ISSN 1061-9348, Journal of Analytical Chemistry, 2009, Vol. 64, No. 12, pp. 1242–1246. © Pleiades Publishing, Ltd., 2009.

ARTICLES

Determination of Inorganic Anions in Mushrooms by Ion Chromatography with Potentiometric Detection[1]

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Received May 30, 2008; in final form, May 14, 2009

Abstract—A simple method for determination of common inorganic anions in mushroom samples has been developed by using suppressed ion chromatography with a pH detection unit. The detection unit which was constructed in such a way that practically no additional dispersion occurred consisted of a flow-through quinhydrone pH sensor and a small reference electrode. Chromatographic separation was performed in the order F^-, Cl^-, NO_2^-, Br^-, PO_4^{3-}, ClO_3^-, NO_3^-, and SO_4^{2-} at room temperature by using Ion Pac AS 9-HC anion exchange column. Anion extracts from dried mushroom samples at room temperature were homogenized and filtered before injection. Under optimized analytical conditions, the detection limits of the method were between 2×10^{-6} and 3×10^{-4} M, depending on the anion studied. The results showed that the concentrations of fluoride and bromide in all mushroom samples were below their limit of detection. Nitrite was found to be the lowest abundant ion, while the most abundant ion was sulfate in all the mushroom samples studied.

DOI: 10.1134/S1061934809120077

Determination of common inorganic anions in environmental waters, such as waste, drinking, ground, and surface waters, is an important field of application of ion chromatography (**IC**) worldwide. IC with a conductivity detector can now be considered a well established, mature technique for the determinations of ionic species in foods, and the number of standard IC methods is growing rapidly. The technique is now applicable to the determination of a wide range of solutes in diverse sample matrices and food analysis, although environmental analysis continues to be the largest application area of IC [1, 2]. However, low detection limits and high measurement sensitivity to ions combined with a small sample volume are needed in a number of applications of ion chromatography. Much attention has been paid to optimizing these factors and various analytical methods have been developed [3–11]. The technology of ion selective electrode (**ISE**) fabrication at low cost, design of miniaturized flow-cells to minimize dead space and carry over sample solutions, low detection limits and low selectivity of some of ISEs have led to an increase in use of ISEs as detectors in ion chromatography [3–6, 12, 13].

Ions are essential to life and play an important role in the cells of living organisms, particularly in cell membranes. As mushrooms may contain highly toxic substances, great effort has been made to evaluate the possible danger to human health from the ingestion of mushrooms. Sulfate ions have caused a number of adverse reactions in hypersensitive individuals, especially asthmatics. The reductions in the intestine of nitrate to nitrite have caused acute methemoglobinemia (when hemoglobin loses its ability to carry oxygen) particularly in infants, resulting from conversion of nitrate to nitrite after consumption [14, 15]. Phosphorus compounds are present in most vegetables; this element is important for skeletal integrity that directly depends on calcium/magnesium/phosphorus ratio. An excess of phosphorus leads to tissue ossification. Fluoride is an important factor for skeletal bone integrity and dental health. Correct daily intake of fluoride is necessary, but excessive consumption causes symptoms of acute and chronic fluoride toxicity (fluorosis). The principal source of fluoride intake is water; nevertheless, other foods can be a source of fluoride. Chloride is one of the most common inorganic anions in foods. Its content is usually related to sodium presence and is very important for metabolic acid-base equilibrium. Chloride have important physiological roles in the central nervous system; the inhibitory action of glycine and action of gamma-aminobutyric acid (in humans acts upon inhibitory synapses in the brain and spinal cord) relies on the entry of Cl^- into specific neurons an. Excess in chloride content creates a series of adverse effects on human health. Bromide content in foods affects central nervous system, brain and eyes. The narrow tolerance limits make the bromide determination very important to avoid risk for human health.

Mushrooms are traditionally used by many countries for food and medicine. Fruit bodies of mushrooms are appreciated, not only for texture and flavour but also for their chemical and high nutritional properties [16].

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[1] The article is published in the original.

Table 1. Retention times (t_R) and detection limits (LOD) of anions

Anion	t_R, min	LOD,* $\times 10^{-5}$ M
Fluoride	4.03 ± 0.01	2.01
Chloride	7.90 ± 0.02	0.21
Nitrite	10.20 ± 0.04	10.30
Bromide	13.89 ± 0.06	9.70
Phosphate	14.80 ± 0.07	12.20
Chlorate	16.27 ± 0.07	14.60
Nitrate	17.19 ± 0.08	31.50
Sulfate	29.19 ± 0.10	6.48

* Signal/noise: 3.

Therefore, it is necessary to examine the ion contents with correct methods.

Although there are many studies on the determination of heavy metals in cultivated and wild-grown edible mushrooms [16–22], there is little information available about common ions in mushrooms. So far, the present paper is the first study purposed for the analysis of common anions in mushrooms. The analysis of common anions (fluoride, chloride, bromide, nitrite, nitrate, phosphate, chlorate and sulfate) in wild-grown edible mushrooms was performed by using ion chromatography with a pH detection unit [23]. The method used in this study can be applied for many other foodstuffs.

EXPERIMENTAL

Instrumentation. Ion chromatography was performed using a Dionex P 680 HPLC pump, Ion Pac AS 9-HC analytical and Ion Pac AG 9-HC guard columns, an anion micro membrane suppressor system (AMMS III) and the flow-through pH detection unit described elsewhere [24]. The mobile phase used for determination of the anions in mushrooms was 4×10^{-2} M $Na_2B_4O_7$ at a flow-rate of 1 mL/min. An Ismatec IPC high precision multi channel dispenser pump was used. The chromatograms were recorded with a Pentium IV computer equipment with a custom built (Molspin Instruments, Newcastle Upon-Tyne, U.K.) analog/output board and electrode interface module controlled by a laboratory written software.

Reagents and standard solutions. Standard anion mixtures were prepared from the 1000 mg/L standards of inorganic anions. The working solitations of anions were prepared daily in the range of 5×10^{-5}–1×10^{-3} M doubly deionized water (18.2 mΩ cm resistivity), obtained from a Milli-Q Millipore water purification system, was used to prepare standards and for cleaning the containers and bottles. All bottles and containers were soaked in Milli-Q water at least overnight and rinsed three times with Milli-Q water prior to use. All reagents used in the preparation of eluents were at least p.a. purity grade.

Sample preparation. Fruiting bodies of eight wild-grown edible mushrooms were provided from the Gaziosmanpasa University Faculty of Art and Science Microbiology Herbarium Laboratory (Herbarium number; *Agaricus bisporus*: Almus-Turk., 1920; *Lepista nuda*: Niksar-Turk., 2104; *Marasmius oreades*: Tokat-Turk., 1710; *Morchella elata*: 2008; *Coprinus comatus*: Tokat-Turk., 1927; *Verpa conica*: Tokat-Turk., 2152; *Morchella esculenta*: Tokat-Turk., 1814; *Pleurotus ostreatus*: Almus-Turk., 1950).

The extractions of anions from 1 g samples of each mushroom dried at room temperature for several days were achieved by heating with Milli-Q water. All the mushroom samples were in an ultrasonic bath for about 15–20 min. In this stage of sample preparation, the existence of protein matter in the extract makes it difficult to obtain a clear solution. To eliminate protein matter, mushroom sample extracts were boiled to denature the protein. To separate particulate matter, filtration with a previous centrifugation step was performed through 0.45 μm syringe filters. Then all samples were adjusted to a volume of 10 ml with Milli-Q water. Samples were stored in cleaned polyethylene bottles until analysis. Three blank samples were treated in the same way.

RESULTS AND DISCUSSION

The determination of inorganic elements such as fluoride, chloride, bromide, nitrite, nitrate, phosphate, chlorate and sulfate in mushroom species are of great concern from the toxicological point of view. In this study, a suppressed ion chromatographic method was successfully applied to the determination of inorganic anions in mushrooms. For selection of eluent composition, a number of ionic standards in aqueous solution were chromatographed using several eluent compositions. The optimum eluent composition was found to be 4×10^{-2} M $Na_2B_4O_7$. With this eluent, the analysis could be performed within 29 min. The migration order of the anions was: F^-, Cl^-, NO_2^-, Br^-, PO_4^{3-}, ClO_3^-, NO_3^-, and SO_4^{2-}, as shown in Fig. 1.

The retention times and detection limits of inorganic anions are given in Table 1. The limits of detection (LODs) were calculated using standard solutions as the concentration corresponding to the signal-to-noise ratio (*S*/*N*) of three.

Calibration graphs were constructed by plotting peak height against concentration of the anions under the optimum conditions (Fig. 2). Linear plots were obtained in the concentration ranges of 5×10^{-5}–1×10^{-3} M of anions. Table 2 shows concentration ranges investigated for each anion, as well as the resulting linear coefficients of determination (r^2) and linearity. The retention time and peak height precisions (expressed

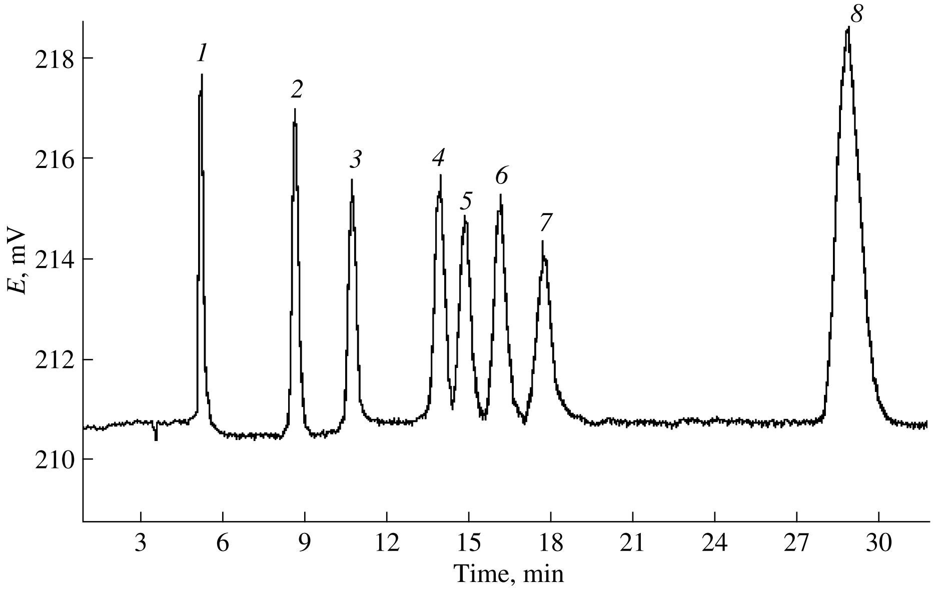

Fig. 1. Suppressed ion chromatographic separation and potentiometric detection of inorganic anions using 5×10^{-4} M standard sample solution of each ion. Eluent: 4×10^{-2} M $Na_2B_4O_7$; Flow rate: 1 mL/min; injection volume: 20 μL; solutes: *1* – fluoride, *2* – chloride, *3* – nitrite, *4* – bromide, *5* – phosphate, *6* – chlorate, *7* – nitrate, *8* – sulfate.

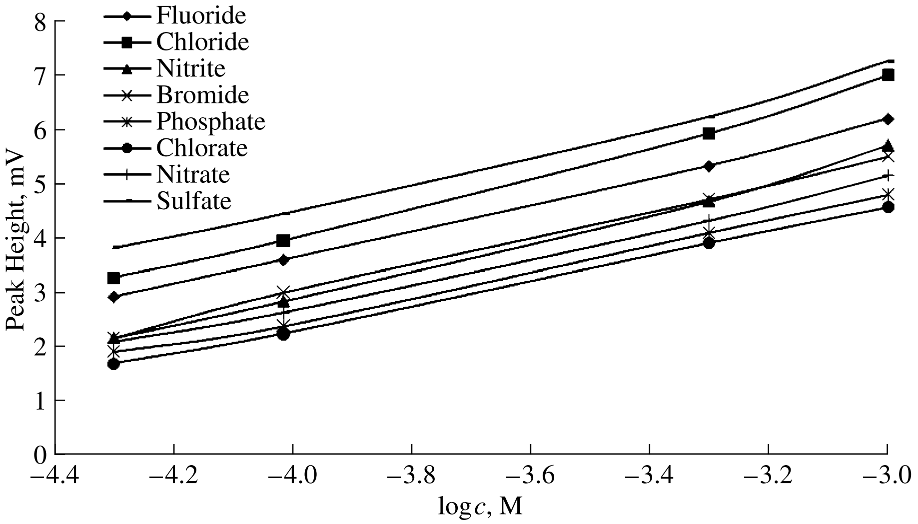

Fig.2. Calibration plots obtained by potentiometric detection in suppressed ion chromatography using flow-through pH detection unit.

as % RSD) were determined from three replicate injections of standard samples of mixed anions. Table 2 also shows typical retention time and peak height precision data that can be obtained for the inorganic anions.

The reproducibility of separation was moderately good; relative standard deviations for the retention time of anions varied from 0.25 to 0.49%. The standard deviations were small, especially when the concentrations of the anions were low.

The concentration of inorganic anions in investigated mushroom samples are given in Table 3. The values are given as mean ± *SD*. The results are means of three replicates. The anions level determined were based on mushrooms dry weight. Fluoride and bromide are nonessential elements in foods, and in mushrooms are mostly derived from various sources of environmental contamination [24]. Our results show that the concentration of fluoride and bromide in all mushroom samples are below their limit of detection. Nitrite has

Table 2. Analytical parameters for inorganic anions studied by the ion chromatographic method

Anions	Concentration range, $\times 10^{-3}$ M	Retention Time Precision, % RSD	Peak Height Precision, % RSD	Linearity	r^2
Fluoride	0.05–1	0.40	0.59	$y = 2.4981x + 13.642$	0.9989
Chloride	0.05–1	0.25	0.63	$y = 2.8456x + 15.437$	0.9965
Nitrite	0.05–1	0.39	0.70	$y = 2.6997x + 13.716$	0.9962
Bromide	0.05–1	0.46	0.54	$y = 2.5353x + 13.113$	0.9989
Phosphate	0.05–1	0.47	0.62	$y = 2.2648x + 11.574$	0.9972
Chlorate	0.05–1	0.43	0.65	$y = 2.2422x + 11.297$	0.9992
Nitrate	0.05–1	0.49	0.77	$y = 2.3556x + 12.156$	0.9974
Sulfate	0.05–1	0.34	0.74	$y = 2.6187x + 15.015$	0.9952

Table 3. Concentrations of inorganic anions in mushroom species obtained by the suppressed ion chromatographic method, mg/g, $n = 3$

Anions	*Agaricus bisporus*	*Lepista nuda*	*Marasmius oreades*	*Morchella elata*	*Coprinus comatus*	*Verpa conica*	*Morchella esculenta*	*Pleurotus ostreatus*
Fluoride	–	–	–	–	–	–	–	–
Chloride	0.16 ± 0.01	0.62 ± 0.04	0.13 ± 0.01	–	0.69 ± 0.03	0.83 ± 0.04	–	0.11 ± 0.01
Nitrite	–	–	0.05 ± 0.001	0.04 ± 0.001	–	–	0.11 ± 0.002	–
Bromide	–	–	–	–	–	–	–	–
Phosphate	0.23 ± 0.01	0.12 ± 0.001	–	–	–	0.41 ± 0.01	–	0.57 ± 0.01
Chlorate	1.52 ± 0.17	28.98 ± 0.53	4.43 ± 0.31	3.19 ± 0.18	6.93 ± 0.22	2.37 ± 0.12	3.11 ± 0.13	3.93 ± 0.26
Nitrate	9.44 ± 0.05	21.32 ± 0.04	66.25 ± 0.21	41.85 ± 0.19	74.35 ± 0.18	3.32 ± 0.20	18.91 ± 0.18	19.50 ± 0.01
Sulfate	25.83 ± 0.32	80.23 ± 0.12	11.15 ± 0.43	30.81 ± 0.14	46.46 ± 0.53	9.42 ± 0.02	14.59 ± 0.09	19.36 ± 0.18

been found to be the lowest abundant ion, while the most abundant ion is chlorate in all mushroom samples studied. However, chloride has not been detected in *Morchella elata* and *Morchella esculenta*, nitrite in *Agaricus bisporus*, *Coprinus comatus*, *Lepista nuda*, *Verpa conica* and *Pleurotus ostreatus*, and Phosphate in *Morchella esculenta*, *Coprinus comatus*, *Marasmius oreades* and *Morchella elata*. As can be seen from Table 3, the contents of sulfate and chlorate in many mushroom species are high. The nitrate concentration in *Marasmius oreades*, *Morchella elata* and *Coprinus comatus* has been found determined highest than other mushroom species.

Chloride is one of the most common inorganic anions in foods [24, 25]. Chloride ranged from 0.11 ± 0.01 (*Pleurotus ostreatus*) to 0.83 ± 0.04 mg/g (*Verpa conica*). The concentration of phosphate which is present in most foods were generally low in nearly all analyzed mushroom samples, ranging from 0.12 ± 0.001 to 0.57 ± 0.01 mg/g, being lowest in *Lepista nuda* and highest in *Pleurotus ostreatus*. Nitrate and sulfate are widely distributed in foods and mushroom spices [24, 25]. The lowest concentration of nitrate was in *Verpa conica* (3.32 ± 0.20 mg/g), while the highest level was in *Coprinus comatus* (74.35 ± 0.18 mg/g). The sulfate contents in mushroom samples were in the range of 9.42 ± 0.02–80.23 ± 0.12 mg/g. The lowest level of sulfate was in *Verpa conica* and the highest was in *Lepista nuda*. Chlorate levels in all mushroom samples were in the range of 1.52 ± 0.17–28.98 ± 0.53 mg/g. Generally, the analyzed mushroom samples contained higher concentrations of sulfate and nitrate relatively to the other inorganic anions.

CONCLUSIONS

Many inorganic anions which have adverse human health effects, such as fluoride nitrite, nitrate, bromide, chloride, sulfate and phosphate can be readily simultaneously determined wild grown mushroom species using ion chromatographic method. Under the chromatographic conditions employed, all inorganic anions were clearly separated from each other. Calibration curves were linear ($r^2 > 0.995$) and precision of the assay was acceptable. The detection limits (LODs) were found to be between 0.21×10^{-5} and 31.50×10^{-5} M. Relative standard deviations of the quantitative results calculated with five injections were between 0.25 and 0.49%.

Finally the method developed here proved to be selective, precise, linear and sensitive being adequate for measuring of inorganic anions in the mushroom species.

ACKNOWLEDGMENTS

The author would like to acknowledge the support of Chemistry Department of Ondokuz Mayis University, additional support of Murat Yolcu in ion chromatographic analysis of samples and discussions, and Ibrahim Turkekul for the gift of mushroom samples.

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