

## Analysis of water extracts from airborne dust samples by capillary isotachopheresis

Petra Sázelová<sup>a</sup>, Václav Kašička<sup>a,\*</sup>, Dušan Koval<sup>a</sup>, Ferenc Kilár<sup>b</sup>, Dietmar Knopp<sup>c</sup>, Gabriel Peltre<sup>d</sup>

<sup>a</sup>*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo 2, CZ-166 10 Prague 6, Czech Republic*

<sup>b</sup>*Central Research Laboratory, Faculty of Medicine, University of Pécs, Szigeti ut. 12, H-7673 Pécs, Hungary*

<sup>c</sup>*Institute of Hydrochemistry, Technical University of Munich, Marchionin str. 17, D-81377 Munich, Germany*

<sup>d</sup>*Ecole Supérieure de Physique et Chimie Industrielle, LECA, Allergie et Environnement, 10 rue Vauquelin, F-75005 Paris, France*

### Abstract

Application of capillary isotachopheresis (CITP) for the analysis of water extracts of the dust samples collected in different periods in air-filtration devices in Prague car traffic tunnels and in Parisian metro station is presented. The extracts were analyzed in cationic mode with a leading electrolyte (LE) of 10 mM KOH, 25 mM acetic acid, pH 4.4, and a terminating electrolyte (TE) of 10 mM  $\beta$ -alanine, adjusted to pH 4.4 with acetic acid, and in anionic mode with LE 10 mM HCl, 20 mM histidine, pH 5.8 and TE 10 mM 2-(*N*-morpholino)ethanesulphonic acid, pH 3.7. Extracted amounts of UV-absorbing substances, including pollen allergens and organic pollutants, the number of the found components and concentrations of some inorganic ions (e.g.  $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ) in the dust samples were determined. It was found that the extracted amounts of anionic components and their number were much higher than those of cationic components. Significant differences have been found in the analyses of the extracts of different origin. Much more material and more components were present in the extracts of samples from the pollen-rich period than from the pollen-free period, especially in anionic CITP mode.

© 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Isotachopheresis; Dust; Air analysis; Pollen; Inorganic anions; Inorganic cations

### 1. Introduction

The number of allergic and asthmatic individuals in human populations in developed countries has significantly increased during the last decades. Allergenic pollens are responsible for a great part of these diseases of the respiratory tract, but there are

some indications that their effect is amplified by some types of air pollution namely its fine and ultrafine particulate load [1].

Gaseous pollutants are now readily detected and quantified by automatic monitoring stations but aerosols of dust or pollen particles are not easily determined. Under the microscope only intact pollen grains picked up in pollen traps could be recognized and counted but this does not reflect the concentration of damaged pollen grains still bearing allergenic molecules. It is thus relevant to measure the concentration of airborne pollen antigens rather than

\*Corresponding author. Tel.: +420-22-018-3239; fax: +420-23-332-3956.

E-mail address: [kasicka@uochb.cas.cz](mailto:kasicka@uochb.cas.cz) (V. Kašička).

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

Pollutants resulting from the burning of fossil fuels and automobile exhaust gas are suspected for causing an increase of allergic airway diseases. Among them diesel exhaust particles (DEPs) are of special interest because of their widespread occurrence [3]. It has been shown that pollen grains bind specifically to DEPs [4]. It was found [5] that extractable polyaromatic hydrocarbons (PAHs) associated with DEP (PAH-DEPs) 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. In mice DEPs have been shown to enhance the IgE response to birch pollen [6] and traffic particulate matter (TPM, collected in Prague tunnel) has been demonstrated to affect both the inflammatory and immunological components of experimental birch pollen allergy [7]. From these facts it follows that the simultaneous detection and quantification of pollen allergens and organic pollutants in the air is necessary for a complex evaluation of the influence of environmental factors on the allergy and asthma and for the early warning to concerned people on the appearance of the elevated concentrations of both allergens and pollutants in the air. Consequently, the final aim of our work is to contribute to building an automated air trap, which will collect the air dust and at regular intervals will extract pollen allergens and organic pollutants and quantify them. The partial aim is to use the capillary electromigration methods for the analysis of these dust extracts.

Different modes of capillary electrophoresis (CE) have developed into high-efficient and high-sensitive analytical methods with a broad application potential [8]. In the recent years CE has been used to analyse various classes of compounds of environmental relevance, including organic pollutants [9,10] and pollen allergens [11]. The application of CE to the analysis of atmospheric aerosol and vehicle emission samples [12] and metal ions in airborne particulate matter after extraction [13] has recently been published. We have demonstrated that capillary electromigration methods, namely zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC), can be used for evaluation of the efficiency

of extraction of pollen allergens and organic pollutants from the airborne dust samples [14].

Capillary isotachopheresis (CITP), another electromigration method, plays a specific role in environmental analysis because of possessing the concentrating effect, i.e. concentration of the sample components in ITP steady state is adapted to that of the leading ion [15]. In this way the originally diluted analyte ions are concentrated into sharply separated zones with stepwise concentration changes. For that reason CITP is often applied as a concentration pretreatment for CZE in environmental analysis. There are many applications of CITP in organic and inorganic environmental trace analysis, e.g. determination of cationic herbicides in river and municipal tap water [16], and nitrate and sulphate [17] in rain water; for a review see [18]. CITP was also applied to the identification of pollen allergens [19] and characterization (profiling) of various allergenic extracts including house dust mites [20].

The aim of this paper was to test the applicability of CITP for analysis of water extracts of the airborne dust samples collected in different periods in air-filtration devices in Prague car traffic tunnels and in a Parisian metro station, to find out the differences in the qualitative and/or quantitative composition of the extracts of the dust collected in the pollen-free and pollen-rich periods both in cationic and anionic CITP modes, and to determine the concentrations of some inorganic anions and cations in the dust samples.

## 2. Experimental

### 2.1. Chemicals

All chemicals used were of analytical reagent grade. Hydrochloric and acetic acids, potassium hydroxide and acetone were from Lachema (Brno, Czech Republic). D,L-Histidine was obtained from Sigma (St. Louis, MO, USA), 2-(*N*-morpholino)-ethanesulphonic acid (MES) from Serva (Heidelberg, Germany) and  $\beta$ -alanine (BALA) from Calbiochem (Luzerne, Switzerland). Buffer solutions were prepared from the deionized and redistilled water and filtered through a 0.45- $\mu$ m membrane

filter (Millipore, Bedford, MA, USA) prior the use in CITP.

## 2.2. Collection of the dust samples

Collection of the Prague tunnels dust samples was performed in the cooperation with the specialists from the National Institute of Public Health, Prague. Dust samples were collected as sediments in the air-filtration devices in the Letná tunnel (samples LT m/yr, m/yr=month/year of collection) and Strahov tunnel (samples ST m/yr), in the centre of Prague, in the regions of an intensive car traffic in the pollen-free period (December, January) and in the pollen-rich period (April, May, July) during the years 1998–2001.

The Parisian metro dust sample collected from the air filtration device in metro station Pasteur in Paris in the August–October period in 1999 was fractionated by mechanical sieving into two fractions, fine dust (PM 1) and fibre-rich dust (PM 2) samples.

## 2.3. Dust extraction procedure

The dust samples were first defatted by treatment with the organic solvent acetone. A 450-mg amount of the dust was treated five times with 25 ml acetone at  $-20^{\circ}\text{C}$ . The yield was 370 mg of defatted, dried dust. Then the following extraction procedure was used: 150 mg of the defatted dried dust sample was suspended in 1 ml of water, extraction was performed in the shaker Elpan, type 357 (Laboratory Instruments, Lubawa, Poland) at ambient temperature  $23^{\circ}\text{C}$  for 1 h followed by centrifugation in the centrifuge Boeco, type U-32 (Boeckel & Co., Hamburg, Germany) at 10 000 rpm ( $r=66$  mm) for 15 min. The yield was 0.850 ml of water extract.

## 2.4. Capillary isotachopheresis

CITP experiments were performed in the Electro-phoretic Analyser EA 101 (Villa Labeco, Spišská Nová Ves, Slovakia) equipped with a column coupling system consisting of two FEP (fluorinated ethylene–propylene copolymer) capillaries. The first, preseparation capillary (160 mm $\times$ 0.8 mm I.D.) is connected with the analytical capillary (180 mm $\times$ 0.3 mm I.D.) via the bifurcation block which enables determination of the sample macrocomponents in the preseparation capillary and determination of microcomponents in the analytical capillary. Contactless conductivity detectors are placed on both columns 40 mm from the outlet ends, and UV-absorption detector (set to the wavelength 254 nm) is situated 30 mm from the outlet end of the analytical column. Sample volumes of 2–15  $\mu\text{l}$  were applied by microsyringe through the septum above the injection valve. The driving currents were 250 and 50  $\mu\text{A}$  in the preseparation and analytical columns, respectively. Separations were performed at ambient temperature  $22$ – $24^{\circ}\text{C}$ . The composition of electrolyte systems used is given in Table 1.

## 3. Results and discussion

CITP has been used for analysis of water extracts of the dust samples collected in metro station in Paris (fine dust fraction PM 1, and fibre-rich dust fraction PM 2) and the dust samples collected in the car traffic Letná and Strahov tunnels in the centre of Prague in the pollen-free period (samples LT 1/98, LT 1/00, ST 1/01) and in the pollen rich period (sample LT 4/99, LT 5/01, ST 7/00). The water extracts were analyzed by CITP both in anionic and cationic modes using the electrolyte systems given in Table 1. The example of conductivity detector

Table 1  
Composition of CITP electrolyte systems

CITP mode	Leading electrolyte (LE)			Terminating electrolyte (TE)		
	Leading ion	Counterion	pH	Terminating ion	Counterion	pH
Anionic	10 mM $\text{Cl}^-$	20 mM $\text{His}^+$	5.8	10 mM $\text{MES}^-$	–	3.7
Cationic	10 mM $\text{K}^+$	25 mM $\text{Ac}^-$	4.4	10 mM $\text{BALA}^+$	$\text{Ac}^-$	4.4

records (integral plot and 1st derivative; cationic and anionic modes) of CITP analyses of water extracts from the fine dust fraction PM 1 collected in air filtration device in Paris metro station are shown in Fig. 1. In addition, to some “classical”, stepwise and well-resolved ITP zones of macrocomponents present in the sample, apparently several zones of minor components are not sufficiently resolved by the contactless conductivity detector due to their short lengths, small differences in conductivity and lower resolution capability of the contactless conductivity detector. For that reason and also taking into account the fact that pollen allergens and organic pollutants are mostly UV-absorbing compounds, the amount of the dust extractable material, including pollen al-

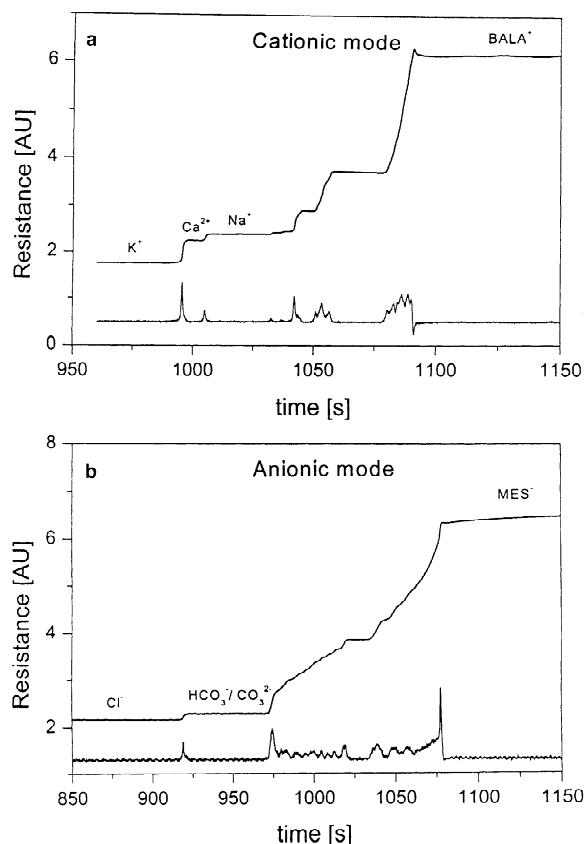


Fig. 1. Conductivity detector records (integral plot and 1st derivative) of CITP analyses of water extracts from the fine dust fraction PM 1 collected in air-filtration device in Parisian metro station: (a) cationic mode; (b) anionic mode (sample volume 4  $\mu$ l; other conditions are given in Section 2.4).

lergens and organic pollutants, has been evaluated from the UV-detector records at 254 nm of CITP analyses of the dust extracts. UV-absorption anionic and cationic isotachopherograms of the water extracts of the dust from Parisian metro station are presented in Fig. 2 and anionic ITP-grams of the extracts of the dust from Prague car traffic tunnels, Letná and Strahov, are shown in Fig. 3. Obviously, the amount of extracted material is proportional to the total area of UV-positive peaks (measured at 254 nm) related to migration velocity of ITP zones. The number of resolvable components has been also estimated from the UV-absorption records of CITP analyses. The results are presented in Table 2.

The substance amounts of ions of the sample, which are identical with the leading ions (chlorides in anionic mode, potassium ions in cationic mode),  $n_L$ , were calculated from the leading ion concentration,  $c_L$ , capillary cross-section,  $S$ , and from the prolongation of the LE zone lengths in the experiments with the samples in comparison with the LE zone length without sample, determined from the prolonged migration time of the boundary between LE and the first sample zone,  $\Delta t$ , and from migration velocity of the LE,  $v$

$$n_L = c_L S v \Delta t$$

The substance amounts of the ions were converted to mass amounts in the injected sample volumes and related to the mass unit of the dried dust sample before water extraction. The substance amounts of sodium and calcium ions in water extracts were determined from the corresponding calibration curves, the equations of which are presented in Table 3. The substance amounts of these ions in applied sample volumes were also converted to mass units and related to mass unit of the dried dust. The concentrations (expressed in ppm units) of all inorganic ions,  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$  and  $Cl^-$ , in the dust samples of different origin are summarized in Table 4.

Determination of the extracted amounts from the different samples, obtained by the same extraction procedure, allowed 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

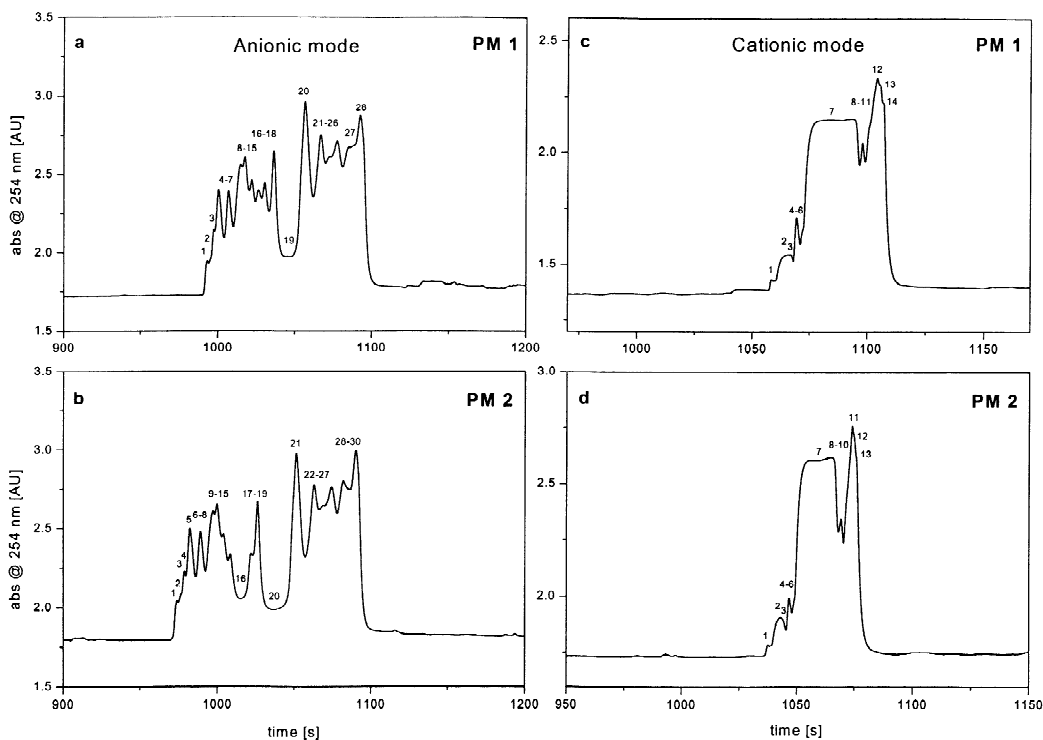


Fig. 2. UV detector records of CITP analyses of water extracts from the fine-dust, PM 1, and fibre-rich dust, PM 2, fractions collected in air-filtration device in Parisian metro station: (a,b) anionic mode; (c,d) cationic mode (sample volumes 4  $\mu$ l; other conditions as in Fig. 1).

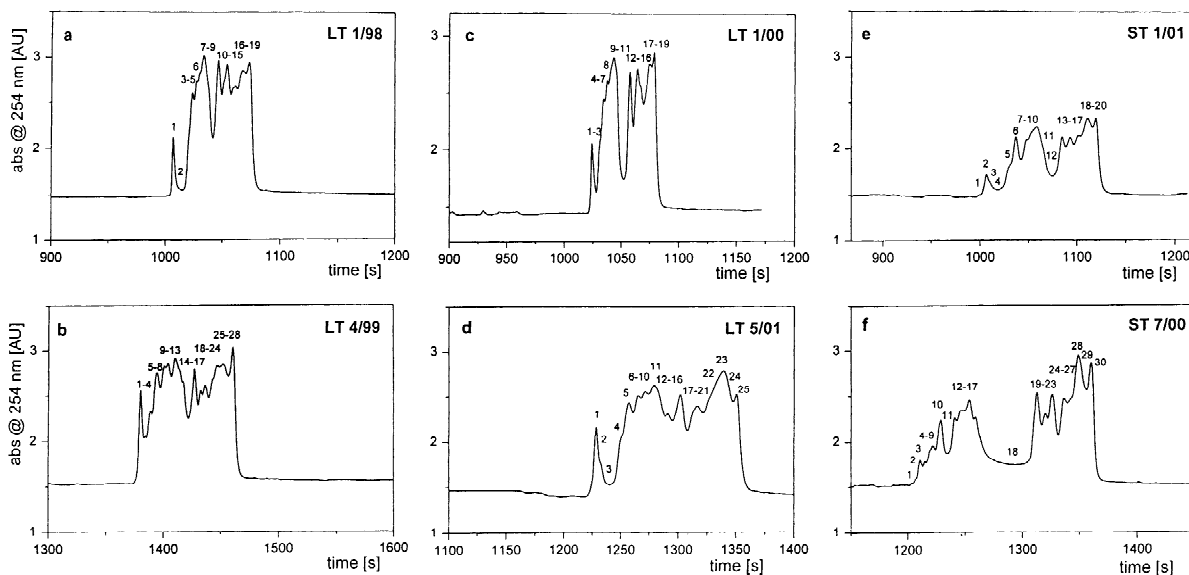


Fig. 3. UV detector records of CITP analyses in anionic mode of water extracts from the dust samples collected in Prague car traffic Letná tunnel (samples indicated as LT m/yr) and Strahov tunnel (samples indicated as ST m/yr) in different periods (month/year): (a,c,e) pollen-free period; (b,d,f) pollen-rich period (sample volumes 10  $\mu$ l; other conditions as in Fig. 1).

Table 2

Extracted amounts (in relative arbitrary units, AU) of UV-absorbing components, including pollen allergens and organic pollutants, and number of components found in the water extracts from Prague traffic tunnels and from Paris metro dust samples

Season	Dust sample	Anionic mode		Cationic mode	
		Extracted amount (AU) <sup>a</sup>	Number of components found	Extracted amount (AU) <sup>a</sup>	Number of components found
Pollen-free	LT 1/98	73.0	19	0.9	3
	LT 1/00	53.7	19	1.3	4
	ST 1/01	118.2	20	0.9	5
Pollen-rich	LT 4/99	133.0	28	1.4	3
	LT 5/01	118.1	25	1.0	5
	ST 7/00	200.5	30	1.3	5
	PM 1	352.9	28	84.0	14
	PM 2	371.4	30	61.0	13

LT m/yr, Letná tunnel dust samples, month/year; ST m/yr, Strahov tunnel dust samples month/year; PM 1, Paris Metro sample, fine dust; PM 2, Paris Metro sample, fibre-rich dust.

<sup>a</sup> Averaged values from two ITP analyses, the values of which differed less than 3–5%.

Table 3

Relative step heights (RSHs) and calibration curves equations of the CITP determination of calcium and sodium ions in cationic electrolyte system given in Table 1

Ion	RSH	Calibration equation	$R_{xy}^2$
Calcium	0.104±0.009	$y = 5.04x - 0.50$	0.989
Sodium	0.128±0.007	$y = 3.20x - 2.82$	0.998

y, zone length (s); x, amount of substance (nmol);  $R_{xy}$ , correlation coefficient.

was extracted and more components of the dust were found in the water extracts analysed in anionic mode than in cationic mode. Only minor differences have been found in the analyses of extracts of fine dust

and fibre-rich dust collected in Parisian metro (see Fig. 2 and Table 2).

Significant differences have been found in the analyses of dust extracts of different origin. Much more material and more components have been found in the extracts of the Prague tunnels dust from the pollen-rich period (April, May, July) (see Fig. 3b,d and f) than from the pollen-free period (January) (see Fig. 3a,c and e and Table 2). These differences are assumed to be caused by the presence of tree and grass pollen allergens in the samples, since in the period of its collection (March, April, July) a lot of tree and grass pollen allergens are present in the air and they are captured in the air traps and filtration devices.

Table 4

Determination of some inorganic ions in the dust samples by CITP

Season	Dust sample	Concentration of selected ions in the dust samples (ppm) <sup>a</sup>			
		K <sup>+</sup>	Cl <sup>-</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>
Pollen-free	LT 1/98	5270	3420	345	936
	LT 1/00	5840	3420	233	961
	ST 1/01	16380	6490	823	476
Pollen-rich	LT 4/99	29940	18100	680	772
	LT 5/01	15440	9560	1125	741
	ST 7/00	30890	31590	581	276
	PM 1	19400	14520	316	130
	PM 2	16200	10080	460	103

LT m/yr, Letná tunnel dust samples, month/year; ST m/yr, Strahov tunnel dust samples month/year; PM 1, Paris Metro sample, fine dust; PM 2, Paris Metro sample, fibre-rich dust.

<sup>a</sup> Averaged values from two ITP analyses, the values of which differed less than 2–3%.

Some similarities have been found in the analyses of extracts from the dust samples originating from Paris metro station and from Strahov tunnel (see Fig. 3f and 2a and b). On the other hand, much more of UV-absorbing components, including pollen allergens and organic pollutants, was extracted from Paris metro dust samples PM 1 and PM 2 than from those from the Prague tunnels, especially in cationic mode (see Table 2).

No special correlation has been found between the inorganic ions contents in the dust extracts and the origin of the dust sample (place and date of collection).

#### 4. Conclusions

CITP has been shown to be a highly efficient and highly sensitive method for analysis of water extracts from the airborne dust and for the estimation of the content of UV-absorbing components, including pollen allergens and organic pollutants in the dust samples of different origin. The extracted amount of UV-absorbing anionic components, including pollen allergens and organic pollutants, and their number were much higher than those of cationic components. Significant differences were found in the analyses of the extracts of the dust collected at different periods and locations. Much more material and more components were present in the extracts of samples originating from pollen-rich period than from the pollen-free period, especially in the anionic CITP mode.

The concentrations of selected inorganic ions ( $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{Na}^+$  and  $\text{Ca}^{2+}$ ) in the dust samples were also determined.

#### Acknowledgements

We wish to acknowledge the financial support of the 4th Framework Program of the European Union, INCO-Copernicus, contract no. ERB-IC15-CT98-0322, from the Ministry of Education of the Czech

Republic, grant No. OK 382, and from the Research Project No. AVOZ 4055905 of the Czech Academy of Sciences. Drs. B. Kotlík and H. Kazmarová are thanked for providing the dust samples from the Prague Letná and Strahov tunnels, while Mrs. V. Lišková and Mrs. C. Mayer are thanked for their skilful technical assistance.

#### References

- [1] J. Ring, B. Eberlein-Koenig, H. Behrendt, *Ann. Allergy Asthma Immunol.* 87 (2001) 2.
- [2] F. Acevedo, O. Vesterberg, C. Bayard, *Allergy* 53 (1998) 594.
- [3] A. Sydbom, A. Blomberg, S. Parnia, N. Stenfors, T. Sandstrom, S.E. Dahlen, *Eur. Resp. J.* 17 (2001) 733.
- [4] R.B. Knox, C. Suphioglu, P. Taylor, R. Desai, H.C. Watson, J.L. Peng, L.A. Bursill, *Clin. Exp. Allergy* 27 (1997) 246.
- [5] D. Diaz-Sanchez, *Allergy* 52 (Suppl. 38) (1997) 52.
- [6] T.E. Steinsvik, H. Ormstad, P.I. Gaarder, I.S. Aaberge, U. Bjonness, M. Lovik, *Toxicology* 128 (1998) 219.
- [7] E. Fernvik, G. Peltre, H. Senechal, B.B. Vargaftig, *Clin. Exp. Allergy* 32 (2002) 602.
- [8] M.G. Khaledi, *High-Performance Capillary Electrophoresis*, Wiley, New York, 1998.
- [9] G.W. Sovocool, W.C. Brumley, J.R. Donnelly, *Electrophoresis* 20 (1999) 3297.
- [10] D. Martinez, F. Borrull, M. Calull, *Trends Anal. Chem.* 18 (1999) 282.
- [11] V. Pacáková, K. Štulík, M. Tichá, *J. Chromatogr. B* 699 (1997) 403.
- [12] E. Dabek-Zlotorzynska, M. Piechowski, M. McGrath, E.P.C. Lai, *J. Chromatogr. A* 910 (2001) 331.
- [13] E. Dabek-Zlotorzynska, R. Aranda-Rodriguez, S.E.J. Buykx, *Anal. Bioanal. Chem.* 372 (2002) 467.
- [14] P. Sázelová, V. Kašička, D. Koval, Z. Prusík, G. Peltre, *J. Chromatogr. B* 770 (2002) 303.
- [15] P. Boček, M. Deml, P. Gebauer, V. Dolnik, *Analytical Isotachopheresis*, VCH, Weinheim, 1988.
- [16] P.A. Walker, J.M. Shaver, M.D. Morris, *Appl. Spectrosc.* 51 (1997) 1394.
- [17] D. Kaniansky, I. Zelensky, P. Havasi, M. Cerovsky, *J. Chromatogr.* 367 (1986) 274.
- [18] F.I. Onuska, D. Kaniansky, K.D. Onuska, M.L. Lee, *J. Microcolumn. Sep.* 10 (1998) 567.
- [19] R. Einarsson, R. Karlsson, *Int. Archs Allergy Appl. Immun.* 68 (1982) 222.
- [20] C.H.M.M. de Bruijn, J.C. Reijenga, G.V.A. Aben, T.P.E.M. Verheggen, F.M. Everaerts, *J. Chromatogr.* 320 (1985) 205.