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Electro membrane extraction of biological anions with ion chromatographic analysis

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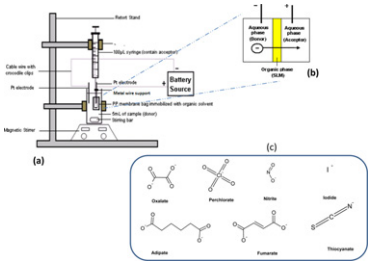
HIGHLIGHTS

- One-step battery-operated electro membrane extraction of anions from biological samples.
- Extraction performance was compared with liquid-phase microextraction.
- Simple and efficient analytical approach with low LODs, good linearity and repeatability.

GRAPHICAL ABSTRACT

. Schematic of (a) battery-operated electro membrane extraction, (b) proposed extraction mechanism and (c) selected target anions.



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ABSTRACT

A simple and sensitive single step electro membrane extraction (EME) procedure was demonstrated for biological organic anions with determination by ion chromatography (IC). Nitrite, adipate, oxalate, iodide, fumarate, thiocyanate and perchlorate were extracted from aqueous donor solutions, across a supported liquid membrane (SLM) consisting of methanol impregnated in the walls of a porous polypropylene membrane bag and into an alkaline aqueous acceptor solution in the lumen of the propylene envelope by the application of potential of 12 V applied across the SLM. The acceptor solution was analyzed by IC. Parameters affecting the extraction performance such as type of SLM, extraction time, pH of the donor and acceptor solution, and extraction voltage were studied. The most favorable EME conditions were methanol as the SLM, extraction time of 5 min, pH of acceptor and sample solutions of 12 and 4, respectively, and a voltage of 12 V. Portable 12 V batteries were used in the study. Under these optimized conditions, all anions had enrichment factors ranging from 3.6 to 36.2 with relative standard deviations ($n = 3$) of between 6.6 and 17.5%. Good linearity ranging from 0.1 to 10 $\mu g\,mL^{-1}$ with coefficients of correlation (r) of between 0.9981 and 0.9996 were obtained. The limits of detection of the EME-IC method were from 0.01 to 0.14 $\mu g\,mL^{-1}$. The developed methodology was applied to amniotic fluid samples to evaluate the feasibility of the method for real applications.

1. Introduction

Considerable efforts have been devoted to the selective sensing of anions because of the important roles that they play in various chemical and biological processes [1].

Anion profiling can help us understand the physiology and biochemistry of many illnesses. Abnormal levels of many organic acids and inorganic anions are indicative of diseases, inborn errors of

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metabolism, toxin exposure and even nutrient insufficiencies detrimental to health [2–5]. For example, Baggot et al. [6] demonstrated the elevation of organic acid markers for Vitamin B_2 deficiency in the amniotic fluid of fetuses diagnosed with Down's syndrome. Other than that, it is of great interest for clinical chemists to understand the concentration levels of inorganic anions for the proper functioning of a living organism and yet responsible for toxicological effects on human beings. Thiocyanate ions are biomarkers to environmental tobacco smoke exposure [7] and nitrite assays in body fluids may be used for monitoring of disease progression [2]. In addition, iodide together with thiocyanate, nitrite and perchlorate analysis are very important in accessing the proper functioning of the thyroid and production of thyroid hormones [3]. This is especially evident from the likely human exposure of widespread perchlorate contamination leading to improvement of thyroid function by competitively inhibiting iodide uptake at the sodium iodide symporter [8]. The United States Environmental Protection Agency has established a human reference dose of 24.5 $\mu g\,L^{-1}$ equivalent of perchlorate in drinking water because of its persistence in food and water matrices [8]. Multi-step solid-phase extraction (SPE) is the most widely used extraction technique for the targeted organic acids followed by ion chromatography (IC) or IC–tandem mass spectrometry [9,10]. There was one work reported on the use of microwave-assisted extraction (MAE) of fumaric acids by an aqueous solution (10 mM) of sulphuric acid. The extracts were subsequently analyzed by ion-exclusion liquid chromatography (IE-LC) associated with photodiode array detection [11].

Other than the use of IC for analyzing organic acids, IE-LC has also been used for the determination of oxalate, thiosulfate, and thiocyanate. The method was successful in separation of these anions on a polymethacrylate-based, weakly acidic cation-exchange resin with detection by means of a glassy carbon (GC) electrode electrochemically modified with polyvinylpyridine, palladium, and iridium oxide [12]. Also, high-performance liquid chromatography (HPLC) has been used to detect perchlorate in tissues of male and female rats [13].

Electromembrane extraction (EME) is an extraction method based on the application of an electrical potential to drive the analytes from an aqueous sample across a suitable supported liquid-membrane (SLM) to another aqueous solution [14,15]. Compared to other liquid-phase microextraction techniques (LPME), EME is a more efficient procedure with higher extraction rates and shorter extraction times. It has been demonstrated successfully on a wide range of biological fluids, for example, in the extraction of drugs directly from untreated human plasma and whole blood [16].

In this study, a total of seven organic acids and inorganic analytes were chosen for anion profiling in human amniotic fluid samples which are usually present at parts per million ($\mu g\,mL^{-1}$) to sub part per billion ($\mu g\,L^{-1}$) levels [2–5]. The primary goal was to develop a single-step, simple and sensitive EME method in determining biological anions in the metabolite profiles of human amniotic fluid samples with the aim of portability in mind. Having a vision of a potential field-portable procedure for battery-operated EME with IC detection may open future developments of portable sample preparation devices for onsite study in clinical applications. A comparison of extraction technique performance was also examined with liquid–liquid–liquid microextraction before applying the optimized system to amniotic fluid samples for anion profiling.

2. Experimental

2.1. Chemicals and materials

HPLC-grade solvents were purchased from Tedia Company (Fairfield, OH, USA). Oxalic acid (99%), adipic acid (99%) and fumaric acid (99.5%) were bought from Sigma Aldrich (Milwaukee, WI, USA). Sodium hydroxide and triethylammonium perchlorate were from Merck (Darmstadt, Germany). Nitrite (99.5%) and iodide (99.5%) standards were purchased from High-Purity Standards (Charleston, SC, USA). Thiocyanate (99%) was obtained from Inorganic Ventures (Lakewood, NJ, USA). Water used for the mobile phase and for preparing stock solutions was generated by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Q3/2 Accurel polypropylene (PP) sheet membrane of 157 μm thickness and 0.2 μm pore size was obtained from Membrana (Wuppertal, Germany). Two rechargeable compact high capacity nickel–cadmium battery packs (12 V each) were bought from a local market. Three cable wires, with a crocodile clip at each end and two platinum wires with a diameter of 0.5 mm used as electrodes were fabricated in-house. A voltmeter was used to monitor and ensure the stability of the potential during the extraction.

2.2. Real samples

Amniotic fluids were obtained from patients by the Department of Obstetrics & Gynecology, National University of Singapore. Samples were handled under approved safety measures following approved ethical guidelines, and disposed of with proper procedures.

2.3. Electromembrane extraction (EME)

A polypropylene membrane was cut into two rectangular pieces (3 cm length × 1 cm width) and overlaid on each other. The two longer and one shorter edges were thermally sealed using a heat sealer, leaving one open side, to form a membrane bag. Each individual membrane bag was cleaned by immersing it in dichloromethane for 15 min. The membrane bag was immediately filled with 100 μL of 10 mM NaOH (acceptor) via a micro syringe. A platinum (Pt) wire electrode (anode) and the tip of the micro syringe were inserted through the open end of the membrane bag. Both were secured to each other with a metal wire support. The SLM was prepared by dipping the membrane bag in the organic solvent for 5 s to impregnate the pores in the polypropylene membrane wall. The EME device, containing the SLM and the acceptor solution, were then placed in the sample vial and secured by the clamps as shown in Fig. 1. A second Pt electrode (cathode) was inserted directly into a 5 mL sample solution. The two electrodes were then coupled to the battery using two cable wires with alligator clips. The electric circuit was monitored by using a voltmeter. Lastly, the entire extraction assembly was placed on a magnetic stirrer which was operated at 500 rpm. After the extraction was completed, the acceptor solution was collected using the micro syringe and transferred to a 0.5 mL flat capped micro centrifuge tube for analysis by the IC system.

A comparison was made between EME and LLLME. The latter was set up exactly as EME as described earlier except no voltage was applied in its operation.

2.4. Ion chromatography

The IC instrument from Metrohm (Herisau, Switzerland) consisting of an 818 IC Pump, 820 Separation Center, 830 Interface, 833 Liquid Handling Unit, a Metrohm Suppressor Module and a 732 conductivity detector, was used for the analysis. Anionic separation was carried out in suppressor mode to enhance analyte conductivity on a Metrosep (Herisau, Switzerland) Anion Dual-2 analytical column (75 mm × 4.6 mm) connected in series with a Metrosep RP guard column. A solution containing a mixture of 1.3 mM Na_2CO_3 and 2 mM $NaHCO_3$ flowing at a rate of 0.8 mL min^{-1} served as the eluent. These are the standard operating conditions