Microchip capillary electrophoresis with a boron-doped diamond electrode for rapid separation and detection of purines

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Abstract

Microchip capillary electrophoresis (CE) coupled with a boron-doped diamond (BDD) electrode has been employed for the separation and detection of several purines and purine-containing compounds. The BDD end-channel amperometric detector offers favorable signal-to-noise (S/N) characteristics at the high detection potential ($+1.3$ V) essential for detecting purine-related compounds. Factors influencing the separation and detection processes were examined and optimized. Five purines (guanine, hypoxanthine, guanosine, xanthine, and uric acid) have been separated within 6 min at a separation voltage of 1000 V using a borate/phosphate run buffer (pH 8.2). Linear calibration plots are observed for micromolar concentrations of the purine compounds. Good stability and reproducibility (R.S.D. $< 5\%$) are obtained reflecting the minimal adsorption of purines at the BDD surface. Applicability for the detection of nucleosides, nucleotides, and oligonucleotides is illustrated. The new microchip protocol offers great promise for a wide range of bioanalytical applications involving assays of purines and purine-containing compounds.
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1. Introduction

The separation and detection of purine bases and purine-containing compounds represents a challenging and important task owing to the importance of these compounds in a variety of biochemical processes. Adenine and guanine are the building blocks of both DNA and RNA that play a crucial role in protein biosynthesis and the storage of genetic information [1]. Hypoxanthine, xanthine, and uric acid are metabolites of purine bases in nucleic acids through a series of biochemical pathways [2]. In addition, hypoxanthine and xanthine have been linked to off-flavors in food and are important markers of fish freshness [3,4]. The concentration of uric acid is an important diagnostic parameter in the evaluation of renal diseases [2]. Accordingly, it is essential to develop a simple and rapid method for the separation and detection of these bioactive purine bases. Liquid chromatography (LC) [5–9] and capillary

electrophoresis (CE) [10–13] have been widely used for the determination of purine bases and related compounds. To our knowledge, there are no reports on the use of CE microchips for the separation and detection of bioactive purines and related compounds.

This article reports on a CE microchip with an electrochemical detector for measurements of purine and purine-containing compounds. Microfabricated fluidic devices, particularly CE microchips, have received growing attention in recent years [14,15]. Such analytical microsystems combine the advantages of high performance, design flexibility, reagent economy, high throughput, miniaturization, and automation [15,16]. Electrochemical detection (ED) offers great promise for CE microchip systems, with features that include high sensitivity, inherent miniaturization (of both the detection and control instrumentation), low cost and power demands, and high compatibility with micromachining technology [17,18]. The performance of CE–ED microchips is strongly influenced by the working-electrode material. Commonly used working–electrode materials for chip-based ED detection, including platinum, gold, and various forms of carbon [19–25], are not suitable for reliable

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detection of purines owing to their limited anodic potential range and surface fouling problems. Boron-doped diamond (BDD) electrodes have attracted considerable recent attention owing to their attractive properties, including a wide potential window, low and stable background currents (and related noise level), and negligible adsorption of organic compounds [26,27]. Such features are particularly attractive for electrochemical monitoring of purine-related compounds whose oxidation occurs at very high anodic potentials and is accompanied by surface accumulation. The use of BDD electrodes for voltammetric analysis of nucleic acids [28] and of purine derivatives [29], as well as for detection in conventional [30] and microchip [31] CE systems has been reported recently. The favorable performance characteristics of the new CE–ED microchip protocol for monitoring purine compounds are discussed and demonstrated in the following sections.

2. Experimental

2.1. Chemicals

Guanine, hypoxanthine, xanthine, uric acid, guanosine, guanosine 5′-monophosphate, sodium hydroxide, and sodium phosphates were all obtained from Sigma (St. Louis, MO, USA). Both $d(G)_6$ and $d(G)_{10}$ were purchased from Invitrogen (Carlsbad, CA, USA). Stock solutions of guanine, guanosine, hypoxanthine, and uric acid (10 mM) were prepared daily in a 50 mM NaOH aqueous solution. Stock solutions of xanthine (10 mM), guanosine 5′-monophosphate (10 mM), $d(G)_6$ (1000 ppm), and $d(G)_{10}$ (2000 ppm) were made in water. The CE running buffer was a mixture of 5 mM sodium borate and 10 mM sodium phosphate buffers (pH 8.2).

2.2. Apparatus

Details of the integrated CE–ED glass chip microsystem were described previously [21]. The simple-cross single-separation channel glass microchip (86 mm × 20 mm × 2 mm) was obtained from Micralyne (model MC-BF4-001, Edmonton, Canada). The detection reservoir was cut off to facilitate the end-column electrochemical detection. The microchip had a four-way injection cross that was connected to the three reservoirs and the separation channel. The chip had a 80 mm long separation channel and a 10 mm long injection channel (between the sample and buffer reservoir). The separation channel had an effective length of 75 mm (from the injection cross to the channel outlet). The channels had a maximum depth of 20 μm and a width of 50 μm (at the top). Short pipette tips were inserted into the fluidic ports of the various reservoirs. A Plexiglas holder was fabricated for housing the separation chip and the detector and allowing their convenient replacement. Platinum wires, inserted into the individual reservoirs on

the holder, served as contacts to the high-voltage power supply. Such homemade power supply had an adjustable voltage range between 0 and +4000 V.

2.3. Electrode preparation

Chemically vapor-deposited boron-doped diamond films were prepared at the Applied Chemistry Department of Tokyo University (Tokyo, Japan) [28]. A piece of diamond film (7 mm × 0.3 mm × 40 μm) was glued onto a 33 mm × 10 mm alumina ceramic plate by a quick-setting nonconductive epoxy (Radio Shack, Forth Worth, TX, USA). The copper wire contact (with the ends scraped to remove the insulating coating) was placed on one end of the BDD band electrode. A conductive epoxy (Chemtronics, Kennesaw, GA, USA) was subsequently applied to the contact point (between the diamond and the copper wire) and was cured at 100 °C for 30 min. Subsequently, a layer of the quick-setting nonconductive epoxy was applied on the cured conductive epoxy. Prior to use, the diamond band electrode was sonicated in 2-propanol for 10 min. The screen-printed carbon electrodes (used for comparison) were printed with a semi-automatic printer (model TF 100, MPN, Franklin, MA, USA) on a similar ceramic plate substrate. A carbon ink (Acheson Colloids, Electrodag 440B, Catolog No. 49AB90, Ontario, Canada) was used for printing the electrode strips. Details of the printing process and dimensions were described elsewhere [21]. The dimensions of the screen-printed bare carbon and diamond electrodes were identical (0.3 mm × 6.0 mm).

2.4. End-column amperometric detection

The working electrode was placed opposite the outlet of the separation channel [21]. The distance between the electrode surface and the channel outlet (∼50 μm) was controlled by a plastic screw and a thin-layer spacer. Amperometric detection was performed with an Electrochemical Analyzer 620 (CH Instruments, Austin, TX, USA) using the "amperometric $i–t$ curve" mode. The electropherograms were recorded with a time resolution of 0.1 s (without software filtration) while applying the desired detection potential (usually +1.3 V). Sample injections were performed after the baseline stabilization. All experiments were performed at room temperature.

2.5. Electrophoretic procedure

The channels of the glass chip were treated before use by rinsing with 0.1 M NaOH and deionized water for 10 min each. The "buffer" reservoirs were filled with the CE running buffer solution, while the "sample" reservoir with the samples. Injections were carried out by applying the desired potential between the "sample" reservoir and the grounded detection reservoir for 3 s (while all other reservoirs floating). Separations were performed by switching the

high-voltage contacts and applying the corresponding separation voltages to the "running buffer" reservoir (with the detection reservoir grounded and all other reservoirs floating).

3. Results and discussion

BDD is a promising electrode material for microchip CE applications owing to its wide potential window, low background currents and related noise level, as well as minimal adsorption of organic compounds [30,31]. Such properties facilitate electrochemical measurements of purine compounds that are commonly hampered by their extreme detection potential and surface accumulation. In the present work, the BDD electrode was coupled with the microchip CE system for the amperometric detection and separation of several purines and purine-containing compounds. The attractive performance of the diamond-electrode microchip detector was demonstrated by comparing its purine response with that observed at a commonly used screen-printed carbon electrode (of identical dimensions). Shown in Fig. 1 are typical electropherograms for a mixture containing guanine (a); hypoxanthine (b); guanosine (c); xanthine (d); and uric acid (e) at the screen-printed carbon (A) and BBD (B) detectors. The BDD electrode displays substantially enhanced signal-to-noise (S/N) characteristics. Note in particular, the significantly lower noise level and stable baseline of the BDD electrode at this (+1.3 V) extreme potential, reflecting its large overvoltage for the solvent decomposition. The favorable signal-to-background characteristics of the BDD electrode are coupled to sharper peaks, and hence to a greatly improved resolution. The half-peak widths of guanine, hypoxanthine, guanosine, xanthine, and uric acid
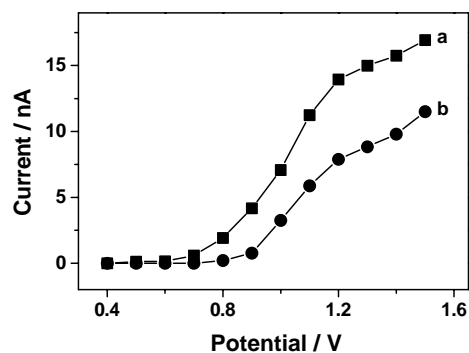
Fig. 2. Hydrodynamic voltammograms for 50 μM guanine (a) and 100 μM guanosine (b) at a BDD-based detector. Conditions: separation voltage, 1500 V; injection voltage, 1500 V; injection time, 3 s; running buffer, 5 mM borate/10 mM phosphate buffer (pH 8.2).

at the BDD electrode are 4.1, 10.2, 10.2, 9.7, and 9.6 s, respectively (compared to 5.1, 7.8, 18.9, 9.7, and 17.4 s at the screen-printed detector).

Fig. 2 displays hydrodynamic voltammograms for 50 μM guanine (a) and 100 μM guanosine (b) at the BDD electrode detector. The curves were recorded pointwise by changing the potential over the +0.4 to +1.5 V range. Both compounds displayed similar HDV profiles, reflecting their oxidation at the N^7 position. The anodic response starts at +0.7 V (guanine) and at +0.8 V (guanosine). Purine nucleotides are known to oxidize at more positive potentials than their free bases [32]. The current rises rapidly up to +1.2 V and then more slowly. Subsequent amperometric detection work employed a detection potential of +1.3 V that offered the most favorable signal-to-noise characteristics. A dramatic increase in the baseline current, its slope, and the corresponding noise level was observed at higher potentials. Different optimal detection potentials would be required in connection to different separation voltages (which may shift the voltammetric profiles).

Fig. 3 shows the influence of the separation voltage upon the separation and detection of guanine (a); guanosine (b); and xanthine (c). As expected, increasing the separation voltage from 1000 to 3000 V (A–E) dramatically decreases the migration time for all three purine-related compounds, from 120 to 39 s (guanine), from 158 to 56 s (guanosine), and from 210 to 69 s (xanthine). Also shown (in the inset) is the effect of the separation voltage upon the separation efficiency, i.e., on the plate number of guanine (N) and resolution (R) of guanosine and xanthine. Both the plate number and resolution decrease upon raising the separation voltage, from 2340 to 501 and from 2.95 to 0.74, respectively. Note also that despite of the high detection potential, flat baselines and low noise levels are maintained even at high separation voltages. Such behavior indicates an effective isolation from the high separation voltage. Significantly larger initial baseline current slopes are common with other electrode materials [21]. Most of the subsequent work employed a separation voltage of 1500 V.
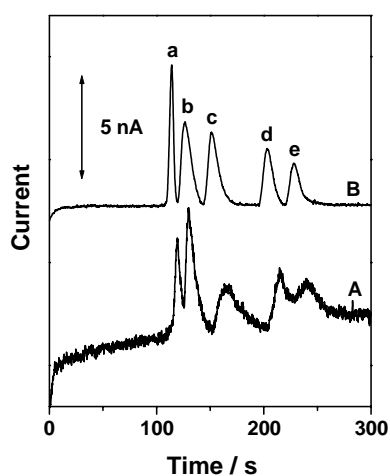
Fig. 1. Electropherograms for mixtures containing 50 μM guanine (a); 100 μM hypoxanthine (b); 100 μM guanosine (c); 100 μM xanthine (d); and 200 μM uric acid (e) at the screen-printed carbon (A) and BDD (B) electrodes. Operation conditions: separation voltage, 1000 V; injection voltage, 1000 V; injection time, 2 s; running buffer, 5 mM borate/10 mM phosphate buffer (pH 8.2); detector potential, +1.3 V (vs. Ag/AgCl wire).
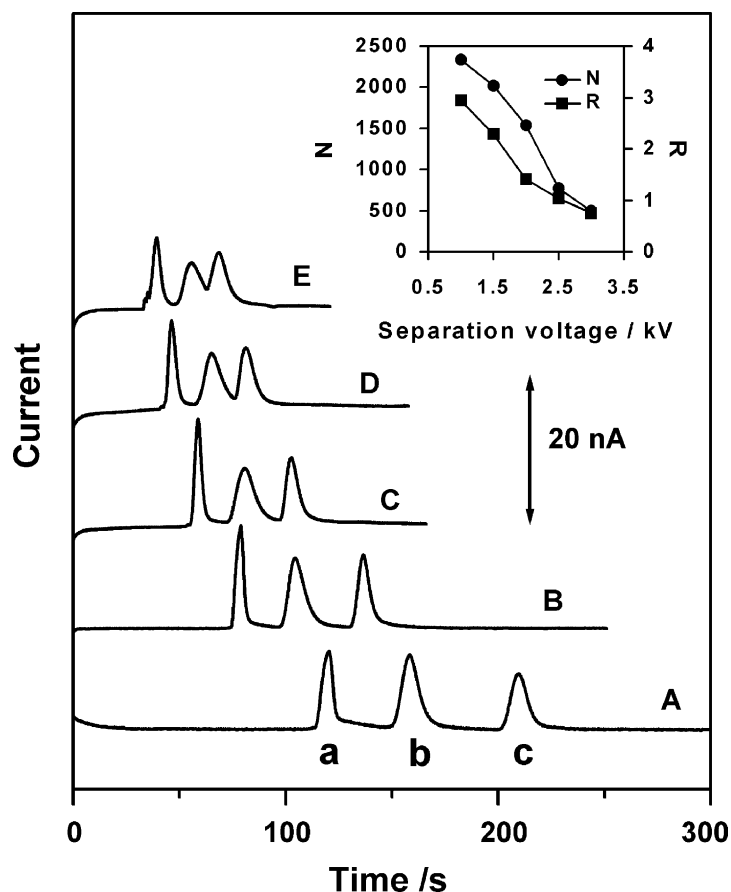
Fig. 3. Influence of the separation voltage on the response of the BDD detector for a mixture containing 50 μM guanine (a); 100 μM guanosine (b); and 100 μM xanthine (c). Also shown (in the inset) is the dependence of the plate number (N) of guanine and the resolution (R) between guanosine and xanthine upon the separation voltage. Separation voltage: (A) 1000 V; (B) 1500 V; (C) 2000 V; (D) 2500 V; (E) 3000 V; other conditions as in Fig. 2.

The amperometric detection at the BDD electrode results in a well-defined concentration dependence. Electropherograms for mixtures containing increasing levels of guanosine (a) and xanthine (b), in 50 μM steps, are shown in Fig. 4(A–E). Defined peaks, proportional to the concentration of both analytes, are observed. The resulting calibration plots (shown as inset) are highly linear with sensitivities of 70.9 and 63.3 nA/mM for guanosine and xanthine, respectively (correlation coefficients, 0.9975 and 0.9982). Also shown (in the second inset) is an electropherogram for a mixture containing 10 μM guanosine and xanthine. The favorable signal-to-noise characteristics of these data indicate detection limits of 2.2 μM guanosine and 2.1 μM xanthine (based on S/N = 3).

Good precision is another attractive feature of the new BDD detector/separation microchip protocol. The precision was examined from a series of eight repetitive injections of a sample mixture containing 50 μM guanine, 100 μM guanosine, and 100 μM xanthine. Reproducible signals were obtained with R.S.D. of 1.3% (guanine), 3.3% (guanosine), and 4.4% (xanthine) for the peak currents. Such good precision reflects the high resistance to fouling of the BDD surface.

The applicability of the CE–ED microchip for the detection of a free purine base, its corresponding nucleotide and nucleoside is illustrated in Fig. 5. This figure shows electropherograms for 50 μM guanine (A); 100 μM guanosine (B); and 200 μM guanosine 5′-monophosphate (C) at the BDD detector. As expected, longer migration times (of 110 and 210 s) are observed when ribose and ribose/phosphate groups are coupled to the free nucleobase. In addition, the peak height and sharpness decrease upon introducing ribose or ribose/phosphate groups to the guanine. The sensitivity is inversely proportional to the size of the biomolecule, reflecting the lower diffusion coefficients and larger hindrance to electron transfer to the guanine oxidation site. Purine nucleotides and nucleosides are known to oxidize at potentials more positive than their parent bases [32]. We also evaluated the detection of purine-based oligonucleotides, such as $d(G)_6$ and $d(G)_{10}$. These yielded broad peaks (with a 50 s width) and similar migration times (not shown). The inclusion of water-soluble polymers in the CE run buffer will be required to facilitate a size-exclusion separation of these oligonucleotides.

In conclusion, we have demonstrated the utility of a CE microchip with a boron-doped diamond electrode detector for the separation and detection of purine and purine-containing compounds. The BDD detector has been shown extremely useful for high-potential detection of
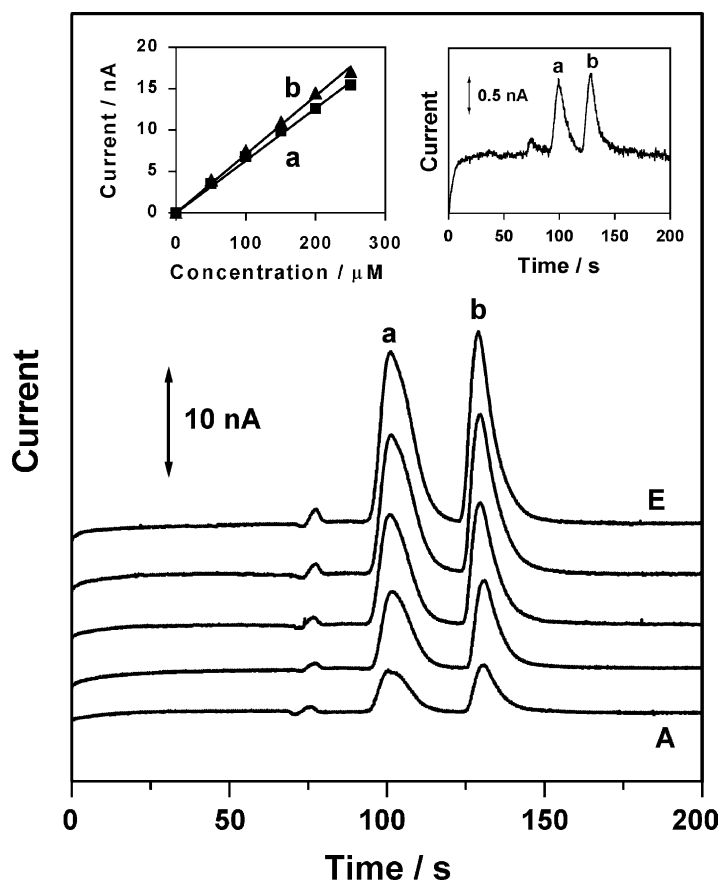
Fig. 4. Calibration data at the BDD detector for mixtures containing increasing levels of guanosine (a) and xanthine (b) in increments of 50 μM (A–E). Also shown (in the insets) are the resulting calibration plots and an electropherogram for a mixture containing 10 μM guanosine (a) and xanthine (b). Detector potential, +1.3 V (vs. Ag/AgCl wire); other conditions as in Fig. 2.

these biomolecules. The method has also successfully been applied for the detection of nucleoside, nucleotide, and an oligonucleotide. The high speed, negligible sample consumption, low power requirements and small size of CE microchip devices offer great promise for the determination of purine and purine-containing compounds in clinical, biotechnological, and food matrices. The BDD detection system also indicates potential for direct DNA analysis (without additional tagging or derivatization).
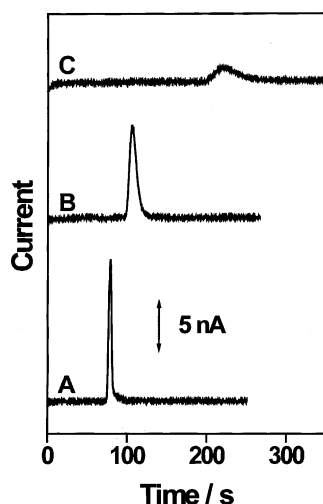
Fig. 5. Electropherograms for 50 μM guanine (A); 100 μM guanosine (B); and 200 μM guanosine 5′-monophosphate (C) at the BDD detector. Detection potential, +1.3 V (vs. Ag/AgCl wire); other conditions as in Fig. 2.

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