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

Zhiguo Li*, Lingling Zhang, Guoe Cheng, Xiaoling Yang, Junbin Zhou, and Yufeng Chen

School of Chemistry Science and Technology, Institute of Physical Chemistry, Zhanjiang Normal University, Zhanjiang 524048, Guangdong, China

E-mail: zgli_zjnu@163.com

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In this article we investigated the effect of potential window, concentration of hexaaammineruthenium (III) chloride (RuHex) and pH value on quantifying surface coverage ($\Gamma_m$) 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 ($1 \text{ mol L}^{-1}$) and the $\Gamma_m$ of DNA-SAMs was calculated in $10 \text{ mmol L}^{-1}$ tris-$\text{HCl}$ (pH 7.4) solution with RuHex. The key problem for quantifying the $\Gamma_m$ 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 $-0.5 \text{ V vs. SCE}$ in CC measurement was sufficient for completely integrating the reduction charge of RuHex adsorbed on DNA-SAMs and could be used to calculate the $\Gamma_m$ of DNA-SAMs. Saturated adsorption of RuHex on DNA-SAMs was achieved for about $20 \mu\text{mol L}^{-1}$ RuHex or bigger concentration. Thus, $20 \mu\text{mol L}^{-1}$ RuHex or bigger concentration should be applied to quantify the $\Gamma_m$. 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 $\Gamma_m$. 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 ($\Gamma_m$) was an especially important parameter for quantificationally characterizing thiol-modified DNA-SAMs on gold. Quantificational methods for the $\Gamma_m$ 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 $\Gamma_m$ of DNA-SAMs due to its simplification, non-destructibility and low cost.

The CC for measuring the $\Gamma_m$ of DNA-SAMs was firstly proposed by Tarlov et al [7]. The $\Gamma_m$ 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 $\Gamma_m$.

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

<table>
<thead>
<tr>
<th>Base amount</th>
<th>pH values</th>
<th>Potential window (V vs. SCE)</th>
<th>Concentration of RuHex (µmol L$^{-1}$)</th>
<th>Preconcentration time (s)</th>
<th>Surface coverage $\Gamma_m$ (10$^{-11}$ mol cm$^{-2}$)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>7.4</td>
<td>0.105 ~ −0.495</td>
<td>5</td>
<td>30</td>
<td>0.18 ~ 0.40</td>
<td>[11]</td>
</tr>
<tr>
<td>30</td>
<td>7.5</td>
<td>−</td>
<td>5</td>
<td>900</td>
<td>1.66</td>
<td>[12]</td>
</tr>
<tr>
<td>20</td>
<td>7.4</td>
<td>−</td>
<td>12</td>
<td>−</td>
<td>0.88(c)</td>
<td>[13]</td>
</tr>
<tr>
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<td>−</td>
<td>50</td>
<td>60</td>
<td>0.31</td>
<td>[14]</td>
</tr>
<tr>
<td>25</td>
<td>7.0</td>
<td>−</td>
<td>50</td>
<td>−(d)</td>
<td>1.2</td>
<td>[15]</td>
</tr>
<tr>
<td>25</td>
<td>7.4</td>
<td>0.1 ~ −0.4</td>
<td>50</td>
<td>−</td>
<td>0.24 ~ 1.66</td>
<td>[7]</td>
</tr>
<tr>
<td>20</td>
<td>7.4</td>
<td>0.1 ~ −0.4</td>
<td>50</td>
<td>−</td>
<td>1.2 ~ 1.8</td>
<td>[16]</td>
</tr>
<tr>
<td>20</td>
<td>7.4</td>
<td>0.07 ~ −0.43</td>
<td>50</td>
<td>30</td>
<td>2.0 ± 0.3</td>
<td>[17]</td>
</tr>
<tr>
<td>14</td>
<td>7.4</td>
<td>0.1 ~ −0.4</td>
<td>50</td>
<td>≥ 5</td>
<td>3.67</td>
<td>[8]</td>
</tr>
<tr>
<td>22</td>
<td>7.4</td>
<td>0.12 ~ −0.48</td>
<td>50</td>
<td>−</td>
<td>2.1</td>
<td>[18]</td>
</tr>
<tr>
<td>36</td>
<td>8.0</td>
<td>0.16 ~ −0.55</td>
<td>50</td>
<td>−</td>
<td>3.67</td>
<td>[19]</td>
</tr>
<tr>
<td>25</td>
<td>7.0</td>
<td>0.1 ~ −0.4</td>
<td>80</td>
<td>120</td>
<td>0.27</td>
<td>[20]</td>
</tr>
<tr>
<td>18</td>
<td>7.4</td>
<td>0.11 ~ −0.39</td>
<td>100</td>
<td>−</td>
<td>0.22 ~ 1.51</td>
<td>[21]</td>
</tr>
<tr>
<td>31</td>
<td>7.4</td>
<td>−0.032 ~ −0.382</td>
<td>500</td>
<td>600</td>
<td>2.16</td>
<td>[22]</td>
</tr>
<tr>
<td>28</td>
<td>−</td>
<td>−</td>
<td>500000</td>
<td>−</td>
<td>0.15</td>
<td>[23]</td>
</tr>
</tbody>
</table>

The potential window was from the initial potential $E_{ip}$ to the final potential $E_{fp}$ set up in the CC experiments. 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. The $\Gamma_m$ was measured from the integrated reduction charge of RuHex adsorbed on DNA-SAMs by cyclic voltammetry (CV)[13]. 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 $\Gamma_m$ 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 $\Gamma_m$ 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 $E_p$ 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 $\Gamma_m$ values and led to the false results. However, the effect of the experimental conditions on the calculation of $\Gamma_m$ values had not been investigated. It was significant to study the effect of different experimental conditions on the quantification of $\Gamma_m$ 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 $\Gamma_m$ 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$_2$SO$_4$/30% H$_2$O$_2$ = 3/1) for 5 min, finally sonicated in ultrapure water for 15 min and electrochemically polished in 0.5 mol L$^{-1}$ H$_2$SO$_4$ 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\(\text{m}\)) 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 \(E_{\text{i.p.}}(+100 \text{ mV})\) to \(E_{\text{f.p.}}(−400 \text{ mV}, −500 \text{ mV} \text{ or } −550 \text{ mV})\) and the pulse period was 500 ms. The Equation for calculating the Γ\(\text{m}\) was:

\[
Γ_{\text{m}} = \left( \frac{zFΓ_0}{m} \right)
\]

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 \(Γ_0\) was the surface density of adsorbed RuHex (mol cm\(^{-2}\)).

\(Γ_0\) was calculated from the integrated Cottrell equation 2 where \(D_0\) was the diffusion coefficient (cm\(^2\) s\(^{-1}\)), \(C_0\) was the bulk concentration of RuHex (mol cm\(^{-3}\)), \(Q_{\text{dl}}\) was the double-layer charging charge (C) and \(Q_{\text{ad}}\) was the reduction charge of surface-adsorbed RuHex. The \(Q_{\text{ad}}\) was equal to \(nFAΓ_0\).

In the presence of RuHex, the CC intercept of \(Q\) vs. \(t^{1/2}\) at \(t = 0\) was the sum of \(Q_{\text{dl}}\) and the reduction charge of surface-adsorbed RuHex \((nFAΓ_0)\); In the absence of RuHex, the CC intercept of \(Q\) vs. \(t^{1/2}\) at \(t = 0\) was the \(Q_{\text{dl}}\). \(Γ_0\) was obtained from the difference of the two intercepts.

\[
Q = \frac{2nFAD_0^{1/2}C_0t^{1/2}}{\pi^{1/2}} + Q_{\text{dl}} + Q_{\text{ad}} \quad (Q_{\text{ad}} = nFAΓ_0)
\]

3. RESULTS AND DISCUSSION

3.1 Effect of the potential window

The measurement of \(Γ_{\text{m}}\) values by CC experiment was based on completely integrating the reduction charge of RuHex saturately adsorbed on DNA phosphate backbone. Complete integral depended on the potential window in CC experiment, which included the initial potential \((E_{\text{i.p.}})\) and the final potential \((E_{\text{f.p.}})\). The \(E_{\text{i.p.}}\) and \(E_{\text{f.p.}}\) might be obtained from the reduction voltammetric wave of
RuHex adsorbed on DNA-SAMs by CV experiment. The $E_{fp}$ should be bigger than the beginning reduction potential and the $E_{ip}$ 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 $\Gamma_m$. 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 $\Gamma_m$, 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, $\Gamma_m$ was calculated to be $4.2 \times 10^{-11}$ mol cm$^{-2}$ for $-0.4$ V $E_{fp}$ and $5.6 \times 10^{-11}$ 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 $\Gamma_m$ of DNA$_{15}$-SAMs.

![Figure 1](image)

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 $\Gamma_m$ 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$_{15}$, DNA$_{25}$ or DNA$_{35}$-SAMs; the $E_{pc}(II)$ for the
reduction of adsorbed RuHex on DNA backbone was negatively shifted to $-0.330 \text{ V}$ and $-0.347 \text{ V}$ with increasing DNA base amount $m$ from 15 to 25 or 35. Furthermore, the peak current of $E_{pc}(\text{II})$ 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 $-0.55 \text{ V}$) for measuring the $\Gamma_m$ by CC. Only the CC plots with $-0.5 \text{ V}$ $E_{fp}$ were showed in Figure 2B. The experimental results showed that the $\Gamma_m$ was smaller for $-0.4 \text{ V}$ $E_{fp}$ and almost the same for $-0.5$ or $-0.55 \text{ V}$ $E_{fp}$ in DNA$_{25}$ and DNA$_{35}$-SAMs. For DNA$_{25}$-SAMs or DNA$_{35}$-SAMs, $\Gamma_m$ was $4.2 \times 10^{-11} \text{ mol cm}^{-2}$ for $-0.4 \text{ V}$ $E_{fp}$ and $6.5 \times 10^{-11} \text{ mol cm}^{-2}$ for $-0.5$ or $-0.55 \text{ V}$ $E_{fp}$. To sum up, $-0.5 \text{ V}$ was enough as the $E_{fp}$ for quantifying the $\Gamma_m$ of DNA-SAMs with base amounts up to 35.

![Graph A](image1.png)

![Graph B](image2.png)

**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 $\mu$mol 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 $\mu$mol 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 $-0.5 \text{ V}$ in CC measurement.

Literatures reported that $E_{pc}(\text{II})$ negatively shifted with the increase of $\Gamma_m$ [24]. Our experiment (Figure 2) obtained that $E_{pc}(\text{II})$ differed much bigger (17 mV) though $\Gamma_m$ of DNA$_{25}$ and DNA$_{35}$-SAMs was the same. Thus, the difference of $\Gamma_m$ was not the essential reason for $E_{pc}(\text{II})$ 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 $E_{pc}(\text{II})$ was almost constant with increasing RuHex concentrations, consistent with literature report[24]. Thus, the shift of $E_{pc}(\text{II})$ was not due to different amount of RuHex adsorbed on DNA-SAMs. We considered that the negative shift of $E_{pc}(\text{II})$ 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 $\Gamma_m$ 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 $E_{ip}$ ranged from $-0.38$ to $-0.55$ V. The bigger $E_{ip}$ than $-0.5$ V in some literatures might be also reasonable because the $\Gamma_m$ 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 $\Gamma_m$ 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 $E_{ip}$ for integrating the reduction charge of RuHex adsorbed on DNA-SAMs (base amount $N \leq 35$) with different $\Gamma_m$ 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 ($R^2 = 0.9969 \sim 0.9998$), which accorded with the Langmuir adsorption model. Based on the $Q_{sat}$, the $\Gamma_m$ of DNA$_{15}$-SAMs was calculated to be $3.1 \times 10^{-11}$ and $6.0 \times 10^{-11}$ mol cm$^{-2}$ for 15 min or 24 h assembly; the $\Gamma_m$ of DNA$_{25}$-SAMs and DNA$_{35}$-SAMs was $5.6 \times 10^{-11}$ and $4.6 \times 10^{-11}$ mol cm$^{-2}$ respectively.
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 \(\Gamma_m\) as 8.8 \(\times\) 10\(^{-12}\) mol cm\(^{-2}\) (20 bases for DNA-SAMs) [13] and about 7 μmol L\(^{-1}\) for \(\Gamma_m\) as 3.67 \(\times\) 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 \(\Gamma_m\) as 2.7 \(\times\) 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 \(\Gamma_m\). Saturated adsorption of RuHex could be achieved with lower concentration of RuHex for DNA-SAMs with lower \(\Gamma_m\). As Figure 4 showed that adsorption saturation of RuHex for DNA\(_{15}\)-SAMs with \(\Gamma_m\) (3.1 \(\times\) 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 \(\Gamma_m\) (6.0 \(\times\) 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 \(\Gamma_m\). 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 \(\Gamma_m\) for DNA-SAMs studied.

\[
\frac{C}{Q_{ad}} = \frac{C}{Q_{sat}} + \frac{1}{KQ_{sat}}
\]

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 $\Gamma_m$. 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 $\Gamma_m$ values was independent of pH (6.4, 7.4 or 8.5).

![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.](image)

**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 $\Gamma_m$ 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 $\Gamma_m$. The conclusions might provide the reference for rightly quantifying the $\Gamma_m$ of DNA-SAMs, which was significant for studying DNA electrochemical sensors (e.g., calculating the hybridization efficiency or hybridization density based on the $\Gamma_m$).

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References


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