

ISOTACHOPHORETIC DETERMINATION OF SOLUBLE NITRATES, NITRITES, SULFATES AND PHOSPHATES IN PLANT MATERIAL

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Testing and statistical evaluation of six operating systems suitable for the isotachophoretic determination of NO_3^- , SO_4^{2-} , NO_2^- and H_2PO_4^- , with the aim of the determination of the mentioned anions in plant material, are described. The proposed sample pretreatment reflecting the effort to minimize the number of unavoidable steps consisted only of extraction and filtration. The attention was paid to quantitative aspects of the extraction and to the possibility of extracts contamination during filtration. Influence of the sample amount on the anion zone lengths and the reproducibility of the determination were investigated too.

Due to the possible toxic effects of nitrates and nitrites at high levels, their content represents an important indicator of the harmlessness of agricultural products for human nutrition^{1,2}. Negative effects on ruminants motivate the monitoring of these anions also in forage^{3,4}. The content of inorganic anions (NO_3^- , SO_4^{2-} , H_2PO_4^-) in plant material is also an important agronomic tool which enables to assess the nutrient status of the plants⁵.

Spectrophotometric determination in the visible region⁶⁻⁸ and determination by means of ion-selective electrodes⁹ are frequently used in practice for the determination of nitrates in agricultural products. Other significant methods include spectrophotometric determination in UV region¹⁰, HPLC¹¹, ion chromatography¹² and capillary isotachopheresis¹³⁻¹⁵. The last three methods enable simultaneous determination of more components during one analysis and often do not require any or only minimum sample pretreatment. The isotachophoretic determination of NO_3^- , SO_4^{2-} and H_2PO_4^- anions in river water¹⁴, as well as in well and surface water¹⁵, mineral water¹⁶ and in combined artificial fertilizers¹³ have been already described. Because of the low differences in the ionic mobilities of NO_3^- and SO_4^{2-} anions in wide pH range¹⁷, retardation of SO_4 anions by complex formation with inorganic cations (Mg^{2+} or Ca^{2+})¹³⁻¹⁵ or with an organic cation (bistrispropane)¹⁴ can be utilized. The pH value of the leading electrolyte lower than 4 forms a prerequisite for nitrite determination, as it prevents mixed zone formation with SO_4^{2-} anions¹⁴.

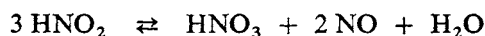
EXPERIMENTAL

For the analyses, the column-coupling isotachophoretic analyzer ZKI 01 (Ústav rádioekologie a využitia jadrovej techniky — Vývojovo výrobný závod prístrojov jadrovej techniky, Spišská Nová Ves) with standard columns and conductivity detectors was used. The employed chemicals were of p.a. grade. Glycine, magnesium chloride, calcium chloride, and citric acid were obtained from Lachema (Brno), hydroxyethylcellulose 4 000 (HEC) and β -alanine were purchased from Serva (Heidelberg, F.R.G.) and bistrispropane (BTP) from Sigma (St. Louis, U.S.A.). Compositions of the operating systems and working conditions are given in Table I.

Dried plant material (*Medicago Varia*, L., cv. Južanka), divided into leaves and stems, was extracted after homogenization with 50 ml of 0.05% solution of Triton X-100 (Koch-Light Labs., Colnbrook Bucks, England) in an ultrasonic bath UC 002 BM1 (Tesla, Vráble). After the extraction, samples were filtered through folded paperfilters (Západočeské papírny, n. p., Perštejn nad Ohří) and introduced directly via the sampling valve into the analyzer.

RESULTS AND DISCUSSION

For each of the operating systems described in Table I, calibration curves were obtained for NO_3^- , SO_4^{2-} , NO_2^- and H_2PO_4^- , resp. Their corresponding parameters from the analytical column are given in Table II. Calibration curves were evaluated in the concentration range 0.1–0.7 mmol l⁻¹. From the slopes of the calibration curves, b , a higher sensitivity is evident in operating systems with glycine as the counterion for NO_3^- , SO_4^{2-} and H_2PO_4^- anions, but a lower one for NO_2^- anions, in comparison to values with β -alanine used. Values corresponding to the intercepts for NO_3^- anions (a) in operating systems D–F were substantially higher than the background. From the values for NO_2^- anions, their loss occurring during separation is evident. The losses of NO_2^- anions and enrichments of the NO_3^- zone, respectively, can be explained by the instability of nitrous acid which reacts as follows:



Due to the self-sharpening effect, NO_3^- anions leave the nitrite zone by shifting the balance of the reaction course to the right¹⁴. Differences between the enrichments of nitrate zones and nitrite losses were approximately in correlation with the real background values.

From the facts mentioned above, it follows that operating systems with β -alanine as a counterion are more suitable for the determination of the mentioned anions than those with glycine, despite the lower detection limits for NO_3^- , SO_4^{2-} and H_2PO_4^- anions. Isotachopherograms of a model mixture of anions in all the operating systems are shown in Fig. 1.

Determination of soluble inorganic anions in plant material was carried out in the operating system A, because the experiments indicated lower probability of the organic anions interference with H_2PO_4^- anions in the sample than in the case of the operating systems B and C. Typical isotachopherograms of leaf and stem extracts

TABLE I

Operating systems (compositions of leading electrolytes) and working conditions. Leading anion Cl^- (10 mmol l^{-1}), c (co-counterion) = 2.5 mmol l^{-1} , additive HEC (1 g l^{-1}), terminating anion citrate (5 mmol l^{-1}), driving current in preparation column $250 \mu\text{A}$, in analytical column $50 \mu\text{A}$

Parameter	Operating system					
	A	B	C	D	E	F
Counterion	$\beta\text{-Ala}^+$	$\beta\text{-Ala}^+$	$\beta\text{-Ala}^+$	Gly^+	Gly^+	Gly^+
Co-counterion	Mg^{2+}	Ca^{2+}	BTP^{2+}	Mg^{2+}	Ca^{2+}	BTP^{2+}
pH	3.62	3.58	3.55	2.97	2.92	2.88
Analysis time, min		22–25			26–29	

TABLE II

Parameters of calibration lines $y = a + bx$; y concentration (in mmol l^{-1}), x zone length in mm, a intercept (in mm), b slope (in mm l mol^{-1}), r correlation coefficient

Parameter	Operating system					
	A	B	C	D	E	F
NO_3^-						
a	2.79	1.88	3.33	11.60	9.48	8.66
b	87.43	94.40	84.40	117.20	145.30	126.19
r	0.9997	0.9998	0.9997	0.9993	0.9997	0.9992
SO_4^{2-}						
a	1.64	1.19	1.83	4.05	0.85	1.17
b	167.05	172.08	165.60	231.19	257.14	232.52
r	0.9999	0.9999	0.9998	0.9993	0.9997	0.9994
NO_2^-						
a	-1.31	-1.69	-1.62	-3.24	-4.19	-4.13
b	80.81	84.64	68.15	61.73	67.54	54.48
r	0.9998	0.9996	0.9994	0.9995	0.9990	0.9983
H_2PO_4^-						
a	0.03	-0.12	0.07	1.45	-0.60	-1.06
b	127.52	133.81	120.18	170.89	202.14	175.38
r	0.9998	0.9999	0.9998	0.9998	0.9998	0.9998

are shown in Figs 2C and 2D, respectively. Isotachopherogram in Fig. 2A shows the analysis of the extraction reagent. The possibility of sample extract contamination during filtration is illustrated in Fig. 2B. From Figs 2A and 2B, resp., it is evident that contamination of the filtrate with NO_3^- , SO_4^{2-} and other anions, which were not identified, occurred. Errors in NO_3^- and NH_4^+ determinations caused by contamination from filter papers during filtration have been also discussed by Sparrow and Masiak¹⁸. We recommend, therefore, some blank analyses of filter papers from each packing. The analysis of the extraction reagent filtrate was taken as the background.

The influence of the extraction time on zone lengths of the corresponding anions at a constant sample amount (0.3 g leaves) was investigated. From the obtained

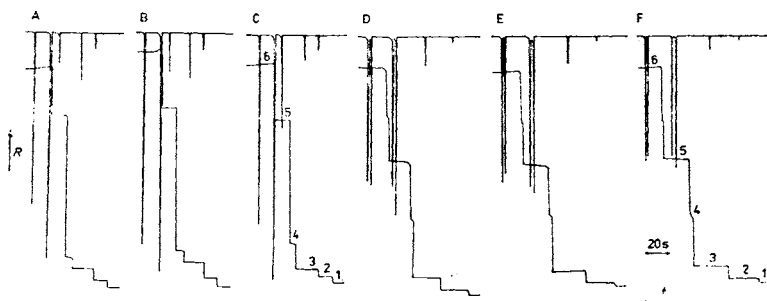


FIG. 1

Isotachopherograms of a model mixture of anions in operating systems A–F. Component: 1 Cl^- , 2 NO_3^- , 3 SO_4^{2-} , 4 NO_2^- , 5 H_2PO_4^- , 6 citrate. R resistance, t time, $c = 0.2 \text{ mmol l}^{-1}$ of each anion

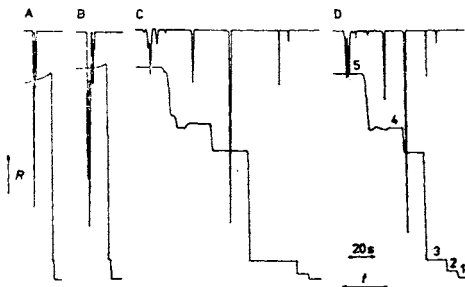


FIG. 2

Determination of inorganic anions in plant material: A extraction reagent; B extraction reagent after filtration; C leaf extract; D stem extract. Components: 1 Cl^- , 2 NO_3^- , 3 SO_4^{2-} , 4 H_2PO_4^- , 5 citrate (other anions were not identified). R resistance, t time, operating system A

results, independence of zone lengths was evident for extraction durations longer than 5 minutes. In order to prevent incomplete extraction, the samples were extracted for 15 minutes.

The investigation of sample amount influence on the zone lengths of the anions reflected a linear course in the ranges 0.05–0.6 g for leaves and 0.10–1.20 g for stems.

The reproducibility of the determination was verified with a sample (0.3 g leaves), which was extracted five times repeatedly and each extract was analyzed three times. From the obtained results (in g kg^{-1} of dry matter): $\text{NO}_3^- = 2.11$ (relative standard deviation, $s_r = 1.9\%$), $\text{SO}_4^{2-} = 9.81$ ($s_r = 0.4\%$) and $\text{H}_2\text{PO}_4^- = 11.37$ ($s_r = 0.5\%$), good reproducibility is evident of the proposed determination. Under the given experimental conditions, the detection limits were as follows (in mmol l^{-1}): $\text{NO}_3^- - 1.1 \cdot 10^{-2}$, $\text{SO}_4^{2-} - 6.0 \cdot 10^{-3}$, $\text{NO}_2^- - 1.2 \cdot 10^{-2}$ and $\text{H}_2\text{PO}_4^- - 8.0 \cdot 10^{-3}$, respectively. Nitrite was not detected in real samples.

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REFERENCES

1. Prugar J. in: *Proceedings of the Conference "Dusičnany v poľnohospodárskych produktoch"*, Nitra, January 29, 1987 (I. Repka, Ed.), p. 18. Agricultural University, Nitra 1987.
2. Hotchkiss J. H., Cassens R. G.: *Food Technol.* **41**, 127 (1987).
3. Míka V.: *Agrochémia* **26**, 178 (1986).
4. Nikolić J. A., Cmilijančić R.: *Acta Veter.* (Beograd) **36**, 107 (1986).
5. Mengel K., Kirkby E. A.: *Principles of Plant Nutrition*, p. 100. International Potash Institute, Bern 1982.
6. Fries J., Getrost H.: *Organische Reagenzien für die Spurenanalyse*, p. 267. E. Merck, Darmstadt 1977.
7. Příběla A.: *Analýza prírodných látok v požívatinách*, p. 130. Alfa, Bratislava 1978.
8. Davídek J., Hrdlička J., Karvánek M., Pokorný J., Seifert J., Velišek J.: *Laboratorní příručka analýzy potravin*, p. 173. SNTL/Alfa, Prague 1981.
9. Radiometer Application 918-314-8408 B. Radiometer, Copenhagen 1984.
10. Heanes D. L.: *Int. Lab.* **4**, 64 (1986).
11. De Kleijn J. P., Hoven K.: *Analyst* **109**, 527 (1984).
12. Jakobsson K., Åberg L.: *In Focus* **10**, 14 (1987).
13. Boček P., Kaplanová B., Deml M., Janák J.: *Collect. Czech. Chem. Commun.* **43**, 2707 (1978).
14. Zelenský I., Zelenská V., Kaniánský D., Havaši P., Lednářová V.: *J. Chromatogr.* **294**, 317 (1984).
15. Vacík J., Muselasová I.: *J. Chromatogr.* **320**, 199 (1985).
16. Boček P., Miedziak I., Deml M., Janák J.: *J. Chromatogr.* **137**, 83 (1977).
17. Hirokawa T., Nishino M., Aoki N., Kiso Y., Sawamoto Y., Yagi T., Akiyama J.: *J. Chromatogr.* **271**, D1 (1983).
18. Sparrow S. D., Masiak D. T.: *Soil Sci. Soc. Am. J.* **51**, 107 (1987).

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