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# Voltammetric and viscometric studies of flavonoids interactions with DNA at physiological conditions

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ABSTRACT

Cyclic voltammetric behaviour of three flavonoids: quercetin, morin and rutin was investigated for their interaction with DNA at pH = 4.7 and pH = 7.4 at body temperature (310 K), using glassy carbon electrode. The diffusion coefficients of the free and DNA bound forms of the flavonoids were evaluated using Randles–Sevcik equation. The binding parameters like binding constant, binding site size and binding free energy were also determined from voltammetric data. Moreover, the binding modes of flavonoids with DNA were evaluated from viscommetric analysis. A comparatively high value of binding constant, binding site size and diffusion coefficients for quercetin reveals its stronger binding with DNA under physiological conditions. The negative values of ΔG indicate the spontaneity of binding of flavonoids with DNA.

## 1. Introduction

Protection of DNA from being damaged has become an important issue in both chemistry and medicinal field. Many physical and chemical factors may induce damages in DNA that introduce deviations from its normal double-helical conformation. These changes include structural distortions which interfere with replication and transcription, as well as point mutations which disrupt base pairs and exert damaging effects on future generations through changes in DNA base sequence [1]. DNA damage caused by oxygen-derived species including free radicals is the most frequent type encountered by aerobic cells which is called oxidative DNA damage [2]. Oxidative DNA damage plays an important role in a number of disease processes such as carcinogenesis, cardiovascular, neurodegenerative and inflammatory diseases [2,3]. Flavonoids are low molecular weight organic compounds composed of a three ring-structure with various substitutions, Figure 1.

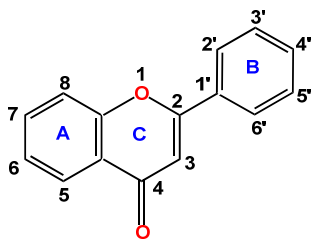


**Figure 1.** Basic structure of flavonoids.

The presence of oxy and hydroxyl groups as well as double bonds at specific positions make them strong antioxidants as they prevent cell damage [4-6] either by direct scavenging of free radicals, or inhibiting the hydroxyl radical production by chelating the transition metals. They may also associate oxidizable substrates like DNA, protein, cell membranes to prevent direct hydroxyl radical damage. Thus they protect the body against free radicals like reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxyl, hydroxyl and reactive nitrogen species (RNS) like peroxynitrite [4,6,7].

The electrochemical interactions of small binding molecules like flavonoids with ds.DNA have biological relevance [8]. In the present work, the electrochemical parameters of flavonoids (quercetin, morin and rutin), binding number, and binding constants with DNA have been obtained using various electrochemical methods at physiological pH of 4.7 and 7.4 and at body temperature i.e., 310 K. Interacting with DNA, flavonoids form a kind of supramolecular complex which has no electrochemical activity and cannot be subsequently oxidized at the electrode surface. Furthermore, the possible binding modes between flavonoids and DNA were determined by viscommetric measurements. Such studies yield information about the mechanism of intercalation and the conformation of flavonoid-DNA complex [9]. These investigations form a theoretic guide for the design of new drugs and chemical treatments of tumor and virus. They are also very valuable for probing the mechanism of the interaction between anticancer drugs and DNA and establishing convenient methods to effectively choose specific anticancer drugs.

## 2. Experimental

### *2.1. Reagents and chemicals*

Quercetin hydrate (Acros Organics), morin (BDH Biochemicals), and rutin trihydrate (Sigma) were of AR grade and used without further purification. DNA was extracted from chicken blood using Falkon method [9]. All conducting samples


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were prepared using (a) 0.1 M acetic acid–sodium acetate buffer solution, containing 50 mM KCl with pH = 4.7 and (b) 0.1 M sodium phosphate buffer solution with pH = 7.4. Stock solution of each flavonoid (1 mM) was prepared in buffered ethanol:water mixture with 1:1 (v:v) ratio. Concentration of chicken blood ds.DNA in distilled water was determined spectrophotometrically at 260 nm using molar extinction coefficient $\varepsilon_{260}$ = 6600 $cm^{-1}M^{-1}$ [9]. The ratio of the absorbance at 260 nm to that at 280 nm, $A_{260}/A_{280}$> 1.8, indicated that DNA was sufficiently pure and free from protein [10].

### *2.2. Instrumentation and procedure*

The electrochemical measurements were carried out in a single compartment cell with a three electrode configuration on an Ecochemie Autolab PGSTAT-12 potentiostat/galvanostat with the electrochemical software package GPES 4.9. A glassy carbon electrode with surface area of 0.071 $cm^2$ was used as the working electrode while platinum wire and saturated calomel electrode SCE [$Hg/Hg_2Cl_2/KCl$] were used as counter and reference electrodes, respectively. The working electrode was polished with alumina powder on a polishing pad and washed with doubly distilled water/test solvent prior to each experiment. Cyclic voltammograms (CVs) of stock solutions of quercetin, morin and rutin (1 mM) were recorded within the potential scan range of -0.1–1.0 V *vs.* SCE at scan rate of 100 mV/s. CVs of flavonoids (1mM) were further recorded by adding varying amount of DNA varying from 1 µM to 6 µM. Viscosity measurements were made on an Anton Paar Stabinger Viscometer SVM 3000. A series of solutions were made with constant concentration of DNA and varying concentration of flavonoids. Cyclic voltammeteric (CVs) and viscosity measurements were carried out at pH = 4.7 (stomach pH) and 7.4 (Blood pH) and at body temperatures i.e., 310 K.

## 3. Results and discussion

### *3.1. Voltammetric studies of DNA-Flavonoid interactions*

The cyclic voltammograms of 1 mM quercetin, morin and rutin in 0.1 M HAc–NaAc (pH = 4.7) buffer solution at a bare glass carbon electrode are shown in Figure 2A(a), 2B(a) and 2C(a), respectively. Cyclic voltammograms showed irreversible one step oxidation process for three flavonoids with the involvement of two electrons and two protons [10-12]. Since pure DNA is electrochemically inactive in the potential range of -0.2 V to -1.6 V [10,11], the interaction flavonoids with DNA can be inferred from the shift in peak potential and decrease in peak heights in the cyclic voltammograms. Addition of different concentration of DNA to the fixed concentration of quercetin and rutin (1 mM) caused a gradual decrease in both the oxidation and reduction peak currents with no shift in both peak potentials, Figure 2 (A and B). However, a slight positive shift in the oxidation peak potential along with gradual decrease in the peak current was observed only with morin, Figure 2C. The decrease in peak current values of flavonoids in the presence of ds-DNA is indicative of decreasing concentration of free flavonoid and formation of FlOH–DNA complex [12-15] which may further be attributed to perpendicular placement of flavonoid molecules between the adjacent base pairs of ds.DNA i.e., intercalation.

The addition of an excess of DNA i.e 6 µM in the flavonoid solution caused the peak currents of both oxidation and reduction peaks of cyclic voltammetric waves to diminish considerably (curve g in Figure 2A, 2B and 2C). These oxidation peak currents decreased to ca. 80.0, 75.7 and 70.5% of those in the absence of DNA, for quercetin, morin and rutin, respectively. Furthermore, high value of % decrease in oxidation peak current of quercetin as compared to morin and rutin reveals that the maximum number of quercetin molecules may intercalate within the DNA. The similar voltammetric trend of three flavonoids with the addition of DNA was observed at pH = 7.4 while the oxidation peak currents decreased to 77.0, 60.7 and 66.5% for quercetin, morin and rutin, respectively. The great decrease in current in CV experiments may also be attributed to the diffusion of flavonoids bound to the large, slowly diffusing DNA with large molecular weight. The changes in current upon addition of DNA can be explained in terms of diffusion of an equilibrium mixture of free and bound flavonoid to the electrode and which can be used to quantify the binding of flavonoids to DNA. The diffusion coefficient of all three flavonoids in the absence and presence of DNA were evaluated by using Randles sevcik equation [9,11,13]:

$$i_p = 2.99\times10^5\, n(\alpha n_a)^{1/2}\, A C_o^{\,*}\, D_o^{\,1/2}\, v^{1/2} \quad (1)$$

$$\left|E_p - E_{p/2}\right| = \frac{47.7}{\alpha n_a}\ mV \quad (2)$$

where; $i_p$ is peak current in amperes (A), $A$ is the area of glassy carbon electrode ($cm^2$), $C_o$ is bulk concentration (1 mM flavonoid), $v$ is scan rate in $Vs^{-1}$ and $D_o$ is diffusion coefficient in $cm^2s^{-1}$, $\alpha$ the transfer coefficient of the oxidation of Morin and $E_{p/2}$ is the potential, where $i = i_{p/2}$ in cyclic voltammograms.

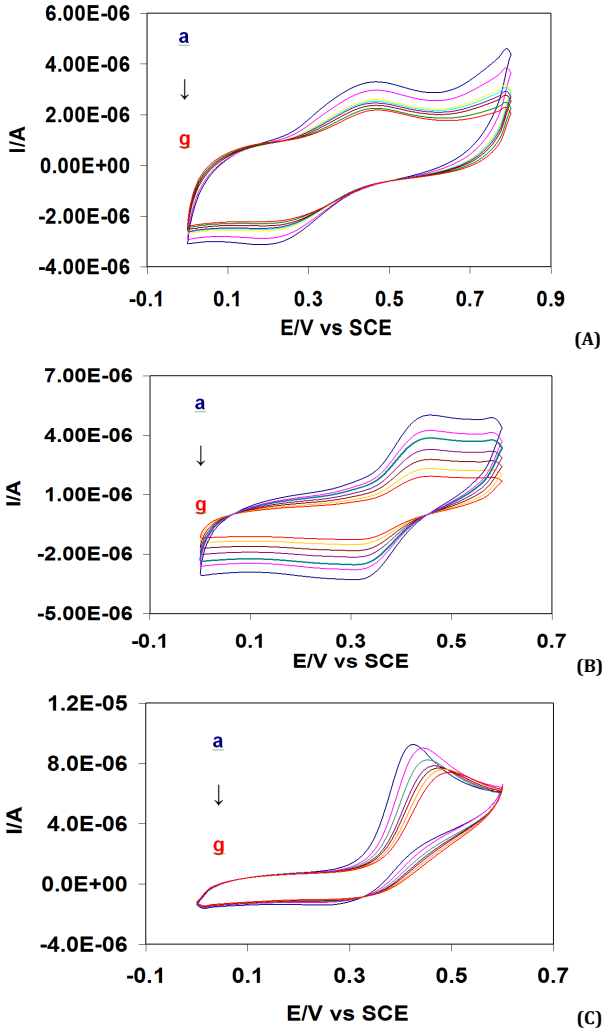


**Figure 2.** Voltammetric responses of 1 mM quercetin (A), rutin (B) and morin (C) without and in the presence of different concentrations of DNA at a scan rate of 100 mV/s, pH = 4.7 and at body temperature (310 K). (a) 1 mM flavonoid, (b-g) after the addition of 1-6 µM DNA.

**Table 1**. Diffusion Coefficients of flavonoid-DNA complex at pH = 4.7 and 7.4 and at body temperature (310 K).

| Flavonoid-DNA complex | pH = 4.7 | | | pH = 7.4 | | |
|---|---|---|---|---|---|---|
| | Binding constant (K, $M^{-1}$) | Free energy (-ΔG, kJ/mol) | Binding site size | Binding constant (K, $M^{-1}$) | Free energy (-ΔG, kJ/mol) | Binding site size |
| Qu-DNA | $8.40\times10^{3}$ | 24.70 | 3.0 | $7.70\times10^{3}$ | 23.10 | 2.9 |
| Mo-DNA | $7.10\times10^{3}$ | 22.80 | 2.5 | $6.55\times10^{3}$ | 21.30 | 2.3 |
| Ru-DNA | $2.10\times10^{3}$ | 19.50 | 1.2 | $1.99\times10^{3}$ | 18.00 | 1.2 |

The average value of $|Ep - E_{p/2}|$ is 34, 43 and 45 mV for quercetin, morin and rutin, respectively. The value of $\alpha n_\alpha$ calculated from equation (2) is 1.40, 1.10 and 1.06 for quercetin, morin and rutin repectively. A possible mechanism for the oxidation of quercetin is shown in Figure 3, which illustrates that two-electrons and two-protons are involved in the oxidation process of flavonoid. Both of the OH of B-ring were oxidized to quinones, which is similar to that of other flavonoids, such as morin and rutin [12,13]. Being electron richer than ring A, the B ring of the flavonoid is an apparent target of any oxidant.

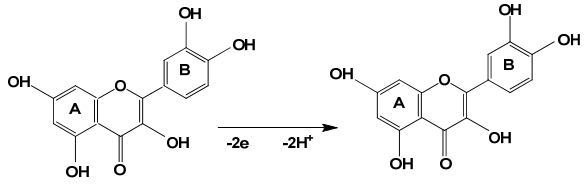


**Figure 3.** Proposed mechanism for the redox reaction of quercetin.

Using equation (1), the diffusion coefficients of quercetin, morin and rutin in the absence of DNA was determined as $8.21\times10^{-5}cm^2s^{-1}$, $1.94\times10^{-6}$ $cm^2s^{-1}$ and $5.41\times10^{-8}cm^2s^{-1}$, respectively. The smaller diffusion coefficient values of rutin as compared to morin and quercetin could be rationalized in terms of its bigger molecular size and the resulting slow diffusion kinetics. Diffusion coefficients of quercetin, morin and rutin in the presence of DNA are given in Table 1.

The values of $D_o$ for flavonoids for the maximum concentration of DNA added lower than that of its absence, indicating slow diffusion processes in the system. The decreasing trend in $D_o$ values may be correlated to stronger interactions in terms of intercalation between the DNA and the flavonoid (FlOH). As the flavonoid becomes more bound to the DNA, the concentration of free electroactive flavonoid at the electrode–solution interface decreases resulting in slow diffusion process due to the formation of bulky FlOH–DNA complex [14,15].

Based upon the decrease in the peak current of flavonoids by the addition of different concentration of DNA, the binding constants, K of flavonoid-DNA complexes were calculated from the following equation [14-16].

$$i_p^{\,2} = \frac{1}{K[DNA]}\,(i_{po}^{\,2} - i_p^{\,2}) + i_{po}^{\,2} - [DNA] \qquad (3)$$

where, $i_{po}$ and $i_p$ are peak currents of flavonoid in the absence and presence of DNA. A plot of $i_p^{\,2}$ vs. $(i_{po}^{\,2} - i_p^{\,2})/[DNA]$ gave a straight line with a slope equal to the reciprocal of the binding constant, K. The binding constant values for three flavonoids are given in Table 2.

The highest value of K for quercetin among the three flavonoids reveals the formation the most stable Qu-DNA complex. A flavonoid molecule consists of two parts; the hydrophilic part (ring-B) and the hydrophobic part, benzopyran-4-one (ring–AC). Since the bases of DNA are hydrophobic in nature, the planner hydrophobic part of the flavonoid molecule will intercalate within the base pairs of the DNA. Since, the interaction of quercetin with solvent molecules is negligibly small as compared to morin where the presence of β-hydroxyls on ring-B facilitates the intermolecular hydrogen bonding [12,13,16]; the possibility of quercetin molecules to intercalate completely within the DNA double helical structure and the formation of stable Qu–DNA complex is greater than for morin. However in the case of rutin, the presence of the bulky sugar moiety may create greater hindrance for benzopyranic moiety to intercalate, hence decreasing its association with the DNA binding sites.

**Table 2.** Binding constant, free energy and binding site size of flavonoid-DNA complexes at physiological conditions.

| Flavonoid-DNA complex | Diffusion coefficient, $cm^2/s$ | |
|---|---|---|
| | pH = 4.7 | pH = 7.4 |
| Qu-DNA | $6.91\times10^{-6}$ | $7.05\times10^{-6}$ |
| Mo-DNA | $4.15\times10^{-7}$ | $5.55\times10^{-7}$ |
| Ru-DNA | $6.01\times10^{-9}$ | $1.01\times10^{-8}$ |

A simple site binding model was used to fit the cyclic voltammetric data acquired from the interaction between flavonoids and DNA. Binding site size of flavonoid-DNA interaction has been evaluated from the following equation [9,15]:

$$C_b/C_f = K\{[DNA] / 2s\} \qquad (4)$$

where, s is binding site per base pairs (bp). $C_b$ is the concentration of bound flavonoid while, $C_f$ is the concentration of free flavonoid, K is the binding constant and can be calculated as [15,16],

$$C_b/C_f = (I - I_{DNA}) / I_{DNA} \qquad (5)$$

where, I and $I_{DNA}$ is peak currents of flavonoid in the absence and presence of DNA. Binding site size are numbers of DNA base pairs covered (or made inaccessible to another molecule) by a binding molecule [9,16]. Binding site sizes of flavonoid (quercetin, morin, and rutin)-DNA complexes were calculated and given in Table 2. The values of the binding-site sizes are closer to that expected for an intercalative mode of binding in which the binding site consists of the intercalating agent sandwiched between two consecutive base pairs. These binding site size values reveal that quercetin covers more base pairs than morin and rutin. The small value of rutin is due to its larger size. The larger value for quercetin further highlights its stronger binding with DNA as compared to morin and rutin.

From the binding constant 'K', the standard Gibbs free energy values, ΔG of flavonoid-DNA complexes, were also calculated, using the following equation,

$$\Delta G = -RT \ln K \ (kJ/mol) \qquad (6)$$

The negative values for the flavonoid-DNA complex as shown in Table 2, indicate spontaneity in binding of flavonoids with DNA and inconsistent to those given in the literature [16]. Comparatively, a more negative ΔG value may manifests enhanced interaction of DNA with quercetin than with morin and rutin, hence conforming to a more stable quercetin-DNA complex and to the above comprehensions.

### *3.2. Viscosity measurements*

In addition to the diffusion coefficients, binding site size, binding constant and Gibbs free energy and viscosity measurements were carried out to provide further evidence about a binding mode between three flavonoids and DNA. Viscommetric technique is an effective tool in clarifying the mode of the interaction of small molecules with DNA. In general, intercalation (in-binding mode) causes an increase in the viscosity of DNA solution due to the lengthening of DNA helix as the base pair pockets are widened to accommodate the binding molecules [17]. The reverse can be taken for electrostatic interaction (out-binding mode). The viscosity measurements were carried out by varying the concentration of the added flavonoid. The values of the relative specific viscosity (η/ηο) of DNA in the presence of flavonoids are plotted against the concentration of flavonoids as shown in Figure 4. Where η and ηο is the viscosity of DNA with and without the flavonoid. Figure 4 reveals that the relative viscosity of DNA increased by increasing the concentration of three flavonoids in both buffer solutions of pH = 4.7 and 7.4. The increase in viscosity is due to intercalation mode which results in increased separation of base pairs at the intercalation sites, and hence an increase in the overall DNA length [17,18].

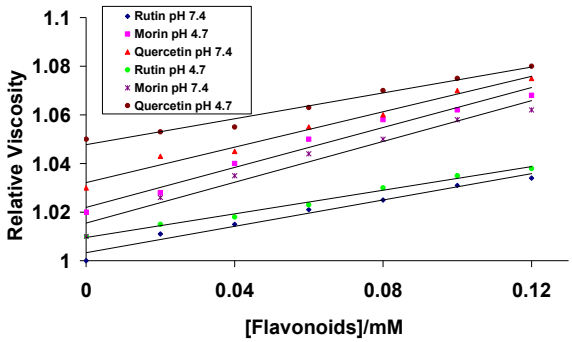


**Figure 4.** Plot of the relative viscosity (η/ηο) vs. concentration of Flavonoids in at pH = 4.7 and 7.4 and at 310 K.

Form the electrochemical and viscommetric data it can be inferred that three flavonoids can intercalate through ds.DNA efficiently, thus distorting DNA structure and inhibiting its replication ability at blood and stomach pH and at body temperature. Most importantly the human body temperature and stomach pH favors the binding of planar molecules like flavonoids with ds.DNA thus helping the physiological prevailing conditions to deter DNA damage. The order of binding of the three flavonoids obtained by cyclic voltammetric and viscommeteric data are as follow; Quercetin > Morin > Rutin which reveals that quercetin forms the most stable complex with the DNA.

## 4. Conclusions

Decrease in the peak current and shift in the peak potential of the three flavonoids in the presence of DNA indicated their mode of interaction to be intercalation. The binding constant, binding site size, diffusion coefficient and viscommetric data further ascertained the enhanced affinity of quercetin with DNA and hence the formation of the most stable quercetin–DNA complex as compared to that with morin and rutin at body temperature. The interaction between flavonoid and DNA is further confirmed by correlating the planarity, hydrophobicity, and electrostatic components of the flavonoids. The negative values of the free energy indicated spontaneity of binding of the three flavonoids with DNA. These investigations showed that electrochemistry could provide a convenient way to characterize both the binding mode and the interaction mechanism of flavonoids binding to DNA, which is important for the design of new anticancer drugs.

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