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ZrO_2 gel-derived DNA-modified electrode and the effect of lanthanide on its electron transfer behavior

Shou-Qing Liu, Jing-Juan Xu, Hong-Yuan Chen*

Laboratory of Mesoscopic Materials and Chemistry, Department of Chemistry, Institute of Analytical Science, Nanjing University, Nanjing 210093, China

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Abstract

A new method of immobilizing deoxyribonucleic acid (DNA) was developed based on sol–gel technique, the resulting DNA-modified electrode was characterized with the cyclic voltammetry. The electrode was used to study the electron transfer of DNA in 1.0 mM potassium ferricyanide system in different concentrations of lanthanum(III), europium(III), and calcium(II). The heterogeneous rate constants of the reduction of $Fe(CN)_6^{3-}$ with and without the above cations were calculated by Tafel equation. The results show that lanthanide ions can increase the electron transfer rate much more than calcium ion. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: DNA immobilization; Zirconia gel; Modified electrode; Lanthanide; Electron transfer

1. Introduction

The interaction between deoxyribonucleic acid (DNA) and other molecules is an important fundamental issue in life sciences which is related to the replication and transcription of DNA in vivo, mutation of genes and related variations of species in character, action mechanisms of some DNA-targeted drugs, origins of some diseases, etc. Great attention was paid to the interaction between DNA and some coordination complexes with redox activity in electrochemistry. For example, Bard studied the voltammetric behaviors of DNA with $Co(phen)_3^{3+}$, $Co(bpy)_3^{3+}$, $Fe(phen)_3^{2+}$ in solution [1,2], immobilized DNA on an aluminum(III) alkanebisphosphonate thin film and detected ss-DNA by electrochemical luminescence [3,4]. Pang and Abruna [5] developed a microscale and surface-based method for investigating the interaction of DNA with Co(III)-tris-chelated complexes and calculated the binding site size, binding constant, etc. The interaction of DNA with other probe molecules [6–8] was also reported.

Sol–gel technique was rapidly developed owing to its applications in electroanalysis, biosensing, electrocatalysis, gas electrodes and energy storage cells. The technique can combine organic molecules with inorganic materials to form organic–inorganic hybrids [9]. Since the biomolecules contains organic groups, the hybrid can be extended to biomolecule-inorganic hybrids, such as the immobilization of glucose oxidase in a silicate composite [10], preparation of immunosensor [11], etc. Zirconia is an inorganic oxide with the thermal stability, chemical inertness, lack of toxicity [12] and affinity for the groups containing oxygen [13,14], so it is an ideal candidate of materials for immobilization of biomolecules with oxygen groups.

Lanthanide and its complexes were often used as a structure and function probe of DNA [15–19] and used as a hydrolytic enzyme for the cleavage of nucleic acid for designing the gene drugs [20]. It is obvious that lanthanide can interact with DNA, however, to the best of our knowledge, the impact of lanthanide ions on the electron transfer of DNA has not been reported. Here, we present a new method of immobilization of DNA, characterize it by cyclic voltammetry and investigate the effect of lanthanide on the electron transfer behavior of DNA.

2. Experimental

2.1. Reagents and apparatus

Calf thymus DNA was obtained from Sino-American Biotechnical. All chemicals (zirconium oxychloride, potassium ferricyanide from Second Chemical Reagent Factory of Shanghai) were of analytical reagent grade without further

* Corresponding author. Tel.: +86-25-3592409; fax: +86-25-3594862.
E-mail address: hychen@netra.nju.edu.cn (H.-Y. Chen).

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purification. All solutions were prepared with doubly distilled water. A glassy carbon (GC) disk electrode of 3.0-mm diameter (from EG&G in USA) was used as a matrix of working electrode. It was polished to a mirror on sand paper and then 0.3- to 0.05-μm alumina. The bare electrode was rinsed with water. Finally, it was cleaned thoroughly in an ultrasonic cleaner with doubly distilled water.

The cyclic voltammetric (CV) experiments were carried out with a CHI660A electrochemical workstation (CH Instrument, Texas, USA) in a three-electrode system at room temperature (about 25 °C). A ZrO_2 gel-derived DNA-modified electrode was used as the working electrode, and a platinum sheet as the counter electrode, a saturated calomel electrode (SCE) as the reference electrode. All potentials are reported vs. SCE.

2.2. Preparation of ZrO_2 gel

ZrO_2 gel was prepared by means of the hydrolysis [21]: 0.10 M $NH3_j{\cdot}H_2O$ was dropped gradually (1 drop per 6 s) into a 45.0 ml 1.0×10^{-2} M $ZrOCl_2{\cdot}8H_2O$ solution under the stirred condition, A ZrO_2 gel can be seen as a white floccus, the dropping was stopped at pH ≈ 9.5. The top light aqueous solution was poured and the floccus at the bottom was remained for the further separation.

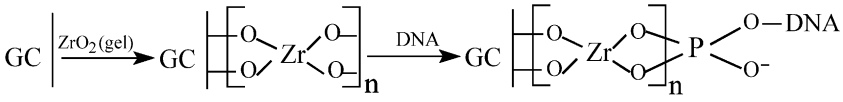
The resulting ZrO_2 gel was transferred into a 10 ml centrifugal tube, then the centrifugal separation was performed for five times at a rate of 2500 cycles per min. For 3 min, before each centrifugal separation, the gel was washed with 8 ml pH 7.0 doubly distilled water. The final gel contained 0.30 M ZrO_2 (percentage of weight of ZrO_2 is 3.69%) with pH 7.0; XRD measurements confirmed that it is amorphous [12,21].

2.3. Preparation of ZrO_2 gel-derived DNA-modified electrode

A 5.0 μl pH 7.0 ZrO_2 gel was added on a bare GC electrode in air for 2 h, and then 5.0 μl 2.0 mg/ml DNA was covered on the ZrO_2 gel surface for 10 h at 4 °C, before used, the electrode was immersed in 5 mM pH 7.10 Tris–HCl with 10 mM EDTA buffer solution for 40 min for the removal of unadsorbed DNA, and kept it in the solutions studied for 5 min for reaching chemical equilibrium.

3. Results and discussion

The cyclic voltammograms of a bare GC electrode, a GC electrode covered with ZrO_2 gel (denoted as GC–ZG electrode) and a GC–ZG electrode modified with DNA (denoted as GC–ZG–DNA electrode) in 1.0 mM $Fe(CN)_6^{3-}$ were shown in Fig. 1, from which we can see that the peak current decreases in the order of GC, GC–ZG and GC–ZG–DNA, and it shows that ZrO_2 gel and DNA were immobilized on the GC and GC–ZG electrodes, respectively. The immobilization process is easily understood that GC electrode is hydrophilic due to the surface with carboxyl and hydroxyl group, and ZrO_2 gel is hydrophilic too, so ZrO_2 gel is readily adsorbed on GC surface. Because ZrO_2 is affinity for phosphoric group [13,14], the scheme can be outlined as follows



The peak current of GC–ZG electrode is less than that of GC electrode due to the ZrO_2 gel taking some sites of the conductive glassy carbon surface, furthermore, the peak current of GC–ZG–DNA electrode is still much less than that of GC–ZG electrode in $Fe(CN)_6^{3-}$ solution. It is clear that DNA is immobilized on the GC–ZG electrode. The decrease of current could be attributed to the electrostatical repulsion of DNA immobilized on GC–ZG electrode to $Fe(CN)_6^{3-}$ owing to both of negative charges [22].

The resulting electrode can be utilized to explore the electron transfer behavior of DNA. The studies show that lanthanide ions can affect strongly the electron transfer rate of DNA in $Fe(CN)_6^{3-}$ solution. Fig. 2 shows the electrochemical change with and without Eu^{3+}, the peak current increased as the concentration of Eu^{3+} was increased gradually. The further investigations revealed that the La^{3+} and Ca^{2+} can also affect the electron transfer behaviors of DNA in $Fe(CN)_6^{3-}$ solution shown in Fig. 3. Under the same condition, the increase of current was different from each other after these metal ions were added, respectively. The relationship between the enhancement of the cathodic peak current and the concentration of these metal ions was shown in Fig. 4. When the concentration of lanthanide is less than 2.5×10^{-5} M, the addition of $Eu^{3+}$and La^{3+} has a great impact on the peak current; while the concentration is more than 2.5×10^{-5} M, the peak current almost keeps unchanged; and the peak current is obviously blunt to the addition of calcium compared with that of lanthanide. The increase order of current is $Eu^{3+} > La^{3+} \gg Ca^{2+}$, which indicates that the lanthanide ions have stronger interaction with the immobilized DNA than calcium ion, and this order is consistent with the ratio of charge to ionic radii [23].

The further studies showed that pH value does not affect obviously the peak current in the pH range of 4.5–7.5 except that peak potential shifts slightly with pH (peak potential shifts positively when pH decreases). In order to

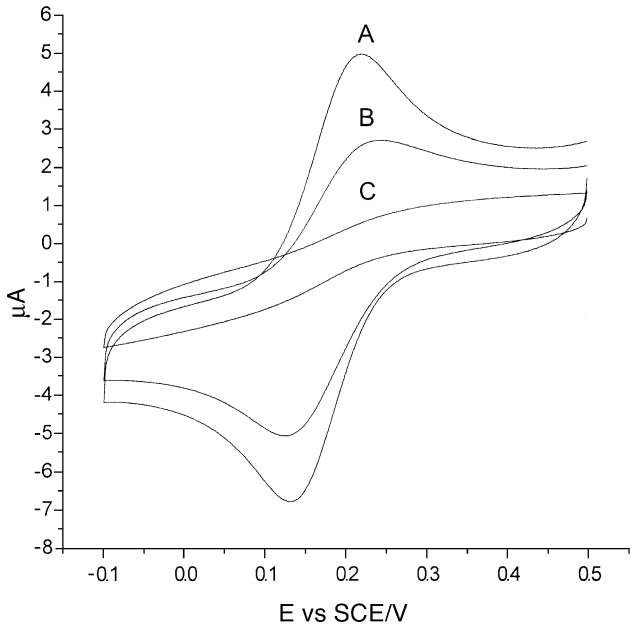


Fig. 1. Cyclic voltammograms of: (A) a GC electrode in 1.0 mM $Fe(CN)_6^{3-}$ + 10 mM NaCl supporting electrolyte solution with 10 mM Tris–HCl pH 7.10 buffer; (B) a GC–ZG electrode in the above solution; (C) GC–ZG–DNA electrode in the above solution. The scan rate was 20.0 mV/s.

prevent from the hydrolysis of these metal ions, the pH values of the solutions studied were adjusted to pH 5.60. To confirm the influence of these metal ions on the electron transfer behavior of the DNA-modified electrode (rather than ZrO_2 modified electrode), a GC–ZG electrode was used for the electrochemical experiments, the results showed that no obvious change of the peak current was

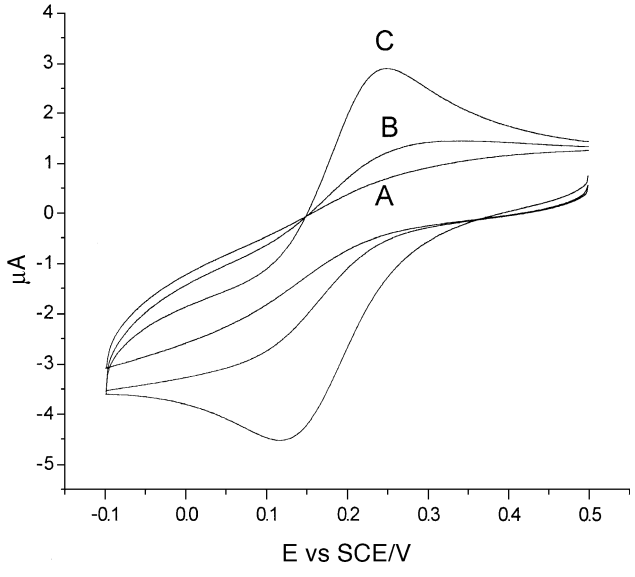


Fig. 2. The effect of lanthanide Eu(III). (A) GC–ZG–DNA electrode in 1.0 mM $Fe(CN)_6^{3-}$ + 10 mM NaAc–HAc pH 5.60 buffer solution; (B) GC–ZG–DNA in solution (A) + 2.5×10^{-6} M Eu^{3+}; (C) GC–ZG–DNA in solution (A) + 3.0×10^{-6} M Eu^{3+}. The scan rate was 20.0 mV/s.

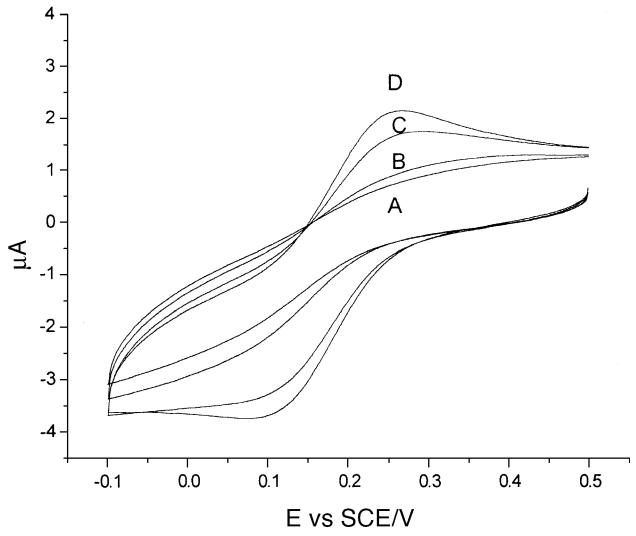


Fig. 3. The effects of different metal cations: (A) cyclic voltammogram of GC–ZG–DNA electrode in 1.0 mM $Fe(CN)_6^{3-}$ solution with 10 mM NaAc–HAc pH 5.60 buffer but without any deliberately added metal cation; (B) in solution (A) + 1.0×10^{-5} M Ca^{2+}; (C) in solution (A) + 1.0×10^{-5} M La^{3+}; (D) in solution (A) + 1.0×10^{-5} M Eu^{3+}. The scan rate was 20.0 mV/s.

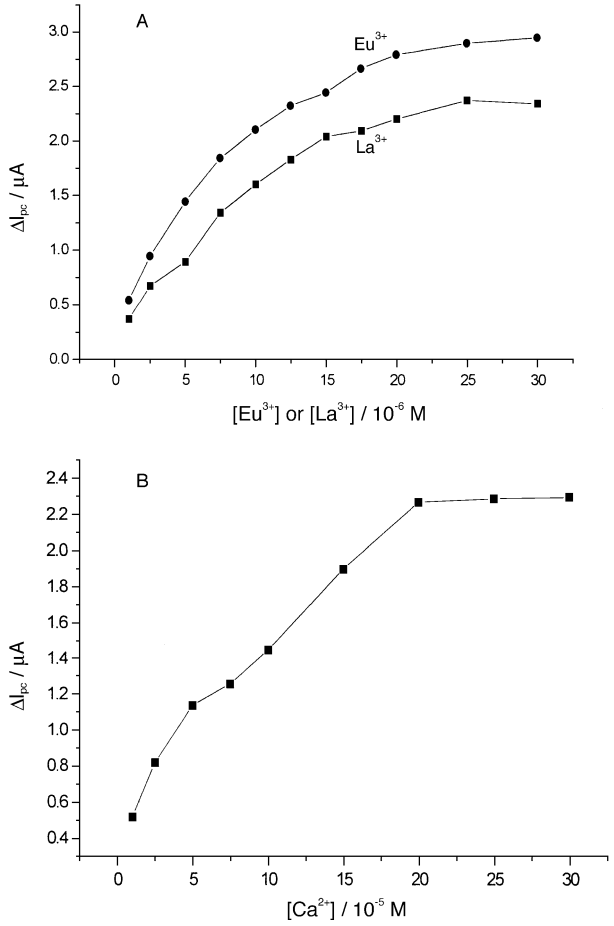


Fig. 4. (A) The relationship between the value of current increment and lanthanide concentration; (B) the relationship between the value of current enhancement and calcium concentration.

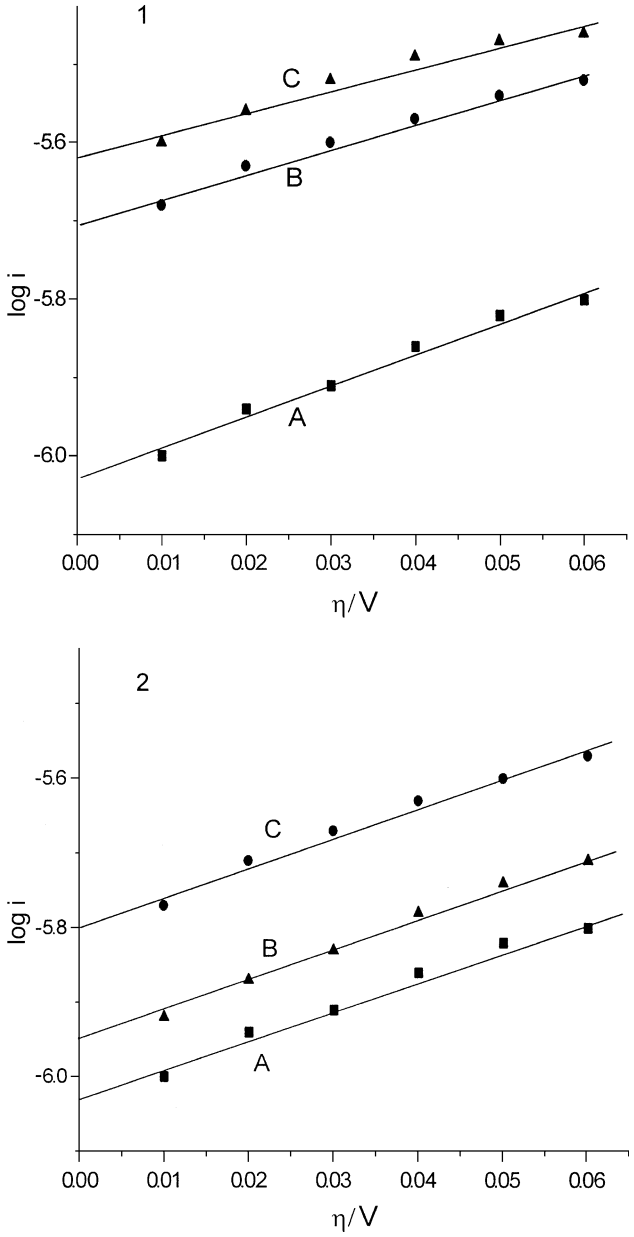


Fig. 5. (1) Tafel plot of GC–ZG–DNA electrode in 1.0 mM $Fe(CN)_6^{3-}$ solution with 10 mM NaAc–HAc pH 5.60 buffer. (A) No Eu^{3+}; (B) containing 5.0×10^{-6} M Eu^{3+}; (C) containing 1.0×10^{-5} M Eu^{3+}. (2) Tafel plot of GC–ZG–DNA electrode in 1.0 mM $Fe(CN)_6^{3-}$ solution with 10 mM NaAc–HAc pH 5.60 buffer. (A) No Ca^{2+}; (B) containing 1.0×10^{-5} M Ca^{2+}; (C) containing 1.0×10^{-4} M Ca^{2+}.

observed in 1.0 mM ferricyanide with and without 3.0×10^{-6} M Eu^{3+}, and the similar behavior was observed for lanthanum and calcium ions; these phenomena showed that the metal ions have a great impact on the electron transfer of DNA-modified electrode, rather than GC–ZG electrode.

In order to estimate quantitatively the effect of these metal on the electron transfer of DNA during the reduction of $Fe(CN)_6^{3-}$, and make a comparison with that of calcium ion, the apparent heterogeneous rate constant k^0 of $Fe(CN)_6^{3-}$

Table 1
k^0 values for the reduction of $Fe(CN)_6^{3-}$

Cations	Concentration (M)	k^0 ($\times 10^{-4}$ cm s^{-1})	k_M^0/k_N^{0a}
No deliberately added metal		1.16	1.00
Ca^{2+}	1.0×10^{-5}	1.68	1.45
La^{3+}	1.0×10^{-5}	2.62	2.26
Eu^{3+}	1.0×10^{-5}	3.37	2.91

[a] k_M^0 is the heterogeneous rate constant of the reduction of $Fe(CN)_6^{3-}$ in the presence of Ca^{2+}, La^{3+} and Eu^{3+}, respectively, k_N^0 is the heterogeneous rate constant of the reduction of $Fe(CN)_6^{3-}$ without these metal ions.

reduction can be calculated by using Tafel plot [24].Assuming $i = i_a + i_c$, $i = i_0\left\{\frac{C_R(0,t)}{C_R^*}e^{(1-\alpha)nf\eta} - \frac{C_O(0,t)}{C_O^*}e^{-\alpha nf\eta}\right\}$, where $\eta = E - E_{eq}$, $f = F/RT$, when the currents are kept low, the surface concentrations do not differ from the bulk values, then the equation becomes

$$I = i_0[e^{(1-\alpha)nf\eta} - e^{-\alpha nf\eta}]$$

if the anodic component of the current (i_a) is negligible when polarity overpotential is negative enough, then

$$I = -i_c = nFAk^0C_O^0\exp(-\alpha nF\eta/RT)$$

or $$\log i = \log i_0 - \alpha nF\eta/2.3RT$$

where η is the overpotential, E_{eq} is the equilibrium potential, equal to the potential of the point at i = zero and i_0 the exchange current.

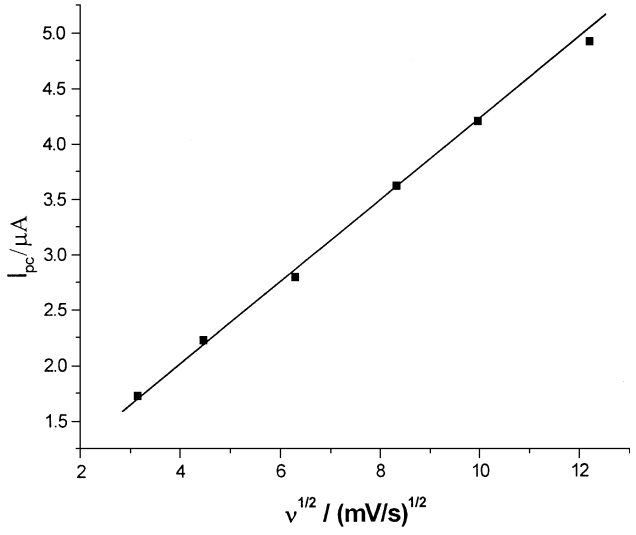


Fig. 6. Relationship between the cathodic peak current and the sweep rate.

Fig. 5 shows the Tafel plot of log i vs. η obtained from the linear sweep voltammetric experiments. To avoid the effect of diffusion limitation, extrapolation of the line of the plot to zero overpotential yields the log i^0.

The values of k^0 at the concentration of 1.0×10^{-5} M for Eu^{3+}, La^{3+} and Ca^{2+}, respectively, can be calculated by the equation $i_c = nFAk^0C_O{}^0$ owing to $C_O = C_R$ [24] and the data are listed in Table 1. By comparing k^0 for lanthanide and calcium with that of no deliberately added metal, it is seen that the rate constant k^0 is greatly increased after these metals were added, respectively. Namely, Eu^{3+}, La^{3+} and Ca^{2+} can expedite the electron transfer rate of DNA in the system by elevation of the rate constant k^0 of heterogeneous electron transfer. The possible reasons for the effect of lanthanide ions for the current enhancement are due to the electrostatic effect and weak coordination of lanthanide ions to DNA. The experiments showed that cathodic peak current of the DNA-modified electrode is proportional to the square root of the sweep rate in the range of 10–150 mV/s in 1.0 mM ferricyanide solution containing 2.0×10^{-5} M Eu^{3+} shown in Fig. 6, that means that $Fe(CN)^{3-}$ cannot be adsorbed to the surface of the electrode in this system. The possible mechanism for the current enhancement after lanthanide ions and calcium ion were added is as follows. First, DNA and these metals formed a non-stable complex (easy dissociation complex, for example, the binding constant K of DNA–Ca^{2+} is small, equal to 0.5–0.6 [25], which can reach to a fast adsorption–desorption equilibrium). Then, the negative charge on the phosphate backbones of DNA was neutralized by the metals with the positive charges, which decreased the repulsion of the DNA-modified electrode surface to $Fe(CN)_6^{3-}$, and led to the current increase. When the sites on DNA were occupied completely with metal ions, the repulsion of the surface to $Fe(CN)_6^{3-}$ decreased to a minimum, that is, the current reached to a maximum. When the amount of metal ions continued to increase, the current did not continue to increase more, and a plateaus appeared (shown in Fig. 4).

4. Conclusion

DNA is immobilized successfully for the first time by the sol–gel method, and the studies of electrochemistry on the immobilized DNA showed that Eu(III), La(III) and Ca(II) can promote the electron transfer rate of DNA during the reduction of ferricyanide by the elevation of rate constant k^0, the order of rate constant k^0 is Eu(III)> La(III)Ca(II) under the same condition.

Acknowledgements

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