Simultaneous monitoring of inorganic cations, amines and amino acids in human sweat by capillary electrophoresis

Takeshi Hirokawa[a,*], Hikaru Okamoto[a], Yoshinori Gosyo[a], Takao Tsuda[b], Andrei R. Timerbaev[c,d]

[a] *Department of Applied Chemistry, Graduate School of Engineering, Hiroshima University, 1-4-1 Kagamiyama 1, Higashi-hiroshima 739-8527, Japan*

[b] *Business Venture Laboratory, Nagoya Institute of Technology, Gokiso, Showa-ku, Nagoya 466-8555, Japan*

[c] *Vernadsky Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, Kosygin St. 19, 119991 Moscow, Russia*

[d] *Institute of Inorganic Chemistry, University of Vienna, Waehringer Str. 42, A-1090, Vienna, Austria*

* Corresponding author. Tel.: +81 824 247610; fax: +81 824 245494.

E-mail address: hiro77@hiroshima-u.ac.jp (Takeshi Hirokawa).

Abstract

The determination of cationic constituents of sweat is widely recognized as a difficult analytical task due to its complex composition and minute sample volumes available for the individual analysis. Capillary electrophoresis (CE) has been evaluated as a simple routine method to measure sweat metal cations, biogenic amines, and amino acids using a sampling procedure previously developed in one of collaborative teams. The carrier electrolyte, which consisted of 10 mM 4-methylbenzylamine, 6.5 mM α-hydroxyisobutyric acid, and 2 mM 18-crown-6 at pH 4.25 allowed the separation of five cations (NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+}) and four amino acids (ornithine, histidine, lysine, arginine) to be completed in about 13 min with a positive polarity of the applied voltage (30 kV). By increasing the sample volume (due to employing hydrodynamic instead of hydrostatic injection mode), it was also possible to detect indirect UV signals of Zn^{2+}, diethanolamine, and trietanolamine. Sweat samples were

collected from the fingers and forearms of three healthy male volunteers and analyzed by CE. A good repeatability and reproducibility of peak area responses based on five intraday and three inter-day assays (average %RSD less than 3.5 and 2.5, respectively) were obtained. The limits of detection were in the range of 3.2-5.8 μM for alkali and alkaline-earth cations (hydrostatic injection) and 0.27-0.79 μM for other target analytes (hydrodynamic injection). The analytical results for particular analytes were found to vary, depending on the sampling spot and individual, but in general correspond well to clinical concentration ranges.

Keywords: Human sweat; Capillary electrophoresis; Sampling; Cations; Amino acids; Amines

1. Introduction

The application of capillary electrophoresis (CE) has increasingly expanded into the area of ionic analyses in biological samples, especially biofluids, as was recently overviewed [1-4]. As one may gather from these comprehensive review works, a variety of physiologically significant small ionic compounds, including inorganic and organic ions, amines, and amino acids, can be routinely determined by CE in serum, urine, and other relevant biological fluids. This is due to the technique's inherent capability of multicomponent analysis. Other related advantages of CE over usual clinical methods in conducting biofluid assays comprise simplicity and cost-efficiency, high tolerance to complex matrices, short analysis times, and small sample requirements. The latter benefit becomes particularly important when the biosample amount is restricted, as is for instance the case of airway surface liquid [5-7] or sweat [8-11] accessible only at a low-microliter level. Nevertheless, the CE usage for analyzing such samples has been relatively seldom reported, most likely, because of difficulties encountered in the development of a suitable sampling procedure and its implementation to the subsequent CE analysis.

The composition of sweat varies greatly between different people and different parts of skin, and is influenced by emotional state, diet, exercise, hereditary factors and, importantly, the state of the health. For example, cystic fibrosis of the pancreas leads to abnormal electrolyte composition of human sweat and can therefore be clinically diagnosed by determining variations in its major cation and anion concentrations [12-14]. Monitoring of certain trace metals in sweat is a critical issue for restricting chronic allergic contact

dermatitis that can be caused by the release of nickel [15] or chromium ions [16] through sweat on skin from steel and other metal-containing products (leather, ceramics, etc.). When dissolved in sweat, toxic metal ions can permeate the skin and trigger different malignances [17]. Both these health issues posed by metals require an advanced control of exposure at workplaces for a number of occupational groups. In this regard, it is important to understand that whatever is the route of potentially toxic elements into the body, it will be accompanied by their appearance in sweat. For healthy individuals, the ionic composition of sweat measured by in vitro techniques can indicate the proper functioning of metabolism (with sweat the sweat glands excrete from the organism the products of protein metabolism, including some amino acids, amines, and ammonium) and – during physical exercise – training possibilities. Likewise, quantifying the ion concentrations in sweat is an effective way of elucidating the exact ionic mechanism of sweat secretion.

As mentioned above, only few references dealing with sweat ion analysis by CE could be traced in the literature. Specifically, a CE method for direct measurement of the pyruvate concentration in human sweat was reported by Jin et al. [8] who used amperometric detection to enable the selective determination of pyruvate. Van Lierde and co-workers applied a powerful CE–inductively coupled plasma-sector field-mass spectrometry technique to follow chemical transformations of chromium species brought into contact with sweat [9] and to assess their ability to permeate through the skin [10]. However, since the simulated sweat was used in these assays, sampling was not of any concern. In a paper of Tsuda's group [11] closely related to the subject of our study, a simple sampling method was developed which allowed quantification of ammonium, alkali, and alkaline-earth cations from sub-microliter volumes of human insensible perspiration (i.e., perspiration that evaporates before it is perceived as moisture on the skin).

In this work, the proposed CE methodology [11] has been the matter of additional development in order to measure a wider array of ionic analytes occurring in sweat. The method was first set up for monitoring relatively abundant sweat analytes, common inorganic cations and amino acids, and next extended to the assessment of minor components – zinc and non-endogenous amines. The suitability of this advanced CE procedure (in terms of the electrolyte composition, separation, detection, and accuracy) was defined with the objective of customized application. Continuing a series of authors' contributions in the area of high-salinity biofluid ion analysis [11,18-20], the results of this article confirm the potential of CE as a reliable technique for routine biomedical research.

2. Experimental

2.1. Instrumentation

The CE experiments were carried out with a CAPI-3300 CE instrument (Otsuka Electronics, Osaka, Japan) equipped with a photodiode-array UV detector and a power supply able to deliver up to 30 kV. Data acquisition and the instrument operation were controlled with the supplier software. The system was thermostated at 25 °C. Samples were introduced by a hydrostatic injection (elevating the sample vial 25 mm for 95 s) or a hydrodynamic injection under 50 kPa pressure for 3 s (injection volume ca. 20 and 130 nL, respectively). The separation voltage applied was 30 kV (under such operating conditions a current of ca. 7 μA was typically measured). Indirect UV detection was achieved at 214 nm.

Bare fused-silica capillaries (Otsuka Electronics) of 75 μm i.d. (375 μm o.d.) and 100 cm total length (87.7 cm to the detector) were used. New capillaries were conditioned by successive flushes with 0.1 M NaOH for 5 min and ultra-pure water for 10 min under a pressure of 50 kPa and finally with separation electrolyte for 60 min by applying a voltage of 30 kV across the capillary. Prior to each sample injection, the capillary was rinsed with water for 2 min and the separation electrolyte for 5 min. Capillaries were purged with water and kept so when not in use.

2.2. Chemicals and solutions

All reagents (metal chloride salts, L-ornitine monohydrochloride, L-lysine, L-arginine, diethatanolamine (DEA), triethanolamine (TEA), 4-methylbenzylamine, citric acid, 18-crown-6, α-hydroxyisobutyric acid (HIBA), methanol) were purchased in analytical-grade form from Katayama Chemicals (Osaka, Japan), except L-histidine and 2-ethyl-n-butyric acid from Sigma-Aldrich (Tokyo, Japan) and Tokyo Kasei (Japan), respectively. The water used throughout was produced by a Milli-Q Gradient A10 water-purification system (Nihon Millipore, Tokyo, Japan). Analyte stock solutions (1.0-5.0 g L^{-1}) were prepared with weighted amounts of the respective reagents and ultra-pure water. Standard working solutions for CE analyses (ranging from 1.0 to 4.0 mg L^{-1}) were made up by successive dilution of the stock solutions. The simulated sweat was an aqueous solution of sodium chloride (58.5 mg L^{-1}), potassium chloride (50.7 mg L^{-1}), ammonium chloride (10.5 mg L^{-1}), calcium chloride (4.4

mg L^{-1}), magnesium chloride (0.95 mg L^{-1}), ornitine (7.9 mg L^{-1}), histidine (3.4 mg L^{-1}), lysine (2.9 mg L^{-1}), and arginine (0.35 mg L^{-1}). Running electrolytes were prepared fresh daily as specified in the text (Section 3.1) and filtered through a 0.45 μm filter (DISMIC-25cs, Toyo Roshi Kaisha, Tokyo, Japan) prior to use. The analytes identity was confirmed by migration time matching.

2.3. Sample collection and handling

Perspiration experiments were performed using a Suzuken Perspiro 201 apparatus (Nagoya, Japan). By applying the sensor of the apparatus to the finger, the dry air (a constant flow) brought the sweat in contact with the humidity sensor (of a capacitor type) and the temperature sensor. From the values of the flow rate and the humidity corrected by temperature, one could continuously monitor the absolute amount of water. Before sampling, both hands were cleaned by washing with a detergent (the hand soap) and then rinsed under cold running water. Both forefingers were additionally washed in three steps using warm water (ca. 40 °C), a 1% ethanol solution, and ultra-pure water (for 1 min each) and finally dried with the paper wipe. The right-hand forefinger was applied to the probe of the apparatus, and the amount of perspiration from the fingertip was continuously monitored (see above). After the amount of perspiration became stable, the left-hand forefinger was washed again with ultra pure water (ca. 10 s) and wiped. A standard 0.6 mL polypropylene sample vial containing 50 μL ultra-pure water was placed between the forefinger and the thumb, and the vial was turned over so that the water came into contact with the surface of the forefinger. The sweat perspiring into the vial was collected for 3 min, and the resultant sample was stored at –20 °C until further use. Before CE analysis, the frozen samples were allowed to thaw at room temperature. The sampling from the forearm was performed in a similar manner with the help of an assistant (the sampling from forefinger can be done by a tester).

3. Results and discussion

3.1. Development of the CE separation method

The initial consideration in devising a CE electrolyte system was that the composition of sweat is similar to that of plasma except that sweat does not contain appreciable amounts of

proteins. In our recent related account [19], the serum samples were subjected to ultrafiltration to remove proteinaceous material prior to CE analysis. Therefore, the fact that sweat resembles a filtrate of plasma gave us rise to expect that a similar complexing electrolyte can be utilized for the separation of cationic constituents of sweat. However, when applied to sweat analysis, the CE system based on using citric acid as a weak complexing agent allowed only a part of target analytes to be resolved from the sweat matrix (Fig. 1; the concentration of citric acid was reduced from 4.5 mM [19] to 3.5 mM to avoid the overlapping of Mg and DEA peaks). For selecting the most suitable electrolyte, special compositional adjustments were therefore required.

In creating conditions for a successful separation of ammonium and potassium cations, 18-crown-6 was first introduced at a 2 mM concentration [11]. Next, it was decided not to add methanol to the electrolyte and to change from citric acid to HIBA which is known to be one of the most efficient ligands for enhancing the CE separation of different group metals [21]. After testing carrier electrolytes containing variable concentrations of HIBA (5-8 mM), the one of 6.5 mM HIBA that afforded the best resolution of metal ions was chosen. Finally, the pH of the electrolyte solution was fine-tuned to furnish the separation of amino acids (pH 4.25). The simulated sweat was employed for an appropriate evaluation of the analysis performance (separation and sensitivity) using this electrolyte. Typical electropherograms are shown in Fig. 2. As can be seen in Fig. 2a, some analytes of interest, such as zinc(II) and TEA, could still not be detected in the hydrostatically introduced sample because of their normally low abundance in sweat. On the other hand, the use large-volume hydrodynamic injection resulted in emerging all the minor analytes as well-resolved, fairly symmetrical (though small) peaks (observe a close-up view of Fig. 2b). However, the gain in detectability took place at the expense of peak resolution and efficiency for alkali and alkaline-earth cations. Note that such a loss of system performance was not an obstacle for quantitative measurements described in the following section, as the major and minor analytes could be determined separately from the same but differently injected sample (see Table 1). Another important remark is that in the CE system developed isotachophoretic sample-induced preconcentration effect [4] can be expected (at least for zinc ion) because of a large surplus of Na^+ in he neighboring zone (Fig. 2).

The above optimization results enabled the 10 mM 4-methylbenzylamine, 6.5 mM HIBA, and 2 mM 18-crown-6 (pH 4.25) electrolyte to be selected for carrying out the CE determination of target analytes in sweat using both injection modes.

3.2. Method validation

Real samples collected as described in Section 2.3 were used to assess the method's practicality regarding sweat analysis. Precision tests based on five consecutive injections of the same sample repeated for three days were performed (Table 1). The average results of the repeatability (expressed as percentage of relative standard deviation, RSD) for migration times and peak areas were 1.0-1.2 and 3.4-4.6%, respectively. The values of the reproducibility testing, which do not exceed 1.9 and 8.2% RSD for migration times and peak areas, respectively, show a satisfactory long-term stability of the method.

The external calibration technique using six concentration levels of each analyte shown in Table 1 was used for evaluating the linearity of the method. A linear least-squares regression was performed for the peak area vs. concentration data, leading to calibration curves statistically significant (at the 99% confidence level) within the concentration ranges reported in Table 1. Correlation coefficients (R) were all 0.99 or better. Also demonstrated in Table 1 are limits of detection determined as a signal-to-noise of 3. Note that in view of that the samples under examination typically encounter fairly dissimilar concentration levels of cationic analytes, these 3s estimates were calculated using different injection modes. In this context, an optimization of detection limits for most of common inorganic cations was not necessary due to their comparatively high concentrations present in sweat.

3.3. Application to sweat analysis

The CE method developed in this work was applied to the analysis of sweat samples obtained from different parts of skin (finger and forearm) of three persons. Three sets of electropherograms, showing the efficient separation and detection of analytes from all six secreted samples, are assembled in Fig. 3. From comparison of the quantitative results of analyte concentrations (Table 2), it is evident that for finger samples the measured cationic components are significantly different in concentration levels (i.e., for more than two orders of magnitude), sodium and potassium, followed by ammonium, being the major components. Calcium, magnesium, and most amino acids occur in the finger sweat at micromolar concentrations, whereas other analytes can be classified as microconstituents. Rather different cationic profiles, also varying from person to person, were monitored for samples taken from the forearm. For the majority of ionic species, sweat perspired from the forearm is less concentrated than forefinger samples. Another interesting observation is that among amino

acids detected, the concentration of ornithine was the highest in sweat from fingers but arginine was the most abundant amino acid in the forearm sweat; this may be related to a higher sensing activity of the finger surface than that of forearms. It should also be noted that DEA and TEA are not protein metabolites but originated from cosmetic lotions.

Since the certified reference materials for sweat analysis are currently unavailable for comparative purposes, accuracy was evaluated by a comparison between the concentrations calculated with the calibration curves and normal clinical concentration ranges for sweat [11] and blood plasma [22,23] (when available). Consistent results were observed for the major target analytes (cf., for instance, reference data for sweat: 5.1±2.4 mM NH_4^+, 39.9±22.1 mM K^+, 54.0±16.5 mM Na^+, 5.5±3.0 mM Ca^{2+}, 1.7±1.0 mM Mg^{2+}; standard deviation shows variation for seven volunteers).

4. Conclusions

A new application of CE for monitoring an array of inorganic and organic species positively charged under acidic electrolyte conditions was demonstrated. Particular attention was devoted to optimization of the separation conditions, taking into account the complex ionic nature of the sample matrix. By introducing into the capillary different (in volume) portions of the same solution, the quantitation of analytes greatly varying in abundances was achieved in secreted sweat using conventional UV detection. With respect to previous work in the field, the major improvement which the procedure developed brought about is the possibility of simultaneously determining different classes of cationic analytes.

A similar method for the determination of anionic components (inorganic anions and organic acids) is under development in our group. This would permit us to develop the CE methodology for complete assaying of the ionic profiles in sweat which might be adopted in routine clinical practice.

Acknowledgements

Partial support for this work was provided by the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Scientific Research (B), 16350045, 2004-2005.

References

[1] A.R. Timerbaev, Analyst 126 (2001) 964.

[2] M. Moini, Methods Mol. Biol. 276 (2004) 253.

[3] B. Baena, A. Cifuentes, C. Barbas, Electrophoresis 26 (2005) 2622.

[4] A.R. Timerbaev, T. Hirokawa, Electrophoresis 27 (2006) 323.

[5] J.C. Transfiguracion, C. Dolman, D.H. Eidelman, D.K. Lloyd, Anal. Chem. 67 (1995) 2937.

[6] K. Govindaraju, E.A. Cowley, D.H. Eidelman, D.K. Lloyd, Anal. Chem. 69 (1997) 2793.

[7] K. Govindaraju, M. Toporsian, M.E. Ward, D.K. Lloyd, E.A. Cowley, D.H. Eidelman, J. Chromatogr. B 762 (2001) 147.

[8] M. Jin, Q. Dong, R. Dong, W. Jin, Electrophoresis 22 (2001) 2793.

[9] V. Van Lierde, C.C. Chéry, L. Moens, F. Vanhaecke, Electrophoresis 26 (2005) 1703.

[10] V. Van Lierde, C.C. Chéry, N. Roche, S. Monstrey, L. Moens, F. Vanhaecke, Anal. Bioanal. Chem. 384 (2006) 378.

[11] J. Terasawa, K. Mitsuya, A. Ishii, T. Tsuda, Bunseki Kagaku 50 (2001) 813.

[12] S.J. Ram, K.L. Kirk, Proc. Nat. Acad. Sci. USA 86 (1989) 10166.

[13] D. Argentini, L. Nardecchia, Boll. Chim. Igien. Part. Sci. 49 (1998) 313.

[14] M.E. Heeley, D.A.Woolf, A.F. Heeley, Arch. Disease Child. 82 (2000) 420.

[15] H. Savolainen, Rev. Environ. Health 11 (1996) 167.

[16] A.R. Halbert, K.A. Gebauer, L.M. Wall, Contact Dermatitis 27 (1992) 214.

[17] A.K. Singhai, L. Tripathi, H.S. Chandel, S. Nayak, Pharma Review 4 (2006) 116.

[18] T. Hirokawa, M. Yoshioka, H. Okamoto, A.R. Timerbaev, G. Blaschke, J. Chromatogr. B 811 (2004) 165.

[19] H. Okamoto, A.R. Timerbaev, T. Hirokawa, J. Sep. Sci. 28 (2005) 522.

[20] Z. Huang, A.R. Timerbaev, B.K. Keppler, T. Hirokawa, J. Chromatogr. A 1106 (2006) 75.

[21] A.R. Timerbaev, J. Chromatogr. 792 (1997) 495.

[22] K. Kunsch, S. Kunsch, Der Mensch in Zahlen, Spektrum, Heidelberg, 2000.

[23] J. Koolman, K-H. Rohm, Taschenatlas der Biochemie, Georg Thieme Verlag, Stuttgart, 1998.

Figure captions

Fig. 1. An electropherogram of finger sweat obtained with the citric acid-based carrier electrolyte. Capillary: 100 cm × 75 μm; electrolyte solution: 10 mM 4-methylbenzylamine, 3.5 mM citric acid, 25% (v/v) methanol, pH 4.05 (adjusted with 2-ethyl-n-butyric acid); sample introduction: pressure 50 kPa for 3 s; applied voltage: 30 kV; detection wavelength: 214 nm; temperature: 25 °C. Peaks: 1 – NH_4^++K^+; 2 – Na^+; 3 – Ca^{2+}; 4 – Mg^{2+}; 5 – DEA; 6 – ornithine; 7 – histidine; 8 – lysine.

Fig. 2. Separation of cationic sweat components from the simulated sweat at optimal electrolyte conditions. Electrolyte solution: 10 mM 4-methylbenzylamine, 6.5 mM HIBA, 2 mM 18-crown-6, pH 4.25 (adjusted with 2-ethyl-n-butyric acid); sample introduction: (a) gravity 25 mm for 95 s; (b) pressure 50 kPa for 3 s. Other conditions as in Fig. 1. Peaks: (a) 1 – NH_4^+; 2 – K^+; 3 – Ca^{2+}; 4 – Na^+; 5 – Mg^{2+}; 6 – ornithine; 7 – histidine; 8 – lysine; (b) 1 – NH_4^+; 2 – K^+; 3, 4 – Ca^{2+}+Na^+; 5 – Mg^{2+}; 6 – Zn^{2+}; 7 – DEA; 8 – TEA; 9 – ornithine; 10 – histidine; 11 – lysine; 12 – arginine.

Fig. 3. Electropherograms from CE analysis of finger (a, c, e) and forearm sweat (b, d, f) of three healthy volunteers. Sample introduction: pressure 50 kPa for 3 s. Other conditions and peak identification as in Fig. 2.

Table 1

Summary of analytical figures of merit

Analyte	Repeatability (%RSD) [b]		Reproducibility (%RSD) [c]		Calibration range (mM) / coefficient of determination (R^2)	Limit of detection (μM)
	Migration time	Peak area	Migration time	Peak area		
NH_4^{+a}	0.9	5.1	0.1	3.4	4.2-100 / 0.9948	1.4
K^{+a}	1.0	5.1	0.2	2.1	2.7-550 / 0.9998	0.9
Na^{+a}	1.3	7.9	0.1	8.2	4.5-80 / 0.9996	1.7
Ca^{2+a}	1.2	5.6	0.1	4.1	4.5-100 / 0.9985	1.5
Mg^{2+a}	1.2	6.3	0.1	5.3	3.0-100 / 0.9992	1.0
Zn^{2+}	1.7	5.1	1.8	1.1	1.2-100 / 0.9918	0.4
DEA	0.4	4.5	1.1	3.3	1.4-100 / 0.9955	0.5
TEA	1.7	3.8	1.8	1.9	0.6-100 / 0.9971	0.2
Ornitine	1.7	2.2	1.9	2.6	0.5-62 / 0.9984	0.2
Histidine	0.4	2.1	1.3	4.5	1.8-26 / 0.9991	0.6
Lysine	1.6	2.8	1.8	1.6	0.6-19 / 0.9982	0.2
Arginine	1.7	4.1	1.9	2.6	0.6-9 / 0.9991	0.2

[a] Hydrostatic injection; other analytes were injected hydrodynamically. [b] $n = 5$; [c] $n = 3$.

Table 2

Measured concentrations (in mM) of sweat components for three individuals (A-C)

Analyte	Finger			Forehand		
	A (0.400 µl[b])	B (0.455 µl[b])	C (0.486 µl[b])	A (0.098 µl[b])	B (0.030 µl[b])	C (0.060 µl[b])
NH_4^{+a}	13.3±1.6	8.61±0.41	12.7±0.4	0.466±0.043	23.8±1.5	0.755±0.035
K^{+a}	53.3±3.4	44.9±0.7	24.9±0.9	0.985±0.043	11.0±0.5	0.784±0.052
Na^{+a}	61.2±5.6	48.6±0.6	38.5±0.9	1.91±0.10	25.8±2.3	1.13±0.04
Ca^{2+a}	2.83±0.15	4.24±0.21	1.86±0.08	2.00±0.07	15.0±1.4	1.07±0.05
Mg^{2+a}	1.01±0.08	0.731±0.045	4.79±0.13	0.398±0.022	3.84±0.20	0.236±0.018
Zn^{2+}	0.185±0.013	0.142±0.005	0.132±0.005	ND[c]	ND	ND
DEA	0.299±0.020	0.202±0.008	ND	ND	ND	ND
TEA	0.157±0.014	ND	ND	ND	ND	ND
Ornithine	7.18±0.08	3.55±0.37	1.26±0.03	0.316±0.016	0.627±0.017	ND
Histidine	2.44±0.21	1.59±0.13	0.673±0.016	2.75±0.18	13.3±0.7	2.75±0.05
Lysine	2.02±0.09	1.45±0.10	0.437±0.011	0.731±0.023	2.15±0.12	0.781±0.048
Arginine	0.321±0.024	0.079±0.002	0.036±0.003	1.34±0.05	4.39±0.26	1.51±0.04

[a] Hydrostatic injection; other analytes were injected hydrodynamically (n = 3).

[b] Volume of the sweat sample collected (3 min). [c] Not detected.

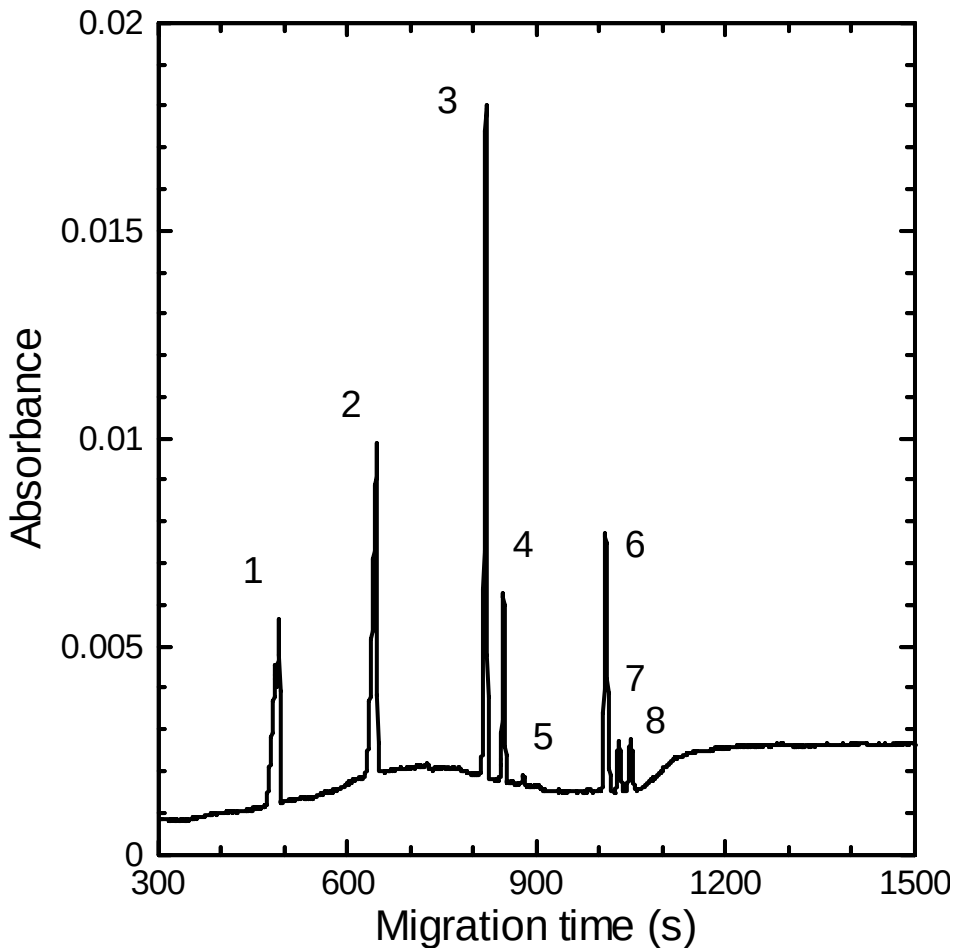
0.02
0.015
0.01
0.005
0
Absorbance
300
600
900
1200
1500
Migration time (s)
1
2
3
4
5
6
7
8

Fig. 1

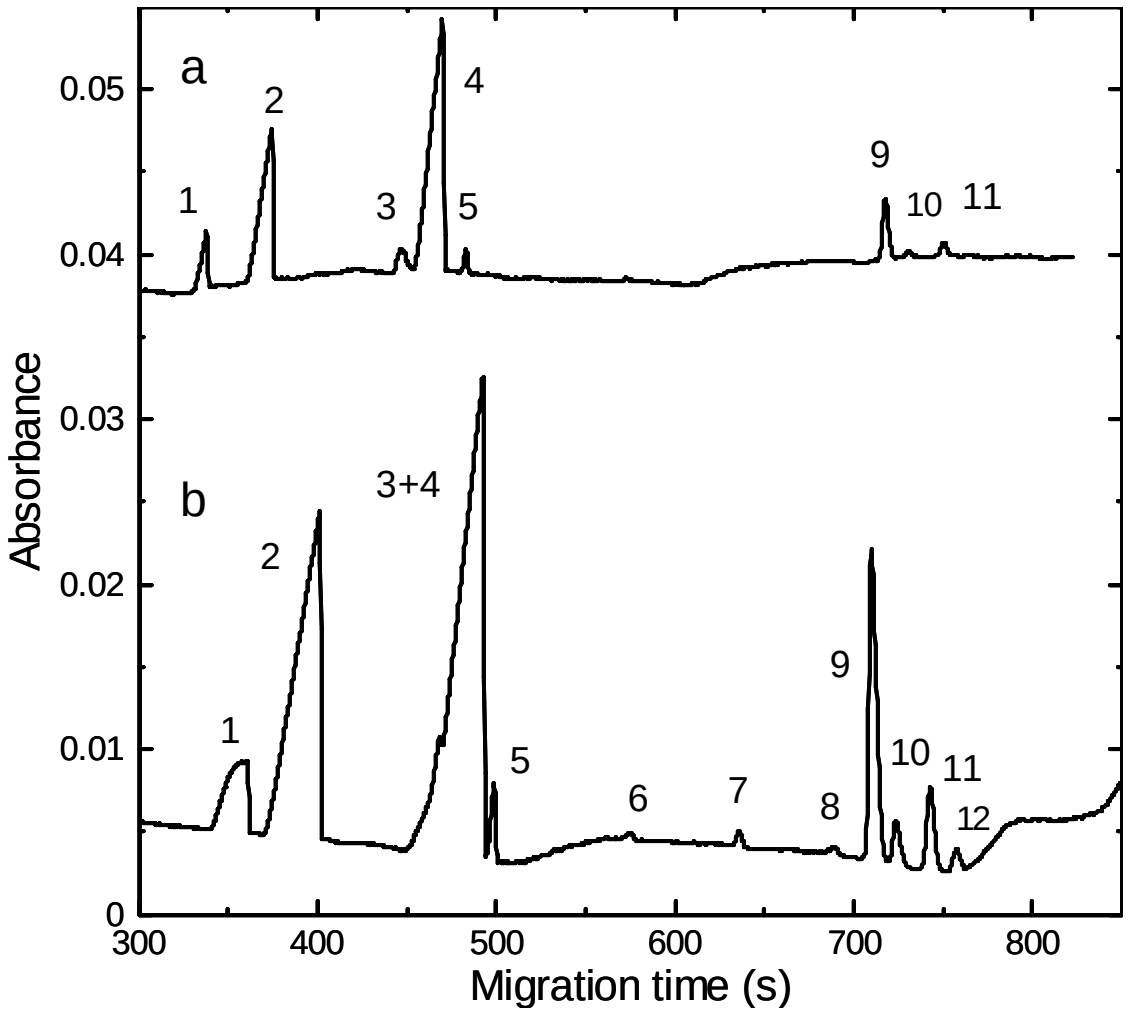
a
b
1
2
3
4
5
9
10
11
3+4
6
7
8
12
Absorbance
Migration time (s)
0
0.01
0.02
0.03
0.04
0.05
300
400
500
600
700
800

Fig. 2

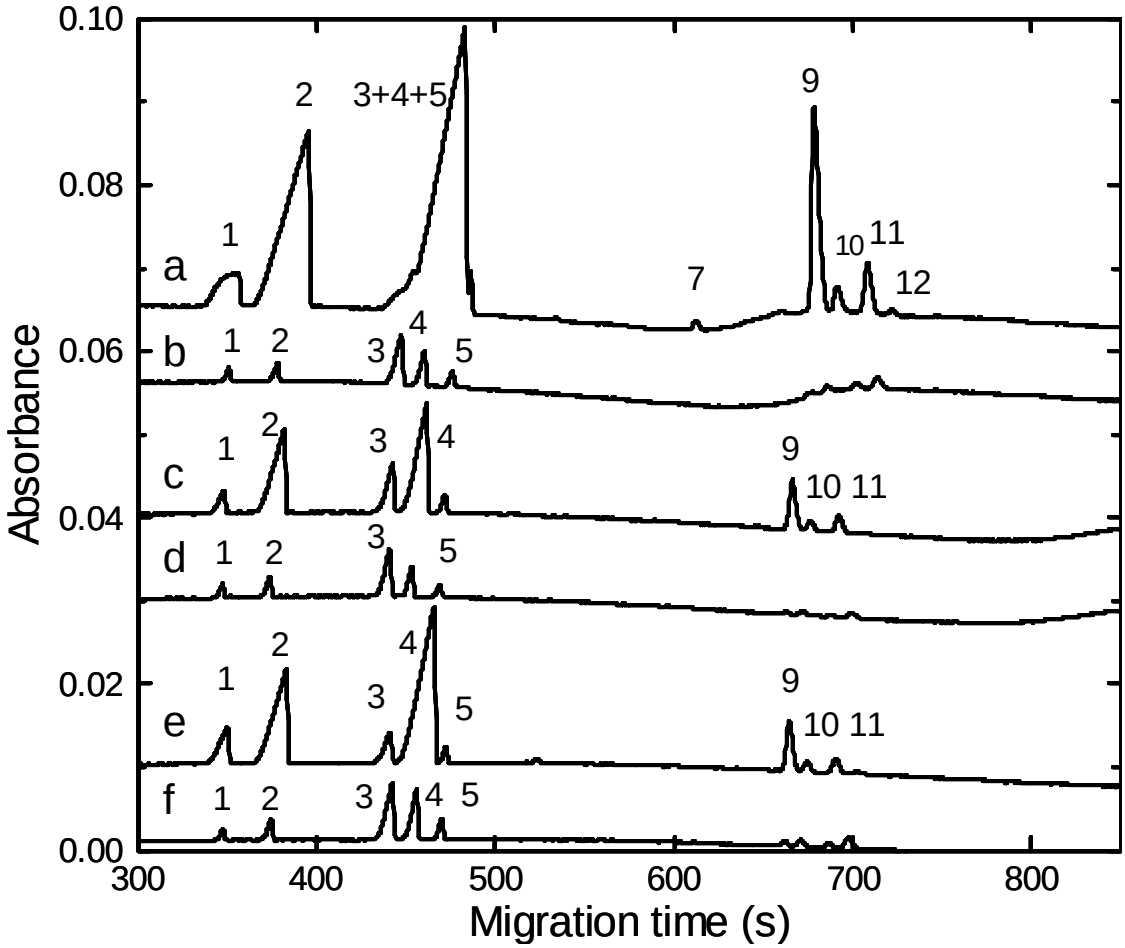
0.10
0.08
0.06
0.04
0.02
0.00
Absorbance
300
400
500
600
700
800
Migration time (s)
a
b
c
d
e
f
1
2
3+4+5
7
9
10
11
12
3
4
5

Fig. 3