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Double opposite end injection capillary electrophoresis with contactless conductometric detection for simultaneous determination of chloride, sodium and potassium in cystic fibrosis diagnosis

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ABSTRACT

A novel approach for diagnosis of cystic fibrosis is presented. A simple and fast procedure to obtain sweat sample was developed. It consists of repeatedly wiping the skin of the forearm with deionized water moisturized cotton swab and extraction in 1 mL of deionized water. Double opposite end injection capillary electrophoresis with contactless conductometric detection is used for the analysis of the extract. Chloride, sodium and potassium as the three target ions that participate in the ion transfer across the cellular membranes, and are affected by CF, are simultaneously determined in approximately 3 min in a background electrolyte containing 20 mM 2-(N-morpholino)ethanesulfonic acid, 20 mM L-histidine and 2 mM 18-crown-6. By using the target ion ratios rather than the concentrations of each individual ion combined with principal component analysis, the diagnosis of CF can be made more accurately and greatly reduce the number of false positive or negative results as is often the case when single ion (chloride) is analyzed.

1. Introduction

Cystic fibrosis (CF) is a relatively rare, but serious genetic disease, affecting 1 in approximately 2500 Caucasians [1,2]. CF is caused by a mutation in transmembrane conductance regulator gene (CFTR) and results in defective ion transfer through the epithelial cellular membranes [3]. The defect in ion transfer causes formation of sticky mucus in the lungs, pancreas and other organs that is manifested by chronic lung infections, excessive inflammatory response to pathogens or gastrointestinal tract problems [4]. Early diagnosis of CF is of high significance—when CF is diagnosed at an early age, its symptoms can be treated and patient prognosis improves significantly. Kulich et al. [5] evaluated a retrospective cohort study of 31,012 subjects and found that the survival rates of patients with cystic fibrosis have improved remarkably between 1985 and 2007, but most of the improvement was limited to patients from 2 to 15 years old. For instance, the average life expectancy has increased from 25 years of age in 1985 to nearly 40 years of age in 2007 [6], which can be also attributed to the newborn screening programs adopted in many countries all around the world [7].

In diagnosis of CF, sweat analysis of chloride is the golden standard [8,9]. The conventional sweat analysis method relies on rather lengthy and uncomfortable sampling procedure, in which pilocarpine is applied to a defined skin area, followed by an application of the electric current (iontophoresis) and collection of the induced sweat. The collection procedure takes typically no less than 30 min. Two collection methods are commonly applied—a Gibson–Cook procedure [10] that uses a filter paper to collect the sweat with subsequent elution from the paper with DI water or a system with microconduit (Macroduct, Wescor, Logan, UT, USA) that is able to collect small amounts of pure sweat (approx 15–30 μL). The sample collection is followed by analysis, typically by colorimetry [11], coulometry [12] or ion selective electrodes (ISE) [13]. Alternative on skin test with ISE has been developed [14] but has not been accepted in clinical practice, due to too many errors associated with the measurements.

When sweat sample is applied in CF diagnosis, the increased concentration of chloride is often used as single indicator, but other ions participate in the ion transfer mechanism as well [15]. Sodium has been also found elevated in CF patients [16] and sometimes the ratio of Cl^-/Na^+ is elevated in CF patients compared to healthy controls [17]. In a paper by Reddy and Quinton [18], K^+ is suggested as a

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possible CF diagnostic ion. None of these alternative markers have however been used in clinical diagnosis. Capillary electrophoresis (CE) is one of the best high performance separation techniques able to cope with minute sample volumes and volumes as low as 1 μL can be used for repeated injections and quantitation. It should thus be one of the most suitable analytical techniques for analysis of biological samples [19]. CE with indirect UV or contactless conductivity detection (C4D) is easily amenable for ion analysis in very short time. Surprisingly, CE has not attained significant interest in sweat analysis and the reports are scarce. Hirokawa et al. [20] reported the analysis of inorganic cations, amines and amino acids in human sweat by CE with indirect UV absorbance. Recently, de Macedo et al. [21] analyzed strong anions (chloride, sulfate, sulfite) in biological samples including urine, plasma and sweat. Jin et al. [22] have analyzed pyruvate in induced sweat samples by CE with amperometric detection. Naruse et al. [23] have attempted to use CE for analysis of chloride in sweat, but conclude later that micro ISE electrode provides reasonable sensitivity and signal output. Unlike other separation techniques, CE offers a unique possibility to analyze anions and cations simultaneously in a single run. This is accomplished by injecting the sample from both capillary ends, a technique pioneered independently by Kubáň and Karlberg [24] and Padarauskas et al. [25] in 1997. After the double-opposite-end injection (DOEI, acronym used first by Durkin and Foley in 2000 [26]) cations and anions migrate in the opposite direction towards the detector placed in an optimized position along the separation capillary.

In this work, we propose a novel approach for CF diagnosis, in which chloride, sodium and potassium are analyzed simultaneously by DOEI-CE with C4D and that allows fast (~3 min) and reliable analysis of ionic content of sweat. A simple, fast and inexpensive "skin wipe" technique was developed for sampling of the sweat from patient's forearm that only takes several seconds and might be suitable as a surrogate to conventional sweat sampling. We demonstrate that by simultaneously quantifying all three ions and applying principal component analysis (PCA) to the concentration ratios of the ions, more relevant information can be obtained and be used to diagnose CF.

2. Experimental

2.1. Materials and methods

2.1.1. Electrophoretic system

A purpose-built CE instrument was employed for all electrophoretic separations. The separation voltage of +15 kV was provided by a high voltage power supply unit (DX250, EMCO high voltages, Sutter Creek, CA, USA). The separation capillaries used were fused-silica (FS) capillaries (50 μm ID, 375 μm OD, 50 cm total length, Microquartz GmbH, Munich, Germany). Prior to the first use, the separation capillary was preconditioned by flushing with 0.1 M NaOH for 30 min, deionized (DI) water for 10 min and background electrolyte (BGE) solution for 10 min. Between two successive injections, the capillary was flushed with BGE solution for 1 min. At the end of a working day, the capillary was washed with DI water for 10 min, followed by applying a vacuum for 5 min to remove any liquid from inside and stored dry overnight. All CE experiments were performed at ambient temperature.

2.1.2. Double opposite end injection

Injection of standard solutions and sweat samples was carried out hydrodynamically in the following sequence: For the injection of standard solution, the cationic standard containing selected concentrations of NH_4^+, K^+, Na^+, Ca^{2+}, Mg^{2+} was first introduced hydrodynamically into the anodic capillary end (20 s injection at 10 cm) followed by the injection of BGE (20 s injection at 10 cm). Then the anionic standard containing selected concentrations of Cl^-, NO_2^-, NO_3^-, SO_4^{2-} was introduced from the cathodic capillary end (20 s injection at 10 cm), resulting in two sample plugs of equal volume being injected into the opposite capillary ends. For sweat and skin wipe samples, exactly the same procedure was applied, except that the same sample was injected from both anodic and cathodic sides.

2.1.3. Detection system

A C4D was used for the detection of the separated analytes. It consisted of an external function generator (GW Instek GFG-8219A, New Taipei City, Taiwan) providing a sinusoidal excitation signal (frequency: 300 kHz, amplitude: 20 V peak-to-peak) to an in-house built detector cell [27] with a pre-amplifier (OPA655, Burr Brown, TX, USA). The amplified cell current was led to an external detector circuitry for further processing. Data were collected using Orca 2800 AD converter (ECOM s.r.o., Prague, Czech Republic).

2.2. Chemicals

2.2.1. Reagents, standards, electrolytes

All chemicals were of reagent grade and DI water (Purite, Neptune, Watrex, Prague, Czech Republic) was used for stock solution preparation and dilutions. 10 mM stock solutions of inorganic anions were prepared from their sodium salts (chloride, nitrate, nitrite, sulfate all from Pliva-Lachema, Brno, Czech Republic). 10 mM stock solutions of inorganic cations were prepared from their chloride salts (potassium, sodium, calcium, magnesium) except for ammonium that was prepared from ammonium fluoride (all from Pliva-Lachema, Brno, Czech Republic). The standard sample solutions used in the analysis were prepared separately for anions and cations by diluting the respective standard solutions to the required concentrations with DI water. BGE for CE measurements was prepared daily by diluting 100 mM stock solutions of L-histidine (HIS, Sigma-Aldrich) and 2-(N-morpholino)ethanesulfonic acid (MES, Sigma-Aldrich) to the required concentration. 18-crown-6 was prepared as 100 mM stock solution and was added to the BGE to yield the final concentration of 2 mM.

2.3. Sample preparation

2.3.1. Sweat samples

Sweat samples were obtained with the patients' informed consent from the Department of Clinical Biochemistry, Children's Medical Center, Brno, Czech Republic. The Gibson-Cook procedure was used to obtain the sweat samples. The filter paper was weighed to calculate the amount of sweat induced and 3 mL of DI water was used to extract the ions from the filter paper. Prior to the analysis the extract was diluted 1:10 with DI water and analyzed by CE. Conventional analysis method in the laboratory of clinical biochemistry was coulometry.

2.3.2. Skin wipe samples

After obtaining an informed consent from the patients and healthy volunteers, the skin wipe samples were obtained as follows: a cotton swab, purchased in local pharmacy, was thoroughly rinsed with DI water to remove residual ion contamination, dried with air and stored in an enclosed vial prior to sampling. Just before the sampling, the cotton swab was wetted with a defined amount of water (typically 200 μL) and a 2 cm × 4 cm skin on an upper side of a forearm was repeatedly (3×) wiped. The cotton swab was then immersed in 800 μL of DI water, let stand for 15 min to extract the analytes and discarded. The extract was then diluted 1:3 with DI