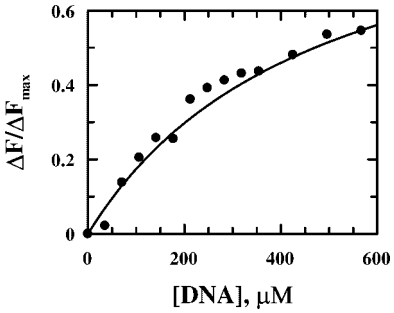

Figure 10. Binding isotherm of Na-Qz-2S and CT DNA and corresponding non-linear fit; $[NaLH_2] = 10\ \mu M$, $[NaCl] = 500\ mM$, $pH = 7.4$, $T = 25\ ^\circ C$

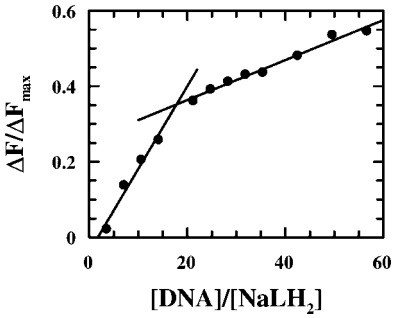

Figure 11. Plot of normalized increase of fluorescence emission intensity as a function of mole-ratio of CT DNA to Na-Qz-2S; $[NaLH_2] = 10\ \mu M$, $[NaCl] = 500\ mM$, $pH = 7.4$, $T = 25\ ^\circ C$

CONCLUSION

Sodium 1,4-dihydroxy-9,10-anthraquinone-2-sulphonate (sodium quinizarin-2-sulphonate), (Na-Qz-2S) a probable analog of the core unit of anthracycline drugs, can be reduced by two electrons in a way similar to that known for the anthracyclines at acidic, neutral and alkaline pH, which may be extrapolated to say that their activity at the cellular level would be similar to that known for the anthracyclines. This was done by carrying out model studies using calf thymus DNA. That the interaction of the compound with calf thymus DNA at physiological pH has very good agreement for values obtained from spectrophotometric and fluorimetric data lends further support to the methods of measurement. The intrinsic binding constant has been evaluated to be $3.44 \times 10^4\ M^{-1}$. The value obtained for the compound used is only an order less than that of the anthracycline drugs being used today which raises hopes that anthraquinones and their analogs can be tried clinically in the near future.

Acknowledgements

SD thanks the Centre of Advanced Studies, Department of Chemistry, Jadavpur University for a grant to purchase chemicals. PSG thanks CSIR, New Delhi, India for a Senior Research Fellowship. PCM acknowledges the Intramural MMDDA project at SINP for its financial support. The authors thank Prof. Subrata Mukhopadhyay, Department of Chemistry, Jadavpur University for kindly providing D_2O for NMR spectroscopy and Dr Pintu Sen of VECC, Kolkata, for helping with the chronocoulometry experiments.

REFERENCES

[1] J. G. Hardman, A. G. Gilman, L. E. Limbird, *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 9th edn, McGraw-Hill, New York **1996**.
[2] K. Reszka, P. Kolodziejczyk, J. A. Hartley, W. D. Wilson, J. W. Lown, *Anthracycline and Anthracenedione based Anticancer Agents*, Elsevier Press, Amsterdam, **1998**.
[3] J. A. Plambeck, J. W. Lown, *J. Electrochem. Soc.* **1984**, *131*, 2556.
[4] F. E. Durr, *Semin. Oncol.* **1987**, *11*, 3.
[5] J. W. Lown, S.-K. Sim, K. C. Matjumdar, R. Chang, *Biochem. Biophys. Res. Commun.* **1977**, *76*, 705.
[6] E. Feinstein, E. Canaani, L. M. Weiner, *Biochemistry* **1991**, *32*, 13156.
[7] D. A. El-Hady, M. I. Abdel-Hamid, M. M. Seliem, N. N. Elmaali, *Talanta* **2005**, *66*, 1207.
[8] M. M. L. Fiallo, A. Gurnier-Suillerot, *Biochemistry* **1986**, *25*, 924.
[9] N. Li, Y. Ma, C. Yang, L. Guo, X. Yang, *Biophys. Chem.* **2005**, *116*, 199.
[10] E. Bachmann, E. Weber, G. Zbinder, *Agents Actions* **1975**, *5*, 383–3393.
[11] V. J. Ferrans, *Cancer Treat. Rep.* **1978**, *62*, 955.
[12] D. Barasch, O. Zipori, I. Ringel, I. Ginsburg, A. Samuni, J. Katzhendler, *Eur. J. Med. Chem.* **1999**, *34*, 597.
[13] M. M. L. Fiallo, A. Garnier-Suillerot, *Biochim. et Biophys. Acta (Genl. Subject)* **1985**, *840*, 91.
[14] S. Das, A. Saha, P. C. Mandal, *Talanta* **1996**, *43*, 95.
[15] W. Priebe, *Molecules* **2000**, *5*, 299.
[16] Y. Kawakami, A. J. Hopfinger, *Chem. Res. Toxicol.* **1990**, *3*, 244.
[17] F. C. de Abreu, P. A. de Ferraz, M. O. F. Goulart, *J. Brazilian Chem. Soc.* **2002**, *13*, 21.
[18] J. Lown, H. Chen, J. A. Plambeck, *Biochem. Pharmacol.* **1982**, *31*, 575.
[19] R. L. Blankespoor, E. L. Kosters, A. J. Post, D. P. VanMeurs, *J. Org. Chem.* **1991**, *56*, 1609.
[20] D. O. Wipf, K. R. Wehmeyer, R. M. Wightman, *J. Org. Chem.* **1986**, *51*, 4760.
[21] H. Lund, M. M. Baizer, *Organic Electrochemistry*, 3rd edn, Marcel Dekker, Inc., New York.
[22] K. Kano, T. Konse, N. Nishimura, T. Kubota, *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2383.
[23] P. He, R. M. Crooks, L. R. Faulkner, *J. Phys. Chem.* **1990**, *94*, 1135.
[24] Y. Hahn, H. Y. Lee, *Arch. Pharm. Res.* **2004**, *27*, 31.
[25] L. M. Ignjatovic, J. M. D. Markovic, D. A. Markovic, J. M. Baranac, *J. Serb. Shem. Soc.* **2002**, *67*, 53.
[26] P. G. Marshall, *J. Chem. Soc.* **1931**, 3206.
[27] P. S. Guin, S. Das, P. C. Mandal, *Int. J. Electrochem. Sci.* **2008**, *3*, 1016.
[28] A. J. Bard, L. R. Faulkner, *Electrochemical Methods: Fundamentals and Application*, John Wiley & Sons, New York.
[29] R. Fakuda, S. Takenaka, M. Takagi, *J. Chem. Soc. Chem. Comm.* **1990**, 1028.
[30] E. C. Long, J. K. Barton, *Acc. Chem. Res.* **1990**, *23*, 271–2273.
[31] C. Cantor, P. R. Schimmel, *Biophysical Chemistry*, vol. *2*, Freeman WH, San Francisco, **1980**.
[32] I. Takenaka, T. Ihara, M. Takagi, *J. Chem. Soc. Chem. Commun.* **1990**, 1485–1487.
[33] X. J. Dang, M. Y. Nie, H. L. Li, *J. Electroanal. Chem.* **1998**, *448*, 61.
[34] A. M. Pyle, J. P. Rehmann, R. Meshoyrer, C. V. Kumar, N. J. Turro, J. K. Barton, *J. Am. Chem. Soc.* **1989**, *111*, 3051–3058.
[35] A. Wolfe, G. H. Shimer, T. Meehan, *Biochemistry* **1987**, *26*, 6392.
[36] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Kluwer Academic, New York, pp. 239–240.
[37] W. Zhong, J. S. Yu, W. L. Huang, K. Y. Ni, Y. Q. Liang, *Biopolymers* **2001**, *62*, 315.
[38] S. Chakraborti, B. Bhattacharyya, D. Dasgupta, *J. Phys. Chem. B* **2002**, *106*, 6947.