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Research Article

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Cyclic Voltametry study of Interaction of Calf Thymus Deoxyribonucleic Acid (ct-DNA) with an Anticancer Analogue Drug 10-Molybdo 2-Vanado Phosphoric Acid

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Abstract In this paper, the electrochemical behavior of $[H_5PMo_{10}V_2O_{40}]$ modified electrode glassy carbon electrode ($[H_5PMo_{10}V_2O_{40}]$/GCE) and its interaction with calf thymus DNA (ctDNA) were investigated using voltammetry. Modified electrode showed two pair of peaks at A_1 (0.00) and A_2 (0.179) during the anodic peaks sweep, while the counterpart to the two cathodic peaks are C_1 (-0.014) and C_2 (0.142) respectively in cyclic voltammetry. The redox property of this modified electrode at various solution pH values and various scan rates were studied using cyclic voltammetry. The linear plot of peak potential E_P versus pH was obtained with corresponding slopes 52 m V/ pH. The apparent charge transfer rate constant, k_s, and transfer coefficient, α, for electron transfer between $[H_5PMo_{10}V_2O_{40}]$ and GCE were calculated as 25.203 ± 3.586 and 0.514 (in pH=3), respectively. Binding constant ($K = 4.65 \times 10^4\ M^{-1}$) and binding site size (s =0.5) of modified electrode by ctDNA were obtained by nonlinear fit analysis of voltammetric data based on an electrochemical equation, which was derived to examine the interaction of DNA and reversibly electroactive compounds.

Keywords ctDNA, 10-Molybdo 2-Vanado Phosphoric Acid, Cyclic Voltametry study, Anticancer

Introduction

The electrochemical methods using chemically modified electrodes have been widely used as sensitive and selective analytical methods for the environmental, clinical and bio-technical analyses [1–8].

Polyoxometalates are early transition metal oxygen cluster. Polyoxometalates have attracted much attention during last decades because of their extensive application to many fields, such as catalysis [9-11], analytical chemistry [12], biochemistry [13,14], medicine [15,16] and materials science [17].

The electrochemical behavior of monomolybdenum-substituted Keggin-type polyoxometalates $[XW_{11}MoO_{40}]^{n-}$ (X＝P, Si, Ge with n＝3, 4) was studied in aqueous and *N*,*N* dimethylformamide (DMF) solution [18].

Because polyoxometalates (POMs) undergo reversible, stepwise, multi-electron transfer at a variety of electrodes, they have generated interest in several fields of chemistry, including electrocatalysis and storage [19,20].

Polyoxometalates, the discrete polymeric anions of early transition metal oxide, form along class of inorganic compound with great molecular diversity and significant potential applications in analytical chemistry, material science, catalysis and medicine. Polyoxometalates are negatively charged inorganic substances which contain early transitional metal ions such as Tungsten (W), molybdenum (Mo), vanadium (V) etc and which make a cluster with the surrounding oxygen atoms.

Same polyoxometalates such as PM-8, PM-17, PM-26 and PM-32 were found to exhibit anti-tumor activities against some of human cancer [21,22].

Various polyoxometalates proved inhibitory to the replication of a number of undeveloped DNA and RNA viruses.

Experimental

Instruments and Reagents

ctDNA was obtained from Sigma and used without further purification. The stock solutions of DNA ($\approx 10^{-3}$ M) was prepared in 0.1 M phosphate buffer + 0.15 M NaCl solution (pH=7.0). Polyvanadomolybdate, $[H_5PMo_{10}V_2O_{40}]$ was prepared according to a published procedure [23]. All chemicals were reagent grade from Merck. The solutions were prepared with double distilled water. The buffer solutions were made up from H_3PO_4 and then adjusting the pH with 2.0 M NaOH.

All electrochemical experiments were carried out using an Autolab potentiostat PGSTAT 30 (Eco Chemie Utrecht, Netherlands) equipped with GPES 4.9 software. The cell used was equipped with a modified glassy carbon electrode as the working electrode, a Pt wire as an auxiliary electrode and with a saturated calomel electrode (SCE) as a reference electrode. All potentials in the text are quoted versus this reference electrode. A personal computer was used for data storage and processing.

Determination of DNA concentration

Stock solution of ct-DNA was prepared by dissolution in 0.1 M phosphate buffer + 0.15 M NaCl solution (pH=7.0) and was stirred for 24 hour below 4°C in the dark and was stored for short periods only. The base-pairs concentration of ct-DNA was determined by its known absorbance measurements using $\varepsilon = 1.32\times10^4$ $L.mol^{-1}.Cm^{-1}$ (i.e. reported in molar base pair) at the absorption maximum of 260 nm [24,25].

Preparation of $[H_5PMo_{10}V_2O_{40}]$/GCE

A glassy carbon electrode (GCE) was polished with emery paper followed by alumina and then washed with twice-distilled water. After being cleaned, the bare electrode was modified by 20 cycles of potential sweep between -0.3 and 1.7 V at 25 mV s^{-1} in 0.1 m M solution of $[H_5PMo_{10}V_2O_{40}]$ in 0.05 M sulfuric acid solution. During this stage, a monolayer of $[H_5PMo_{10}V_2O_{40}]$ is bound to the surface of bare electrode.

Results and Discussion

Electrochemistry of $[H_5PMo_{10}V_2O_{40}]$-GCE

Fig. 1 shows cyclic voltammograms of in 0.1 M phosphate buffer solution (pH 3.0). In the potential rang from -0.3 – 0.6 V/s, $[H_5PMo_{10}V_2O_{40}]$ displayed two waves A_1 (0.00) and A_2 (0.179) during the anodic peaks sweep, while the counterpart to the two cathodic peaks are C_1 (-0.014) and C_2 (0.142) respectively. The peak potential separation, ΔE_p values ($\Delta E_p = E_{p.a} - E_{p.c}$) for all redox couple were larger than 65 mV, so that these redox processes were all quasi reversible.

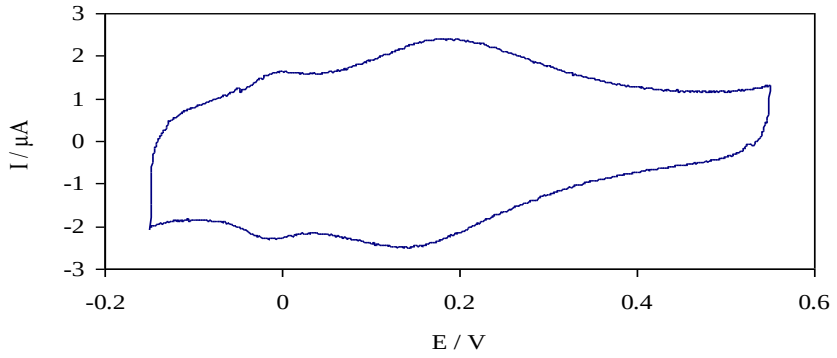

Figure 1: Cyclic voltammograms of modified electrode in 0.1 M phosphate buffer solution (pH=3)

We found that the E_p values are proportional to the logarithm of the scan rate, for scan rates higher than 5.5 V s^{-1} (Fig. 2 part C).

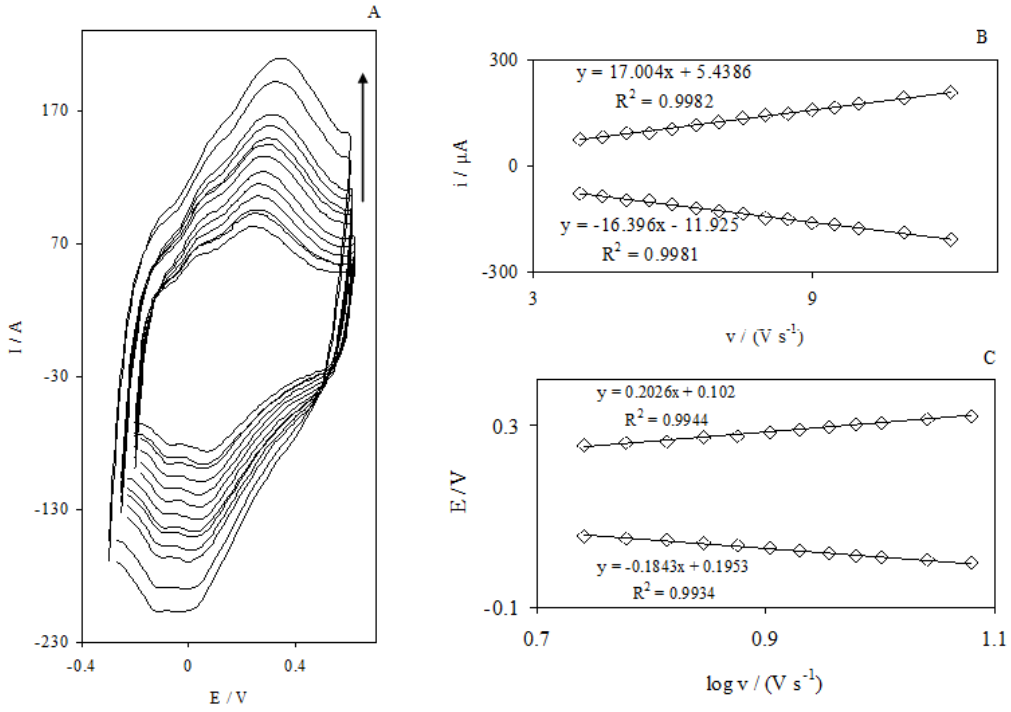

Figure 2: A) Cyclic voltammograms of modified electrode in 0.1 M phosphate buffer (pH=3) in different scan rate. The numbers correspond to 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11 and 12 V s^{-1} respectively. B) Variation of anodic and cathodic peak currents versus of scan rate. C) Variation of E_P versus the logarithm of the scan rate.

Fig. 2 shows the cyclic voltammograms of the $[H_5PMo_{10}V_2O_{40}]$-GCE in 0.1 M phosphate buffer solution (pH=0.3) at various scan rates. According to Fig. 2 part B, the peak currents were directly proportional to the scan rate. The heterogeneous charge transfer rate constant, k_s, and charge transfer coefficient, α, of a surface-confined redox couple can be evaluated from cyclic voltammetric experiments and using the variation of anodic and chatodic peak potentials with scan rate, according to the procedure of Laviron [26]. Part C of Fig. 2 shows the variations of peak potentials (E_p) as a function of potential scan rate.

Under these conditions, the following equation can be used to determine the electron transfer rate constant between $[H_5PMo_{10}V_2O_{40}]$ and GCE [26]:

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log(\frac{RT}{nFv}) - \alpha(1-\alpha)\frac{n_\alpha F\Delta F_P}{2.3Rt} \quad \text{(eq 1)}$$

where $(1-\alpha)n_\alpha =$, $n = 2$, $\Delta E_p = E_{p.a} - E_{p.c}$, v is the sweep rate and all other symbols have their conventional meanings. From the values of ΔE_p corresponding to different sweep rates, an average value of k_s was found to be 25.203 ± 3.586. Also the average value obtained for the charge transfer coefficient, from the slopes of the inset C plots, was found to be 0.514.

Also, we study the scan rate effect on modified electrode in biological pH (6,7) and evaluated α and k_s in these pHs. The table 1 represent the result of α and k_s in pH=3, 6 and 7. Fig. (3) shown cyclic voltammograms of the

modified electrode in 0.1 M phosphate buffer solution with biological pHs (pH=6,7). The effect of pH on modified electrode, $[H_5PMo_{10}V_2O_{40}]$-GCE, was investigated by cyclic voltammetry using 0.1 M buffer solutions with various pH values, ranging from 3 to 9 (Fig. 4). The electrochemical behavior of $[H_5PMo_{10}V_2O_{40}]$-GCE was found to be pH dependent in a wide pH range. The potentials shifted negatively and the peak currents changed significantly with increasing pH of supporting electrolyte solution. The linear plot of peak potential E_p versus pH were obtained with corresponding slopes -53.661 m V/ pH. From the slope value, it could be concluded that uptake of electron was accompanied by an equal number of proton in both electrode reactions. As can be seen in Fig. 5, the formal potential, $E^{0'} = E_{p.a} - \alpha(E_{p.a} - E_{p.c})$ [27], of $[H_5PMo_{10}V_2O_{40}]$-GCE was pH-dependent.

Table 1: α and k_s in pH=3, 6

pH	α	k_s / s^{-1}
3	0.514	25.203 ± 3.586
6	0.513	50.676 ± 7.134
7	0.470	37.134 ± 2.756

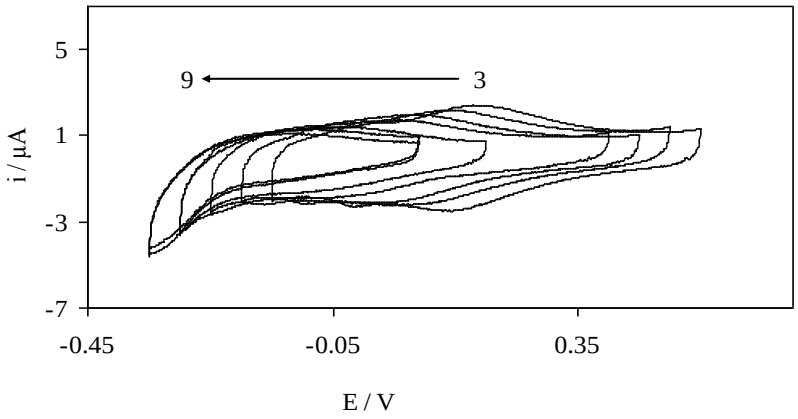

Figure 3: Voltamograms of modified electrode in 0.1 M phosphate buffer with different pH

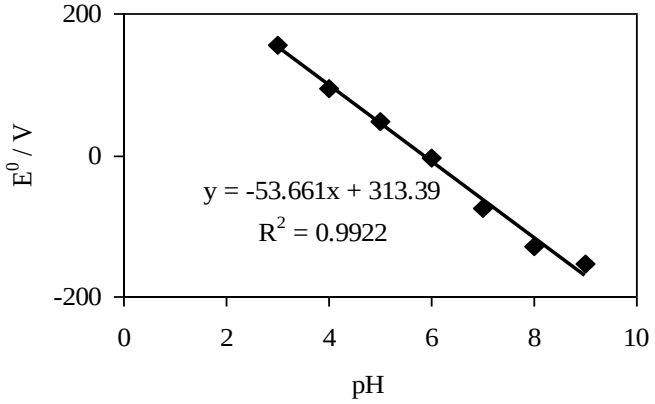

Figure 4: Dependence on pH of formal potential, $E^{0'}$, for modified electrode in 0.1 M phosphate buffer solution.

Interaction of $[H_5PMo_{10}V_2O_{40}]$-GCE with DNA

Considering the sensitivity, we choose the oxidation peak P_1 as focus to investigate the interaction between $[H_5PMo_{10}V_2O_{40}]$-GCE and DNA. DVP technique provides higher sensitivity and better peak resolution than CV for studying the electrochemical behavior of biological systems [28], so we use DVP in the following experiments. The

purpose of the part of this work is the study of different modes of $[H_5PMo_{10}V_2O_{40}]$ binding to DNA by the method of the DVP and the determination of K and n (K-binding constant, n- number of bases corresponding to one site of binding). To prevent DNA from acidic or basic denaturing, pH 7.1 phosphate buffer was chosen as supporting electrolyte in this part. The ionic strength was adjusted to 0.15 M. Differential pulse voltammograms of modified electrode in the presence and absence of DNA shown in Fig. (5). In the presence of DNA, peak current, I_p decrease obviously. However, E_p not shift and no reduction peak is found.

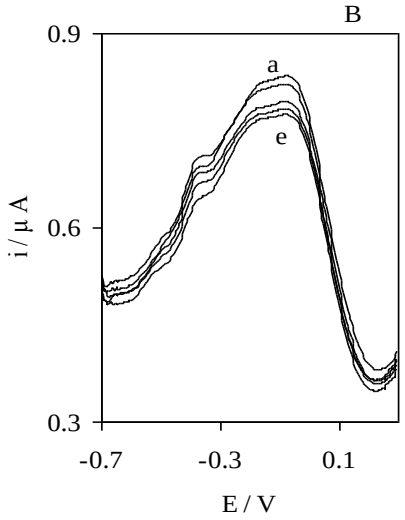

Figure 5: DVP for modified glassy carbon electrode (a-e) in present different constant of DNA, 0, 0.5, 0.1, 0.13 and 0.195 m M respectively

The interaction of anticancer drugs with DNA can be described using the following equation:

$$drug + DNA \Leftrightarrow drug - DNA$$

The distribution of bound and free forms depends on the square of the measured current, so we can therefore calculate the mole fraction of bound complex as [29]:

$$X_b = (i^2 - i_{sat}^2)/(i_0^2 - i_{sat}^2) \quad \text{(eq 2)}$$

where i_{sat} ($=i_b$) is the current when all of the metal complex is bound to DNA and i_0 ($=i_f$) is the current when no DNA has been added. A plot of the current expression for C_b/C_t (eq) versus the DNA concentration was constructed from the voltammograms and is shown in Fig. (6).

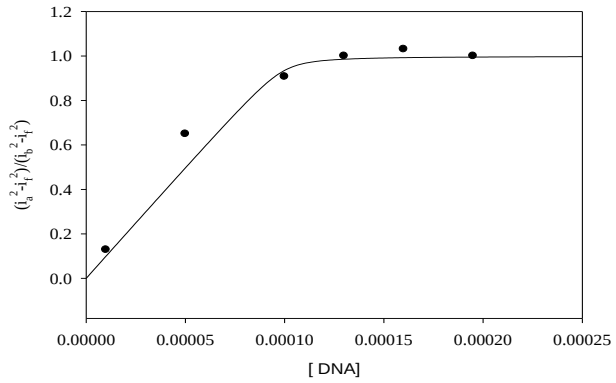

Figure 6: Binding isotherm from simulated cyclic voltammograms

For relatively low binding affinities (K < 10 000 M^{-1}), the mole fraction bound can be fit as

$$X_b = K[DNA]/(K[DNA]+1) \quad \text{(eq 3)}$$

The DNA dependence was not fit well by the weak binding isotherm (eq 3), which was expected because at these binding constants, neighbor exclusion must be considered. Neighbor exclusion occurs when the extent of binding is limited by coverage of a finite number of base pairs by binding of a single complex, which prohibits binding of an additional complex in the same site. Binding isotherms in this regime are generally fit to a strong binding equation [29-31]

$$\frac{C_b}{C_t} = \frac{b - (b^2 - \frac{2K^2 C_t[DNA]}{s})^{1/2}}{2KC_t}$$

$$b = 1 + KC_t + K[DNA]/2s$$

where *s* is the site size in base pairs excluded by the bound metal complex. Fiiting the simulated isotherm to eq 2 gave a very good fit. The fit of data with two parameter gave K= 465 × 10^4 M^{-1} and s=0.5.

Conclusion

The results obtained in these studies show that the electrochemical behavior of modified electrode is pH dependent. Interactions between DNA and $[H_5PMo_{10}V_2O_{40}]$-GCE were studied by voltammetry method. In the presence of DNA, peak current, I_p decrease obviously. However, E_p not shift and no reduction peak is found. For this binding K= 465 × 10^4 M^{-1} and s=0.5.

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