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Monitoring of nitrite, nitrate, chloride and sulfate in environmental samples using electrophoresis microchips coupled with contactless conductivity detection

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ABSTRACT

This report describes the development of an analytical methodology on microchip electrophoresis (ME) devices coupled with capacitively coupled contactless conductivity detection (C^4D) to monitor inorganic anions in environmental samples. The buffer composition as well as detection operating parameters were optimized to achieve the best separation selectivity and detector sensitivity, respectively. Electrophoretic separations of Cl^-, NO_3^-, SO_4^{2-} and NO_2^- were successfully performed within 60 s using a running buffer composed of 30 mmol L^{-1} latic acid and 15 mmol L^{-1} L-histidine (His). The best detectability levels were found applying a sinusoidal wave with 1100-kHz-frequency and 60-V_{pp} amplitude. Quantitative analyzes of inorganic anions were carried out in the presence of $Cr_2O_7^{2-}$ ion as internal standard (IS), which ensured great repeatability in terms of migration times (< 1%) and peak areas (6.2–7.6%) for thirty consecutive injections. The analytical performance revealed a linear behavior for concentration ranges between 0–120 μmol L^{-1} (Cl^-, NO_2^- and NO_3^-) and 0–60 μmol L^{-1} (SO_4^{2-}) and limits of detection (LODs) varying from 2.0 to 4.9 μmol L^{-1}. The concentration levels of anionic species were determined in aquarium, river and biofertilizer samples with recovery values between 91% and 105%. The nitrification steps associated with conversion of ammonium to nitrite followed by the conversion of nitrite to nitrate were successfully monitored in a simulated environment without fishes during a period of twelve weeks. Lastly, the monitoring of anionic species was carried out during eight weeks in an aquarium environment containing ten fishes from *Danio rerio (Ciprynidae)*. The recorded data revealed the absence of nitrite and a gradual increase on the ammonium and nitrate concentration levels during eight weeks, thus suggesting the direct conversion of ammonium to nitrate. Based on the data herein reported, the proposed analytical methodology can be used for routine environmental analysis.

1. Introduction

In the last years, considerable attention has been dedicated to the development of analytical methods capable of identifying, monitoring and quantifying inorganic and organic pollutants potentially harmful to the environment [1–5]. The nitrogen cycle is one of the most essential cycles in aquatic and terrestrial ecosystems and it comprises four microbiological processes, especially nitrification and denitrification. Nitrification is an important step in the nitrogen cycle because of the oxidation from ammonium to nitrite followed by its conversion to nitrate. The monitoring of concentration levels of all nitrogen species is of paramount importance once they may lead to significant water quality problems and accelerate eutrophication, promoting, for example, noticeable increases in aquatic plant growth and changes in the types of plants and animals that live in the stream [6–11].

Environmental monitoring of nitrogen species, mainly nitrite and nitrate, is commonly performed by standard analytical techniques including spectrophotometry and ion chromatography (IC) [5,12]. This latter is the regulatory method adopted by different agencies or communities including the U.S. Environmental Protection Agency (EPA), the American Society for Testing and Materials (ASTM) and the Association of Analytical Communities (AOAC) International. While IC methods allow simultaneous determination of nitrogen species, spectrophotometric approaches require a previous reduction of nitrate to nitrite to be then determined through Griess reaction based on the formation of an azo compound [12]. Besides nitrite and nitrate, the monitoring of

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ammonium concentration levels is quite relevant, once it is considered the most severe pollutant causing eutrophication of aquatic ecosystems. The determination of ammonium has been performed using often photometric, fluorescence, and chemiluminescence and electrochemical detectors coupled with IC, flow injection analysis and capillary electrophoresis (CE) techniques [13–15]. However, most of the mentioned analytical techniques require derivatization reactions, which are considered laborious and, consequently, time-consuming.

For this reason, the feasibility of rapid, simple and reliable analytical methods for the simultaneous monitoring of inorganic species in environmental samples is highly desirable. As recently reported in a few review articles, microchip electrophoresis (ME) devices have appeared as a promising technology for this purpose, particularly when coupled with electrochemical detection modes [2–4]. ME devices offer short analysis time, low sample consumption and waste generation, capability for high-throughput analysis and compatibility to be integrated with other analytical steps on a single platform [16,17]. These devices have been fabricated on a broad variety of substrate materials including glass [18–20], polymeric [21–23] and toner-based [24,25] platforms. However, due to its chemical similarity to fused silica capillaries, glass substrate has been one of the most popular platforms for electrophoretic applications [20].

Capacitively coupled contactless conductivity detection (C^4D) has become a popular detection mode to be coupled with ME devices due to their noticeable advantages for applications in analytical and bioanalytical chemistry [26–30]. Likewise ME devices, C^4D has also received growing attention to monitor on-chip separations due to remarkable features when compared to contact conductivity measurements. Basically, the positioning of sensing electrodes outside the electrophoresis channels eliminates problems associated with bubble generation, electrode surface fouling and electrical interferences from the electric field applied during the electrophoretic run commonly seen in electrochemical detection systems based on faradaic signal [26–30]. Sensing electrodes for C^4D measurements have been often fabricated in different arrangements through the deposition of thin metal layers [31–33], patterning of conductive surfaces like printed circuit boards [34], use of metallic adhesive tapes [35,36], hand-drawing of pencil electrodes on paper [37] or even filling of perpendicular channels to separation channel with metal alloys [38,39] and ionic solutions [40,41]. The attractive features provided by the coupling of C^4D on ME systems have ensured a great increase on the number of publications as well as the spread of this technology that can be applied in a wide range of target compounds as well as sensing purposes [42–44]. Furthermore, the easy integration of electrodes and fluidic channels makes the production of fully portable devices possible, which enables their use for on-site analysis.

In this way, the main goal of this current study is to describe the use of ME-C^4D devices to determine the concentration levels of inorganic anions (chloride, sulfate, nitrate and nitrite) in aquarium, river and biofertilizer samples. For this purpose, the separation and detection conditions were optimized to achieve the best selectivity and sensitivity. Lastly, due to satisfactory analytical performance of ME-C^4D devices for the separation and detection of anions, this portable analytical tool was also explored to monitor the nitrogen cycle in aquarium environments in the absence and presence of fishes. The nitrification steps associated with the conversion of ammonium to nitrite and nitrate were successfully monitored in a simulated environment in a period of 12 weeks.

2. Materials and methods

2.1. Chemicals and samples

Sodium hydroxide, L-histidine (His), lactic acid, sodium chloride, sodium sulfate, sodium nitrate, sodium nitrite, potassium dichromate and ammonium hydroxide solution were purchased from Sigma Aldrich Co. (Saint Louis, MO, USA). Running buffer was prepared containing lactic acid and His prepared in different proportions. Potassium dichromate was used as internal standard (IS) at concentration of 200 μmol/L. Stock solutions of all anionic species were prepared at concentration of 10 mmol L^{-1} each from their corresponding sodium salts. Stock and buffer solutions were prepared weekly with ultrapure water (resistivity 18 MΩ cm) and filtered through nylon filters with 0.22 μm pore diameter. All experiments were performed at 23 ± 1 °C.

2.1.1. Sample preparation

Aquarium water was taken directly from dirty aquarium. Meia Ponte river water samples were collected in the geographic coordinates 16°39'26.4"S and 49°12'26.7"W using empty polymeric bottles. Biofertilizer samples were donated by the Institute of Biological Phosphate (IFB Biotecnologia, Goiania, Brazil). Prior to analysis, Meia Ponte river and aquarium water samples were diluted at the proportions of 1:20 (v:v) and 1:2 (v:v), respectively. For the biofertilizer sample, the extraction of the anionic species was performed with ultrapure water. Basically, 10 g of biofertilizer was dispersed in 12 mL of deionized water resulting in a shiny appearance solution. The sample was then kept at room temperature overnight and then filtered and diluted at the proportion of 1:100 (v:v). Lastly, samples from fish tanks were collected periodically for the monitoring of the nitrification steps.

2.2. Instrumentation

A Quad HV microchip electrophoresis system (model ER455) supplied by Edaq (Denistone East, NSW, Australia) was used to carry out electrophoretic separations. The instrument comprises two high-voltage sequencers, a C^4D data system and a platform to settle the electrophoresis chip. Electrophoretic separations were performed on commercial borosilicate glass microchips (model ET145–4) acquired from Micronit Microfluidics B.V. (Enschede, Netherlands). Glass chips with integrated electrodes consisted of two channels arranged in double-T geometry with gap of 100 μm. All channels were 100 μm wide and 10 μm deep. The electrophoresis chip consisted of a 40 mm long separation channel and 0.7 cm long side arms. The effective length of the separation channel was 33 mm (from the intersection to the sensing electrodes). Two parallel electrodes measuring 200 μm wide × 500 μm long × 200 nm thick spaced by 250 μm were used for C^4D measurements. Fig. S1 (available in supplementary material) displays the layout and dimensions of the commercial glass ME-C^4D devices. As it can be seen in Fig. S1, ME chips contain four circular points for electrical contacts with C^4D circuitry. Two contacts are used as excitation and receiver electrodes while the other two act as reference electrodes to minimize the stray capacitance.

2.3. Electrophoresis procedures

Prior to use, microchannels were electrokinetically conditioned with 0.1 mol L^{-1} NaOH, water and running buffer during 20, 10 and 20 min, respectively. Then, 50-μL-aliquots of sample or buffer were added in the sample (SR), sample waste (SW), buffer (BR) and buffer waste (BW) reservoirs. Gated injection mode was performed by applying voltages of −0.8 kV and −1.0 kV to the SR and BR, respectively, keeping other reservoirs grounded. In order to