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Effect of Potential Window, Concentration of Hexaammineruthenium (III) and pH on Electrochemical Quantification of Thiol-Modified DNA Monolayers on Gold

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In this article we investigated the effect of potential window, concentration of hexaammineruthenium (III) chloride (RuHex) and pH value on quantifying surface coverage (Γ_m) of thiol-modified single-stranded DNA self-assembled monolayers (SAMs) on gold by cyclic voltammetry (*CV*) and chronocoulometry (*CC*). Thiol-modified DNA (DNA base amount m = 15, 25 or 35) was assembled on gold in the solution with high ionic strength (1 mol L^{-1}) and the Γ_m of DNA-SAMs was calculated in 10 mmol L^{-1} tris-HCl (pH 7.4) solution with RuHex. The key problem for quantifying the Γ_m was that reduction charge of RuHex adsorbed on DNA-SAMs should be integrated completely. The potential window was from the initial potential E_{ip} to the final potential E_{fp}, which was set up in the *CC* experiment. Results indicated that applying the E_{fp} for −0.5 V vs. SCE in *CC* measurement was sufficient for completely integrating the reduction charge of RuHex adsorbed on DNA-SAMs and could be used to calculate the Γ_m of DNA-SAMs. Saturated adsorption of RuHex on DNA-SAMs was achieved for about 20 μmol L^{-1} RuHex or bigger concentration. Thus, 20 μmol L^{-1} RuHex or bigger concentration should be applied to quantify the Γ_m. In addition, changing the pH value from 7.4 to 6.4 or 8.5 in tris-HCl solution did not influence the quantification of Γ_m. The conclusions provided the important reference for electrochemical quantification of DNA-SAMs on gold rightly.

Keywords: thiol-modified DNA, self-assembled monolayer, gold, electrochemical quantification

1. INTRODUCTION

Thiol-modified DNA self-assembled monolayers (SAMs) on gold were often used for studying the mechanism of electron transfer through DNA chains and designing DNA sensor [1−6]. Surface coverage (Γ_m) was an especially important parameter for quantificationally characterizing thiol-modified DNA-SAMs on gold. Quantificational methods for the Γ_m of DNA-SAMs included

electrochemical techniques (chronocoulometry, *CC*; cyclic voltammetry, *CV*), spectroscopic techniques (surface plasmon resonance, *SPR*; X-ray photoelectron spectroscopy, *XPS*; infrared spectroscopy, *IR*; fluorescence or radioactive tagging) and other techniques (scanning tunneling microscopy, *STM*; quartz crystal microbalance, *QCM*; molecular simulation). Among these, *CC* was the preferred method for measuring the Γ_m of DNA-SAMs due to its simplification, non-destructibility and low cost.

The *CC* for measuring the Γ_m of DNA-SAMs was firstly proposed by Tarlov et al [7]. The Γ_m was calculated based on the reduction charge of RuHex saturatedly adsorbed on DNA phosphate backbone, which was obtained from the difference of *CC* intercepts in *Q* vs. $t^{1/2}$ of *CC* plots in the presence or absence of RuHex [7]. Afterwards, the *CC* method was widely used for calculating the Γ_m.

Table 1. Parameters in *CC* experiments for measuring surface coverage (Γ_m) of DNA-SAMs on gold from literatures

Base amount	pH values	Potential window (V vs. SCE) [(a)]	Concentration of RuHex (μmol L^{-1})	Preconcentration time (s)	Surface coverage Γ_m (10^{-11} mol cm^{-2}) [(b)]	References
51	7.4	0.105 ~ −0.495	5	30	0.18 ~ 0.40	[11]
30	7.5	–	5	900	1.66	[12]
20	7.4	–	12	–	0.88[(c)]	[13]
31	7.4	–	50	60	0.31	[14]
25	7.0	–	50	–[(d)]	1.2	[15]
25	7.4	0.1 ~ −0.4	50	–	0.24 ~ 1.66	[7]
20	7.4	0.1 ~ −0.4	50	–	1.2 ~ 1.8	[16]
20	7.4	0.07 ~ −0.43	50	30	2.0 ± 0.3	[17]
14	7.4	0.1 ~ −0.4	50	–	2.1	[18]
22	7.4	0.12 ~ −0.48	50	≥ 5	3.67	[8]
36	8.0	0.16 ~ −0.55	50	–	–	[19]
25	7.0	0.1 ~ −0.4	80	120	0.27	[20]
18	7.4	0.11 ~ −0.39	100	–	0.22 ~ 1.51	[21]
31	7.4	−0.032 ~ −0.382	500	600	2.16	[22]
28	–	–	50000	–	0.15	[23]

[a]The potential window was from the initial potential E_{ip} to the final potential E_{fp} set up in the *CC* experiments. [b]The buffer solution was 10 mmol L^{-1} tris-$HClO_4$ in Ref. [11] and the buffer solutions in other literatures were 10 mmol L^{-1} tris-HCl. [c]The Γ_m was measured from the integrated reduction charge of RuHex adsorbed on DNA-SAMs by cyclic voltammetry (*CV*)[13]. [d]The equilibrium time for RuHex saturated adsorption on DNA-SAMs was only seconds in the case of 50 μmol L^{-1} RuHex and tens of minutes in the case of 3.5 μmol L^{-1} RuHex[15].

The correctness of Γ_m value calculated by *CC* method had been validated by ^{32}P radioactive tagging technique or electrochemically integrating the oxidation or reduction charge of redox probes covalently linked with DNA [8−10]. We summarized the literature reports [7,8,11–23] about measuring the Γ_m of DNA-SAMs by *CC* method (Table 1) and obtained that the experimental

conditions (potential window, concentration of RuHex, preconcentration time of RuHex, pH of solutions) were not consistent. The E_{fp} of potential window was from −0.4 V to −0.55 V (vs. SCE); the concentration of RuHex was from 5 μmol L^{-1} to 50 mmol L^{-1}; the preconcentration time of RuHex was from 5 s to 900 s and the pH value was from 7.0 to 8.0 (Table 1). The difference of experimental conditions possibly influenced the quantification of Γ_m values and led to the false results. However, the effect of the experimental conditions on the calculation of Γ_m values had not been investigated. It was significant to study the effect of different experimental conditions on the quantification of Γ_m values.

In this article, we fixed the preconcentration time of RuHex for 60 s in *CC* experiments when considering the efficiency of experimental measurement and studied the effect of three experimental conditions (potential window, concentration of RuHex, pH of solution) on the quantification of Γ_m values of DNA-SAMs by *CV* and *CC*. The influencing effect was discussed as compared with literature reports.

2. EXPERIMENTAL SECTION

2.1 Chemicals and apparatus

Thiol-modified single-stranded DNA: HS-$(CH_2)_6$-5'-CTC TCG TAA GCT GTG-3' (15 bases, abbreviated by DNA_{15}), HS-$(CH_2)_6$-5'- CTC TCG TAA GCT GTG ATG GCA CTT G-3' (25 bases, abbreviated by DNA_{25}), HS-$(CH_2)_6$-5'-CTC TCG TAA GCT GTG ATG GCA CTT GAG TCA GTA TG-3' (35 bases, abbreviated by DNA_{35}) were purchased from Takara biotechnology (Dalian) Co. Ltd. Tris(hydroxymethyl)nomethane (tris, ≥99.9%, Sigma), hexaammineruthenium (III) chloride (RuHex, 98%, Aldrich). Other chemicals were analytical grade from Sinopharm Chemical Reagent Co. Ltd. Ultrapure water (18 MΩ cm) was used in all experiments. Experiments were performed using CHI660D (CH Instruments, USA) electrochemical workstation. Three-electrode system was applied, which included a working electrode (polycrystalline gold, 2 mm diameter, CH Instruments), a counter electrode (platinum electrode) and a reference electrode (saturated calomel electrode, SCE). Solutions were deaerated with high-purity nitrogen and nitrogen atmosphere was maintained over the solutions.

2.2 Pretreatment of gold electrodes

The gold electrodes were hand-polished on microcloth pads with alumina slurries (1.0, 0.3 and 0.05 μm), sonicated in ultrapure water for 10 min, then dipped into newly prepared piranha solution (Concentrated H_2SO_4/30% H_2O_2 = 3/1) for 5 min, finally sonicated in ultrapure water for 15 min and electrochemically polished in 0.5 mol L^{-1} H_2SO_4 solution from −0.4 to +1.5 V at 0.1 V s^{-1} until reproducible voltammograms were obtained. The gold electrodes were then rinsed with ultrapure water and blown to dry with 99.999% high-purity nitrogen. The real surface area *A* of gold electrodes was determined by integrating the charge of reduction peak with 400 μC cm^{-2} as a monolayer of chemisorbed oxygen [5,6].

2.3 Preparation of SAMs

The pretreated gold electrode surface was covered with a 30 μL drop of assembly solutions, and then enveloped with a 0.5 mL centrifugal tube to protect the solution from evaporation. The assembly solution was 10 μmol L^{-1} thiol-modified single-stranded DNA (ss-DNA), 1 mol L^{-1} $MgCl_2$ and 5 mmol L^{-1} sodium phosphate buffer solution (pH 7.0) and the assembly time was 24 h. When the assembly finished, the electrodes were taken out and rinsed with 50 mmol L^{-1} NaCl and 5 mmol L^{-1} phosphate buffer solution (pH 7.0) prior to use.

2.4 Electrochemical characterization

Cyclic voltammetry (*CV*) was used to investigate the electrochemical response of RuHex in 10 mmol L^{-1} tris-HCl solution (pH 6.4, 7.4 or 8.5) from 0.1 V to −0.5 V at a scan rate of 0.1 V s^{-1}.

According to Tarlov's method [7], chronocoulometry (*CC*) was used to calculate the surface coverage (Γ_m) of DNA-SAMs on gold. The experiments were performed in 10 mmol L^{-1} tris-HCl solution (pH 6.4, 7.4 or 8.5) and 100 μmol L^{-1} RuHex. The preconcentration time of RuHex was fixed as 60 s. The potential window was from E_{ip} (+100 mV) to E_{fp} (–400 mV, –500 mV or –550 mV) and the pulse period was 500 ms. The Equation for calculating the Γ_m was:

$$\Gamma_m = \frac{z\Gamma_o}{m} \qquad (1)$$

Where z was the charge of RuHex (z = 3), m was the number of bases in DNA (m = 15, 25, 35 for DNA_{15}, DNA_{25}, DNA_{35} respectively) and Γ_o was the surface density of adsorbed RuHex (mol cm^{-2}).

Γ_o was calculated from the integrated Cottrell equation 2 where D_o was the diffusion coefficient (cm^2 s^{-1}), C_o was the bulk concentration of RuHex (mol cm^{-3}), Q_{dl} was the double-layer charging charge (C) and Q_{ad} was the reduction charge of surface-adsorbed RuHex. The Q_{ad} was equal to $nFA\Gamma_o$. In the presence of RuHex, the *CC* intercept of Q vs. $t^{1/2}$ at t = 0 was the sum of Q_{dl} and the reduction charge of surface-adsorbed RuHex ($nFA\Gamma_o$); In the absence of RuHex, the *CC* intercept of Q vs. $t^{1/2}$ at t = 0 was the Q_{dl}. Γ_o was obtained from the difference of the two intercepts.

$$Q = \frac{2nFAD_o^{1/2}C_o t^{1/2}}{\pi^{1/2}} + Q_{dl} + Q_{ad} \quad (Q_{ad} = nFA\Gamma_o) \qquad (2)$$

3. RESULTS AND DISCUSSION

3.1 Effect of the potential window

The measurement of Γ_m values by *CC* experiment was based on completely integrating the reduction charge of RuHex saturatedly adsorbed on DNA phosphate backbone. Complete integral depended on the potential window in *CC* experiment, which included the initial potential (E_{ip}) and the final potential (E_{fp}). The E_{ip} and E_{fp} might be obtained from the reduction voltammetric wave of

RuHex adsorbed on DNA-SAMs by *CV* experiment. The E_{ip} should be bigger than the beginning reduction potential and the E_{fp} should be smaller than the ending reduction potential for the reduction voltammetric wave of RuHex adsorbed on DNA-SAMs, as indicated by the arrows in Figure 1A.

Figure 1A showed the *CV* plots of DNA_{15}-SAMs in 10 mmol L^{-1} tris-HCl (pH 7.4) and 100 μmol/L RuHex. In the presence of RuHex, two reduction peaks appeared at about −0.190 V for E_{pc}(I) and −0.316 V for E_{pc}(II), which were ascribed to the reduction of RuHex freely in solution or adsorbed on DNA_{15} phosphate backbone respectively[7,8,13,15]. The reduction wave at E_{pc}(II) received special attention because its integral charge could be used to calculate the Γ_m. From the *CV* plots (Figure 1A), we obtained that the E_{ip} might be bigger than –0.05 V and the E_{fp} should be equal or smaller than –0.50 V in *CC* measurement for exploring the reduction of RuHex freely in solution or adsorbed on DNA phosphate backbone fully. In order to investigate the effect of E_{fp} on the calculation of Γ_m, we fixed the E_{ip} as 0.1 V and adjusted the E_{fp} (−0.4, −0.5 or −0.55 V) to perform the *CC* measurement (Figure 1B). Based on the intercept charge of *CC* plots, Γ_m was calculated to be 4.2×10^{-11} mol cm^{-2} for −0.4 V E_{fp} and 5.6×10^{-11} mol cm^{-2} for −0.5 or −0.55 V E_{fp}. Thus, –0.5 V was sufficient as the E_{fp} to quantify the Γ_m of DNA_{15}-SAMs.

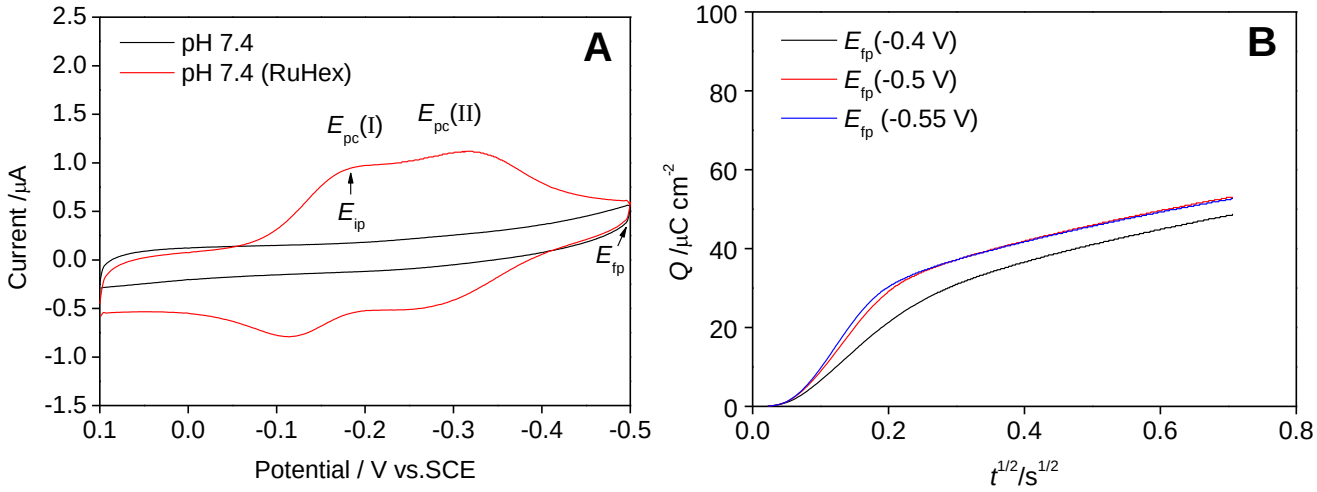

Figure 1. (A) *CV* plots of DNA_{15}-SAMs on gold in 10 mmol L^{-1} tris-HCl (pH 7.4) and 100 μmol L^{-1} RuHex. The 10 mmol L^{-1} tris-HCl (pH 7.4) solution was abbreviated as pH 7.4; the 100 μmol L^{-1} RuHex with 10 mmol L^{-1} tris-HCl (pH 7.4) solution was abbreviated as pH 7.4 (RuHex). (B) *CC* plots (background subtraction) of DNA-modified gold electrode in 10 mmol L^{-1} tris-HCl (pH 7.4) and 100 μmol L^{-1} RuHex. The E_{fp} was –0.4 V, –0.5 V and –0.55 V respectively in *CC* measurement.

Base amount *m* of thiol-modified DNA from literature reports usually ranged from 15 to 35. Then, whether –0.5 V was sufficient as the E_{fp} to calculate the Γ_m of DNA_{25} or DNA_{35}-SAMs was not known. Thus, we investigated the electrochemical behaviors of RuHex in DNA_{25} and DNA_{35}-SAMs. Figure 2 showed the *CV* and *CC* plots of DNA_{15}, DNA_{25} and DNA_{35}-SAMs in 10 mmol L^{-1} tris-HCl (pH 7.4) and 100 μmol L^{-1} RuHex. The *CV* plots (Figure 2A) for all DNA-SAMs appeared two reduction peaks including E_{pc}(I) and E_{pc}(II). The E_{pc}(I) was the diffusion wave of RuHex through DNA-SAMs and located at about 0.190 V for DNA_{15}, DN_{A25} or DNA_{35}-SAMs; the E_{pc}(II) for the

reduction of adsorbed RuHex on DNA backbone was negatively shifted to −0.330 V and −0.347 V with increasing DNA base amount *m* from 15 to 25 or 35. Furthermore, the peak current of E_{pc}(II) in Figure 2A (as well as the integral charge of RuHex adsorbed on DNA backbone in Figure 2B) increased with increasing DNA base amount *m* from 15 to 35. It was understandable because DNA_{35} had more phosphate group and could adsorb more RuHex than DNA_{15} or DNA_{25}. Similarly, we adjusted the E_{fp} (−0.4, −0.5 or −0.55 V) for measuring the Γ_m by *CC*. Only the *CC* plots with −0.5 V E_{fp} were showed in Figure 2B. The experimental results showed that the Γ_m was smaller for −0.4 V E_{fp} and almost the same for −0.5 or −0.55 V E_{fp} in DNA_{25} and DNA_{35}-SAMs. For DNA_{25}-SAMs or DNA_{35}-SAMs, Γ_m was 4.2×10^{-11} mol cm^{-2} for −0.4 V E_{fp} and 6.5×10^{-11} mol cm^{-2} for −0.5 or –0.55 V E_{fp}. To sum up, −0.5 V was enough as the E_{fp} for quantifying the Γ_m of DNA-SAMs with base amounts up to 35.

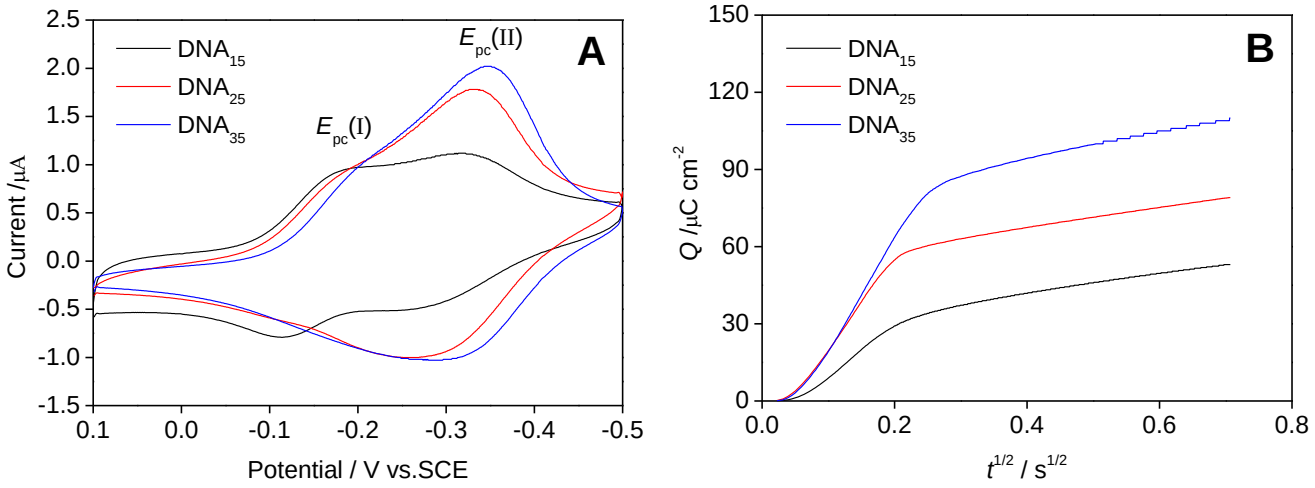

Figure 2. (A) *CV* plots of DNA_{15}, DNA_{25} and DNA_{35}-SAMs on gold in 10 mmol L^{-1} tris-HCl (pH 7.4) with 100 μmol L^{-1} RuHex; (B) *CC* plots (background subtraction) of DNA-modified gold electrode in 10 mmol L^{-1} tris-HCl (pH 7.4) with 100 μmol L^{-1} RuHex. In order to compare the *CV* and *CC* plots of DNA_{15}-SAMs with DNA_{25} and DNA_{35}-SAMs, we also included the *CV* and *CC* plots of DNA_{15}-SAMs in Figure 2, which were the same as those in Figure 1. The E_{fp} was −0.5 V in *CC* measurement.

Literatures reported that E_{pc}(II) negatively shifted with the increase of Γ_m [24]. Our experiment (Figure 2) obtained that E_{pc}(II) differed much bigger (17 mV) though Γ_m of DNA_{25} and DNA_{35}-SAMs was the same. Thus, the difference of Γ_m was not the essential reason for E_{pc}(II) shift. Furthermore, we performed the *CV* experiments of DNA_{25}-SAMs on gold in the solution with different RuHex concentrations (Figure 3). It was found that E_{pc}(II) was almost constant with increasing RuHex concentrations, consistent with literature report[24]. Thus, the shift of E_{pc}(II) was not due to different amount of RuHex adsorbed on DNA-SAMs. We considered that the negative shift of E_{pc}(II) might be due to the increasing hindrance for pumping $tris^+$ cation into DNA-SAMs or the increasing binding constants for oxidized and reduced RuHex (i.e., $Ru(NH_3)_6^{3+}$ and $Ru(NH_3)_6^{2+}$) in DNA-SAMs [24,25] with higher Γ_m and longer DNA chains.

Table 1 showed that the E_{ip} from literatures was at about 0 or 0.1 V, which was suitable for *CC* measurement. However, the E_{fp} ranged from −0.38 V to −0.55 V. The bigger E_{fp} than −0.5 V in some literatures (Table 1) might be also reasonable because the Γ_m of DNA-SAMs studied by the literatures was usually smaller. In our experiment, 1 mol L^{-1} $MgCl_2$ was used to assemble the DNA-SAMs on gold. Mg^{2+} neutralized the negative charge of DNA phosphate backbone much better than monovalent cations (e.g. Na^+) and could permit the close-packing of DNA-SAMs on gold more. The maximal Γ_m of DNA_{15}, DNA_{25} or DNA_{35}-SAMs on gold might be obtained in our experiment. Therefore, –0.5 V was suitable as the E_{fp} for integrating the reduction charge of RuHex adsorbed on DNA-SAMs (base amount $N \leq 35$) with different Γ_m completely.

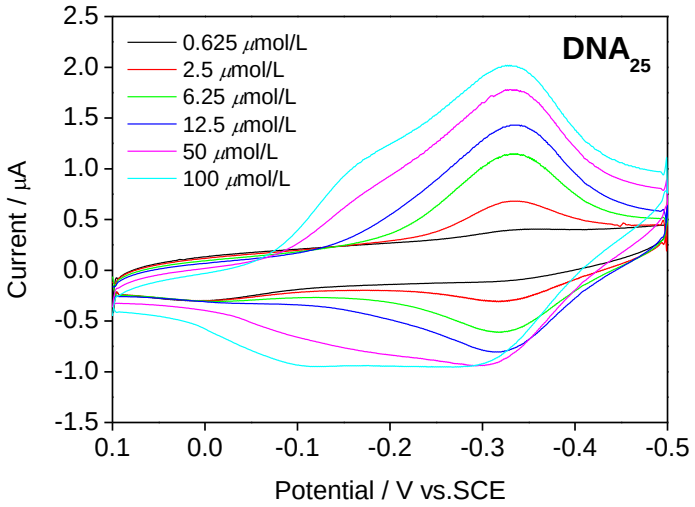

Figure 3. *CV* plots of DNA_{25}-SAMs on gold at 0.1 V s^{-1} in 10 mmol L^{-1} tris-HCl (pH 7.4) with different RuHex concentrations from 0.625 to 100 μmol L^{-1}.

3.2 Effect of RuHex concentration

Figure 4A showed the adsorption isotherm of RuHex on DNA_{15}, DNA_{25} or DNA_{35}-SAMs on gold. For DNA_{15}, DNA_{25} or DNA_{35}-SAMs assembled for 24 h in 1 mol L^{-1} $MgCl_2$ solution, adsorption saturation of RuHex was achieved with about 20 μmol L^{-1} or larger concentration; For DNA_{15}-SAMs assembled for 15 min in 1 mol L^{-1} $MgCl_2$ solution, adsorption saturation of RuHex was achieved with about 7 μmol L^{-1}. Furthermore, we analyzed the adsorption isotherm of RuHex based on Equation 3 from the Langmuir adsorption isotherm model (Figure 4B). The C was the concentration of RuHex, Q_{ad} was the reduction charge of adsorption RuHex with different concentrations, Q_{sat} was the reduction charge of RuHex with saturated adsorption, K was the association constant of RuHex with DNA. The $C/Q_{ad} \sim C$ plot (Figure 4B) had well linear relationship (R^2 = 0.9969 ~ 0.9998), which accorded with the Langmuir adsorption model. Based on the Q_{sat}, the Γ_m of DNA_{15}-SAMs was calculated to be 3.1×10^{-11} and 6.0×10^{-11} mol cm^{-2} for 15 min or 24 h assembly; the Γ_m of DNA_{25}-SAMs and DNA_{35}-SAMs was 5.6×10^{-11} and 4.6×10^{-11} mol cm^{-2} respectively.

$$\frac{C}{Q_{ad}} = \frac{C}{Q_{sat}} + \frac{1}{KQ_{sat}} \qquad (3)$$

The adsorption isotherm of RuHex on DNA-SAMs had been reported in literatures. Yu et al obtained that saturated adsorption of RuHex was achieved with 5 μmol L^{-1} RuHex for Γ_m as 8.8×10^{-12} mol cm^{-2} (20 bases for DNA-SAMs) [13] and about 7 μmol L^{-1} for Γ_m as 3.67×10^{-11} mol cm^{-2} (22 bases for DNA-SAMs) [8]; Tarlov et al [7,26] and Bartlett et al [20] obtained that saturated adsorption of RuHex was achieved with about 40 μmol L^{-1} RuHex (25 bases for DNA-SAMs) and 80 μmol L^{-1} RuHex for Γ_m as 2.7×10^{-12} mol cm^{-2} (25 bases for DNA-SAMs) respectively. The reason arousing the different concentrations for RuHex saturated adsorption was not known. However, it was assured that the concentration for RuHex adsorption saturation in DNA-SAMs was dependent of Γ_m. Saturated adsorption of RuHex could be achieved with lower concentration of RuHex for DNA-SAMs with lower Γ_m. As Figure 4 showed that adsorption saturation of RuHex for DNA_{15}-SAMs with Γ_m (3.1×10^{-11} mol cm^{-2}) was achieved with about 7 μmol L^{-1}, smaller than 20 μmol L^{-1} RuHex for DNA_{15}-SAMs with Γ_m (6.0×10^{-11} mol cm^{-2}). Furthermore, the preconcentration time had not been reported in some literatures, which possibly influenced the concentration of RuHex saturated adsorption. From our experiment, it was concluded that 20 μmol L^{-1} or larger concentration of RuHex was sufficient for neutralizing the negative charge of DNA-SAMs and could be used to calculate the Γ_m. Most of RuHex concentrations for *CC* measurement from literature reports (Table 1) were bigger than 20 μmol L^{-1}, consistent with our experimental results. The smaller RuHex concentrations for *CC* measurement in literatures [11–13] might be due to lower Γ_m for DNA-SAMs studied.

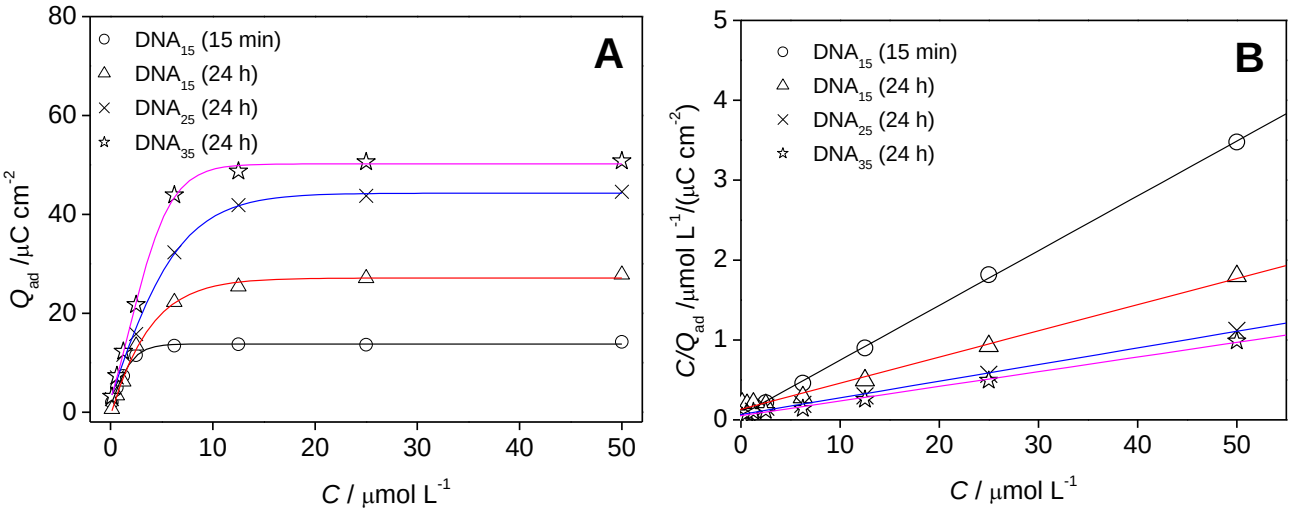

Figure 4. (A) Adsorption isotherm of RuHex on DNA_{15}-SAMs assembled for 15 min or 24 h and on DNA_{25}-SAMs or DNA_{35}-SAMs assembled for 24 h. (B) The plots of C/Q_{ad} with C, where C was the concentration of RuHex in solution and Q_{ad} was the reduction charge of adsorbed RuHex on DNA-SAMs. The Q_{ad} was measured by *CC* experiment with E_{ip} and E_{fp} as 0.1 and −0.5 V respectively.

3.3 Effect of pH

Table 1 showed that the pH values of tris buffer solutions from literature reports were from 7.0 to 8.0. Thus, we studied the effect of different pH values (pH 6.4, 7.4, 8.5) on the quantification of

DNA-SAMs. Figure 5 showed the *CV* plots of DNA_{15}-SAMs in 10 mmol L^{-1} tris-HCl (pH 6.4, 7.4, 8.5) and 100 μmol L^{-1} RuHex. The *CV* plots in the solutions with different pH values were almost superposable, which indicated the same effect of pH values on the calculation of Γ_m. The pK_a of phosphate group for DNA molecule was close to 1.0[27], thus it would be negatively charged in the solutions with pH values (6.4, 7.4, 8.5). The adsorption quantity of RuHex on DNA backbone might be the same for pH 6.4, 7.4 or 8.5. So the calculation of Γ_m values was independent of pH (6.4, 7.4 or 8.5).

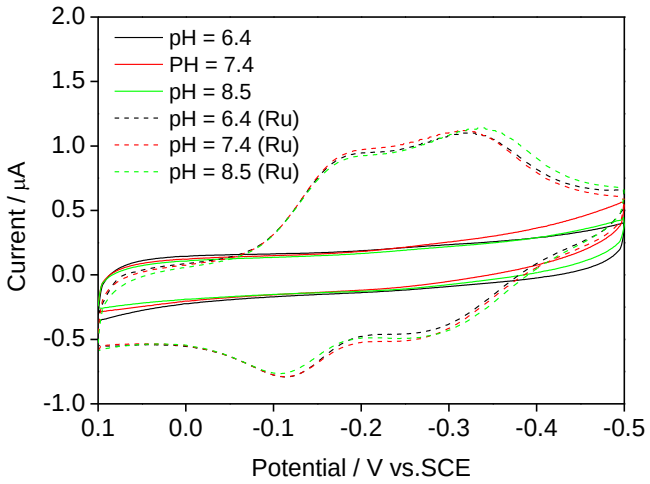

Figure 5. *CV* plots at 0.1 V s^{-1} of DNA_{15}-SAMs in 10 mmol L^{-1} tris-HCl (pH 6.4, 7.4, 8.5) and 100 μmol L^{-1} RuHex. In the Figure, the 10 mmol L^{-1} tris-HCl (pH 6.4, 7.4, 8.5) solutions were abbreviated as pH 6.4, pH 7.4 and pH 8.5 respectively; the 10 mmol L^{-1} tris-HCl (pH 6.4, 7.4, 8.5) with 100 μmol L^{-1} RuHex solutions were abbreviated as pH 6.4 (RuHex), pH 7.4 (RuHex) and pH 8.5 (RuHex) respectively.

4. CONCLUSIONS

Effect of potential window, concentration of RuHex and pH on electrochemical quantification of DNA-SAMs was investigated by *CV* and *CC*. The E_{fp} (−0.5 V) and 20 μmol L^{-1} RuHex were sufficient for calculating the Γ_m of DNA-SAMs with base amount up to 35. Adjusting the pH values (pH 6.4, 7.4, 8.5) in 10 mmol L^{-1} tris-HCl solution did not influence the quantification of Γ_m. The conclusions might provide the reference for rightly quantifying the Γ_m of DNA-SAMs, which was significant for studying DNA electrochemical sensors (e.g., calculating the hybridization efficiency or hybridization density based on the Γ_m).

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