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Capillary electrophoresis of adenosine phosphates using boron-doped diamond electrodes

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Abstract. A capillary electrophoresis coupled with electrochemical detection using boron-doped diamond electrode was developed for simultaneous detection of adenosine phosphates, i.e. adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP). In phosphate buffer solution pH 7, these three adenosine phosphates have similar oxidation potentials at around +0.9 V (vs. Ag/AgCl), which indicated that the oxidation occurred at the same moiety. Capillary electrophoresis, which was then performed using fused silica capillary (dia. 0.05 mm) at an applied potential of 10 KV can separate ATP, ADP and AMP with the retention times of 848 s, 1202 s, and 1439 s, respectively. Linear calibration curves with the limits of detection of 0.59 μM, 0.56 μM and 1.78 μM, respectively, can be achieved, suggested that capillary electrophoresis with electrochemical detector is promising for simultaneous detection of adenosine phosphates.

Keywords: capillary electrophoresis, adenosine phosphates, electrochemical detection, boron-doped diamond

1. Introduction

Adenosine phosphate is a nucleotide composed of pentose, adenine bases (purine), and phosphates. Adenosine phosphates have a various number of phosphates, that are adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP). Among these three types of adenosine phosphates, only ATP is used as a medical treatment for amyotrophia disease, cerebral hemorrhage and hepatitis [1]. Accordingly, a sensitive method to measure ATP in the mixture of those adenosine phosphates is necessary. Generally, HPLC is employed to determine the adenosine phosphates. However, HPLC is not a cheap instrument. A capillary electrophoresis method, which is performed based on the movement of the charged molecules through a fluid under an electric field influence, can be used as an alternative for the separation process of adenosine phosphates. Then, by combining the electrophoresis with a detector, the separated adenosine phosphates can be quantified.

The detectors commonly used in capillary electrophoresis are UV-visible and laser induced fluorescence [2]. UV-visible has the disadvantage in the detection limit, while the laser induced fluorescence is an expensive instrument. Meanwhile, boron-doped diamond (BDD) is established as an alternative of solid electrodes due to its superior properties among other electrodes, including its wide potential window, low background current, and high surface stability [3-5]. BDD have been investigated for electrochemical detection of adenosine phosphates [1], showing similar oxidation

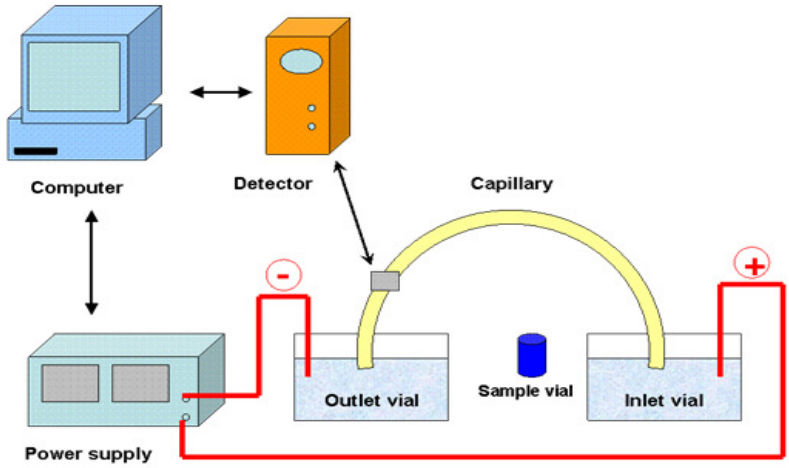

Figure 1. Scheme of the electrophoresis cell.

potentials at around +0.9 V for those three adenosine phosphates. In this work, BDD was employed as the detector for capillary electrophoresis of AMP, ADP, and ATP. Succesfull separation with good detection limits could be achieved, suggested that the method was promising for the real applications.

2. Materials and methods

Prior to use, the BDD film was cleaned subsequently by ultrasonication in 1-propanol and water for 15 min each. Cyclic voltammetry was performed in one-container electrochemical cell to study the electrochemical behaviour of adenosine phosphates in phosphate buffer solution (PBS).

The capillary electrophoresis cell was self-fabricated and modified from the design of Shin *et al.* [2]. This cell was composed of a couple of platinum electrodes immersed in two separate containers. The electrodes were connected to a power supply to provide negative and positive poles, while between the containers, a silica fused capillary tube (i.d. 0.05 mm) was placed. A BDD film was arranged at the end of the capillary tube in the negative pole container and connected to a potensiostat with a Pt wire as the counter electrode and an Ag/Ag system as the reference electrode. The scheme of capillary electrophoresis cell is displayed in figure 1.

3. Results and discussion

Cyclic voltammograms (CVs) of 0.1 M PBS pH 7 in the absence and in the presence of AMP (figure 2a) in comparison with those of ADP and ATP (figure 2b and figure 2c, respectively) at BDD electrode show an oxidation peak of all types of adenosine phosphates at the potential around +0.90 to +0.95 V (vs. Ag/AgCl). It can be seen that the more phosphate groups contained in the adenosine phosphates, the higher oxidation potential was required. However, the oxidation potential was observed in the narrow range between +0.90 to +0.95 V, suggested that the oxidation occurred at the same moieties. Adenine, the nitrogenous bases with electroactive properties, is the most possible moiety to undergo the oxidation reaction [1]. Furthermore, there was no reduction peak observed in the potential range between -1.0 to +1.5 V indicated that the oxidation reaction was irreversible.

Influence of the pH was also studied. Linear relationships were observed in the pH range between pH 2 and pH 8 for all adenosine phosphates with a slope of around -0.59 mV/pH (data not shown) suggested that the reaction involving the same numbers of proton and electron. Furthermore, linear calibration curves showed good linearity of the adenosine phosphates in the concentration range of 50 µm to 500 µm, indicated that the electrode can be applied for detection of all adenosine phosphates.

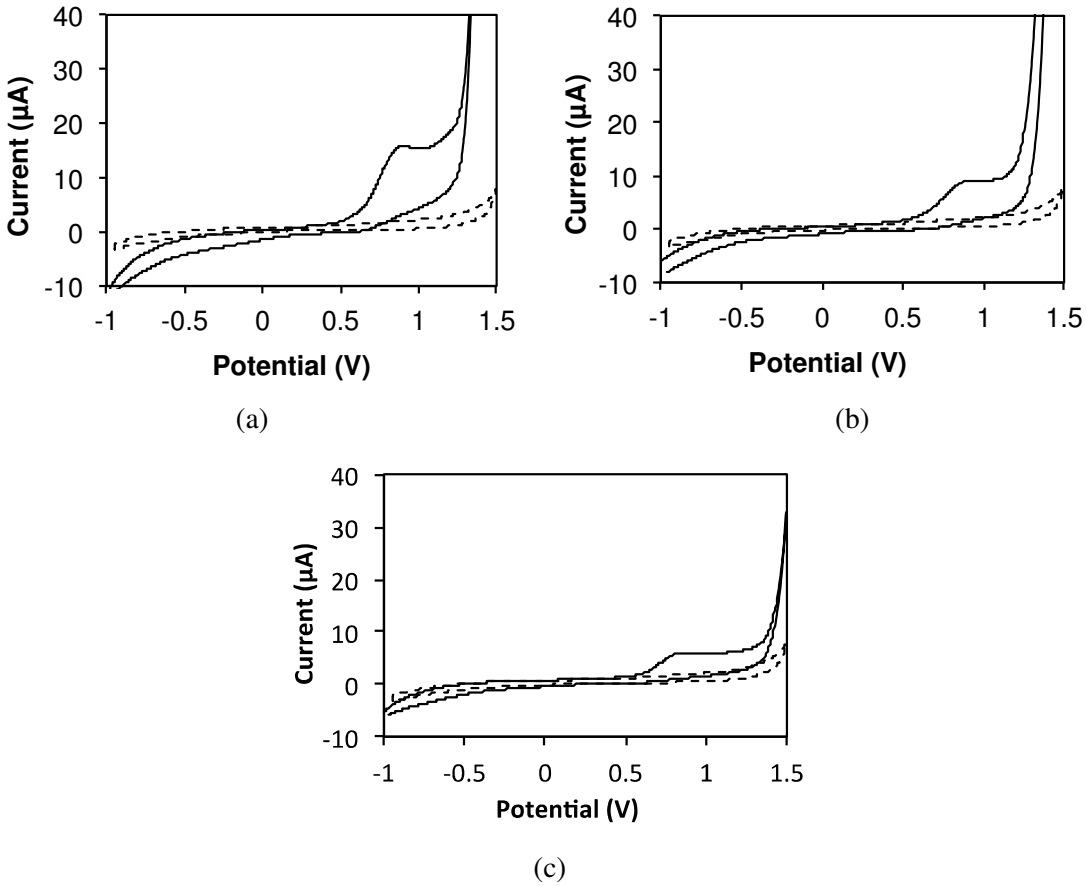

Figure 2. Cyclic voltammetry of 0.1 M phosphate buffer solution in the absence (dashed line) and in the presence (solid line) of 0.05 mM (a) AMP, (b) ADP, and (c) ATP in pH 7 at BDD electrodes.

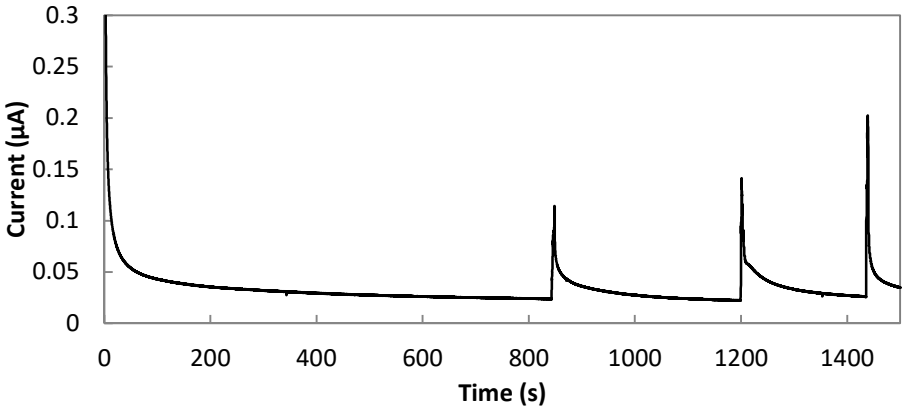

Figure 3. Typical amperogram of the mixture of 0.05 mM AMP, 0.05 mM ADP, and 0.05 mM ATP in 0.1 M phosphate buffer solution pH 7 after separating process using capillary electrophoresis method. Separating voltage was 10 KV. BDD film was used as the electrochemical detector at an applied potential of +0.95 V.

Capillary electrophoresis of the adenosine phosphates was performed at the separating potential of 10 KV. At the end of capillary tube in the negative pole container, BDD film was placed.

Amperommetry technique at an applied potential of +0.95 V was applied and all types of adenosine phosphate was added into the sample container with PBS pH 7 was used as the mobile phase. Figure 3 shows the amperogram of a mixture solution of 0.05 mM ATP, 0.05 mM ADP, and 0.05 mM AMP in 01 M PBS pH 7. The peaks of ATP, ADP and AMP were observed at the retention times of 848 s, 1202 s, and 1439 s, respectively. ATP appeared as the first peak due to its higher charge, followed by ATP and AMP. Further, investigation of the mixture with various concentrations of adenosine phosphates can achieve linear calibration curves with the limits of detection of 0.59 μM, 0.56 μM and 1.78 μM, respectively. The results suggested that electrophoresis with electrochemical detector is promising for the simultaneous detection of adenosine phosphates.

4. Conclusions

BDD electrode was successfully applied in the electrochemical detection using a capillary electrophoresis method for mixtures solution of ATP, ADP, and AMP. The peaks of ATP, ADP and AMP were observed at the retention times of 848 s, 1202 s, and 1439 s, respectively, with the limits of detection of 0.59 μM, 0.56 μM and 1.78 μM.

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