

## Sensitive Electrochemical Determination of Adenosine-5'-triphosphate with 1-Butyl-2,3-dimethylimidazolium Hexafluorophosphate Based Carbon Paste Electrode

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A new voltammetric procedure was established for the analysis of adenosine-5'-triphosphate (ATP) with ionic liquid 1-butyl-2,3-dimethylimidazolium hexafluorophosphate (BMMImPF<sub>6</sub>) based carbon paste electrode (CPE). The modification of high conductive BMMImPF<sub>6</sub> in CPE decreased the interfacial resistance and provided good electrocatalytic activity to accelerate the oxidation of ATP with the increment of the oxidation peak current. A well-defined irreversible oxidation peak of ATP was observed at 1.42 V in a pH 3.0 Britton-Robinson (B-R) buffer solution with an adsorption-controlled process, which was due to the interaction of ATP with the imidazolium group of ionic liquid used. The experimental conditions were optimized with the accumulation time of 80 s and the accumulation potential of 0.3 V. Under the optimum conditions with differential pulse voltammetry, the oxidation peak current showed a good linear relationship with ATP concentration from 0.3 to 700.0 μmol/L with the detection limit as 0.912 nmol/L (3 S/N). This method was not disturbed by the interferences from commonly coexisting compounds, which was applied to detect the drug samples with good recovery.

**Keywords:** Carbon paste electrode; Adenosine-5'-triphosphate; Voltammetry; 1-butyl-2,3-dimethylimidazolium hexafluorophosphate

### 1. INTRODUCTION

Carbon paste electrode (CPE) is often acted as working electrode in electroanalytical chemistry due to its excellent properties including cheap fabrication price, easy modification and moderate sensitivity with renewable electrode interface [1]. Liquid paraffin or mineral oil is often chosen as the binder in graphite powder with a hydrophobic surface formed. However the conductivity is limited

with the nonconductive paraffin inside carbon paste. Ionic liquids (ILs) are defined as melting salts, which are consisted of large organic cation with a weakly coordination organic or inorganic anion. ILs have many specific physiochemical properties including high chemical and thermal stability, negligible vapor pressure, high ionic conductivity and wide electrochemical windows [2]. ILs have been applied in the fields of electrochemistry and chemically modified electrodes such as the supporting electrolyte or the modifier [3,4]. Opallo et al. checked the ion transfer process across ILs/aqueous solution interface using various kinds of electrodes [5-7]. Maleki et al. indicated that IL modified CPE exhibited the superiority than different kinds of carbon based electrodes [8]. The incorporation of IL as the modifier on the electrode interface exhibited better properties with the high ionic conductivity, good anti-fouling ability, wide potential windows and inherent catalytic activity [9,10]. Our group also applied several IL based CPE as the basal electrode in electroanalytical chemistry or electrochemical biosensors [11-15]. IL-CPE has been widely used to analyze various targets with good electrochemical responses and fast electron transfer rate [16,17].

As an extracellular and intracellular signal transfer molecule, adenosine-5'-triphosphate (ATP) is important in the metabolic processes, which supplies energy source for daily activities [18]. It can promote the repair and regeneration of various cells in the body and enhance the metabolic activity of cells. The concentration of ATP is also related to some diseases including angiocardiopathy, malignant tumors, ischemia, Parkinson's disease etc. [19]. Therefore, analysis of ATP is very important in chemistry and clinical diagnosis. Different methods have been proposed for ATP quantitation such as electrophoresis, high-performance liquid chromatography, aptamer and spectrofluorimetry [20-23]. Although enough sensitivity and stability can be achieved, some of them are time-consuming with long time for sample pretreatment. So new and simple methods are still need to be explored for ATP detection. Electrochemical methods have been proposed as an efficient procedure for the analysis of ATP, which show advantages including high sensitivity, excellent selectivity and simplicity with small dimensional and cheap instruments [24, 25].

In this paper 1-butyl-2,3-dimethylimidazolium hexafluorophosphate (BMMImPF<sub>6</sub>) was used as binder for the modification of CPE and the electrooxidation of ATP on BMMImPF<sub>6</sub> based CPE was checked in detail. ATP yielded an irreversible oxidation peak on IL-CPE with enhanced oxidation peak current, indicating a typical electrocatalytic effect. Voltammetric behaviors of ATP on this IL-CPE were carefully studied and an electroanalytical sensor was developed for ATP detection with relatively wider linear range and lower detection limit.

## 2. EXPERIMENTAL

### 2.1. Instruments and Reagents

A CHI 1210B voltammetric analyzer (Shanghai CH Instrument, China) was equipped with a three-electrode cell including IL-CPE ( $\Phi = 4.0$  mm) as working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode. The parameters of differential pulse voltammetry (DPV) were set as follows: increment potential 0.008 V, pulse amplitude 0.05 V, pulse width 0.05 s, sample width 0.0167 s, pulse period 0.2 s, quiet time 30 s.

Graphite powder (Shanghai Colloid Chemical Co., China), BMMImPF<sub>6</sub> (Lanzhou Institute of Chemical Physics, CAS, China) and ATP (Shanghai Kayon Biological Technology Ltd. Co., China) were used without further purification. 0.2 mol/L B-R buffer solutions (pH 2.0~5.5) were chosen as supporting electrolytes. All other chemicals were of analytical grade with doubly distilled water used thoroughly the experiments.

## 2.2 Preparation of the working electrode

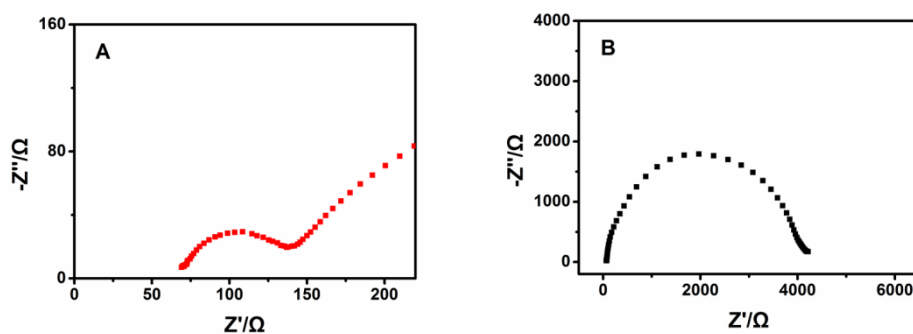
Preparation of CPE: 1.6 g graphite powder and 0.5 mL liquid paraffin were ground in an agate mortar for 1 h, and the resulted homogeneous carbon paste was packed into glass electrode tube ( $\Phi = 4.0$  mm). A copper wire was put in the opposite end to obtain CPE with its surface polished into a mirror on the weighing paper before use.

Preparation of IL-CPE: 1.5 g graphite powder and 1.5 g BMMImPF<sub>6</sub> were ground in an agate mortar for 2 h to get the homogeneous IL modified paste, which was further packed into the same diameter glass electrode tube with a copper wire used as the conductive wire to obtain IL-CPE.

## 3. RESULTS AND DISCUSSION

### 3.1 Electrochemical characterization

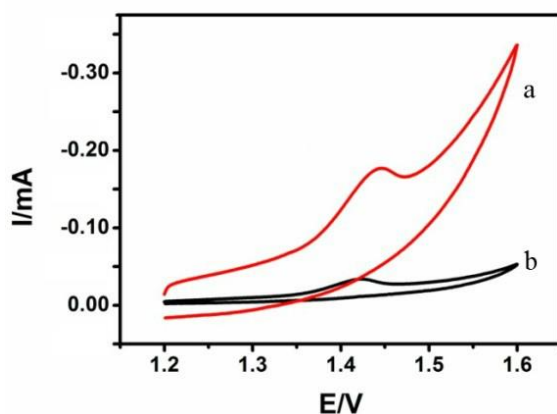
Electrochemical impedance spectra (EIS) of IL-CPE (A) and CPE (B) were measured in a 10.0 mmol/L [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> and 0.1 mol/L KCl mixed solution with curves present in Fig. 1. It can be calculated from the two impedance curves that the electron transfer resistance (Ret) of the conventional CPE (4.1 K $\Omega$ ) is much larger than that of IL-CPE (92  $\Omega$ ), which proved the high conductive BMMImPF<sub>6</sub> could enhance the whole conductivity with the interfacial resistance decreased in CPE. Therefore the Ret value is decreased greatly, which also proved the advantage of IL-CPE with high surface conductivity.



**Figure 1.** EIS of (A) IL-CPE and (B) CPE in a 10.0 mmol/L [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> and 0.1 mol/L KCl solution with the frequencies from 10<sup>5</sup> to 1 Hz.

### 3.2 Voltammetric behaviors of ATP

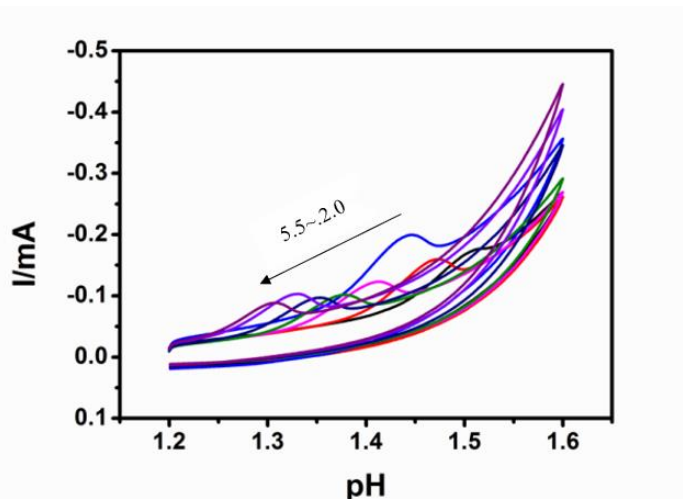
Voltammetric behaviors of 0.1 mmol/L ATP on IL-CPE (curve a) and CPE (curve b) were carefully investigated in pH 3.0 B-R buffer with cyclic voltammograms present in Fig. 2. An oxidation peak could be observed without reduction peak, which was the typical oxidation process of adenine group of ATP on the working electrode [26]. ATP can undergo a two-electrons and two-protons oxidation mechanism on the electrode with the adenine group to a 2-hydroxyadenine group [27]. On IL-CPE (a) and CPE (b), the oxidation peak potential ( $E_{pa}$ ) were located at 1.42V and 1.44V with the oxidation current ( $I_{pa}$ ) of 59.78  $\mu$ A and 18.2  $\mu$ A, respectively. The oxidation peak current of ATP on IL-CPE was 3.28 times higher than that of CPE, indicating that IL-CPE gave an excellent electrocatalytic activity toward the oxidation reaction of ATP due to the presence of IL in CPE. IL-CPE have been proved to exhibit higher ionic conductivity with the presence of IL on the electrode interface, which can accumulate ATP inside the IL film on the electrode and the interaction of imidazolium group of IL with ATP.



**Figure 2.** Cyclic voltammograms of 0.1 mmol/L ATP on IL-CPE (a) and CPE (b) in pH 3.0 B-R buffer, Scan rate: 100 mV/s.

### 3.3 Effect of buffer pH

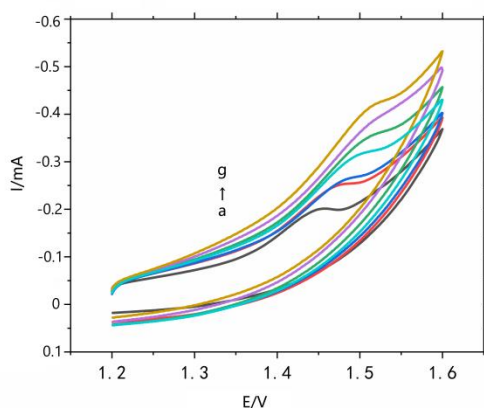
The effect of buffer pH on the oxidation process of ATP was studied from 2.0 to 5.5 with the curves present in Fig. 3. It can be observed that buffer pH value showed obvious influence on the oxidation responses and the shape of oxidation peak changed greatly. In the pH range a stable irreversible oxidation peak could be obtained with well-defined shape and the relationship of the electrochemical responses with pH value was constructed. The biggest current value was observed at pH 3.0, which was selected in the following experiments. The relationship between  $E_{pa}$  and the buffer pH were further plotted with good linear relationship. The linear regression equation was  $E_{pa}$  (V) = -0.060 pH + 1.62 ( $n=13$ ,  $\gamma=0.998$ ) and the slope value (60 mV/pH) was the same as the theoretical value (59 mV/pH) of Nernst equation, indicating that same proton and electron transfer took place in the electrode reaction. The results were in good agreement with the reported value in reference [28].



**Figure 3.** Cyclic voltammograms of 0.1 mmol/L ATP on IL-CPE with different buffer pH (2.0, 2.5, 3.0., 3.5, 4.0, 4.5, 5.0, 5.5).

### 3.4 Effect of scan rate

The influence of scan rate on the oxidation of ATP was studied with the curves overlapped in Fig. 4. The increase of scan rate led to the increase of the oxidation peak current gradually with the shift of the oxidation peak potential positively. A good linear relationship between the oxidation peak current ( $I_{pa}$ ) and scan rate ( $v$ ) was got as  $I_{pa}(\mu A) = 399.1 v (V/s) + 27.195$  ( $n=13$ ,  $\gamma = 0.998$ ) within the range from 0.05 to 0.35 V/s, indicating an adsorption-controlled process of ATP on the IL-CPE. The results may be due to the interaction of ATP with IL on the electrode surface that adsorbed ATP on the electrode surface.



**Figure 4.** Cyclic voltammograms of 0.1 mmol/L ATP on IL-CPE at different scan rates (a-g: 0.05, 0.11, 0.15, 0.19, 0.23, 0.27, 0.31 V/s).

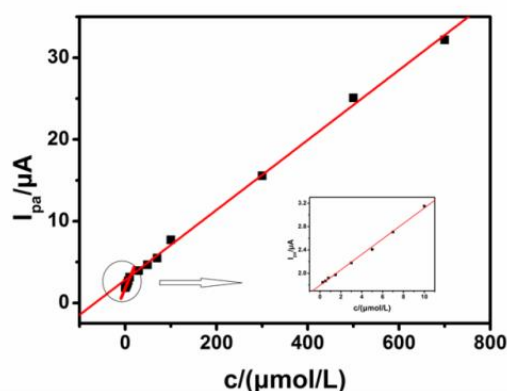
### 3.5 Effects of accumulation conditions

Due to the adsorption-controlled process, the accumulation conditions including electrode potential and time had significant impacts on the oxidation signal of ATP, and then enhanced the

analysis sensitivity. The results indicated that the maximum current values occurred at the accumulation potential and time of 0.3 V and 80 s, proving the saturated adsorption of ATP on the electrode. Therefore 0.3 V and 80 s were set as the accumulation potential and accumulation time for ATP analysis.

### 3.6 Calibration curve

Under the selected conditions, a series of ATP standard solutions were determined on IL-CPE by DPV to construct the calibration curve, which gave a sensitive peak with good separation and better resolution than cyclic voltammetry. As shown in Fig. 5, the oxidation peak currents of ATP displayed two good linear relationships with its concentration in the range from  $3.0 \times 10^{-7}$  to  $1.0 \times 10^{-5}$  mol/L and  $1.0 \times 10^{-5}$  to  $7.0 \times 10^{-4}$  mol/L with regression equations as  $I_{pa} (\mu A) = 0.132 C (\mu mol/L) + 1.794$  ( $n=8$ ,  $\gamma=0.998$ ) and  $I_{pa} (\mu A) = 0.043 C (\mu mol/L) + 2.749$  ( $n=5$ ,  $\gamma=0.999$ ). The detection limit was calculated to be  $9.12 \times 10^{-8}$  mol/L ( $3\sigma$ ), which was got by the equation ( $C_L = K S_o / S$ ).



**Figure 5.** Linear relationship of the oxidation peak current ( $I_{pa}$ ) and ATP concentration.

**Table 1.** Comparison of the analytical performances for ATP detection by various electrodes

Electrodes	Technique	Linear range ( $\mu\text{mol/L}$ )	Detection limit ( $\mu\text{mol/L}$ )	Reference
GOD/hexokinase electrode	DPV	50.0~500.0	10.0	24
Carboxyl based IL-CPE	DPV	5.0~1000.0	1.67	27
CTS-GR/MW-CPE	DPV	$1.0 \times 10^{-3}$ ~700.0	$0.342 \times 10^{-3}$	28
MW-CPE	DPV	0.1~2000.0	0.0128	29
CTS-GR/CILE	DPV	1.0~1000.0	0.311	30
GN-ABA/Au	DPV	0.015~4000.0	/	31
NG/ITO	OSWV	0.1~10.0	0.1	32
IL-CPE	DPV	0.3~700.0	0.912	This work

In this equation  $C_L$  is the detection limit,  $K$  is a constant of the confidence level (based on the rule of IUPAC,  $K=3$  at 99% confidence),  $S_o$  is the standard deviation of the blank measurements ( $n=9$ ) and  $S$  is the slope of the calibration curve. Also the analytical parameter of this IL-CPE for ATP

detection was compared with the other reported values with the data listed in table 1. It can be seen that a relatively wide linear range and low detection limit could be achieved with the use of one kind of commercial available IL.

### 3.7 Interference

The influences of ions and amino acids to the analysis of 0.1 mmol/L ATP was checked with the results listed in table 2, which indicated that these coexisting molecules had less interference to the determination with the relative error less than 4.0%. Therefore, this IL-CPE had good selectivity for ATP detection.

**Table 2.** Influences of coexisting molecules on the analysis of 0.1 mmol/L ATP (n=3).

Coexisting molecules	Concentration (mol/L)	Relative error (%)
L-alanine	$5.0 \times 10^{-4}$	1.04
L-cystine	$5.0 \times 10^{-4}$	0.83
L-arginine	$5.0 \times 10^{-4}$	2.96
L-valine	$5.0 \times 10^{-4}$	1.07
L-proline	$5.0 \times 10^{-4}$	1.31
L-tryptophan	$5.0 \times 10^{-4}$	-0.18
L-aspartic acid	$5.0 \times 10^{-4}$	1.17
Threonine	$5.0 \times 10^{-4}$	3.65
Ba <sup>2+</sup>	$5.0 \times 10^{-4}$	2.45
Ca <sup>2+</sup>	$5.0 \times 10^{-4}$	2.45
Cr <sup>3+</sup>	$5.0 \times 10^{-4}$	2.37
K <sup>+</sup>	$5.0 \times 10^{-4}$	1.61
Ni <sup>2+</sup>	$5.0 \times 10^{-4}$	2.93

### 3.8 Sample analysis

**Table 3.** Analysis results of ATP in the injection sample (n=3)

Sample	Concentration (μmol/L)	Found (μmol/L)	Added (μmol/L)	Total (μmol/L)	RSD (%)	Recovery (%)
1	82.5	82.4	200.0	282.2	0.1	99.90
2	165.0	163.0	200.0	362.0	0.3	99.50
3	330.0	327.0	200.0	525.3	0.2	99.20

This IL-CPE was further used to the analysis of ATP injection sample with the concentration calculated by the calibration curve and the recovery analyzed by the standard addition method. As listed in table 3, the results were satisfactory with the recovery from 99.20% to 99.90%, proving the practical applications of this IL-CPE for ATP analysis in drug sample.

#### 4. CONCLUSION

IL-CPE was home-made by using 1-butyl-2,3-dimethylimidazolium hexafluorophosphate as a modifier and binder, and used to study the electrochemical behaviors of ATP in detail. This IL-CPE had a significant catalytic effect on the oxidation reaction of ATP with an irreversible peak and the peak currents were enhanced in pH 3.0 B-R buffer. Under the optimized experimental conditions, the oxidation peak current of ATP and its concentration showed a good linear relationship from 0.3 to 700.0  $\mu\text{mol/L}$  with the detection limit of 0.912 nmol/L. The modified electrode was applied to the determination of actual injection sample with satisfactory results, proving the real applications of this IL based electrode.

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