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Short communication

Evaluation of the efficiency of extraction of ultraviolet-absorbing pollen allergens and organic pollutants from airborne dust samples by capillary electromigration methods

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Abstract

Capillary electromigration methods, zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC), have been used for evaluation of the efficiency of different extraction agents applied to the extraction of pollen allergens and organic pollutants from dust samples collected during different periods (before, during and after pollen seasons) and in different locations in air-filtration devices (car-traffic tunnel in Prague and a metro station in Paris). Water and acetic acid extracts were analyzed by CZE using acetic acid as background electrolyte (BGE). Water and alkaline water–SDS–buffer extracts were analyzed by MEKC in Tris–phosphate BGE with anionic detergent sodium dodecylsulfate (SDS) micellar pseudophase. More material was extracted and more components were found in the water–buffer extracts than in the water extracts, and better resolution of the components was achieved by MEKC than by CZE. Significant differences have been found in the analyses of dust extracts of different origin. More material and more components have been found in the extracts of the dust collected in the pollen-rich period (March, April) than in the pollen-free period (December, January).

Keywords: Airborne dust; Pollen allergens; Organic pollutants

1. Introduction

The air quality in urban areas is of great importance. Many sources of air pollution are threatening the quality of life and public health. The number of allergic and asthmatic individuals has significantly increased during the last decades. It seems that air pollutants can constitute an important trigger in the development of allergic diseases [1].

Gaseous pollutants are now well detected and quantified by automatic monitoring stations but aerosols of dust or pollen particles are not easily analyzed. Aero-allergens in outdoor air are mainly from plant origin, namely grass, weed or tree pollens. In pollen traps, only intact pollen grains could be recognized by counting them under a microscope. The data are not available early enough for prophylactic measures. Furthermore ordinary pollen counts do not reflect the concentration of damaged pollen grains, i.e. microparticles still bearing allergenic molecules. It is thus relevant to measure the concentration of airborne pollen antigens rather than

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that of pollen grains for prevention of allergic reactions in man [2].

Pollutants resulting from burning of fossil fuels and automobile exhaust gas are especially suspicious of causing an increase in allergic airway diseases. Among them diesel exhaust particles (DEP) are of special interest because of their widespread occurrence [3]. It has been shown that pollen grains bind specifically onto DEPs [4]. Latex allergens were found in aerosols originating from the wear and tear of truck tyres [5]. In hu-PBL-SCID mice (human in vivo model), DEPs have been shown to enhance the IgE response to birch pollen [6]. It was found [7] that extractable polyaromatic hydrocarbons (PAH) associated with DEP (PAH-DEP) can enhance B-cell differentiation, and by initiating and elevating IgE production, may play an important role in the increased incidence of allergic airway diseases. For the above reasons the simultaneous detection and quantitation of pollen allergens and organic pollutants in the air is very desirable.

Capillary electrophoresis (CE) has developed into a powerful analytical technique with a broad application potential [8]. In the past few years, CE has been used to analyze various classes of compounds of environmental relevance, including organic pollutants [9,10] and pollen allergens [11], but the widespread use of CE as a routine quantitative tool for environmental analysis is still relatively limited.

The final aim of our work is to contribute to build an automated air trap which will collect air dust and at regular intervals will extract pollen allergens and organic pollutants and quantify them.

The aim of this paper was to test the applicability of capillary electromigration methods, zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC), for analysis of the liquid extracts of airborne dust samples collected in the filtration devices at different places and during different periods in Prague and in Paris, to evaluate the efficiency of different extraction agents for the extraction of UV-absorbing components, including pollen allergens and organic pollutants, from airborne dust samples by CZE and MEKC, and to find out if there are any differences in the qualitative and/or quantitative composition of the liquid extracts of the dust collected in pollen-free and pollen-rich periods.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent-grade. Phosphoric acid and acetic acid (HAc) were from Lachema (Brno, Czech Republic). Sodium dodecylsulfate (SDS) and tris(hydroxy-methyl) aminomethane (Tris) were from Serva (Heidelberg, Germany). Buffer solutions were prepared from deionized and redistilled water and filtered through a 0.45-μm membrane filter (Millipore, Bedford, USA) prior to use in CZE and MEKC.

2.2. Collection of the dust samples

Collection of the dust samples was performed in cooperation with specialists from the State Institute of Health, Prague. Two different dust samples were collected as a sediment in the air-filtration device in the Letná tunnel in the center of Prague, in the region of intensive car traffic, in the pollen-free period (December, January)—sample LT1, and in the pollen-rich period (March, April)—sample LT4.

The dust sample collected from the air filtration device in a metro station in Paris in the autumn period was fractionated by mechanical sieving into two fractions, fine dust (PM1) and fibre-rich dust (PM2) samples.

2.3. Dust extraction procedure

After the organic solvent (acetone) treatment of the dust samples (450 mg of the dust+5×25 ml of acetone at −20 °C) to defat the collected material (yield: 370 mg of the defatted dust), three different extraction agents have been tested for the extraction of pollen allergens and organic pollutants: (i) water, (ii) acid water solution (0.5 mol/l acetic acid, pH 2.5), (iii) alkaline water buffer solution with anionic detergent (20 mmol/l Tris, 5 mmol/l H_3PO_4, 50 mmol/l sodium dodecylsulfate (SDS), pH 8.7).

In all three cases, 150 mg of the dust sample was suspended in 1 ml of extracting agent, extraction was performed in the shaker at ambient temperature 23 °C for 1 h followed by centrifugation at 10 000 rpm (r=66 mm) for 15 min. The yield was 0.850 ml of liquid extract.

2.4. Capillary electrophoresis experimental conditions

CZE and MEKC experiments were performed in lab-made apparatus equipped with a UV photometric detector monitoring absorbance at 206 nm [12]. Fused silica capillary (I.D. 50 μm, O.D. 200 μm, total length 315 mm, effective length 200 mm) was supplied by the Institute of Glass and Ceramics Materials, Czech Academy of Sciences (Prague, Czech Republic). Separations were performed at an ambient temperature of 22–24 °C. Sample solution was applied by hydrostatic pressure (induced by a 50-mm height difference in capillary tips) for 5–25 s. The applied voltage (constant) was 8.0–12.0 kV and current 10–25 μA. The background electrolytes (BGEs) were 0.5 mol/l acetic acid, pH 2.5 (HAc) for CZE, and 20 mmol/l Tris, 5 mmol/l H_3PO_4, 50 mmol/l SDS, pH 8.7 (TP-SDS) for MEKC.

3. Results and discussion

Capillary electromigration methods, CZE and MEKC, have been used for analysis of water and water–buffer extracts of the dust samples collected in a metro station in Paris (fine dust fraction PM1 and fiber-rich dust fraction PM2) and the dust samples collected in the Letná car traffic tunnel in the center of Prague in the pollen-free period (sample LT1) and in the pollen-rich period (sample LT4). The water extracts and the acetic acid–water solution extracts were analyzed by CZE in the acid background electrolyte (BGE), HAc (Figs. 1a,b–4a,b). The water extracts and alkaline water–SDS–buffer extracts were analyzed by MEKC using TP-SDS as BGE (50 mmol/l SDS formed the micellar pseudophase of this BGE) (Figs. 1c,d–4c,d).

The amount of extractable material containing the pollen allergens and organic pollutants has been evaluated by integration of the electropherograms and chromatograms from CZE and MEKC analyses of the dust extracts. The amount of extracted UV-absorbing pollen allergens and organic pollutants is supposed to be proportional to the total area of UV-positive peaks (measured at 206 nm). The number of resolvable components in different extracts has also been estimated approximately from the corresponding records of CZE and MEKC analyses (Figs. 1–4). The results are summarized in Tables 1 and 2.

Comparing the extracted amounts from the same sample obtained by the different extraction agents, the efficiency of the extraction of UV-absorbing components, including pollen allergens and organic pollutants, from the airborne dust samples was evaluated. Determination of the extracted amounts from the different samples, obtained by the same extraction procedure with the same extraction agent, allowed us to estimate and compare the content of UV-absorbing components, including pollen allergens and organic pollutants, in the dust samples of different origin.

Generally, more material has been extracted and more components of the dust have been found in the water–buffer extracts than in the water extracts and more components of the dust extracts have been resolved in the records of MEKC analyses than in those of CZE analyses (Figs. 1–4; Tables 1 and 2). The regions of unresolved peaks, labelled ‘‘x’’ in the records, and corresponding to mixed zones in the capillary, have not been included in the number of components found.

Only minor differences have been found both in the CZE and MEKC analyses of extracts of fine dust and fibre-rich dust samples collected in the Parisian metro (Figs. 1 and 2; Table 1).

Significant differences have been found in the analyses of the dust extracts of different origin. Much more material and more components have been found in the extracts from the Prague dust sample from the pollen-rich period (March, April—LT4) than from the pollen-free period (December, January—LT1) (Figs. 3 and 4; Table 2). These differences are assumed to be caused by the presence of tree pollen allergens in the LT4 sample, since in the period of its collection (March, April) a lot of tree pollen allergens, mainly those originating from birch are present in the air and they are captured in the air traps and filtration devices.

Some similarities have been found in the analyses

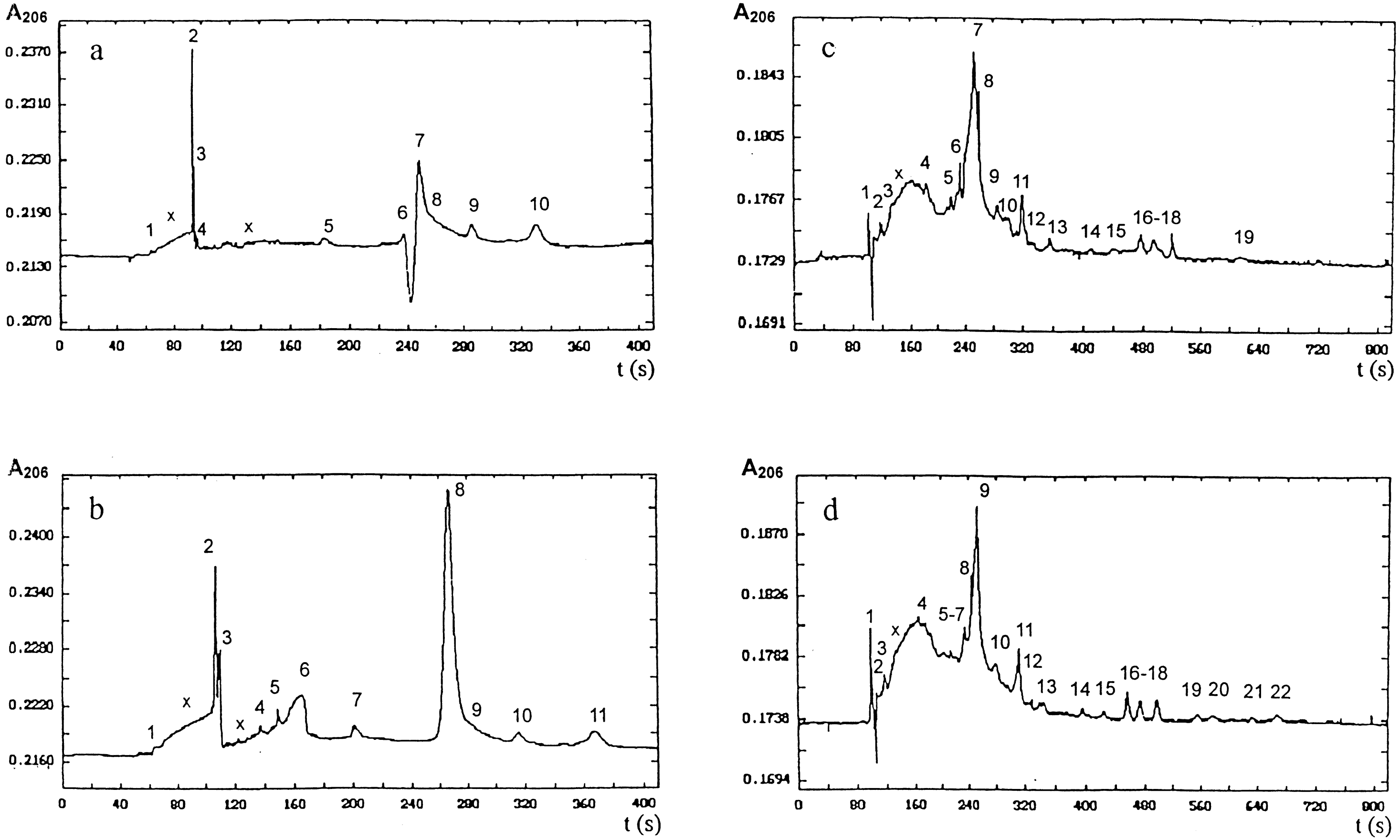

Fig. 1. CZE and MEKC analyses of the liquid extracts from the fine dust fraction of the dust collected in the air filtration device in the Parisian metro station—sample PM1: (a) water extract, (b) HAc extract—both analyzed by CZE using HAc as BGE, (c) water extract, (d) TP-SDS extract—both analyzed by MEKC using TP-SDS as BGE. HAc=0.5 *M* acetic acid; TP-SDS=20 m*M* Tris, 5 m*M* H_3PO_4, 50 m*M* SDS, pH 8.7. A_{206}, absorbance at 206 nm; ×, region of unresolved peaks, resolved peaks indicated by the numbers. For other conditions see the text, Sections 2.2–2.4.

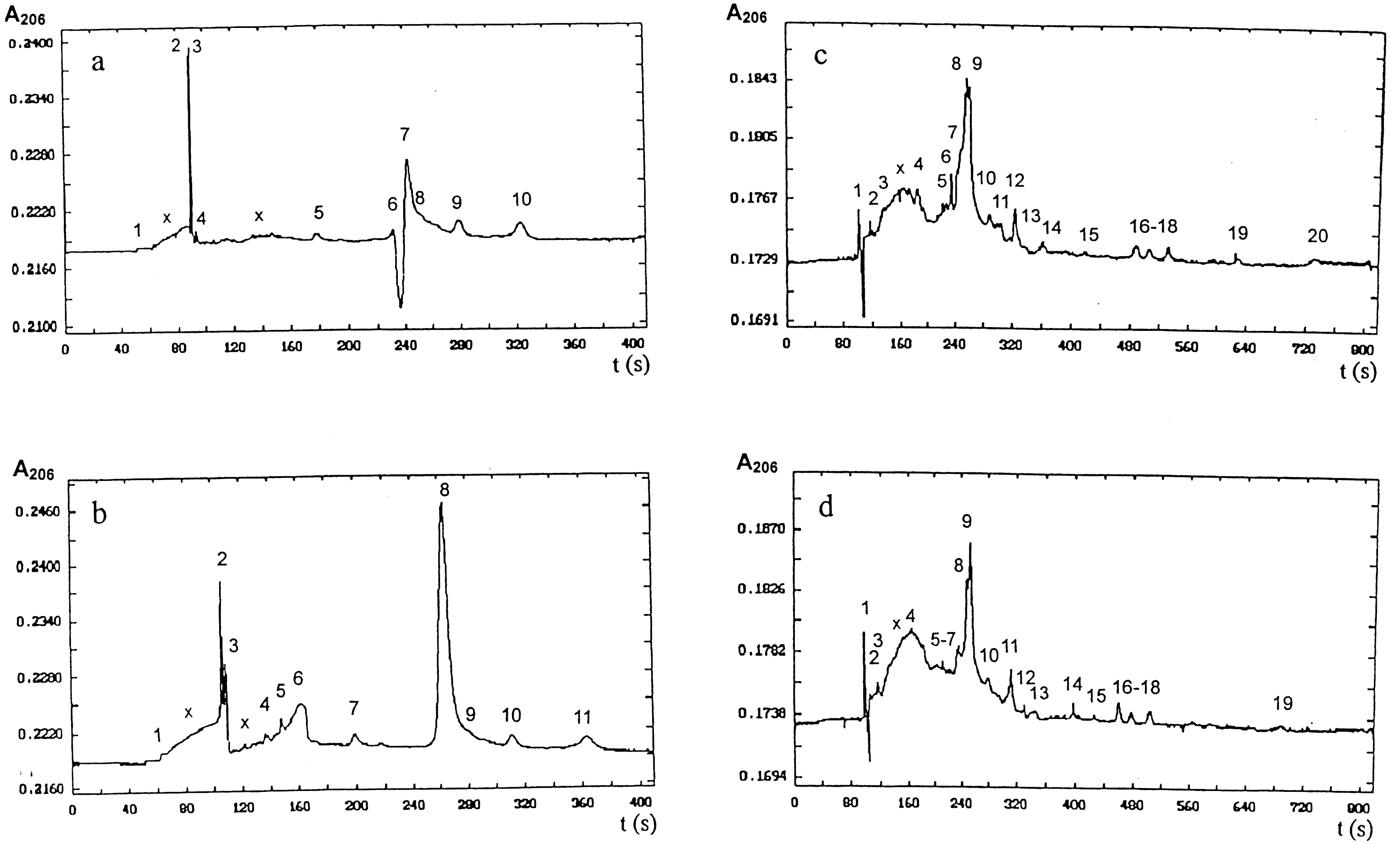

Fig. 2. CZE and MEKC analyses of liquid extracts from the fibre-rich fraction of dust collected in the air filtration device in the Parisian metro station—sample PM2: (a) water extract, (b) HAc extract—both analyzed by CZE using HAc as BGE, (c) water extract, (d) TP-SDS extract—both analyzed by MEKC using TP-SDS as BGE. Other conditions as in Fig. 1.

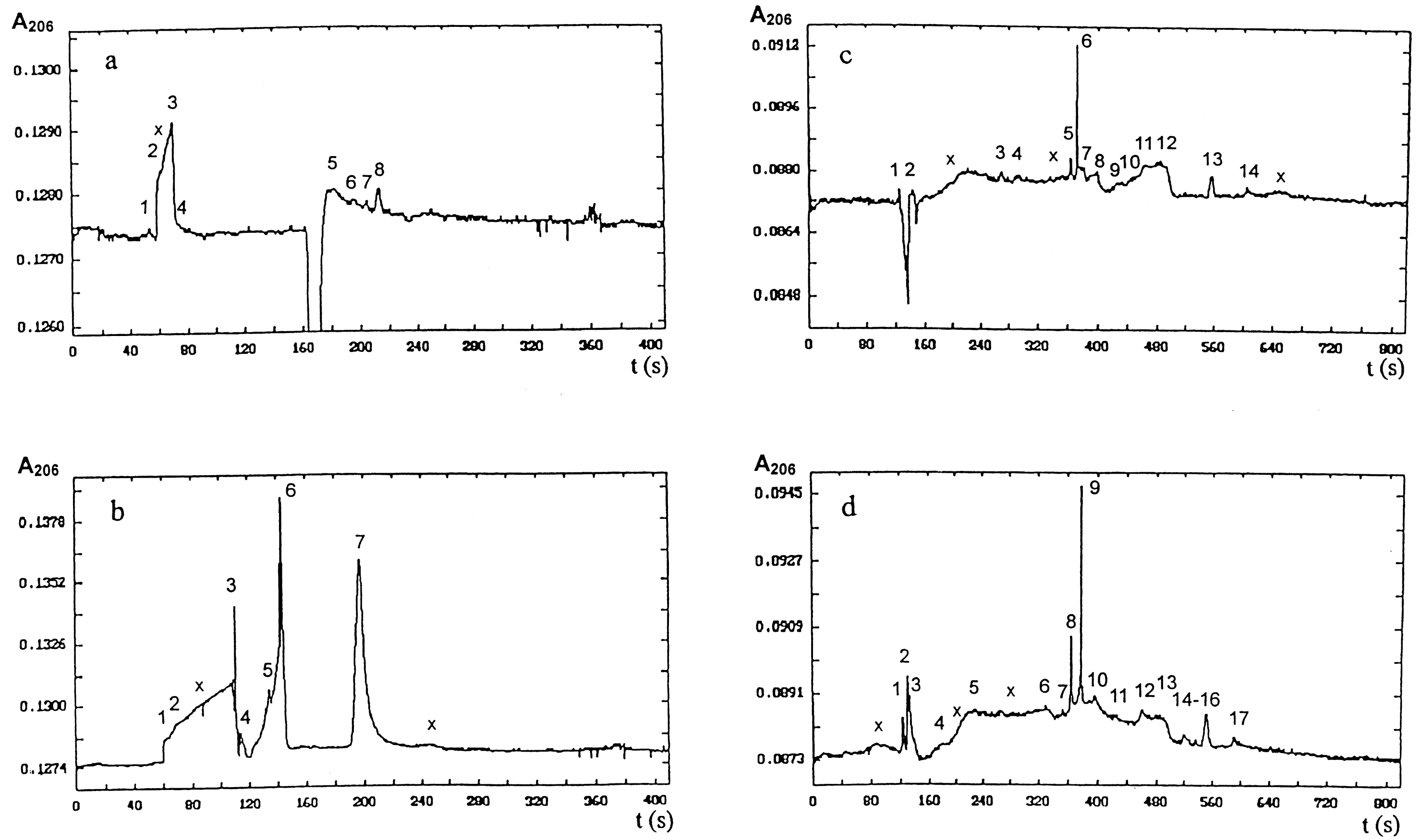

Fig. 3. CZE and MEKC analyses of the liquid extracts from the Prague Letná tunnel dust collected in pollen-free season (December, January)—sample LT1: (a) water extract, (b) HAc extract—both analyzed by CZE using HAc as BGE, (c) water extract, (d) TP-SDS extract—both analyzed by MEKC using TP-SDS as BGE. Other conditions as in Fig. 1.

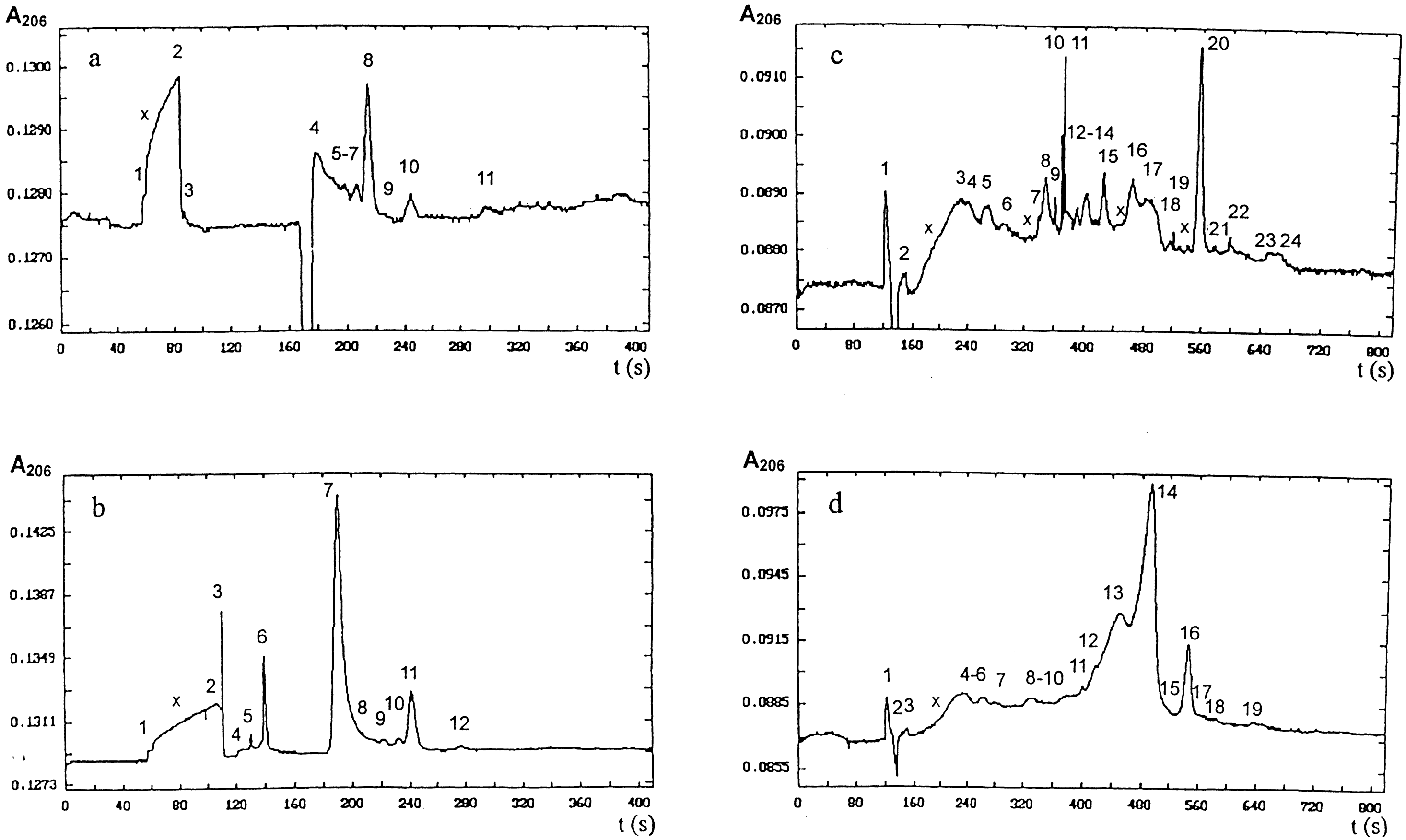

Fig. 4. CZE and MEKC analyses of the liquid extracts from the Prague Letná tunnel dust collected in pollen-rich season (March, April)—sample LT4: (a) water extract, (b) HAc extract—both analyzed by CZE using HAc as BGE, (c) water extract, (d) TP-SDS extract—both analyzed by MEKC using TP-SDS as BGE. Other conditions as in Fig. 1.

Table 1
Evaluation of the efficiency of extraction—Parisian metro dust samples

Extracting agent	CE-mode	BGE	Extracted amount (AU)		Number of components found	
			PM1	PM2	PM1	PM2
Water	CZE	HAc	151	160	10	10
HAc	CZE	HAc	485	484	11	11
Water	MEKC	TP-SDS	425	379	19	20
TP-SDS	MEKC	TP-SDS	441	357	22	19

Extracted amount (in relative arbitrary units, AU) of UV-absorbing components, including pollen allergens and organic pollutants, and number of components found in the extracts from the Parisian metro dust samples extracted by different extraction agents and analyzed by CZE and MEKC methods using HAc and TP-SDS as BGEs, respectively. HAc=0.5 *M* acetic acid; TP-SDS=20 m*M* Tris, 5 m*M* H_3PO_4, 50 m*M* SDS, pH 8.7. PM1, fine dust fraction; PM2, fibre-rich dust fraction.

of extracts from the dust samples originating from the Parisian metro station and from the Prague tunnel (Figs. 1–4).

4. Conclusions

Capillary electromigration methods, CZE and MEKC, have been shown to be highly efficient and highly sensitive methods suitable for evaluation of the efficiency of extraction of UV-absorbing components, containing pollen allergens and organic pollutants from the airborne dust, and for the estimation of the content of these components in dust samples of different origin. Using these methods, it was possible to select the suitable extraction agent and to distinguish dust samples collected during different periods, pollen-free and pollen-rich seasons.

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Table 2
Evaluation of the efficiency of extraction—Prague tunnel dust samples

Extracting agent	CE-mode	BGE	Extracted amount (AU)		Number of components found	
			LT1	LT4	LT1	LT4
Water	CZE	HAc	41	69	8	11
HAc	CZE	HAc	234	302	7	12
Water	MEKC	TP-SDS	231	459	14	24
TP-SDS	MEKC	TP-SDS	500	975	17	19

Extracted amount (in relative arbitrary units, AU) of UV-absorbing components, including pollen allergens and organic pollutants, and number of components found in the extracts from Prague traffic tunnel dust samples extracted by different extraction agents and analyzed by CZE and MEKC methods using HAc and TP-SDS as BGEs, respectively. HAc=0.5 *M* acetic acid; TP-SDS=20 m*M* Tris, 5 m*M* H_3PO_4, 50 m*M* SDS, pH 8.7. LT1, dust from the pollen-free season; LT4, dust from the pollen-rich season.

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