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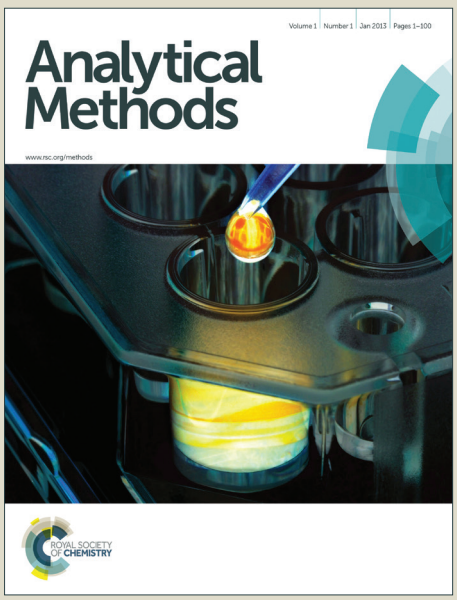

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Selective recognition and sensing for adenosine triphosphate by label free electrochemistry based on its inclusion with per-6-ammonium-β-cyclodextrin

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Abstract:

A novel method for selective sensing of ATP was developed using ferrocenecarboxylic acid (FcA) as an electrochemical probe, based on the competitive reaction between ATP (or ADP, AMP) and FcA with Per-6-ammonium-β-cyclodextrin (*p*ABCD).

*P*ABCD has been synthesized in four steps from β-cyclodextrin by an improved method referring to literatures. In the *p*ABCD molecule, the primary hydroxyl groups at position 6 of β-cyclodextrin have been substituted by amino groups. The presence of amino groups increased the binding ability. *p*ABCD showed the strongest binding ability towards the adenosine triphosphate (ATP), due to not only the host-guest inclusion of the cavity of *p*ABCD with the adenosine base but also the positively charged ammonium of *p*ABCD with the phosphate anion moiety.

FcA, as an excellent electroactive probe, can be included in the *p*ABCD cavity and produce a decreased oxidation signal. However when ATP was added to *p*ABCD-FcA system, the oxidation peak currents increased dependently on the concentration of ATP. In this way, *p*ABCD-FcA system can recognize and sense the ATP through the competitive interaction of ATP and FcA with *p*ABCD. The increased signal of FcA with the addition of ATP in FcA-ABCD sysytem was studied by differential pulsed voltammetry (DPV). The inclusion constant of *p*ABCD with FcA, ATP, ADP and AMP have been evaluated with DPV by application of the Langmuir equation $1/\Delta I = 1/\Delta I_{max} + 1/(kc\Delta I_{max})$.

Based on these results, under the optimized experimental conditions, the oxidation current of the FcA of the FcA-*p*ABCD system responding to the concentration of ATP, was

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linear in the range of 3.12×10^{-7}–1.68×10^{-6}mol/L, with a correlation coefficient of 0.9978 and the detection limit (*S/N* = 3) of 1.43×10^{-7} mol/L. These results demonstrate that this method is highly selective and sensitive for determination of ATP.

Keywords: Per-6-ammonium-β-cyclodextrin, Adenosine triphosphate, Ferrocenecarboxylic acid, Inclusion complexes, Cyclic voltammetry, Differential pulse voltammetry, Inclusion constant, Competitive interaction

1. Introduction:

Selective recognition and sensitive detection of ATP is of great significance because ATP is one of the most important metabolites in biological systems to support general cellular works necessary for survival and reproduction[1] and the level of ATP is closely related to variety of common physiological diseases such as cardiovascular disease, Parkinsonism, and Alzheimer's[2–4]. Therefore, recently, great efforts have been dedicated to develop effective methods that can selectively recognize and sense nucleotides, especially the three basic anionic adenosine phosphates, AMP, ADP and ATP.

Nowadays, different techniques have been reported for the determination of ATP, such as fluorescence[5], UV–Vis reflectance spectrum[6], high performance liquid chromatography (HPLC) [7] and electrochemiluminescence (ECL) [8]. Until now, there is fewer electrochemical methods reported to determine ATP due its sluggish electrochemical activity, that is, it is hard to be reduced or oxidized directly on electrodes to produce detectable current signal.

The development of novel system for indirect recognizing and detecting ATP becomes more crucial. According to the literature, nucleotide sensors consist of the receptor moieties and the signaling units. Existing artificial nucleotide receptors can be classified as the multiple amino compounds, polyammoniums[9], peptides, dendrimers, metallic complexes, and cyclodextrin derivatives.

β-cyclodextrin (β-CD), having its special conic structure containing a hydrophobic internal cavity and a hydrophilic external surface[10,11], are being explored to be as adaptors to recognize the molecule of interest. Since application of natural β-CD is limited because there only exists weak hydrophobic-bonding interaction with guest molecules. In order to strength the recognition ability and selectivity of β-CD to ATP, based on the knowledge about the

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binding ability of β-amino-cyclodextrin to phosphate anions and nucleotides reported[12-16], we synthesized the homogeneous pABCD by substitution of the all the primary hydroxyl groups at position 6 with amino pendant groups. Compared with native β-CD, the pABCD is expected to manifests the combined hydrophobic, hydrogen bonding and electrostatic interaction with guest molecules due to the introduction of the amino groups, which may form a crown of positive charges on the opening of the cavity to recognize the anionic guest feasibly in comparison with unmodified β-CD.

In order to investigate the binding ability of homemade *p*ABCD with ATP by electrochemical methods, FcA was employed as a probe to study the binding ability of *p*ABCD to ATP by cyclic voltammetry (CV) and DPV based on the competitive reaction of ATP and FcA with *p*ABCD because FcA is a good electroactive probe[17], which can produce a reversible and sensitive redox CV response in solution, and but also its current signal is affected by the inclusion effect of *p*ABCD.

Base on this principle and the pre-research, we investigated about the interaction of the ATP and the *p*ABCD. We found that, when FcA coexist with *p*ABCD in solution, there is steady current signal from the oxidation of free FcA. The signal will increased with the addition of ATP due to the competitive binding of ATP and FcA with *p*ABCD and more free FcA expelled out from the cavity. Under the optimal experimental conditions, the oxidation current of the FcA in the FcA-*p*ABCD system responds to the concentration of ATP. As well, these results demonstrated the developed method is selective and highly sensitive for determination of adenosine disodium triphosphate. The mechanism was also proposed based the comparison of inclusion effect of ATP with β-CD and *p*ABCD, *p*ABCD with ATP, ADP and AMP, $HPO_4^{2-}$and AMP with *p*ABCD. The results show, the *p*ABCD can recognize ATP selectively is due to not only inclusion ability of the hydrophobic cavity and also the electro static attraction between ammonium ions in *p*ABCD and phosphate anions in ATP.

2. Experimental

2.1 Reagents

β-CD (purchased from Aldrich) was dried at 120 ºC overnight under vacuum before use. Adenosine triphosphate was obtained from Sigma-Aldrich (Shanghai, China). DMF was dried over $CaCl_2$ and distilled. Ferrocenecarboxylic acid and other reagents were purchased from

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Aldrich and were of the best commercial quality available. All inorganic salts were analytical grade and used without further purification. All solutions were freshly prepared with Distilled water which was further purified via an E-Pure 4-Holder system (Barnstead).

2.2 Instruments

^{1}H and ^{13}CNMR spectra were determined with Varian INOVA-400M spectrometer working at 400 MHz and 100 MHz for ^{1}H and ^{13}C nucleus, respectively. Chemical shift are given in ppm from tetramethylsilane(TMS) as internal standard. ESI-MS spectra were recorded in a Thermo Finnigan-LTQ-XL spectrometer. Fourier transforms infrared (FT-IR) spectra of each β-cyclodextrin derivatives were recorded with a Bruker TENSOR-27 spectrometer between wavenumber of 400-4000 cm^{-1}. The pH measurements were performed on a pHs-3B pH meter (Dazhong, Shanghai, China). Electrochemical measurements were carried out on a CHI660C Electrochemical Workstation (ChenHua Instruments Co., Shanghai, China). A three-electrode system was used in the experiment with the glassy carbon electrode (GCE, 3 mm in diameter) as the working electrode. A saturated calomel electrode (SCE) and a Pt wire electrode were used as reference and counter electrodes, respectively.

2.3 Preparation of stock solutions

The solution of *p*ABCD (5×10^{-3} mol/L) was prepared by dissolving 0.346 g *p*ABCD of in 50mL water. The solutions of Ferrocenecarboxylic acid (2.50×10^{-3} mol/L) was prepared by dissolving 0.575 g Ferrocenecarboxylic acid in 100mL water. Solutions (0.25 mol/L) of Adenosine disodium triphosphate, adenosine disodium diphosphate and adenosine disodium monophosphate were prepared by dissolving proper amount them in water.

2.4 pretreatment of the GCE

The glassy carbon electrode (GCE) was polished with 0.05 mm alumina slurry and rinsed thoroughly with doubly distilled water between each polishing step. Then, it was washed successively with 1 mol/L H_2SO_4, acetone and doubly distilled water in an ultrasonic bath. Finally, the electrode was dried in the infrared drying oven.

2.5 Electrochemical measurement

CV and DPV measurements were performed in 8 mL of 0.1 mol/L Tris-HCl (pH 7.0) buffer solution and CV and DPV curves were scanned from -0.2 to 0.8 V. The parameters

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were set as following, scan rate 0.1V/s, pulse width 0.2 s, pulse period 0.5 s and pulse amplitude of 5 mV.

3 Results and Discussion

3.1 The preparation of *p*ABCD

To synthesis *p*ABCD efficiently and economically, an improved method was developed based on the literature[18], the structure (**Scheme 1**) and purity of the target product is characterized by means of ^{1}HNMR, ^{13}CNMR, IR and MS.

(**Scheme 1**)

3.2 The interaction of ferrocenecarboxylic acid with per-6-ammonium-β-cyclodextrin

Firstly, the electrochemical behavior of FcA on GCE was studied, and then the inclusion effect of *p*ABCD was investigated by means of CV. FcA exhibited a pair of redox peaks within the potential window from -0.2 to 0.8 V. Separate solutions with a constant concentration of FcA (1.56×10^{-4} mol/L) with different concentration of *p*ABCD were prepared and their CV curves were recorded with CHI660C Electrochemical Station. The results (in **Fig.1)**, shown that the CV response of FcA depends upon the concentration of *p*ABCD, the peak currents gradually decreased upon the addition of *p*ABCD, although the concentration of total FcA was kept constant. This is because, in the presence of *p*ABCD, the formation of inclusion complexes of FcA and *p*ABCD lead to the decrease of concentration of free FcA than that of FcA in absence of *p*ABCD in the solution, making the less FcA move to the GCE surface, thus resulting in current signal decrease, which supports for the formation of inclusion complexes between *p*ABCD and FcA.

The corresponding relationship between the anodic peak current and the concentration of added *p*ABCD are shown in the inset plots. From these figures, the anodic peak currents decrease gradually with the increase of *p*ABCD concentration, and then approach a plateau after the amount of *p*ABCD added was more than 5 times of equivalent of FcA, which implied an almost completion of inclusion of FcA with *p*ABCD.

(Fig.1)

To study the interaction of the host-gust inclusion further, DPV is also employed to investigate the inclusion constant between FcA with *p*ABCD. As shown in **Fig. 2A**, the

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decreased DPV signals are directly related to the concentration of added *p*ABCD. The inclusion constant has been determined by the Langmuir equation:

$$1/\Delta I = 1/\Delta I_{max} + 1/(kc\Delta I_{max})^{19} \quad (1)$$

where ΔI is the change of peak current upon the addition of pABCD, ΔI_{max} *is* the maximum change of peak current, *c* is the concentration of *p*ABCD and *K* is the inclusion constant. The plots of $1/\Delta I$ *versus* $1/c$ (Inset of **Fig. 2**) were linear ($1/\Delta I$ (μA) = 0.628+0.738 ($1/c$) (mM)) with a good correlation coefficient of 0.9997. Eq. (1) allows the *K* were calculated from the ratio of intercept to slope of the double reciprocal curve $1/\Delta I$ versus $1/c$ plot to be 850.5 L/mol (283 K)

(Fig.2)

3.3 The binding of adenosine triphosphate (ATP) and ferrocenecarboxylic acid (FcA) with per-6-ammonium-β-cyclodextrin (*p*ABCD)

In order to recognize and sense ATP, further investigation was carried out on electrochemical response of ATP to the FcA-*p*ABCD system. Firstly, the solution of 2.5×10^{-6} mol/L *p*ABCD and 5.0×10^{-7} mol/L FcA was prepared and scanned by the DPV method, then after 9.4×10^{-7} mol/L ATP was added the DPV was recorded as well.

The results shown in **Fig.3**, there is no peak on the DPV curve when only ATP exsits or *p*ABCD and ATP coexist in the solution since *p*ABCD and ATP are none electroactive substance. Compared with 5.0×10^{-7} mol/L FcA and 2.5×10^{-6} mol/L *p*ABCD solution without ATP, the peak current increased when ATP solution was added. A conclusion can be drawn, the FcA-*p*ABCD system can response to the addition of ATP, which may be caused by the competitive interaction ATP and FcA with *p*ABCD to expel out more FcA from its *p*ABCD complex (see **Scheme 2**).

To compare the binding ability of β-CD, we also repeat the above experiment just using β-CD instead of *p*ABCD. As can be shown in the inset plots, the effect of β-CD either on the oxidative current of FcA or on the binding of ATP is weaker than the *p*ABCD on current. It indicated the *p*ABCD has a stronger binding ability with ATP than β-CD. It inferred that the amino or ammonium group contributed to the effect due to the hydrogen bonding or the static attraction force between the ammonium cations and the ATP phosphate anion.

Based on this phenomenon, the *p*ABCD/FcA complex may play as an electrochemical

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probe for the selective sensing of ATP. The selective and sensitive determination methods may be established for sensing of ATP. Therefore the relationship of the rise of the current and the concentration of the ATP added was investigated further.

(Fig.3)

(Scheme 2)

3.4 The interaction of adenosine triphosphate (ATP) with per-6-ammonium-β-cyclodextrin (*p*ABCD)

To get the fundamental data about the binding function of *p*ABCD with ATP, the interaction constant of ATP with *p*ABCD was determined by the DPV using FcA as a probe. As shown in **Fig. 4**, the increased DPV signals are directly related to the concentration of ATP. The plot of $1/\Delta I$ *versus* $1/c$ (Inset of **Fig. 4**) fits to a linear relationship ($1/\Delta I$ (μA) = 0.717 + 1.196 ($1/c$) (mM))) with correlation coefficient of 0.9987. The interaction constant was calculated from the ratio of intercept to slope of $1/\Delta I$ *versus* $1/c$ plot to be 599.6 L/mol (283 K). Meanwhile the constant of ADP and AMP with *p*ABCD were also investigated and respectively found to be 255.5 L/mol (283 K) (**Fig. 5**) and 141.1 L/mol (283 K) (**Fig. 6**). It means that, the interaction of *p*ABCD with ATP is much stronger than with ADP or AMP.

(Fig.4)

(Fig.5)

(Fig.6)

3.5 Optimization of the Determination Conditions

Based on the response of FcA-*p*ABCD to the addition of ATP, to achieve the better sensing performance, the experimental parameters such as pH, reaction time, scan rate, have been optimized.

3.5.1 Optimization of the pH

The oxidative signal of FcA was investigated by CV under different pH of 0.1 mol/L Tris-HCl buffer solutions with 0.5×10^{-3} mol/L FcA, 2.5×10^{-3} mol/L *p*ABCD and 2.5×10^{-3} mol/L ATP. As shown in **Fig. 7A**, the peak currents increased with the pH of the buffer solution in the range of 5.0-7.0. Peak current reaches to the maximum at pH = 7.0. However when the pH was further increased, the peak decreased significantly.

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At different pH, the response of FcA-*p*ABCD system to addition of ATP also was investigated. The results show, the increase of the peak current due to the same concentration ATP added have something with pH. At pH = 7.0, the increment of oxidative peak current is much more evident.

This is may be explained that the pH effect on the existing forms of FcA and *p*ABCD, further on the binding ability of *p*ABCD to ATP. Since the p*K*a of FcA is 4.2[20], when pH is more than 4.2, the anionic form of FcA is predominant, which benefits the transfer and the oxidation of FcA on electrode. However, if the pH is too large, it doesn't favor to the protonation of amino group in *p*ABCD because the p*K*a values of the *p*ABCD were found to be 7.0, 7.7, 7.8, 8.4, 9.0, 9.3 and 12.4 from potentiometric titration curves obtained as described by Martell and Motekaitis, a charged form of the *p*ABCD with a value between 6^+ and 7^+ is assumed to be present in aqueous solutions at neutral pH = 7.0[21]. To compromise, the 0.1 mol/L Tris-HCl buffer solution of pH = 7.0 was used as the optimal conditions of supporting electrolyte in the following voltammetric determination.

3.5.2 Optimization of the Reaction time

After mixing of particular concentration of ATP with 0.5×10^{-3} mol/L FcA and 2.5×10^{-3} mol/L *p*ABCD for some time, the CV scan was performed and the CV graph was recorded. As can be shown in **Fig. 7B**, the peak current increasesed significantly with the increase of mixing time at first and then approached a plateau after 40 min. It indicated that when ATP was added, it took some time to interact with *p*ABCD competitively to drive FcA out of the cavity, which is an electroactive probe and produces oxidative signal.

Therefore, an optimum mixing (reaction) time of 40 min was adopted in the following experiments.

(Fig.7)

3.5.3 Optimization of the Scan Rate

The effect of the scan rate for redox peak current was investigated. As shown in **Fig. 8**, the redox peak currents gradually increase when the scan rate in the range from 0.03 to 0.3 V/s. The redox peak currents increase with the scan rates, The relationship between the redox peak current and the square root of scan rate is well fitted to linear equation (Inset **A** of **Fig.8**), and the regression equation are expressed as i_{pa} (μA) = 0.108 + 13.194 $v^{1/2}$ (V/s) (R = 0.9987)

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and i_{pc} (μA) = 0.228 − 11.714 $v^{1/2}$ (V/s) (R = 0.9991), which suggested that this electrochemical redox processes are diffusion-controlled.

Meanwhile, the relationship between the redox peak potential and the scan rates was also investigated (Inset **B** of **Fig.8**). The redox peak potentials kept constant with the scan rates in the range of 0.03-0.15 V/s. When the scan rate exceeds 0.15 V/s, the reduction peak potentials shift negative, and the oxidation peak potentials shift positive. The redox process becomes a little irreversible, which is not good to the following measurement. Therefore, a scan rate of 0.1 V/s was adopted in the following experiments.

(Fig.8)

3.6 Determination of Adenosine triphosphate (ATP)

Under the chosen conditions, solutions with a constant concentration of 2.50 × 10^{-6} mol/L *p*ABCD and 0.50 × 10^{-6} mol/L FcA upon the addition of different concentration of ATP were measured by means of DPV measurements. The result was shown in **Fig. 9**, there is a linear relationship between the current and ATP concentration of 3.12 × 10^{-7} − 1.68 × 10^{-6} mol/L. The regression equation is ΔI (nA) = 42.20 + 4.00 c (μM) with a correlation coefficient of 0.9978 (Inset of **Fig. 9**), and the detection limit of ATP is 1.43 × 10^{-7} mol/L (S/N = 3).

(Fig.9)

3.7 Selectivity and Repeatability

The Selectivity of this method was examined by detecting the effect of fourteen potential interference agents: Na^+, Fe^{3+}, Ca^{2+}, Cu^{2+}, K^+, NO_3^-, Cl^-, CO_3^{2-}, SO_4^{2-}, PO_4^{3-}, $H_2PO_4^-$, HPO_4^{2-}, AMP, ADP. As shown in **Fig.10**, fourteen interfering agents led to some signal change, in contrast to ATP, the addition of Na^+, Fe^{3+}, Ca^{2+}, Cu^{2+}, K^+, NO_3^-, Cl^-, CO_3^{2-}, SO_4^{2-}, PO_4^{3-}, produced less than 1.89% of the peak current produced by the equivalent ATP. So it indicated that their interferences can be negligible. However, equivalent HPO_4^{2-}, AMP, ADP only produce less than about 16.7% of the peak current of ATP.

Meanwhile, the reproducibility of the method was evaluated by detecting the current response of 9.37 × 10^{-7} mol/L ATP respectively. The relative standard deviation (RSD) is found to be 2.4%, indicating an acceptable accuracy.

3.8 Discussions on the inclusive mechanism of *p*ABCD

Base on the experimental data, a possible mechanism is proposed as Scheme 2. Firstly,

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the inclusion effects of natural β-CD（without any amino group）and *p*ABCD with ATP was compared, the inclusion ability of *p*ABCD is stronger the β-CD, it indicates the ammonium cations play a important role in the inclusion process. Secondly, the inclusion effects of *p*ABCD with ATP, ADP and AMP, which have 3, 2 or only 1 phosphate anion(s) is decreasing with number of charge on the basic anionic adenosine phosphates, it implied the electrostatic attraction between negative charges and positive charges on ammoniums play an important role in the inclusion. Thirdly, the inclusion effect of *p*ABCD with HPO_4^{2-} and AMP was also compared, although the same negative charge on both of them, the inclusion effect of *p*ABCD to AMP is stronger than HPO_4^{2-} ,it indicated that the base moiety also can be included in the hydrophobic cavity of *p*ABCD[9].

In a word, in the inclusion of *p*ABCD with ATP, the inclusive effect of the hydrophobic cavity with the adenosine base group and the electrostatic static attraction between the polyammonium cations and the negatively charged phosphate moieties in the ATP both play a very important synergic effect, which makes the recognition more selectively and the sensing more sensitively.

(Fig.10)

4. Conclusions

Using own synthesized per-6-ammonium-β-cyclodextrin as a receptor and ferrocenecarboxylic acid as an electrochemical probe, the inclusion effect of ATP with *p*ABCD was studied. Based on the result, a selective and sensitive electrochemical sensing method to ATP was developed. This method has the advantage of excellent selectivity, high sensitive, fast detection and excellent reproducibility, which are proved to be a unique method for ATP detection.

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Notes

Electronic Supplementary Information (ESI) available: Synthesis and characterization of

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the *p*ABCD.

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Figure captions

Scheme 1. The structure of *p*ABCD.

Scheme 2. The interaction mechanism of ATP and FcA with *p*ABCD

Fig.1. The CV responses for FcA (1.56×10^{-4} mol/L) with different concentration of *p*ABCD: (a) 0.00, (b) 0.16, (c) 0.32, (d) 0.47, (e) 0.63, (f) 0.78, (g) 0.94×10^{-3} mol/L. Inset: The corresponding relationship between the peak current and *p*ABCD the concentration. In 0.1 mol/L Tris-HCl (pH, 7.0) buffer solution.

Fig.2. The DPV responses for FcA (1.56×10^{-4} mol/L) with different concentration of *p*ABCD: (a) 0.00, (b) 0.16, (c) 0.31, (d) 0.47, (e) 0.63, (f) 0.78×10^{-3} mol/L. Inset: The plots of $1/\Delta I$ *versus* $1/c$.

Fig.3. The CV responses for different CD solutions: 0.94×10^{-6} mol/L ATP + 2.50×10^{-6} mol/L CD (blue curve), 0.50×10^{-6} mol/L FcA + 2.50×10^{-6} mol/L CD (red curve), 0.50×10^{-6} mol/L FcA + 2.50×10^{-6} mol/L CD + 0.94×10^{-6} mol/L ATP (green curve), 0.50×10^{-6} mol/L FcA (black curve). (A) CD = *p*ABCD, (B) CD = the native β-CD

Fig.4. The DPV responses to different concentration of ATP: (a) 0.00, (b) 0.13 , (c) 0.50, (d) 1.00, (e) 1.50, (f) 1.68, (g) 2.00×10^{-3} mol/L. Inset: The plots of $1/\Delta I$ *versus* $1/c$. in 0.1 mol/L Tris-HCl (pH, 7.0) buffer solution containing 0.50×10^{-3} mol/L FcA + 2.50×10^{-3} mol/L *p*ABCD.

Fig.5. The DPV responses to different concentration of ADP: (a) 0.00, (b) 0.13, (c) 0.50, (d) 0.75, (e) 1.00, (f) 1.25, (g) 2.00×10^{-3} mol/L Inset: The plots of $1/\Delta I$ *versus* $1/c$. in 0.1 mol/L Tris-HCl (pH, 7.0) buffer solution containing 0.50×10^{-3} mol/L FcA+2.50×10^{-3} mol/L *p*ABCD.

Fig.6. The DPV responses to different concentration of AMP: (a)0.00, (b) 0.50, (c) 0.56, (d) 1.00, (e) 1.25, (f) 1.50, (g) 1.88×10^{-3} mol/L. Inset: The plots of $1/\Delta I$ *versus* $1/c$. The experiments have been done in 0.1 mol/L Tris-HCl (pH, 7.0) buffer solution containing 0.50×10^{-3} mol/L FcA + 2.50×10^{-3} mol/L *p*ABCD.

Fig.7. (A) The effect of the pH values on the peak current. (B) The relationship between the

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peak current and the reaction time. In Tris-HCl buffer solution containing 0.50×10^{-3} mol/L FcA + 2.50×10^{-3} mol/L *p*ABCD + 2.50×10^{-3} mol/L ATP.

Fig.8. The relationship between the peak currents and the scan rates: (a) 0.03, (b) 0.05, (c) 0.10, (d) 0.15, (e) 0.20, (f) 0.25, (g) 0.30 V/s. Inset (A): The linear relationship between the scan rates and the peak currents. Inset (B): The relationship between the logarithm of scan rates and the changes of peak potentials. In Tris-HCl buffer solution containing 0.50×10^{-3} mol/L FcA + 2.50×10^{-3} mol/L *p*ABCD + 2.50×10^{-3} mol/L ATP.

Fig.9. The DPV responses to different concentration of ATP: (a) 0.00, (b) 0.15, (c) 0.30, (d) 1.25, (e) 2.50, (f) 3.75, (g) 7.50, (h)1.00, (i)1.25, (j)1.68×10^{-6} mol/L. Inset: The corresponding relationship between the anode peak currents and the concentrations. 0.1 mol/L Tris-HCl (pH, 7.0) buffer solution containing 0.50×10^{-6} mol/L FcA + 2.50×10^{-6} mol/L *p*ABCD.

Fig.10. The increment of peak current(ΔI) in different ions of 0.1 mol/L Tris-HCl (pH 7.0) buffer solution containing 0.50×10^{-6} mol/L FcA + 2.50×10^{-6} mol/L *p*ABCD. ATP (9.37×10^{-7} mol/L) and various ions (1.80×10^{-6} mol/L)

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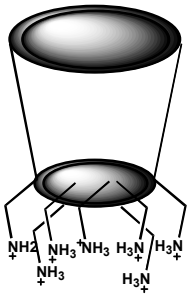
Scheme 1.



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Scheme 2.

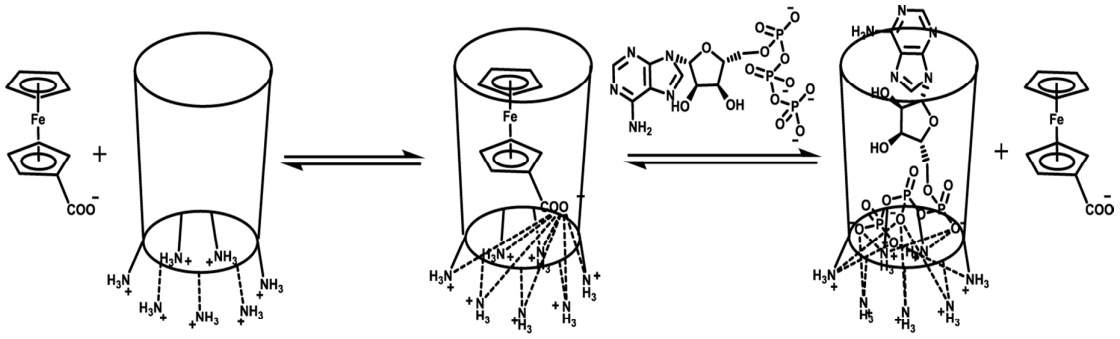

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Fig 1 .

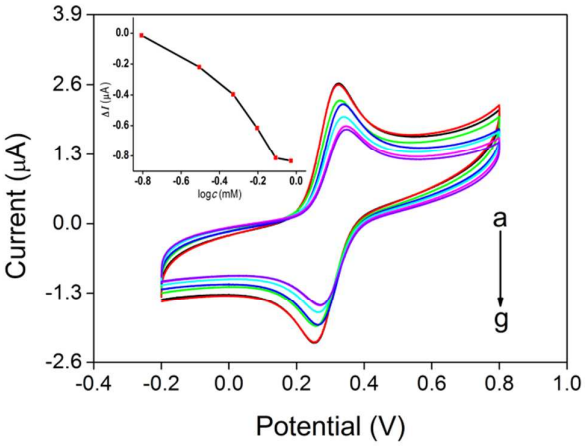

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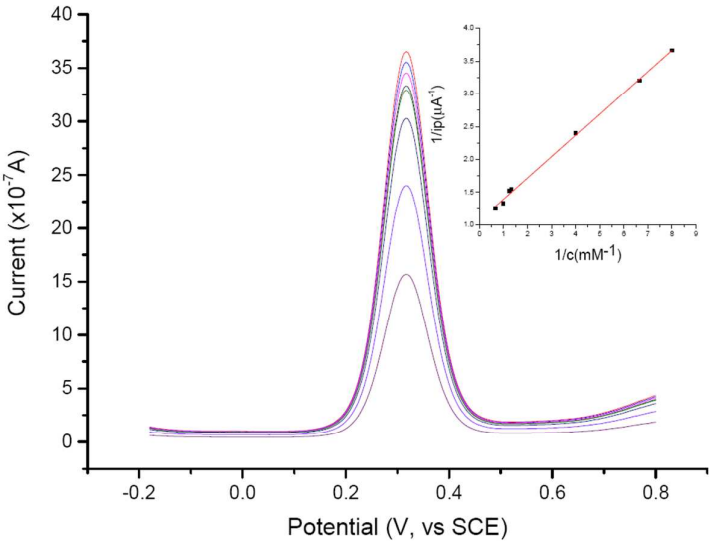
Fig 2.



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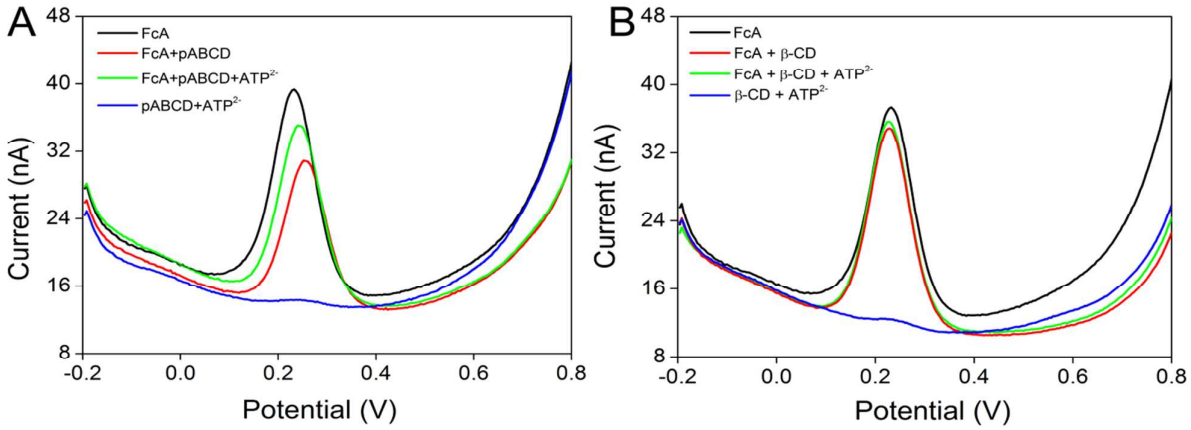

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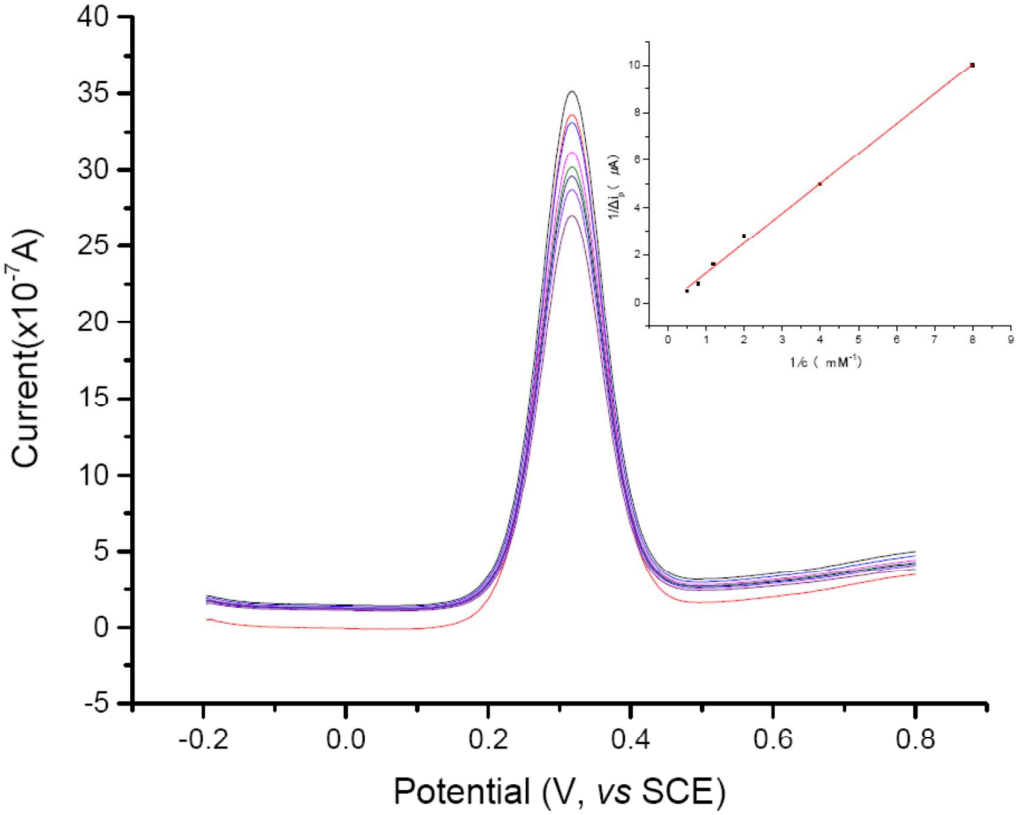

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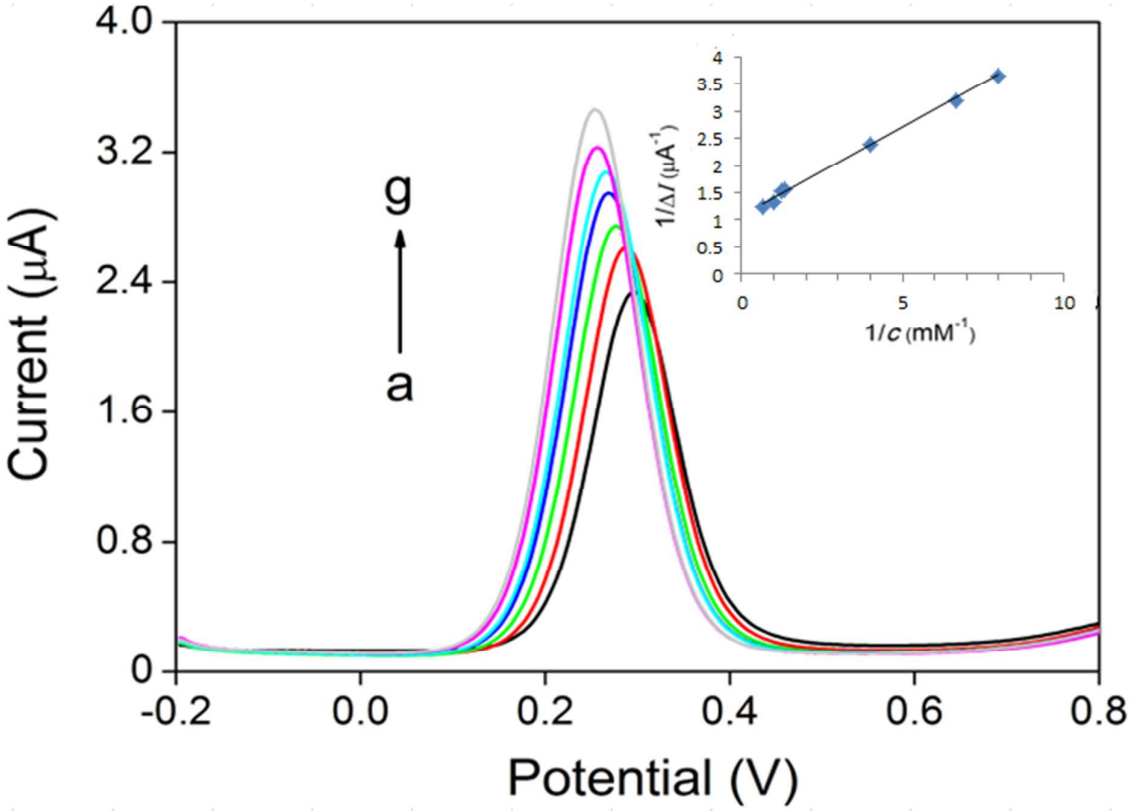

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Fig 6.

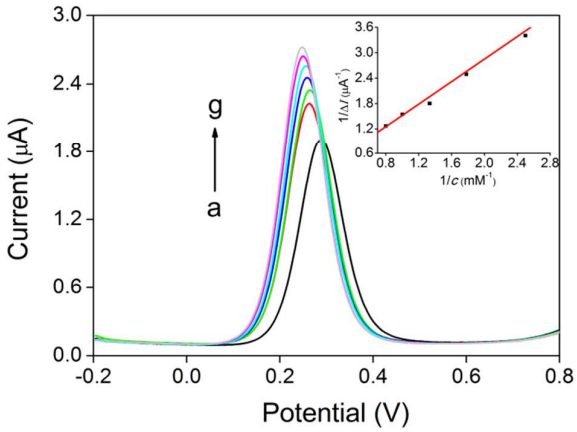

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Fig 7.

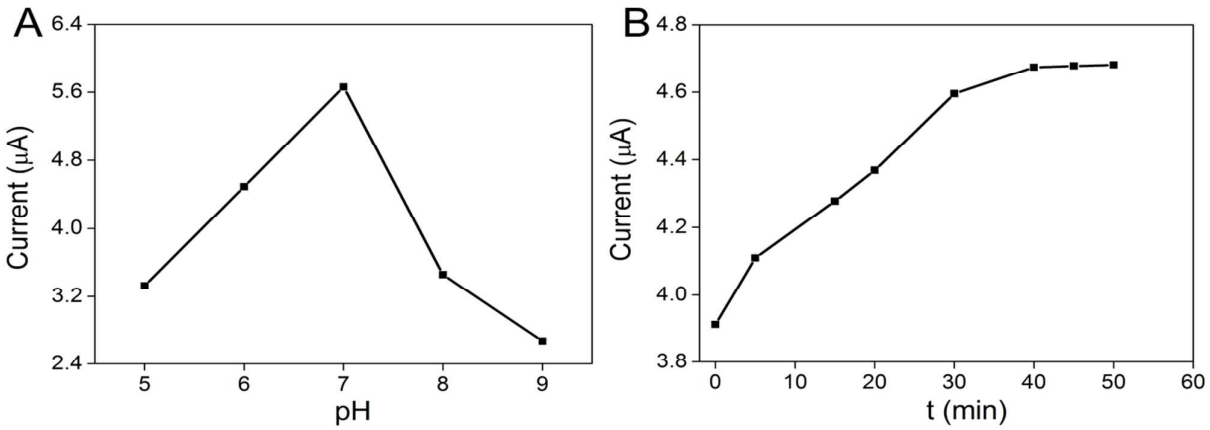

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Fig 8.

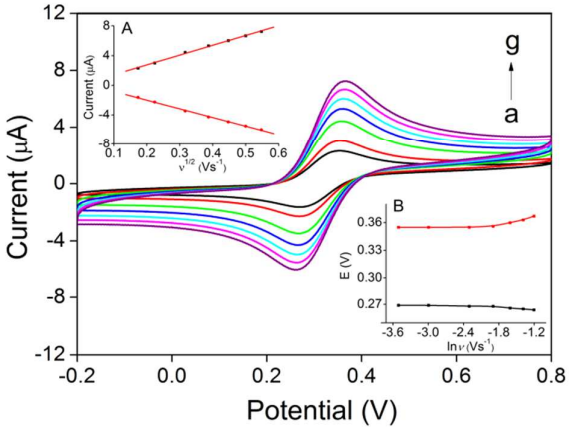

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Fig 9.

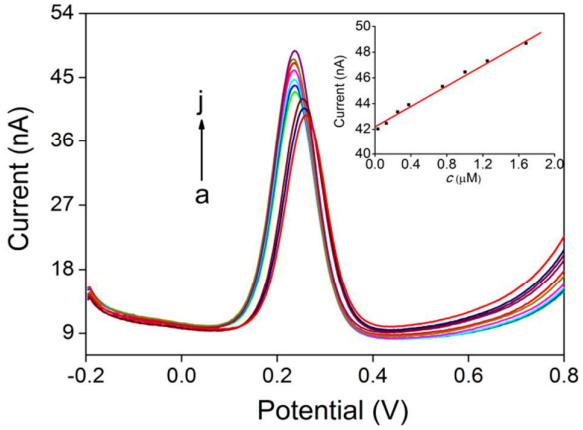

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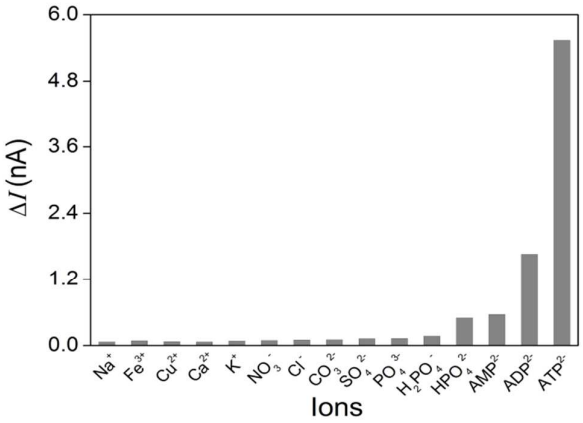
Fig 10.



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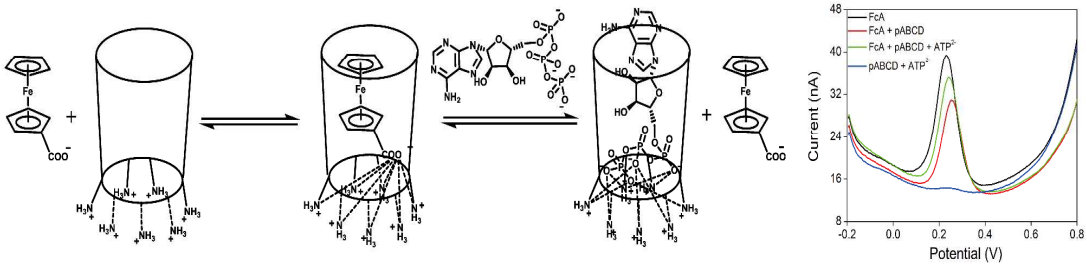
Graphical Abstract



The illustration diagram for the competitive inclusion of adenosine triphosphate to displace ferrocenecarboxylic acid from its inclusion complex with per-6-ammonium-β-cyclodextrin and the DPV curves.

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