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Microchip flow-injection analysis of trace 2,4,6-trinitrotoluene (TNT) using mercury-amalgam electrochemical detector

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Abstract

This paper reports on a microfluidic device for the flow-injection/electrochemistry analysis of nitroaromatic explosive. The response is very fast (150 assays/h), highly sensitive (detection limit 7.0 μg L^{-1}), reproducible and stable (R.S.D. = 2.0%; n = 30) and linear (over 20–100 μg L^{-1} range). Relevant experimental parameters have been optimized. The new microsystem offers great promise for on-site monitoring of TNT, with significant advantages of speed/warning, sample size, efficiency and cost. Most favorable S/N characteristics were obtained at the Hg/Au-amalgam end-channel detector.

Keywords: Explosives; Microfluidics; Flow-injection analysis; Electrochemistry

1. Introduction

In response to recent terrorist activities, there are growing needs for developing field screening methods for detecting TNT and related explosive compounds [1]. “Lab-on-a-chip” technology offers great promise for obtaining the desired forensic information in a faster, simpler and cheaper matter compared to conventional laboratory-based techniques [2–4]. Microchip-based flow-injection analysis (μFIA) use electroosmotic flow (EOF) to move samples through the microchip manifolds. μFIA with electroosmoticaly driven flow overcomes many problems associated with the conventional FIA systems, such as portability and robustness since there are no moving mechanical parts. Moreover, the amounts of reagents required for analysis are vastly reduced as flow rates and sample volumes in the nanolitre region are used. Usefulness of microchip-based FIA has been demonstrated on determination of nitrite [5], nitrate [6] and phosphates [7] with sample rates ranging from 30 to 60 assays/h. Described μFIA studies [5–7] were performed with somehow bulky spectrophotometric detection, which is incompatible with truly portable “lab-on-a-chip” format. Contrary to the spectrophotometric detection, the electrochemical one offers a great advantages for microchip-based systems, with attributes of high sensitivity, inherent miniaturization, low power requirements and low cost.

Several papers have demonstrated the utility of electrophoresis microchips for the separation and amperometric detection of nitroaromatic explosives utilizing different working electrode materials, including carbon [8–10] and gold [11].

The goal of this effort is to develop fast-responding flow-injection microchip device for the electrochemical detecting trace levels (down to low ppb-level) of 2,4,6-trinitrotoluene.

2. Materials and methods

2.1. Chemicals

A stock solution of 2,4,6-trinitrotoluene (TNT, 1000 mg L^{-1}) was obtained from Cerilliant (Austin, TX, USA). Sodium borate 10-hydrate was received from J.T. Baker Chemical Company (Phillipsburg, NJ, USA). Standard solutions of TNT were prepared daily (using proper safety precautions) in the running buffer. The mercury(II) stock solution (4000 mg L^{-1}) in 0.1 M HCl was received from Aldrich.

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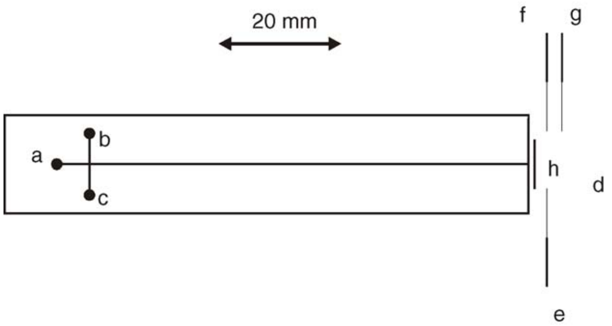

Fig. 1. Microchip system for FIA with electrochemical detection: (a) run buffer reservoir, (b) sample reservoir, (c) unused/second sample reservoir, (d) detection reservoir, (e) platinum cathode for μFIA, (f) Ag/AgCl wire reference electrode, (g) platinum counter electrode and (h) detection electrode.

All the chemicals were used without any further purification.

2.2. Apparatus

The glass chip was fabricated by Micralyne (Model MC-BF4-001, Edmonton, Canada) by means of wet chemical etching and thermal bonding techniques. The 86 mm × 16 mm chip, shown in Fig. 1, consisted of a four-way injection cross, with a 74 mm long main channel and side arms of 5 mm long each. The end of the chip (containing the outlet reservoir) was cut off, leaving the channel outlet at the end side of the chip, to facilitate the end-column amperometric detection [8,12]. The channels were 50 μm wide and 20 μm deep.

The homemade high-voltage power supply had an adjustable voltage range between 0 and 5000 V. A plexiglas holder was fabricated for housing the glass chip and the detector and allowing their convenient replacement. Short pipet tips were inserted into the three reservoir holes on the glass chip for providing solution contact between the channel on the chip and corresponding reservoir on the chip holder. The amperometric detector placed in the waste reservoir (at the channel outlet side), consisted of a Ag/AgCl wire reference (f), a platinum wire counter (g) and a working (h) electrodes. Different working electrodes were tested. The disc gold and mercury/gold working electrodes, housed in the plastic screw, were placed opposite to the channel outlet, at 50 μm distance [12]. The screen-printed working electrode was placed opposite to the channel outlet, at a 50 μm distance (controlled by a plastic screw and a spacer of the desired thickness) [8].

Platinum wires, inserted into the individual reservoirs, served as contacts to the high-voltage power supply. Amperometric detection was performed with an electrochemical analyzer CH 621 (CH Instruments, Austin, TX, USA) using the “amperometric *i*–*t* curve” mode.

2.3. Electrodes fabrication

The gold disc electrode was received from CH Instruments. Prior to use, the gold working electrode was polished with 3 and 0.05 μm alumina (10 min each) to achieve reproducible response [12]. The amalgam mercury/gold electrode was prepared by preplating mercury, using the 100 mg L^{-1} Hg(II)/0.1 M HCl solution onto highly polished gold surface for 8 min at −0.8 V.

The thick-film carbon electrodes were printed with a semiautomatic printer (Model TF 100, MPM, Franklin, MA). An Acheson carbon ink (Electrodag 440B; Acheson Colloids, Ontario, CA, USA) was used for printing the electrode strips. Details of the printing process and dimensions were described elsewhere [8].

2.4. Procedure

The channels of the microchip were treated before use by rinsing with 0.1 M NaOH and deionized water for 10 min each. Usually, the “buffer” reservoirs (a and c) were filled with the electrophoresis running buffer solution, while the “sample” reservoir (b) with the TNT solution. After the initial sample loading in the injection channel, the sample was injected by applying field strength +190 V/cm for 3 s between the “sample” reservoir (b) and the grounded detection reservoir (d). This drove the sample “plug” into the main channel through the intersection. Flow-injection analysis was usually carried out by applying high-voltage to the running buffer reservoir (a) with detection reservoir (d) grounded and all other reservoirs floating. For “carry-over” experiments, reservoir (a) was filled with the running buffer and reservoirs (b and c) with samples of different concentrations. These samples were subsequently injected applying a voltage of +190 V/cm between reservoirs (b and d) or (c and d), respectively.

The electropherograms were recorded with a time resolution of 0.1 s while applying the selected detection potential (usually −0.6 V) onto the working electrode (versus Ag/AgCl wire). Sample injections were performed after stabilization of the baseline. All experiments were performed at room temperature.

2.5. Safety considerations

The high-voltage power supply should be handled with extreme care to avoid electrical shock. 2,4,6-Trinitrotolune is a toxic/suspected carcinogen; skin and eye contact and accidental inhalation or ingestion should be avoided.

3. Results and discussion

Nitroaromatic compounds, such as TNT, are readily reduced at various electrodes [13–15]. Reduction of nitrotoluenes occurs in single four-electron step to form hydroxylamines, which is followed by two-electron reduction to corresponding amine [13,16–18]. The electrochemical reactivity of TNT, and thus performance of working electrode is strongly influenced by the type of the electrode. Fig. 2 displays the flow-injection amperometric response for a 200 ppb TNT solution using three different electrode materials. All three working electrodes displays single peak corresponding to the TNT reduction. The thick-film carbon electrode resulted in a larger background (Fig. 2A), compared to gold (Fig. 2B) and amalgam mercury/gold (Fig. 2C) electrodes. Most favorable signal-to-noise characteristics were observed on

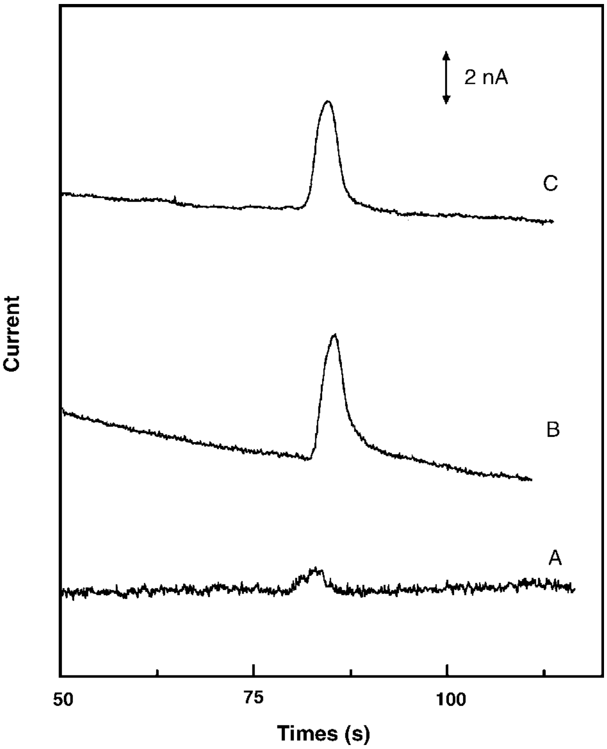

Fig. 2. FIA microchip response of different detector materials for a 200 ppb of TNT solution. (A) Carbon thick-film electrode, (B) gold disc electrode and (C) amalgam mercury/gold disc electrode. Conditions: μFIA and injection field strength, +190 V/cm; detection potential, −0.6 V; running buffer, borate buffer (15 mM, pH 9.2).

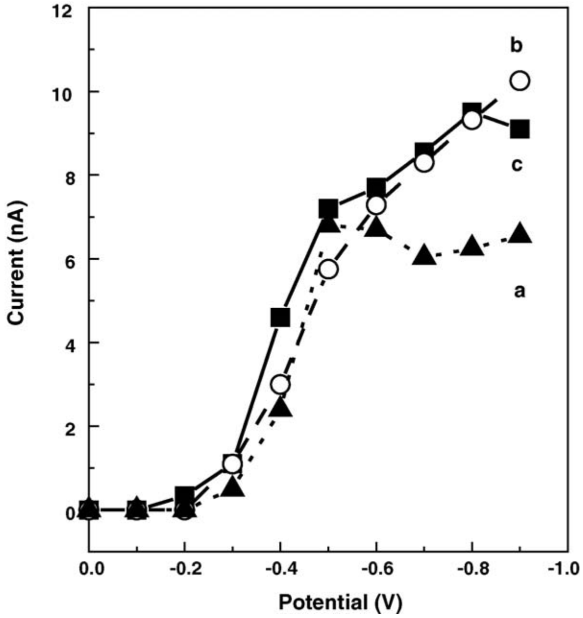

Fig. 3. Hydrodynamic voltammograms for TNT using thick-film carbon (a), gold disc (b) and amalgam mercury/gold disc (c) electrodes. TNT concentration 5 ppm (a) and 1 ppm (b and c). Conditions as in Fig. 2.

amalgam mercury/gold electrode that was used in all subsequent work.

Hydrodynamic voltammograms were used to assess the redox properties of TNT at the carbon, gold and amalgam mercury/gold detection electrodes (Fig. 3). The curves were developed point-wise by making 100 mV changes in the applied potential from 0 to −0.9 V. Defined waves are observed for all electrodes, starting at −0.3 V (a–c) and leveling off at −0.5 (a) or −0.8 V (c). Due to the rising background signal, all subsequent work was carried out at a potential of −0.6 V, which offered the most favorable signal-to-noise characteristics.

The effect of the applied field strength upon the sensitivity and speed of the microchip-based FIA TNT assay at the Hg/Au detector is shown in the Fig. 4. As expected, increasing the "flow-injection" field strength from +127 to +633 V/cm dramatically decreases the elution time from 147 to 24 s. The peak height is increasing rapidly between +127 and +633 V/cm. Increasing the field strength has a minimal effect upon the background noise. However, a larger initial baseline can be observed for field strengths ranging from +380 to +633 V/cm, indicating an incomplete isolation of detector from high-field strengths [19,20]. Note that 150 μFIA assays/h can be realized at field strength +633 V/cm.

The attractive performance of the flow-injection microchip amperometric detection system is demonstrated in Fig. 5. For example, Fig. 5A displays portion of the response for TNT solutions of different concentrations (60 ppb (a) and 180 ppb (b)). Well-defined current signals are observed for these $\mu g\, L^{-1}$ changes in the level of TNT. The response is reversible, with no apparent "carry-over". Fig. 5B shows the high reproducibility of the amperometric response for 30 repetitive injections of a 350 ppb TNT solution at a field strength +633 V/cm. This series result in mean peak current of 39.5 nA with relative stan-

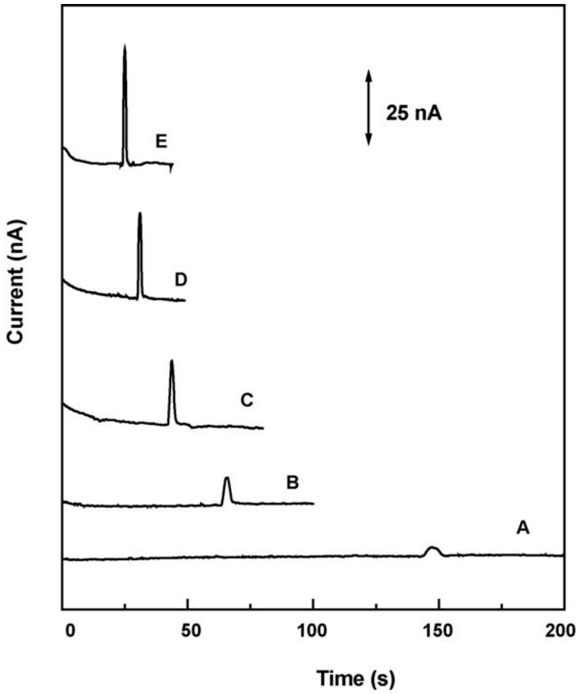

Fig. 4. Influence of flow-injection field strength upon the elution time and response of 350 ppb of TNT using amalgam mercury/gold electrode. Fields strengths +127 V/cm (A), +253 V/cm (B), +380 V/cm (C), +506 V/cm (D) and +633 V/cm (E). Other conditions as in Fig. 2.

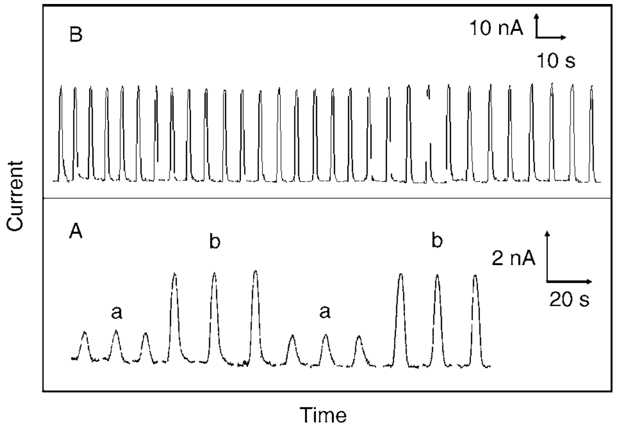

Fig. 5. (A) Amperometric response to injections of TNT solution of varying concentrations of (a) 60 ppb and (b) 180 ppb using amalgam mercury/gold electrode. (B) Amperometric response to 30 successive injections of a 350 ppb-TNT solution using amalgam mercury/gold electrode (only sections of the electrophoregrams are shown). Field strength +190 V/cm (A) and +633 V/cm (B). Other conditions as in Fig. 2.

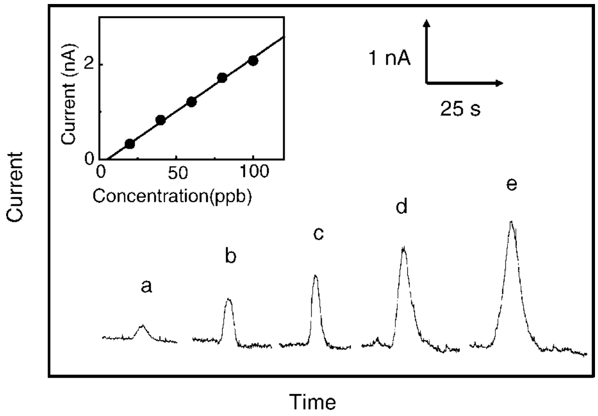

Fig. 6. Microchip FIA response for 20 ppb (a), 40 ppb (b), 60 ppb (c), 80 ppb (d) and 100 ppb (e) TNT using amalgam mercury/gold electrode (shown the sections of electrophoregrams). Also shown (as inset) is corresponding calibration graph. Other conditions as in Fig. 2.

dard deviation (R.S.D.) of 2.00%. Hg/Au electrode showed a good mid-term (9 h) reproducibility in microfluidic system with R.S.D. of 4.34% ($n = 80$; concentration of TNT, 180 ppb). Different Hg/Au electrodes also displayed a good precision indication no passivation of the detection electrode.

The Hg/Au amperometric microchip detector displays well-defined concentration dependence. A calibration experiment involving samples containing increasing levels of TNT in 20 ppb steps yielded well-defined peaks, proportional to analyte concentration (Fig. 6a–e). The resulting calibration plot was linear with a sensitivity of 22.0 pA/ppb, intercept of −0.09 nA and correlation coefficient 0.9984. The favorable signal-to-noise characteristics for the 20 ppb TNT solution indicated an extremely low detection limit of 7 ppb (based on S/N 3); this corresponds to 9.25×10^{-17} mol in the 3 nL injected sample. Analogous microchip electrophoresis-electrochemistry assay had detection limit 24 ppb utilizing gold electrode [11].

4. Conclusion

In conclusion, we have demonstrated the high rapid and sensitive measurements of TNT can be accomplished using on-chip flow-injection amperometry with Hg/Au detecting electrode. The new microfluidic μFIA device allows high-throughput sampling (about 150 samples/h) with minimal sample consumption (3 nL). Such rapid screening in combination with continuous sampling world-to-chip interface [21] will ultimately lead to the creation of portable and field disposable lab-on-a-chip analyzer for providing timely and sensitive detection of 2,4,6-trinitrotoluene.

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