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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

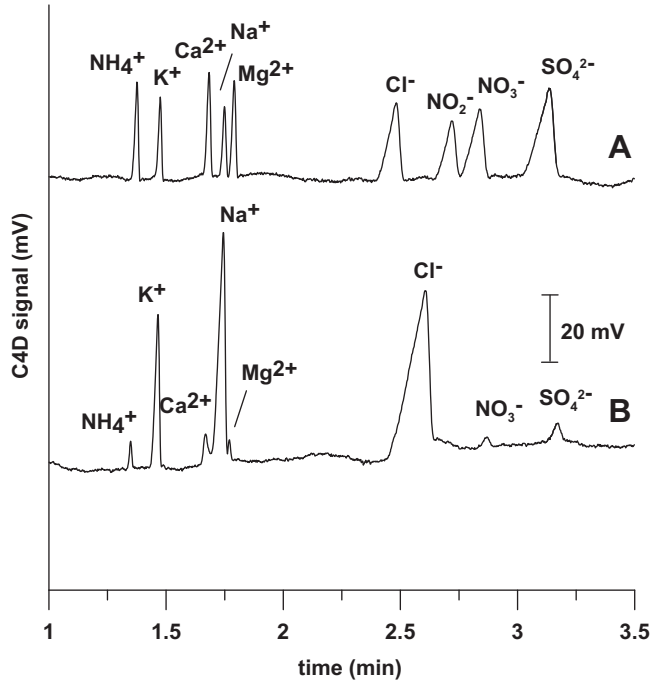

Fig. 1. DOEI-CE separation of 9 ion mixture (A) and a sweat sample (B). CE conditions: Voltage 15 kV, C4D detection, HD injection of sample/BGE/sample: 20/20/20 s from the height of 10 cm. Ion concentrations in mixture (A) 50 μM each.

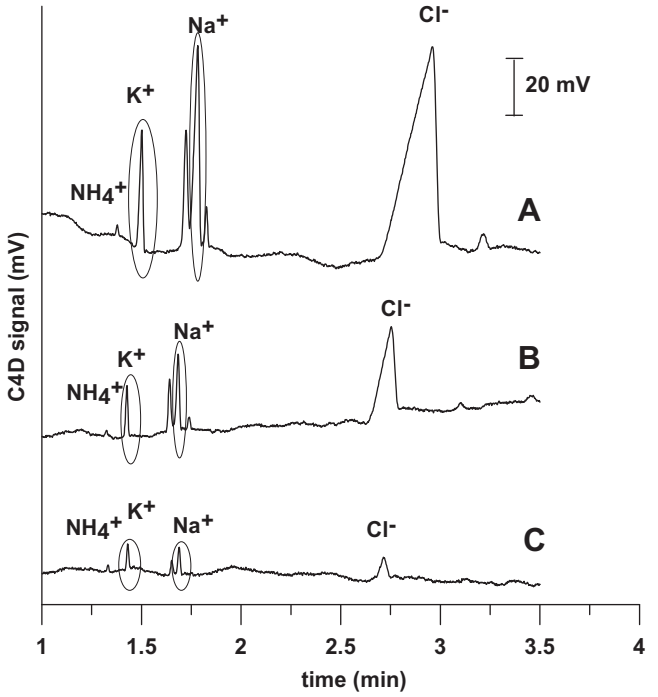

Fig. 2. DOEI-CE separation of skin wipe of a person with dF508 and G551D mutation before (A) and 14 days after (B) the treatment with Kalydeco. Trace (C) is a comparison with skin wipe from a healthy volunteer. CE conditions: the same as in Fig. 1.

water and analyzed by CE. All samples were stored at −20 °C when not in use.

3. Results and discussion

3.1. Selection of separation electrolyte

In DOEI-CE, the separation electrolyte should allow migration of both anions and cations injected into the opposite capillary ends towards the detector placed approximately in the middle of the separation capillary. Typically, the EOF is suppressed or reversed using cationic surfactants to allow the analysis of both fast and slow migrating anions. In this work, for the purpose of CF diagnosis, the analysis of slowly migrating anions from sweat sample was not required. Thus the selection and optimization of the separation electrolyte was very simple. We have used a 20 mM MES/HIS at pH 6, with 2 mM 18-crown-6 electrolyte without an EOF modifier. In this electrolyte, the separation of anions (injected at the cathodic side) is counter-electroosmotic and allows only the anions of strong acids to be separated and detected in reasonable time. On contrary, cations (injected at the anodic side) migrate in the direction of EOF and for their efficient separation, the capillary length must be sufficient. The position of the detector cell is important for the apparent selectivity, as discussed by Macka et al. [28]. Thus, the detector was placed 30 cm from the anodic end and 20 cm from the anodic end. Four anions and 5 cations were separated in 3 min and the separation of a model solution of 9 ions is shown in Fig. 1A.

3.2. Analytical parameters of the method

The analytical parameters of the developed CE method were assessed and are shown in Table 1. The data on repeatability are based on the above described, manual, hydrodynamic injection from both capillary ends. It is apparent that the repeatability of migration times was excellent with RSD values less than 1% ($n = 7$). Also the repeatability of the peak areas was very good (below 6%) that is the value commonly encountered with manual HD injection. Thus, the triple injection sequence does not induce any additional error. The five point calibration graphs were constructed by preparing the calibration solutions of all ions in DI water in the concentration range between 0 and 100 μM and the corresponding calibration parameters are also listed in Table 1. Limits of detection were in the low micromolar range for all analyzed ions (2.3–4.2 μM). The values are higher than those obtained in carefully optimized CE separation systems [29] or by using commercial C4D detectors, but are sufficient for the current application. On the other hand, the performance is better than what is achieved for small inorganic ions by indirect absorption detection with commercial UV–vis detectors.

3.3. Analysis of sweat and skin wipe samples

The suitability of the developed method was tested in analysis of sweat and "skin wipe" samples. Fig. 1B shows an electropherogram of the sweat sample, obtained from the patient with diagnosed CF. The determined concentration of chloride by CE was 29.3 ± 0.6 mmol/L, which corresponds well to the values obtained by coulometry in the clinical laboratory of Children's Medical Center (32.0 and 35.8 mmol/L obtained by Macroduct device and the Gibson-Cook filter paper methods, respectively). It is apparent that the developed DOEI-CE method is able to rapidly analyze the sweat content and allows the quantitation of not only chloride but all other ions of interest (Na^+, K^+). We have noticed that in sweat samples, the ratio of Na^+ and K^+ in CF patients was significantly higher than 1, as is also apparent from the peak heights in the electropherogram in Fig. 1B.

Because the procedure of obtaining the sweat sample by conventional methods is rather tedious, we have developed a simpler, cheaper and faster method for sample collection. In this method, a defined area of skin on the forearm was swept by a moist cotton swab as described in Section 2. Fig. 2 shows a result of skin

Table 1
Analytical parameters of the method, $n = 10$.

	RSD (tM)%[a]	RSD (PA)%[a]	LOD (μM)	Linear regression equation[b]	R^2
Chloride	0.25	3.76	3.49	$y = 1.4267x + 0.8486$	0.9990
Nitrite	0.26	2.47	4.16	$y = 1.4227x + 1.3149$	0.9995
Nitrate	0.25	5.24	3.85	$y = 1.2635x - 1.7235$	0.9975
Sulfate	0.24	3.78	2.30	$y = 2.6201x + 1.9442$	0.9991
Ammonium	0.66	3.92	2.67	$y = 0.6872x - 0.8943$	0.9989
Potassium	0.68	5.32	3.67	$y = 0.4921x - 0.0825$	0.9990
Calcium	0.77	5.44	3.08	$y = 0.7567x - 0.7352$	0.9991
Sodium	0.76	6.02	4.17	$y = 0.4757x - 0.1488$	0.9991
Magnesium	0.76	4.85	3.21	$y = 0.8279x - 1.5022$	0.9977

tM, Migration time; PA, peak area.
[a] $n = 7$.
[b] Calibration range 0–100 μM.

wipe sampling of a person with diagnosed CF (trace A). This person has a specific mutation (dF508/G551D) for which a new medication (Kalydeco, Vertex Pharmaceuticals Inc., USA [30]) has been recently made available. The trace B in Fig. 2 shows a skin wipe of the same person analyzed 2 weeks after the Kalydeco therapy. It is clearly evident that the concentration of all analyzed ions, but most notably chloride, sodium and potassium has significantly decreased. The measured decrease in chloride concentration was 4.2-fold between the traces A and B, that correlates quite well with the results obtained from sweat tests before and after the treatment (the concentration of chloride using the sweat test decreased 3.2–3.6 fold). What is also noticeable in Fig. 2 are the concentration ratios of Na^+ and K^+ that did not change significantly from the trace A and are higher than 1. Trace C in Fig. 2 shows the skin wipe sample obtained from a healthy volunteer. Except that much lower total concentrations of all ions are observed, which is in accordance to the normal ion transport through the sweat glands, the ratio of Na^+/K^+ is much closer to 1. The altered ion ratios, i.e. the ratio of Na^+/K^+ higher than 1 in CF patients and equal or lower than 1 in healthy subjects was subsequently found in all measured samples. The measurement of all ions in sweat or skin wipe samples thus may provide an alternative means for CF diagnosis and the ion ratios (for instance, the ratios of Na^+/K^+, Cl^-/Na^+ or Cl^-/K^+) could be used for differential analysis rather than using chloride concentration only. Further, ion ratios are less prone to the sampling error and no standardization of the skin prior to sampling was required.

3.4. Comparison of skin wipe samples from CF patients and healthy volunteers

To confirm the hypothesis on the importance of ion ratios, we have obtained a "skin wipe" sample from 10 healthy volunteers and 10 patients diagnosed with CF. All skin wipe samples were obtained as described in Section 2.3 with no prior cleaning/preparation of the forearm's skin and analyzed by DOEI-CE with C4D. Fig. 3 shows the graph with the obtained concentrations of selected ions from the group of healthy individuals and patients with CF. The leftmost part of Fig. 3 shows a comparison of chloride values of the healthy group and the patients with CF. It is clear that the average concentration of chloride in CF group was much higher than in the healthy group. However, it is also apparent that the values of chloride alone can lead to some false positive or false negative results. This is a common pitfall of sweat chloride analysis, as documented in several papers [11,17]. The concentration of chloride in sweat is also age dependent [31] and thus there are currently different border values for Cl^- in infants and adults to be used as CF diagnosis values [7]. Apparently, the absolute value of chloride may not provide a 100% proof diagnostic method. The other ions (Na^+, K^+) alone have very little diagnostic value. This applies both for standardized sweat sampling and the newly developed "skin wipe" sampling.

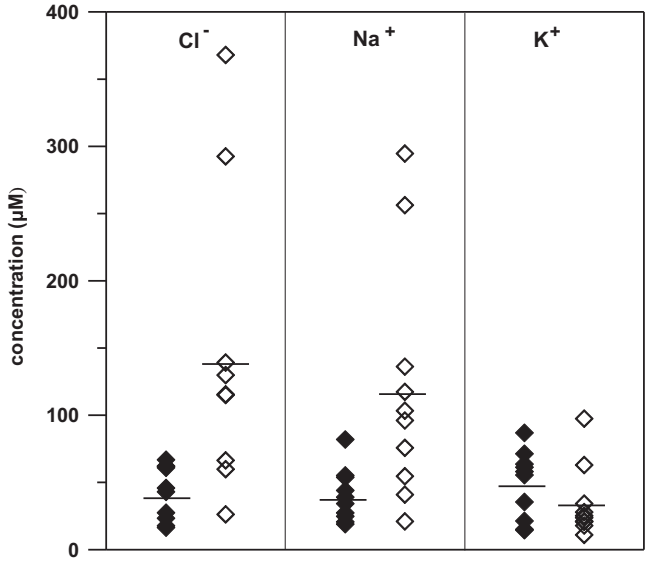

Fig. 3. The concentrations of chloride, sodium and potassium in a group of healthy individuals (♦, $n = 10$) and patients with cystic fibrosis (◊, $n = 10$). The horizontal lines indicate the average concentration of the particular ion in each group. Samples analyzed by DOEI-CE, CE conditions: the same as in Fig. 1.

However, when looking at Fig. 4, where the ion ratios rather than the total concentrations of ions are plotted, apart from the fact that the values of Cl^-/Na^+ do not provide any clue, it is clear that, especially the ratio of Cl^-/K^+ and also the ratio of Na^+/K^+ provide rather clear distinction between the group of healthy and the group of CF patients, regardless of the absolute concentrations of the relevant ions. We thus suggest that rather than using the value of chloride alone, the ion ratios can be better used for CF diagnosis.

3.5. Principal component analysis (PCA) in CF diagnosis

Chemometric approaches, such as PCA, are known to simplify the data when straightforward concentration data alone are not sufficient for interpretation. That is why in the next step, we have applied PCA to the measured data. In PCA, the matrix of measured data (here the concentrations or the peak areas of anions and cations) is simplified by a mathematical procedure to provide a model, in which each sample is represented as a point in an n-dimensional space. The n is the number of significant principal components that are used to explain the variation in the data. Typically, 1–3 principal components (PCs) are the most significant and explain most of the variance. As we have previously noticed that peak ratios provide more distinction of the selected groups of individuals, peak areas ratios of the most important ions participating in the ion transport (Cl^-, Na^+, K^+) were subjected to PCA analysis. The

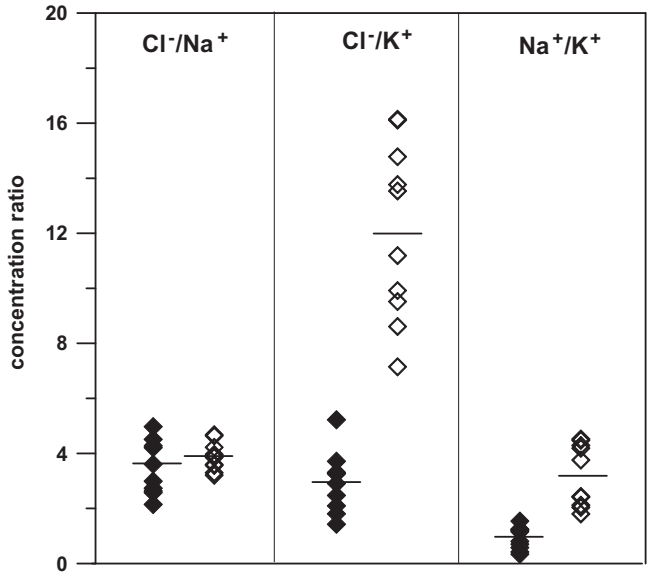

Fig. 4. The concentration ratios of chloride, sodium and potassium in a group of healthy individuals (♦, $n = 10$) and patients with cystic fibrosis (◊, $n = 10$). The horizontal lines indicate the average of concentration ratios of the selected ions in each group Samples analyzed by DOEI-CE, CE conditions: the same as in Fig. 1.

resulting table consisted of 20 rows (samples of different individuals) and 3 columns of corresponding ion peak area ratios. The table was subjected to mean centered PCA procedure. The "leave-one-out" cross-validation procedure was used to determine the number of significant principal components. This procedure revealed 2 significant principal components that accounted for 99.61% of the total variance. In Fig. 5, the PCA plot demonstrates that when using peak ratios, there is distinct cluster formation for the healthy and CF patients groups (marked in green and red). Thus the use of peak area ratios in conjunction with PCA seems to be a robust, yet

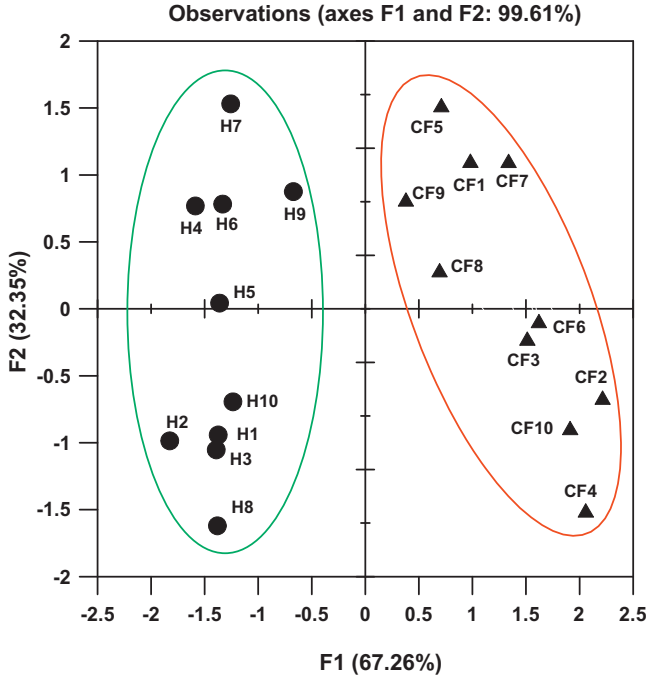

Fig. 5. Peak area ratios data from all samples analyzed by standard principal component analysis (PCA). H_n, denotes each individual in the healthy group, CF_n, denotes each individual in the group of patients with cystic fibrosis.

simple way of diagnosing CF and classifying the person's sweat ionic fingerprint. We are currently performing a detailed study with large number of CF patients, including those with low chloride values to assess a full diagnostic potential of the newly developed method. We assume the extension to newborn screening program in which this simple, 10 s skin wipe sampling, might replace the pilocarpine iontophoresis tedious procedure. In this context, use of portable CE instrumentation [32] for on-site sweat analysis would be clearly an asset.

4. Conclusions

In this work, we have demonstrated that the developed DOEI-CE method is a useful tool for analysis of target ions in sweat and "skin wipe" samples for diagnosis and monitoring of CF. The method allows simultaneous analysis of all ions that participate in the ion transfer across the cellular membranes. In particular simple and fast, "skin wipe" technique for sampling of sweat is of a high significance, because it can considerably simplify the sampling procedure, decrease the analysis time and provide a completely non-invasive mode of sampling that can be for instance used in new-born screening programs. By combination of selected information obtained from the electropherograms and PCA 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. This is owing to the simultaneous analysis of oppositely charged small ions using the unique DOEI approach that shows a great potential of CE in real world analysis. With a prospective use of portable CE instrumentation, the diagnosis results could be obtained in very short time which is another asset of the developed method.

However, to employ the developed sampling and analysis method in clinical practice, a detailed study with large cohort of healthy individuals and CF patients, the assessment of normal and elevated ranges of ions and ion ratios, repeatability, inter- and intra-subject, variability, etc., needs to be performed. Such a study is currently under way in cooperation with the University Hospital in Brno.

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References

[1] B. Wilcken, V. Wiley, G. Sherry, U. Bayliss, Neonatal screening for cystic fibrosis: a comparison of two strategies for case detection in 1.2 million babies, J. Pediatr. 127 (1995) 1229–1256.
[2] R.J. Massie, M.B. Delatycki, A. Bankier, Screening couples for cystic fibrosis carrier status: why are we waiting? Med. J. Aust. 183 (2005) 501–502.
[3] B. Kerem, J.M. Rommens, J.A. Buchanan, D. Markiewicz, T.K. Cox, A. Chakravarti, M. Buchwald, L.C. Tsui, Identification of the cystic fibrosis gene: genetic analysis, Science 245 (1989) 1073–1080.
[4] J.C. Davies, E.W.F.W. Alton, A. Bush, Cystic fibrosis, Br. Med. J. 335 (2007) 1255–1259.
[5] www.lung.org/assets/documents/publications/solddc-chapters/cf.pdf, Accessed May 8, 2014.
[6] http://www.lung.org/lung-disease/cystic-fibrosis/understanding-cystic-fibrosis.html, Accessed May 8, 2014.
[7] L.M. Bender, S.W. Cotten, M.S. Willis, Kids in America: newborn screening for cystic fibrosis, Lab Med. 42 (2011) 595–601.
[8] M. Beauchamp, L.C. Lands, Sweat-testing. A review of current technical requirements, Ped. Pulmonol. 39 (2005) 507–511.
[9] A. Mishra, R. Greaves, J. Massie, The relevance of sweat testing for the diagnosis of cystic fibrosis in the genomic era, Clin. Biochem. Rev. 26 (2005) 135–153.

[10] L.E. Gibson, R.E. Cooke, A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis, Pediatrics 23 (1959) 545–549.
[11] J.M. Kirk, M. Keston, I. McIntosh, S. Al Essa, Clinical evaluation of the macroduct sweat collection system and conductivity analyzer in the diagnosis of cystic fibrosis, Ann. Clin. Biochem. 29 (1992) 145–152.
[12] M. Itano, E. Nussbaum, J.L. Symmons, Coulometric titration of sweat collected with the Webster collection system, Respiration 47 (1985) 220–224.
[13] V.A. LeGrys, J.R. Yankaskas, L.M. Quittell, B.C. Marshall, P.J. Mogayzel, Diagnostic sweat testing: the cystic fibrosis foundation guidelines, J. Pediatr. 151 (2007) 85–89.
[14] P.T. Bray, G.C.F. Clark, G.J. Moody, J.D.R. Thomas, Sweat testing for cystic fibrosis: characteristics of a combination chloride ion-selective electrode, Clin. Chim. Acta 77 (1977) 69–76.
[15] P.M. Quinton, Cystic fibrosis: a disease in electrolyte transport, FASEB J. 4 (1990) 2709–2717.
[16] B.J. Rosenstein, G.R. Cutting, The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel, J. Pediatr. 132 (1998) 589–595.
[17] A. Green, P. Dodds, C. Pennock, A study of sweat sodium and chloride, criteria for the diagnosis of cystic fibrosis, Ann. Clin. Biochem. 22 (1985) 171–176.
[18] M.M. Reddy, P.M. Quinton, Cytosolic potassium controls CFTR deactivation in human sweat duct, Am. J. Physiol. Cell Physiol. 291 (2006) C122–C129.
[19] A.R. Timerbaev, Inorganic analysis of biological fluids using capillary electrophoresis, J. Sep. Sci. 31 (2008) 2012–2021.
[20] T. Hirokawa, H. Okamoto, Y. Gosyo, T. Tsuda, A.R. Timerbaev, Simultaneous monitoring of inorganic cations, amines and amino acids in human sweat by capillary electrophoresis, Anal. Chim. Acta 581 (2007) 83–88.
[21] A. Nori de Macedo, M.I.Y. Jiwa, J. Macri, V. Belostotsky, S. Hill, P. Britz-McKibbin, Strong anion determination in biological fluids by capillary electrophoresis for clinical diagnostics, Anal. Chem. 85 (2013) 11112–11120.
[22] M. Jin, Q. Dong, R. Dong, W. Jin, Direct electrochemical determination of pyruvate in human sweat by capillary zone electrophoresis, Electrophoresis 22 (2001) 2793–2796.
[23] S. Naruse, H. Ishiguro, K. Shirota, M. Nanakuki, A. Yamamoto, T. Kondo, Sweat chloride measurement with a highly sensitive electrode, Pancreas 33 (2006) 100.
[24] P. Kubáň, B. Karlberg, Simultaneous determination of small cations and anions by capillary electrophoresis, Anal. Chem. 70 (1997) 360–365.
[25] A. Padarauskas, V. Olsauskaite, G. Schwedt, Simultaneous separation of inorganic anions and cations by capillary zone electrophoresis, J. Chromatogr. A 800 (1998) 369–375.
[26] D. Durkin, J.P. Foley, Dual-opposite injection electrokinetic chromatography for the unbiased, simultaneous separation of cationic and anionic compounds, Electrophoresis 21 (2000) 1997–2009.
[27] L. Zhang, S.S. Khaloo, P. Kubáň, P.C. Hauser, Analysis of electroplating baths by capillary electrophoresis with high voltage contactless conductivity detection, Meas. Sci. Technol. 17 (2006) 3317–3322.
[28] M. Macka, J. Hutchinson, A. Zemann, Z. Shusheng, P.R. Haddad, Miniaturized movable contactless conductivity detection cell for capillary electrophoresis, Electrophoresis 24 (2003) 2144–2149.
[29] P. Kubáň, C.J. Evenhuis, M. Macka, P.R. Haddad, P.C. Hauser, Comparison of different contactless conductivity detectors for the determination of small inorganic ions by capillary electrophoresis, Electroanalysis 18 (2006) 1289–1296.
[30] http://www.kalydeco.com, Accessed May 8, 2014.
[31] A. Mishra, R. Greaves, K. Smith, J.B. Carlin, A. Wootton, R. Stirling, J. Massie, Diagnosis of cystic fibrosis by sweat testing: age-specific reference intervals, J. Pediatr. 153 (2008) 758–763.
[32] M. Ryvolová, J. Preisler, D. Brabazon, M. Macka, Portable capillary-based (non-chip) capillary electrophoresis, Trends Anal. Chem. 29 (2010) 339–353.