VILNIUS UNIVERSITY

Svetlana Nabiyeva

WATER-BASED HEADSPACE SINGLE-DROP MICROEXTRACTION AND CAPILLARY ELECTROPHORESIS FOR THE DETERMINATION OF VOLATILE INORGANIC COMPOUNDS IN COMPLEX MATRICES

Summary of doctoral dissertation

Physical sciences, chemistry (03 P)

Vilnius, 2010

This dissertation was carried out in Vilnius University in the period of 2006-2010.

Scientific supervisor:

prof. habil. dr. Audrius Padarauskas (Vilnius University, physical sciences, chemistry - 03 P)

Evaluation board:

Chairman:

prof. dr. Stasys Tautkus (Vilnius University, physical sciences, chemistry - 03 P)

Members:

prof. dr. Jurgis Barkauskas (Vilnius University, physical sciences, chemistry - 03 P)

dr. Benedikta Lukšiene (Center of Physical Sciences and Technology, Institute of Physics, physical sciences, chemistry - 03 P)

prof. habil. dr. Algimantas Undzenas (Center of Physical Sciences and Technology, Institute of Physics, physical sciences, chemistry - 03 P)

doc. dr. Aušra Valiuniene (Vilnius University, physical sciences, chemistry - 03 P)

Opponents:

prof. dr. Gintaras Denafas (Kaunas University of Technology, technological sciences, environmental engineering and landscaping - 04 T)

prof. dr. Vida Vickackaite (Vilnius University, physical sciences, chemistry - 03 P)

The official discussion will be held at 14 p.m. 3 December 2010 in the meeting of the Council of Chemistry science direction at the Auditorium of Inorganic Chemistry of the Faculty of Chemistry of Vilnius University.

Address: Naugarduko 24, LT-03225 Vilnius, Lithuania.

The summary of doctoral dissertation was mailed on the 3 November 2010.

The dissertation is available at the library of Vilnius University and at the library of Institute of Chemistry.

VILNIAUS UNIVERSITETAS
FTMC CHEMIJOS INSTITUTAS

Svetlana Nabiyeva

MIKROEKSTRAKCIJA IŠ VIRŠERDVES VANDENS LAŠU–KAPILIARINE ELEKTROFOREZE LAKIU NEORGANINIU JUNGINIU NUSTATYMUI SUDETINGOSE MATRICOSE

Daktaro disertacijos santrauka
Fiziniai mokslai, chemija (03 P)

Vilnius, 2010

Disertacija rengta 2006-2010 metais Vilniaus universitete.

Mokslinis vadovas:

prof. habil. dr. Audrius Padarauskas (Vilniaus universitetas, fiziniai mokslai, chemija – 03 P)

Disertacija ginama Vilniaus universiteto Chemijos mokslo krypties taryboje:

Pirmininkas:

prof. dr. Stasys Tautkus (Vilniaus universitetas, fiziniai mokslai, chemija – 03 P)

Nariai:

prof. dr. Jurgis Barkauskas (Vilniaus universitetas, fiziniai mokslai, chemija – 03P)

dr. Benedikta Lukšiene (Fiziniu ir technologijos mokslu centro Fizikos institutas, fiziniai mokslai, chemija – 03P)

prof. habil. dr. Algimantas Undzenas (Fiziniu ir technologijos mokslu centro Fizikos institutas, fiziniai mokslai, chemija – 03P)

doc. dr. Aušra Valiuniene (Vilniaus universitetas, fiziniai mokslai, chemija – 03P)

Oponentai:

prof. dr. Gintaras Denafas **(**Kauno technologijos universitetas, technologijos mokslai, aplinkos inžinerija ir kraštotvarka – 04T)

prof. dr. Vida Vickackaite (Vilniaus universitetas, fiziniai mokslai, chemija – 03 P)

Disertacija bus ginama viešame Chemijos mokslo krypties tarybos posedyje 2010 m. gruodžio men. 3 d. 14 val. Vilniaus universiteto Chemijos fakulteto Neorganines chemijos auditorijoje.

Adresas: Naugarduko 24, LT-03225, Vilnius, Lietuva.

Disertacijos santrauka išsiuntineta 2010 m. lapkricio men. 3 d.

Disertacija galima peržiureti Vilniaus universiteto ir Chemijos instituto bibliotekose.

1. INTRODUCTION

Despite unprecedented growth in analytical techniques over the last few decades, complete non-invasive measurements are still not possible in most cases. More often than not, one or more sample pre-treatment steps are necessary prior to analysis. Consequently, sample preparation becomes a major part of modern analysis, capable of taking up to 75% of the total time of a complete analytical process and is typically the primary source of errors. Ideally, sample-preparation techniques should be selective, fast, easy to use, inexpensive and compatible with a range of analytical instruments. Extraction with its various modes is the most popular sample pre-treatment method. However, it is time-consuming, tedious and uses large amounts of potentially toxic organic solvent that is usually expensive because of its high purity necessary for analytical applications. Therefore, much effort has been devoted in recent years to exploring the possibilities of miniaturization of the extraction procedures to minimize or eliminate the limitations of the sample preparation.

In order to overcome above mentioned problems, simple, inexpensive single-drop microextraction (SDME) technique was introduced in the mid-1990s. In this technique, a small drop of a water-immiscible solvent suspended at the tip of a microsyringe is directly immersed in (direct SDME) or placed above (headspace SDME) a stirred aqueous sample solution. After an optimized period of time, the drop is retracted back into the microsyringe and analyzed by conventional gas chromatography or liquid chromatography techniques. In contrast to conventional liquid-liquid extraction, SDME is not an exhaustive extraction technique, and only relatively small fraction of analyte is extracted/preconcentrated for analysis. Consequently, there are additional possibilities to enhance the extraction efficiency and thus to improve the sensitivity by proper selection of the extraction conditions. However, till now the selection of extraction conditions in SDME is performed on a trial-and-error basis. Clearly, in order to effectively employ these possibilities, the main parameters affecting the extraction performance should be identified and quantitatively evaluated.

Although SDME technique is very simple, fast and inexpensive, it is mainly used for the extraction of organic compounds with subsequent analysis using gas chromatography or liquid chromatography techniques. Several studies dealing with SDME of metal

complexes before electrothermal volatilization spectrometric determination are rather exceptions to the rule. Furthermore, the organic solvents used for SDME which are immiscible with water, are not compatible with conventional analytical techniques such as capillary electrophoresis (CE), ion chromatography and some others. An alternative way of minimizing some of these problems is to use headspace SDME. In this approach a microdrop of appropriate solvent is placed in the headspace of the sample solution to extract volatile analytes. Headspace SDME has similar capabilities in terms of precision and speed of analysis compared to direct SDME but has an advantage to choice of a wider variety of solvents. Unlike direct SDME, water can be also used as the solvent to extract volatile and water soluble analytes. This significantly enhances the range extractable analytes as well as the range of analytical methods that can be coupled with SDME.

The aim of the present work was systematic investigation of water-based headspace SDME technique, its conjunction with capillary electrophoresis, and application for the analysis of volatile inorganic compounds.

The main tasks set to achieve the aim were as follows:

1. Theoretical evaluation and experimental verification of the main parameters affecting the extraction performance in headspace SDME.
2. Optimization of water-based headspace SDME and CE techniques for the preconcentration and determination of cyanide and ammonia.
3. Development of a new approach involving ligand-exchange displacement, headspace SDME and CE for the determination of weak acid dissociable cyanide.
4. Application of the developed systems in the analysis of real samples.

Statements for defence:

1. Water-based headspace SDME significantly enhances the application areas of microextraction techniques.
2. Alteration of the analyte volatility by its chemical modification in the sample and/or in the acceptor phase is the most effective way to enhance the performance of headspace SDME.
3. Ligand-exchange displacement is more effective and selective than techniques currently used for the determination of weak acid dissociable cyanide.
4. Developed water-based headspace SDME-CE methods are fast, simple and selective.

2. EXPERIMENTAL

Separations were performed on a P/ACE 2100 apparatus (Beckman Instruments Inc., Fullerton, CA, USA) equipped with a UV detector with wavelength filters (200, 214, 230 and 254 nm). Fused silica capillary (Polymicro Technology, Phoenix, AZ, USA) of 75 μm i.d. was used. Samples were injected in the hydrodynamic mode by overpressure (3.43×10^3 Pa). System Gold software (Beckman Instruments Inc.) was used for data acquisition. All CE separations were conducted at 25 °C with a liquid thermostated capillary cartridge.

A 50-μL Hamilton microsyringe with a fixed beveled-point needle was used for extraction. Headspace SDME was carried out from 5 mL of sample solution placed in a 10 mL vial closed with the silicone rubber septum containing cap. Extraction was carried out as follows. A appropriate amounts of sample (2.0-4.5 mL) and buffer solutions (final volume 5 mL) were placed in a sample vial. After uptake of 5 μL of acceptor solution the needle of the syringe was then inserted into the headspace of sample solution. The syringe plunger was depressed and a microdrop of acceptor phase was suspended from the needle tip. After an optimized period of time, the plunger was withdrawn and the microdrop was retracted back into the syringe. The needle was removed from the headspace and its content was introduced to a CE microvial for subsequent analysis.

All electrolyte solutions were filtered through a 0.45 μm membrane filter degassed by ultrasonication. Each new fused-silica capillary was flushed with 1 mol/L NaOH for 30 min and then with deionized water for 15 min. Between all electrophoretic separations the capillary was rinsed with carrier electrolyte for 1 min.

3. RESULTS AND DISCUSSION

3.1. Headspace SDME and CE for free cyanide analysis

SDME principle. In the present study, a new method involving headspace single-drop microextraction with in-drop derivatization and capillary electrophoresis was investigated for the pre-concentration and determination of cyanide (Fig. 1). An alkaline aqueous microdrop (5 μL) containing Ni(II)-NH_3 (as derivatization agent) and ammonium pyromellitate (as internal standard) was used as the acceptor phase.

Extracted volatile hydrogen cyanide forms stable $Ni(CN)_4^{2-}$ complex which is then determined by CE.

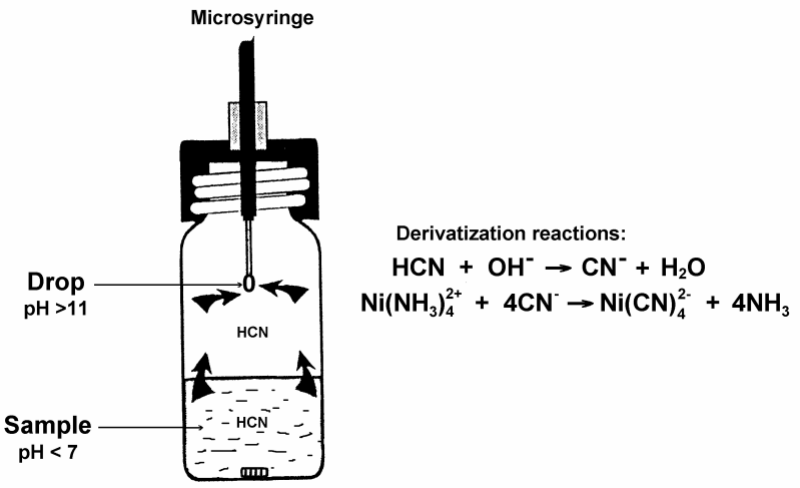

Fig. 1. Schematic of headspace single-drop microextraction for cyanide analysis.

Choice of CE conditions. The use of an internal standard is often necessary in order to compensate the relatively poor repeatability observed in SDME and CE techniques, and hence to achieve an acceptable method precision. For quantitative analysis, we have selected pyromellitate anion as an internal standard. This anion meets the main requirements for internal standard: it is compatible with the acceptor phase, chemically stable, nonvolatile, absorbs at 254 nm, and has a sufficiently high mobility. Since the electrophoretic mobilities of highly charged $Ni(CN)_4^{2-}$ and pyromellitate anions are large enough to cause them to move toward anode without the need to reduce or reverse the electroosmotic flow (EOF), initial CE experiments were performed under counter-EOF conditions. For optimum peak shapes, the mobility of the electrolyte co-ion in CE must be as close as possible to the mobility of the analytes. In addition, buffering of the electrolyte is essential for reproducible and rugged separations. For this purpose, we tested three carrier electrolytes containing 20 mmol/L of HCl, H_2SO_4, or H_3PO_4 neutralized with Tris to pH 8.3. The best separation performance in respect to peak efficiency and separation time was obtained for a Tris-sulfate electrolyte. Furthermore, in order to shorten analysis time additionally the effective capillary length was reduced to a minimum (18 cm) and the EOF velocity was lowered by adding 1 mmol/L hexamethonium bromide to the carrier electrolyte. Fig. 2 shows the separation of

$Ni(CN)_4^{2-}$ and pyromellitate anions under the CE conditions chosen in this work. As can be observed, an excellent separation of both ions was obtained in about 3 min. It should be noted that the use of short-end injection (effective capillary length of 7 cm) allowed an ultra-rapid separation in less than 1 min without any loss in separation performance.

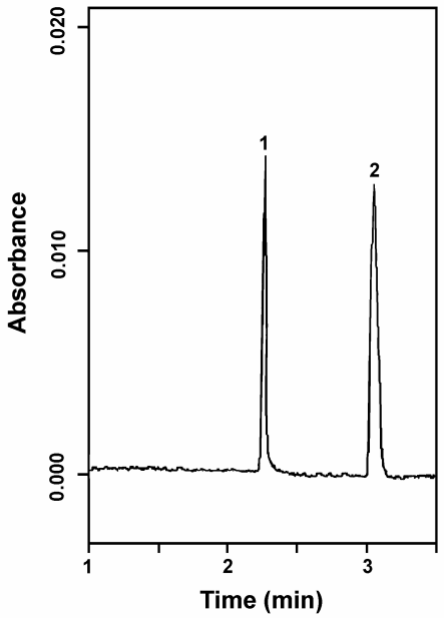

Fig. 2. Electropherogram obtained for a standard (0.1 mmol/L of each compound) solution under optimized CE conditions. Electrolyte, 20 mmol/L H_2SO_4 adjusted to pH 8.3 with Tris, 1 mmol/L hexamethonium bromide; voltage, -10 kV; capillary, 18 cm (effective length) × 75 μm i.d.; detection, UV at 254 nm. Peaks: (1) $Ni(CN)_4^{2-}$, (2) pyromellitate.

Theoretical considerations. In the headspace SDME system, the distribution of the analyte A between the sample solution, the headspace and the acceptor phase (microdrop) takes place until equilibrium is reached. This process may be illustrated with the following equation:

$$A_s \; ? \; A_h \; ? \; A_a \qquad (1)$$

where subscripts *s*, *h* and *a* represent the sample solution, the headspace, and the acceptor phase, respectively.

Since the total amount of the analyte should remain the same during the extraction as the initial amount, we have:

$$c_0V_s = c_sV_s + c_hV_h + c_aV_a \qquad (2)$$

where c_0 is the initial concentration of the analyte in the sample. c_s, c_h, and c_a are the equilibrium concentrations of the analyte in the sample, the headspace, and the acceptor phase, respectively. V_s, V_h, and V_a are the volumes of the sample solution, the headspace, and the acceptor phase, respectively.

At equilibrium, partition coefficients both between the headspace and the sample ($K_{h/s}$) as well as between the acceptor phase and the headspace ($K_{a/h}$) have to be considered:

$$K_{h/s} = c_h/c_s \qquad (3)$$

$$K_{a/h} = c_a/c_h \qquad (4)$$

Based on Eqs. 2-4, the equilibrium concentration (c_a) of the analyte *A* in the acceptor phase can be calculated by the following equation:

$$c_a = \frac{K_{h/s} K_{a/h} c_0 V_s}{K_{h/s} K_{a/h} V_a + K_{h/s} V_h + V_s} \qquad (5)$$

Finaly, the enrichmet factor (*E*) for the analyte *A* is calculated as follows:

$$E_A = \frac{c_a}{c_0} = \frac{K_{h/s} \cdot K_{a/h} \cdot V_s}{K_{h/s} \cdot K_{a/h} \cdot V_a + K_{h/s} \cdot V_h + V_s} \qquad (6)$$

It should be noted, that the above equations are defined under equilibrium conditions and the kinetic parameters such as stirring rate and extraction time are not reflected by these equations.

Eqs. 5 and 6 indicate that there are five parameters which affect the extraction performance of a particular analyte: the volume of the acceptor phase, V_a; the volume of the sample solution, V_s; the volume of the headspace, V_h; partition coefficients both between the headspace and the sample ($K_{h/s}$) as well as between the acceptor phase and the headspace ($K_{a/h}$).

Volume of the acceptor phase. In the headspace SDME technique the volume of acceptor solution in most cases ranges between 1 and 10 µL. Larger drops are dificult to manipulate and are less reliable. Such a narrow volume range is the main reason why the V_a is not considered as an important parameter for the enhancement of the extraction performance. It is clear from Eq. 5 that the absoliute amount of the extracted analyte increases with V_a, whereas the analyte concentration in the acceptor phase (c_a) does not change with V_a. Consequently, an improvement in the extraction efficiency using larger V_a will be obtained only when total amount of the final acceptor phase is subjected to further analysis.

Volumes of the sample solution and the headspace. Usually the SDME is performed from fixed volume sample vials and an increase in the sample volume reduces the headspace volume and *vice versa*. Consequently, the influence of both these parameters should be discussed together. Furthermore, when sample volume is very large (i.e., $V_s >> V_h$ and $V_s >> V_a$), the first two terms in the denominator of Eq. 5 can be eliminated resulting in:

$$c_a = \frac{K_{h/s} \cdot K_{a/h} \cdot c_0 \cdot V_s}{V_s} \approx K_{h/s} \cdot K_{a/h} \cdot c_0 \qquad (7)$$

In this case the concentration of the analyte in the acceptor phase is independent of the volume of the sample.

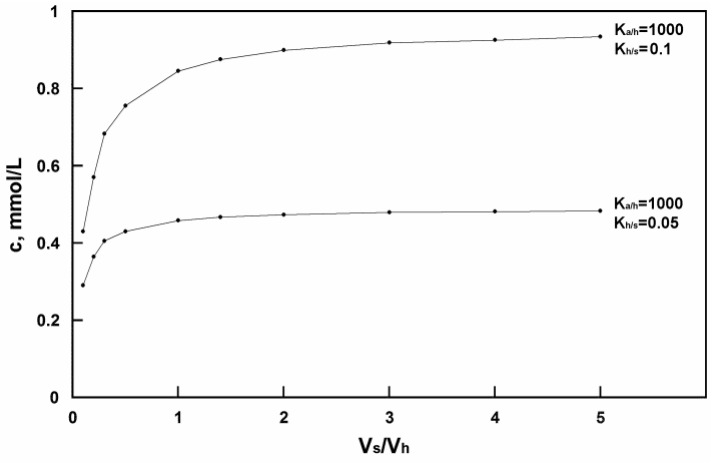

Fig. 3. Theoretically calculated dependence of analyte concentration in the acceptor phase on the sample/headspace volume ratio for virtual analyte with two different $K_{h/s}$ values ($K_{a/h}$=1000). Initial analyte concentration - 0,01 mmol/L. Drop volume – 5 µL. Total sample and headspace volume – 12 mL.

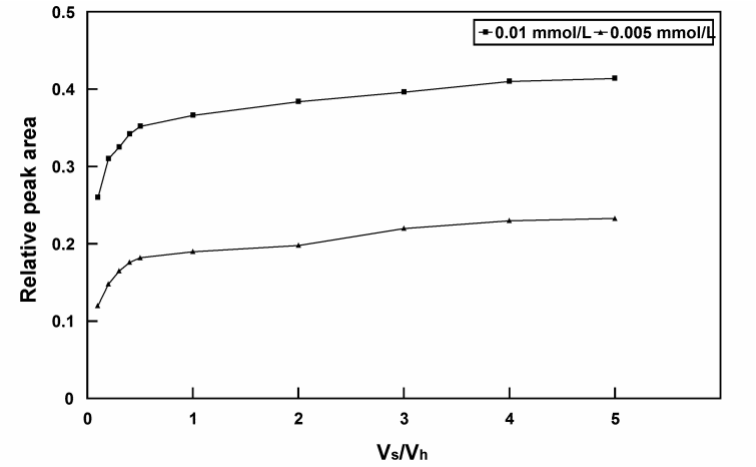

Fig. 4. Experimentally measured dependence of analyte concentration in the acceptor phase on the sample/headspace volume ratio for two initial cyanide concentrations. Drop volume – 5 µL. Total sample and headspace volume – 12 mL.

Fig. 3 illustrates the effect of sample/headspace volume ratio on the c_a for a virtual analyte calculated using Eq. 5 for different $K_{h/s}$ and $K_{a/h}$ values. As can be observed, an increase in sample/headspace volume ratio leads to an increase of analyte amount extracted. As expected, for larger sample volumes the extraction performance does not change significantly as the sample/headspace volume ratio becomes higher. Moreover,

these results suggest that for less volatile analytes with smaller $K_{h/s}$ values the extraction performance is less affected by the sample/headspace volume ratio. Theoretically obtained data were in a good agreement with experimental results presented in Fig. 4.

Analyte partition coefficients. In headspace SDME, an increase in analyte partition coefficient between the headspace and the sample ($K_{h/s}$) leads to an increase of analyte concentration in the headspace, and helps to improve extraction sensitivity. Generally, the $K_{h/s}$ values vary from ~0.0001 to ~10 for medium and highly volatile compounds, respectively. Unfortunately, in practice there are only a few ways to enchance $K_{h/s}$ value for particular analyte and thus to improve the extraction performance. Generally, by increasing the temperature and/or the sample ionic strength, $K_{h/s}$ also increases but this increase is very limited. Fig. 5 illustrates the effect of temperature on the extraction efficiency obtained for two different cyanide concentrations (0.02 and 0.01 mmol/L). As can be seen, the extraction ability gradually increases, with increasing extraction temperature, up to 55 ^{o}C, due to the increasing distribution constant of analyte between the aqueous phase and headspace. However, a slight decrease in extracted HCN amount was observed when the extraction temperature was set higher than 55 ^{o}C. Furthermore, when extraction temperature was set at 70 ^{o}C or higher, the microdrop became unstable and tended to detach from the needle tip. Although the maximum peak areas were obtained at 55 ^{o}C, room temperature was adopted to ensure a stable, more reproducible and simple operation without decreasing the sensitivity of the method significantly.

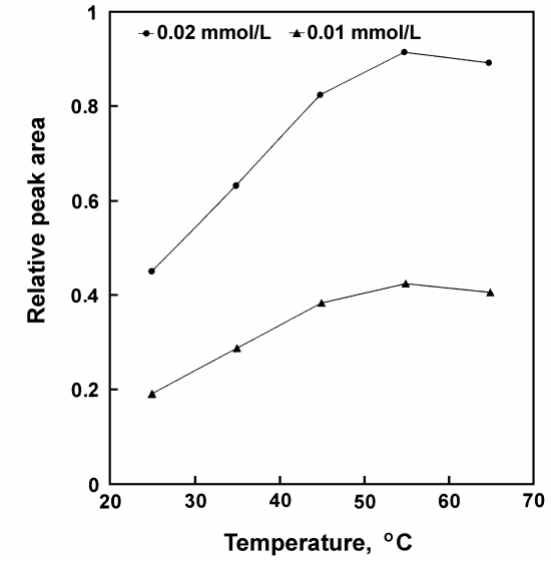

Fig. 5. Effect of temperature on the extraction efficiency. Acceptor phase, 100 mmol/L NH_3, 0.1 mmol/L $NiCl_2$, 0.2 mmol/L ammonium pyromellitate. Extraction time, 10 min.

Another and perhaps most effective way to increase $K_{h/s}$ for particular analyte is its conversion by appropriate chemical reaction to more volatile specie. However, this approach can be used only for limited number of the compounds. As an example, Fig. 6

illustrates the effect of sample pH on the extraction efficiency obtained for two different cyanide concentrations (0.02 and 0.01 mmol/L). As expected, at pH above 7.5-8.0, the signal response dramatically decreases indicating that the deprotonation of the analyte takes place. The above results show that the headspace SDME of free cyanide can be performed in the wide pH range and the acidification of the sample is not necessary.

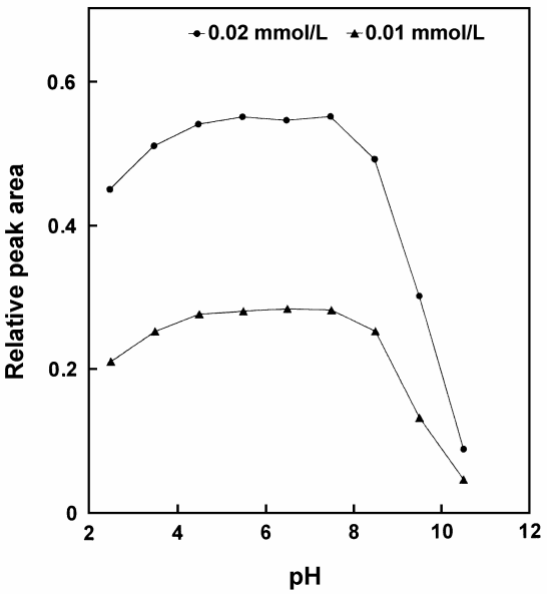

Fig. 6. Effect of sample pH on the extraction efficiency. Extraction conditions: extraction time, 15 min; extraction temperature 25 ºC. Acceptor phase, 100 mmol/L NH_3, 0.1 mmol/L $NiCl_2$, 0.2 mmol/L ammonium pyromellitate.

In practice, the $K_{a/h}$ value for a particular analyte can be effectively enhanced by a proper selection of the acceptor phase solvent. Under headspace SDME conditions the acceptor phase does not contact with the sample solution. Consequently, there are no limitations for solvent of the acceptor phase. However, this was not proved in our study because CE technique is not compatible with organic solvents.

Finally, chemical modification of the extracted compound in the acceptor phase should be an effective approach to enchance $K_{a/h}$ value for a particular analyte. Large $K_{a/h}$ can be achieved when by appropriate chemical reaction (e.g., protonation/deprotonation, complexation, etc.) analyte in the acceptor phase is converted to nonvolatile compound. In this way, back extraction of analyte from the acceptor phase to the headspace is prevented. In general, such derivatization process may be illustrated by simple equation:

$$A + R \;?\; AR \qquad (8)$$

where R represents the derivatizing reagent added to the acceptor phase and AR is nonvolatile reaction product formed.

In this case, the distribution coefficient $K_{a/h}$ for the analyte A can be expressed as:

$$K_{a/h} = \frac{[A]_a}{c_h} \qquad (9)$$

where $[A]_a$ – concentration of the analyte *A* in the acceptor phase at equilibrium; c_h –total concentration of the analyte *A* in the headspace at equilibrium. Total concentration c_h is used in this case because in the headspace analyte does not participate in any secondary equilibrium.

The formation constant of the compound *AR* in the acceptor phase can be expressed by the following equation:

$$K_{AR} = \frac{[AR]}{[A]_a \cdot [R]} \qquad (10)$$

Concentration of the analyte *A* in the acceptor phase can be expressed as:

$$[A]_a = \frac{[AR]}{K_{AR} \cdot [R]} \qquad (11)$$

Consequently, the derivatization reaction in the acceptor phase should decrease of free analyte concentration in the acceptor phase, and thus should increase extraction efficiency. As can be observed from Eq. 11, higher decrease in $[A]_a$ will be observed for the higher K_{AR} and $[R]$ values.

In our system two derivatization reactions take place. The first one is deprotonation of the extracted HCN and the second reaction is complexation of the cyanide with Ni^{2+}:

$$HCN + OH^- \; ? \; CN^- + H_2O \qquad (12)$$

$$4CN^- + Ni^{2+} \; ? \; Ni(CN)_4^{2-} \qquad (13)$$

As follows from the above discussion, the second reaction should additionally enhance the extraction performance of cyanide. This effect is illustrated by the results in Fig. 7, where the extraction efficiency of cyanide obtained without and with the second derivatization reaction is compared. As can be seen, by using an additional complexation step significantly higher amounts (about 17-20-times) of cyanide were extracted.

Analytical performance. The enrichment factor, defined as the ratio of relative peak areas after extraction and that before extraction, was used to evaluate the extraction efficiency. With hydrodynamic sample injection for 10 s cyanide was enriched by a factor of approximately 58. The calibration curve was linear for concentrations of CN^- in

the range from 0.25 to 20 μmol/L (R^2 = 0.997). The limit of detection (S/N = 3) was estimated to be 0.08 μmol/L of CN^-. Such detection sensitivity suggests a high potential for monitoring free cyanide in common environmental and physiological samples by headspace SDME-CE. The relative standard deviations (RSDs) obtained after six consecutive extractions of cyanide standard at two concentration levels (1 and 10 μmol/L) were calculated to be 6.8 and 4.3%, respectively.

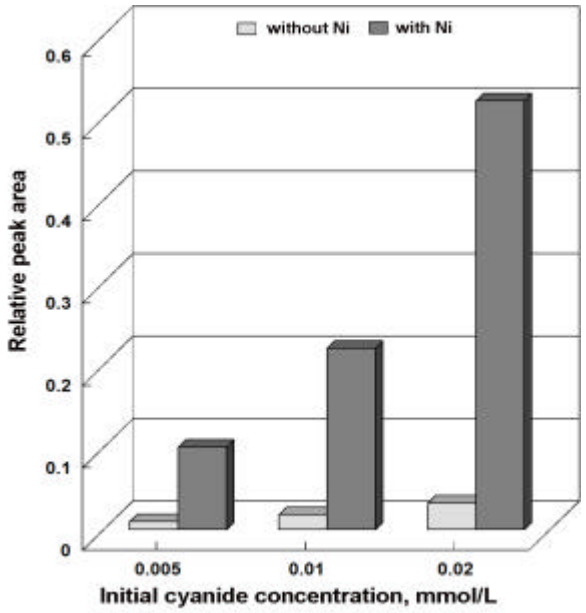

Fig. 7. Effect of the second derivatization reaction (complexation with Ni^{2+}) in the acceptor phase on the extraction efficiency of cyanide.

To finish the evaluation of headspace SDME method, free cyanide was determined in nonsmoker and smoker urine and saliva samples. After the collection, samples were stored at 4 ºC and cyanide was determined within one day. Determinations were performed using standard addition technique. For each sample, the extraction was repeated three times. Typical electropherogram of the smoker urine sample is shown in Fig. 8 and the results obtained are summarized in Table 1. As expected, higher cyanide levels were determined in the urine and saliva samples collected from tobacco smoker. Although the cyanide in the nonsmoker urine was detected its concentration was too low for the quantitative determination.

Finally, recovery testing was carried out with spiking 1.0 and 2.5 μmol/L cyanide standard to saliva and urine samples. The percent recoveries were between 92-106% for both cyanide concentration levels, indicating the method accuracy.

In addition to the high cyanide preconcentration, headspace SDME also accomplished a substantial sample clean-up; in spite of the relatively universal detection wavelength no other peaks were present in the electropherograms. Macromolecules and most small organic and inorganic compounds are nonvolatile under extraction conditions employed and therefore remained in the sample solution. As already mentioned, the main problem in the determination of cyanide in physiological samples is the thiocyanate, which may form additional cyanide during sample acidification procedure used to extract volatile HCN. Using headspace SDME of free cyanide from neutral samples this problem was completely avoided.

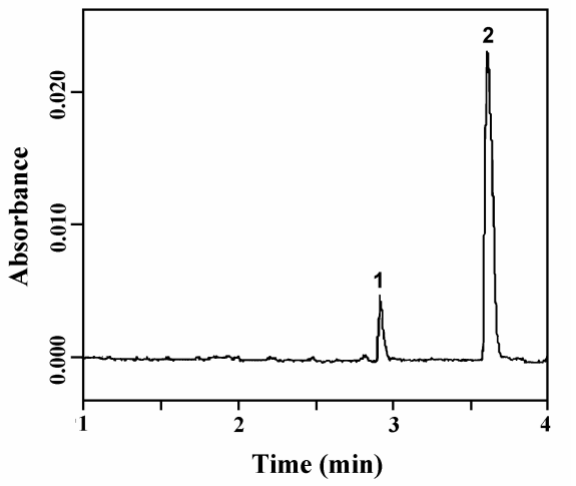

Fig. 8. Electropherogram of urine sample from a smoker. Extraction conditions: extraction time, 15 min; extraction temperature 25 ºC. Acceptor phase, 50 mmol/L NH_3, 1 mmol/L sodium carbonate, 0.1 mmol/L $NiCl_2$, 0.01 mmol/L ammonium pyromellitate (pH 11). Peaks: (1) $Ni(CN)_4^{2-}$, (2) pyromellitate.

Table 1. Results of the cyanide in physiological samples (n = 3)

Subject (male)	Sample	c(CN^-), µmol/L	RSD, %
Nonsmoker	urine	ND[a]	-
	saliva	0.38	7.9
Smoker	urine	0.42	8.4
	saliva	0.76	7.5

[a] Not determined

This study shows that water-based headspace SDME with in-drop derivatization and subsequent capillary electrophoretic analysis is a useful technique which complements existing methodologies in the analysis of free cyanide. The main advantage of this method is that sample clean-up, preconcentration, and derivatization procedures can be completed in a single step.

3.2. SDME-CE for the determination of weak-acid-dissociable cyanide

The cyanide present in the environment is mostly classified into three groups: free cyanide, weak acid dissociable (WAD) cyanide and total cyanide. The term "free cyanide" is considered the sum of cyanide anions (CN^-) and hydrogen cyanide (HCN), WAD cyanide includes free cyanide and moderately and weakly complexed metal cyanides, such as $Zn(CN)_4^{2-}$, $Cd(CN)_4^{2-}$, $Cu(CN)_3^{2-}$, $Ag(CN)_2^-$, $Ni(CN)_4^{2-}$ and $Hg(CN)_4^{2-}$, whereas total cyanide includes free cyanide, WAD cyanide plus the relatively non-toxic iron cyanide complexes. From an environmental point of view, the "toxicologically significant" or "ecologically important" form of cyanide is the WAD cyanide. The sample pre-treatment to release cyanide from the metal-cyanide complexes is perhaps the most important aspect in cyanide analysis. In this part of the study a new approach was developed for the rapid and inexpensive determination of WAD cyanide. The method combines ligand-exchange displacement of cyanide from the metal-cyanide complexes with headspace single-drop microextraction and capillary electrophoretic determination of the released cyanide.

Headspace SDME without ligand displacement. Standard solutions of different metal-cyanide complexes at 0.01 mmol/L of cyanide were dissolved in 0.4 mol/L sodium phosphate buffer at pH 6.5, extracted for 15 min and then analyzed by CE technique. To be a viable method for determination of WAD cyanide, the used procedure should give complete recoveries of cyanide from the less stable complexes and no recoveries from very stable iron cyanide complexes that are considered as non free cyanide producing ones. For this reason, the decomposition of the $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ complexes was also investigated. Table 2 summarizes the species-dependent cyanide recoveries and RSD values. Complete cyanide recoveries were obtained only from the labile and relatively unstable complexes of Zn(II) and Cd(II). For $Hg(CN)_4^{2-}$ and $Cu(CN)_3^{2-}$, the obtained recoveries were about 42% and 29%, respectively, and no or very low recoveries were obtained from the $Hg(CN)_2$, $Ag(CN)_2^-$, $Ni(CN)_4^{2-}$ and iron cyanide complexes.

Table 2. Stability constants of the metal-cyanide species and species-dependent cyanide recoveries obtained by headspace SDME-CE from 0.4 mol/L sodium phosphate buffer (pH 6.5)

Species (10 µmol/L as CN^-)	logβ_n	Recovery, %
$Cd(CN)_4^{2-}$	5.5; 10.6; 15.3; 18.9	103 (2.4)[b]
$Zn(CN)_4^{2-}$	5.3; 11.0; 16.7; 21.6	99 (2.8)
$Cu(CN)_3^{2-}$	21.7; 26.8[a]	29 (3.6)
$Ag(CN)_2^-$	20.9[a]	4.0 (3.9)
$Hg(CN)_4^{2-}$	18.3; 34.7; 38.5; 41.5	42 (4.2)
$Hg(CN)_2$	18.3; 34.7	0
$Ni(CN)_4^{2-}$	30.3[a]	2.3 (5.5)
$Fe(CN)_6^{4-}$	36.9[a]	0
$Fe(CN)_6^{3-}$	43.9[a]	0
Method blank		0

[a] The stability constants of lower complexes are too small to be determined.
[b] Relative standard deviations (%; n = 3) are given in parentheses.

Evaluation of ligand-displacing reagents. Ethylenediamine (En), dithizone and polyethyleneimine (PEI) were investigated for this purpose. En and PEI were prepared as 1% (wt) solutions in 0.01 mol/L NaOH and less soluble dithizone was prepared as 0.01% (wt) solution in 0.01 mol/L NaOH. Prior to the extraction, to a 2.5 mL of standard solution, 0.5 mL of ligand-displacing reagent was added and the solution was mixed for 5 min. After this, 2 mL of 1 mol/L sodium phosphate buffer (pH 6.5) was added to the sample (final sample volume 5 mL), the released cyanide was extracted and analyzed. As expected, the use of ligand-displacing reagents significantly enhanced the decomposition of the WAD cyanide complexes. However, none of the reagents used alone releases cyanide completely from all WAD complexes. Incomplete cyanide release was obtained from Hg(II) and Ag(I) cyanide complexes using En and PEI ligands, whereas dithizone was less effective at displacing cyanide from $Ni(CN)_4^{2-}$. No recoveries of CN^- were obtained from the $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ complexes. The higher RSD values obtained with PEI ligand most likely can be explained by the precipitation of the polymer after addition of phosphate buffer and/or by the changes in solution viscosity. From the obtained results it is evident that at least two ligand-displacing reagents should be used to release completely cyanide from WAD complexes. For further studies we selected En

and dithizone. In order to simplify and shorten the analysis, we made an attempt to integrate the cyanide displacement and extraction processes. Since En exhibit suitable buffering properties at neutral pHs, instead of the sodium phosphate buffer we have employed ethylenediamine chloride buffer (pH 6.5). In this case both En and chloride (for Hg(II) and Ag(I) complexes) act as ligand-displacing reagents. Furthermore, the extraction was performed immediately after addition of the sample aliquot to the 0.4 mol/L En-chloride (pH 6.5) buffer containing 0.001% (wt) dithizone. Recovery tests performed with metal-cyanide standards at three concentration levels are shown in Table 3. As can be seen, acceptable (97-104%) recoveries were found from all WAD cyanide species.

Table 3. Species-dependent cyanide recoveries (%) at three cyanide concentration levels

Species	Concentration (µmol/L as CN^-)		
	1	5	10
$Cd(CN)_4^{2-}$	104 (6.0)[a]	100 (2.5)	99 (3.3)
$Zn(CN)_4^{2-}$	102 (5.2)	98 (3.9)	101 (2.6)
$Cu(CN)_3^{2-}$	97 (5.5)	97 (3.2)	99 (2.4)
$Ag(CN)_2^-$	104 (5.8)	99 (4.0)	98 (1.9)
$Hg(CN)_4^{2-}$	103 (6.2)	96 (3.3)	99 (3.6)
$Hg(CN)_2$	98 (4.9)	103 (4.1)	101 (2.8)
$Ni(CN)_4^{2-}$	98 (5.3)	102 (4.4)	97 (2.5)

[a] Relative standard deviations (%; n = 3) are given in parentheses.

Sample analysis. The proposed headspace SDME-CE method was tested by analyzing three real samples for their WAD cyanide content. As the industrial effluent and wastewater samples contained relatively large amounts of cyanide, they were diluted before analysis. Concentration of WAD cyanide in the samples was measured using the external calibration method. The results obtained from the analysis of real samples can be seen in Table 4. The samples were then spiked with known amounts of WAD metal-cyanide complexe mixtures at two different concentrations. In all cases, acceptable recoveries were obtained as presented in Table 4. The recovery tests demonstrate that WAD cyanides are quantitatively recovered and that this technique can be used successfully for WAD cyanide monitoring of industrial effluents.

Table 4. Results of the ligand-exchange displacement SDME-CE determination of WAD cyanide in real samples

Sample	CN^- found, µmol/L	Spiked concentration, µmol/L	Recovery, %
Effluent from the electroplating plant 1 (1:25 dilution)	161 (3.8)[a]	25.0 100	96 (3.5) 99 (2.9)
Effluent from the electroplating plant 2 (1:10 dilution)	39 (4.4)	10.0 25.0	97 (4.2) 96 (3.6)
River water	ND[b]	2.0 10.0	105 (6.2) 103 (4.4)

[a] Relative standard deviations (%; n = 3) are given in parentheses.
[b] Not determined.

From the above discussion, it is obvious that the ligand displacement in combination with headspace SDME-CE is a useful technique which complements existing methodologies in the analysis of weak acid dissociable cyanide. The use of equilibrium-based headspace microextraction avoids the acidification and heating of the sample and thus is much less susceptible to interferences compared to existing methods. Other advantages of the proposed approach include simplicity and compatibility with other analytical techniques such as high-performance liquid chromatography and headspace gas chromatography.

3.3. SDME-CE for the determination of ammonia

Choice of CE conditions. Since the analyte studied exhibits any significant absorbance in the UV range, indirect detection mode at 214 nm using conventional imidazole containing carrier electrolyte was employed for CE separations. For quantitative analysis, we have selected potassium cation as an internal standard. Potassium meets the main requirements for internal standard: it is compatible with the acceptor phase, chemically stable, nonvolatile, and has a sufficiently high mobility. Because the separation of NH_4^+ and K^+ is difficult due to their identical electrophoretic mobilities at a neutral and acidic pH, 18-crown-6 was added to the carrier electrolyte.

This crown ether reduces the mobility of potassium ion without any significant changes in the migration time for ammonium. Thus, initial CE separations were performed in the carrier electrolyte containing 5 mmol/L of imidazole neutralized with CH_3COOH to pH 4.3 and 3 mmol/L of 18-crown-6. Under the conventional CE conditions both cations were well resolved in about 3 min, but the peaks obtained were relatively broad and poorly shaped. In order to shorten analysis time and, consequently, to improve peak efficiency the electroosmotic flow velocity was increased by a bilayer capillary coating with oppositely charged ionic polymers. The capillary coating protocol used in this work involves flushing the capillary with 0.1% (w/v) aqueous poly(diallyldimethylammonium chloride) (PDADMA) solution for 5 min followed by flushing the capillary with water for 1 min. Next, the capillary was flushed with 0.1% (w/v) aqueous poly(sodium-4-styrenesulfonate) (PSS) solution for 5 min. In addition, for best performance between all electrophoretic separations the capillary was rinsed with PSS solution for 1 min followed by flushing with carrier electrolyte for 1 min. Using this coating procedure, the second negatively charged PSS layer yielded very fast and stable cathodal electroosmotic flow. At pH 4.3 the cathodal EOF was about 5-times greater for coated capillary than for uncoated one. Fig. 9 shows the electropherograms of a test mixture of two cations separated under conventional CE conditions (Fig. 9a), and with a PDADMA/PSS-coated capillary (Fig. 9b). As can be observed, significantly better separation performance in respect to peak efficiency and separation time was obtained for coated capillary.

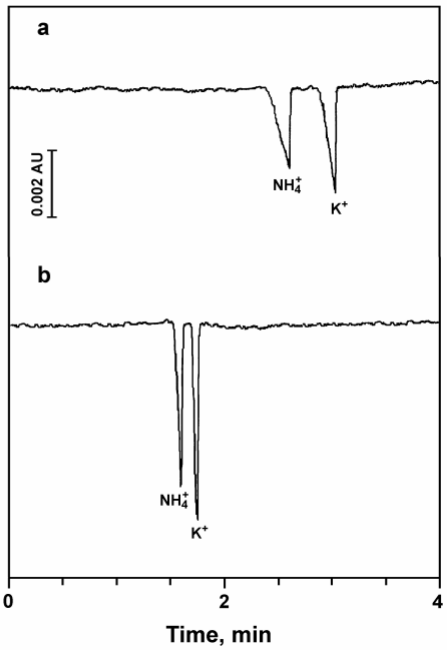

Fig. 9. Separation of ammonium and potassium in (a) the uncoated capillary and (b) the PDADMA/PSS coated capillary. Electrolyte, 5 mmol/L imidazole adjusted to pH 4.3 with CH_3COOH, 3 mmol/L 18-crown-6; voltage, 25 kV; capillary, 50 cm (effective length) × 75 µm i.d.; detection, indirect UV at 214 nm.

Microextraction performance. All the SDME experiments were performed with 5 mL of sample solution and 5 µL of aqueous acceptor phase containing 0.5 mmol/L KH_2PO_4 as an internal standard.

Since ammonium cation is weakly acidic (pK_a = 9.25), the pH of both the sample solution and the acceptor phase should be very important parameter in headspace SDME of ammonia. Basically, the sample solution should be alkaline enough in order to promote deprotonation of the weakly acidic NH_4^+ while the acceptor phase should be acidic in order to convert extracted analyte into the nonvolatile ammonium ion. The effect of sample pH was examined in the pH range between 8.0 and 13.0 by headspace extraction at room temperature for 10 min. Ammonium standards were diluted in 0.1 mol/L KH_2PO_4 electrolytes neutralized with KOH to desired pH. Three independent experiments for each pH value have been carried out. The results obtained for two different analyte concentrations (0.1 and 0.05 mmol/L) are demonstrated in Fig. 10. As expected, in the pH range 8.0-10, the extraction efficiency dramatically increases indicating that the deprotonation of the analyte takes place. The peak areas are maximum and do not depend on pH in the pH range 11–13. Therefore, a sample pH of 12 was selected for further experiments.

The pH of the acceptor phase was varied in the pH range from 3 to 8 by neutralization of initial 1.0 mmol/L H_3PO_4 and 0.5 mmol/L KH_2PO_4 solution with NaOH to desired pH. An increase of the acceptor phase pH from 3 to about 7 did not show any significant changes in the extraction efficiency. By further increase in the pH, a gradual decrease in extracted NH_3 amount was observed due to reextraction of deprotonized ammonia from the drop. On the basis of the above experiments, aqueous solution containing 1 mmol/L H_3PO_4 and 0.5 mmol/L KH_2PO_4 was selected as the acceptor phase for further studies.

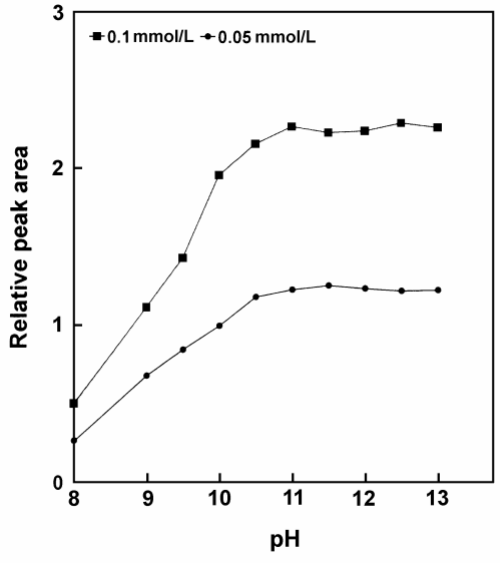

Fig. 10. Effect of sample pH on the extraction efficiency obtained for two ammonium concentrations. Extraction conditions: extraction time, 10 min; extraction temperature 25 ºC. Acceptor phase, 1.0 mmol/L H_3PO_4 and 0.5 mmol/L KH_2PO_4.

SDME is an equilibrium-based technique and there is a direct relationship between the amount extracted and the microdrop exposure

time in the headspace of the sample. In order to obtain better repeatability and higher sensitivity of the analysis an equilibrium between the sample, headspace and the acceptor phase should be reached. Extraction time effect was studied in the range of 5–30 min at 25 ºC. A plot of relative peak area versus extraction time (Fig. 11) shows that ammonia amount extracted increases with sampling time in the range of 5-15 min, reaches equilibrium after about 15 min, and then stays almost constant until 30 min. Therefore, an extraction time of 15 min was chosen for further studies.

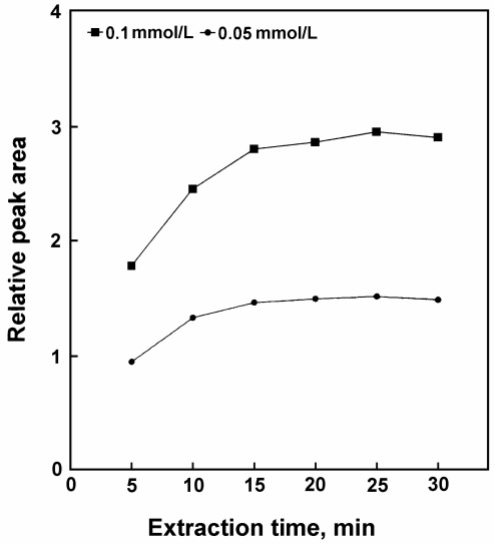

Fig. 11. Effect of extraction time on the extraction efficiency.

Analytical performance. To evaluate the practical applicability of the proposed SDME technique, several analytical performance characteristics such as enrichment factor, linearity, limit of detection (LOD) and repeatability were investigated under optimized conditions.

The enrichment factor, defined as the ratio of relative peak areas after extraction and that before extraction, was used to evaluate the extraction efficiency. With headspace SDME at room temperature for 15 min ammonia was enriched by a factor of approximately 14. The calibration curve was linear for concentrations of NH_4^+ in the range from 5 to 100 μmol/L (R^2 = 0.996). The LOD (S/N = 3) was estimated to be 1.5 μmol/L of NH_4^+.

The relative standard deviations (RSDs) obtained after six consecutive extractions of ammonia standard at two concentration levels (10 and 50 μmol/L) were calculated to be 7.5 and 5.3%, respectively. The repeatability was acceptable and comparable with other microextraction techniques reported in the literature. These data support the suitability of the proposed method for its application to common environmental and biological samples.

To finish the evaluation of headspace SDME method, ammonia was determined in human blood, seawater and milk samples. Typical electropherogram of the blood sample is shown in Fig. 12 and the results obtained are summarized in Table 5. The method accuracy was validated by spiking natural samples with known amounts of ammonia and evaluating the recovery (Table 5).

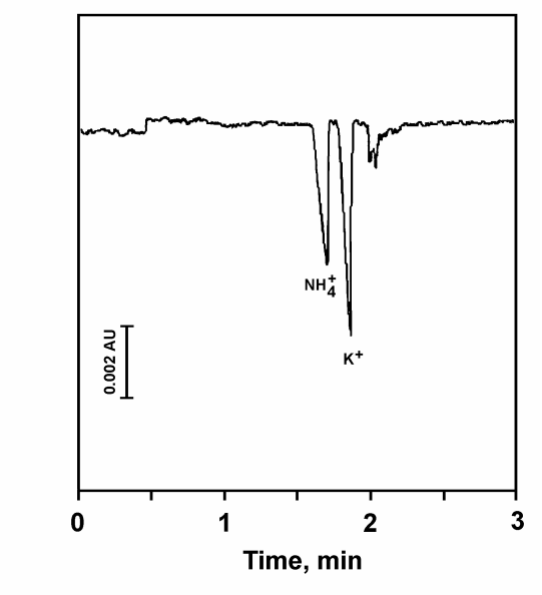

Fig. 12. Electropherogram of blood sample.

The main advantage of this method is that sample clean-up and preconcentration procedures can be completed in a single step. Macromolecules and most small organic and inorganic compounds are nonvolatile under extraction conditions employed and therefore remained in the sample solution. The main problem in the determination of ammonia in biological samples is the formation additional ammonia during sample pretreatment (deproteinization, alkaline distillation, derivatization, etc.) procedures. Using rapid and simple headspace SDME technique this problem was completely avoided.

Table 5. Results of the ammonium in real samples (n = 3)

Sample	Found, µmol/L mean ± SD[a]	Added, µmol/L	Found total, µmol/L mean ± SD	Recovery (%)
Human blood	34.1 ± 2.7	10.0	44.8 ± 3.2	107
		25.0	59.7 ± 2.9	102
Seawater	18.3 ± 1.0	10.0	28.1 ± 1.6	98
		25.0	43.0 ± 2.6	99
Milk	262 ± 16	50.0	310 ± 14	96
		125	384 ± 19	98

[a] Standard deviation.

CONCLUSIONS

1. Theoretically evaluated and experimentally confirmed that the alteration of analyte volatility by its chemical modification in the sample and/or in the acceptor phase is the most effective way to enhance extraction performance in headspace SDME. In this way the enrichment factor may be increased up to several hundred times. The enrichment factor depends on the equilibrium constant value of the derivatization reaction and on the concentration of the added reagent.
2. Maximum extraction efficiency was observed in the pH range 4.5–7.5, where cyanide anion is completely transferred into volatile HCN. At lower pHs the extraction efficiency decreases due to a drop in the pH of the acceptor phase caused by co-extraction of the phosphoric acid from the sample and/or by faster evaporation of ammonia from the acceptor phase. This may be avoided by the proper buffering of the acceptor phase.
3. Without ligand-exchange displacement complete cyanide recoveries were obtained only from the labile and relatively unstable complexes of Zn(II) and Cd(II). For $Hg(CN)_4^{2-}$ and $Cu(CN)_3^{2-}$, the obtained recoveries were 42% and 29%, respectively, and no or very low recoveries were obtained from the $Hg(CN)_2$, $Ag(CN)_2^-$, $Ni(CN)_4^{2-}$ and iron cyanide complexes. Cyanide recoveries correlate well with the stability constants of metal-cyanide complexes.
4. Among the three different ligand-exchange reagents (ethylenediamine, dithizone and polyethileneimine) studied none of the reagents used alone releases cyanide completely from all WAD cyanide complexes. Complete recoveries (=96%) were obtained by using two ligand-exchange reagents (ethylenediamine and dithizone).
5. Recovery tests showed that higher than 0.1 mmol/L sulfide concentrations significantly hinder the extraction efficiency of hydrogen cyanide. However, this is not a serious problem since sulfide anions can be precipitated with lead carbonate before extraction.
6. Maximum extraction efficiency was observed by the extraction of ammonia from alkaline samples (pH=11) with neutral or slightly acidic acceptor phase (pH 3-7).

7. Compared to existing methods water-based headspace SDME is significantly faster and simpler. In addition, proposed technique does not require any sample pre-treatment (deproteinization, acidic/alkaline distillation, etc.) and thus is much less susceptible to interferences.
8. Water-based headspace SDME significantly enhances the application areas of microextraction techniques. It offers possibility to choice of a wider variety of solvents. This enhances the range extractable analytes as well as the range of analytical methods that can be coupled with SDME.

The List of Original Publications by the Author

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1. **S. Jermak**, B. Pranaityte, A. Padarauskas. Headspace single drop microextraction with in-drop derivatization and capillary electrophoretic determination for free cyanide analysis. *Electrophoresis,* Vol. 27 (2006) 4538-4544.
2. **S. Jermak**, B. Pranaityte, A. Padarauskas. Ligand displacement, headspace single-drop microextraction, and capillary electrophoresis for the determination of weak acid dissociable cyanide. *Journal of Chromatography A,* Vol. 1148 (2007) 123-127.
3. B. Pranaityte, **S. Jermak**, E. Naujalis, A. Padarauskas. Capillary electrophoretic determination of ammonia using headspace single-drop microextraction. *Microchemical Journal,* Vol. 86 (2007) 48-52.

Published contributions to academic conferences

1. **S. Jermak**, B. Pranaityte, A. Padarauskas. Single drop microextraction and capillary electrophoresis for cyanide preconcentration and analysis. Konferencijos "Neorganiniu medžiagu chemija ir technologija" pranešimu medžiaga, Kaunas, 2006, p. 64-65.
2. **S. Jermak**, B. Pranaityte, V. Vickackaite, A. Padarauskas. Ligand-exchange displacement, headspace single-drop microextraction, and capillary electrophoresis for the determination of weak-acid-dissiciable cianide. Thesis of Ninth International Symposium on Advances in Extraction technologies, Alesund, Norway, 2007, p. 147.
3. **S. Jermak**, B. Pranaityte, A. Padarauskas. Ligand-exchange displacement, headspace single-drop microextraction, and capillary electrophoresis for the speciation of weak-acid-dissociable and total cyanide. Thesis of the 4th Nordic Separation Science Society International Conference, Kaunas 26-29 August 2007, p. 66.

CURRICULUM VITAE

Svetlana Nabiyeva

2000-2004 Bachelor studies at Vilnius University – Bachelor degree in chemistry.

2004-2006 Master studies at Vilnius University – Master degree in chemistry.

2006-2010 Post-graduate studies at the Department of Analytical and Environmental chemistry, Faculty of Chemistry of Vilnius University.

Acknowledgements

I would like to thank Lithuanian State Science and Studies Foundation for financial support.

MIKROEKSTRAKCIJA IŠ VIRŠERDVES VANDENS LAŠU–KAPILIARINE ELEKTROFOREZE LAKIU NEORGANINIU JUNGINIU NUSTATYMUI SUDETINGOSE MATRICOSE

SANTRAUKA

Šioje daktaro disertacijoje apibendrintu moksliniu tyrimu tikslas – nuodugniai ištirti mikroekstrakcijos iš viršerdves vandens lašu metoda, apjungti ji su kapiliarine elektroforeze (KE) bei pritaikyti lakiu neorganiniu junginiu analizei. Tyrimams buvo pasirinktos dvi pakankamai aktualios analiciu/meginio matricos sistemos: cianidas ir amonis aplinkos ir/arba biologiniuose objektuose.

Skaiciavimais parodyta ir eksperimentiškai patvirtinta, kad efektyviausias budas mikroekstrakcijos iš viršerdves efektyvumui pagerinti – analites lakumo didinimas chemiškai modifikuojant ja prieš ekstrakcija ir/arba jos lakumo mažinimas modifikuojant ja akceptorineje fazeje. Tai leidžia padidinti sukoncentravimo laipsni nuo keliolikos iki keliu šimtu kartu. Sukoncentravimo laipsnis priklauso nuo vykstancios reakcijos/reakciju susidarymo konstantos dydžio ir nuo reagento koncentracijos. Maksimalus cianido mikroekstrakcijos efektyvumas pasiekiamas pH srityje 4,5- 7,5, kur cianidas kiekybiškai pervedamas i laku HCN. Rugštesneje terpeje ekstrakcijos efektyvumas sumažeja del rugšties koekstrakcijos iš meginio ir NH_3 nugaravimo iš akceptorines fazes, sumažinanciu akceptorines fazes pH. Ekstrakcijos efektyvumo sumažejimo rugšcioje terpeje išvengiama padidinus akceptorines fazes buferine talpa.

Ištyrus cianido mikroekstrakcija tirpiklio lašu iš $Ag(CN)_2^-$, $Ni(CN)_4^{2-}$, $Hg(CN)_2$, $Cd(CN)_4^{2-}$, $Cu(CN)_3^{2-}$, $Hg(CN)_4^{2-}$, $Zn(CN)_4^{2-}$, $Fe(CN)_6^{3-}$ ir $Fe(CN)_6^{4-}$ tirpalu neutralioje terpeje (pH 6,5) nustatyta, kad be papildomo poveikio cianidas ekstrahuojasi pilnai tik iš palyginti nepatvariu ir labiliu Zn(II) ir Cd(II) kompleksu. Tuo tarpu iš $Hg(CN)_4^{2-}$ ir $Cu(CN)_3^{2-}$ kompleksu išekstrahuota tik atitinkamai 42% ir 29% cianido, o iš likusiu kompleksu cianidas nesiekstrahuoja. Cianidu išgavos gerai koreliuoja su metalu cianidiniu kompleksu patvarumo konstantomis. Nors papildomas poveikis konkuruojanciu ligandu (etilendiaminu, ditizonu arba polietileniminu) pagerina metalu cianidiniu kompleksu (išskyrus Fe kompleksus) suardyma, taciau nei vienas iš triju ligandu pilnai nesuardo visu kompleksu. Kiekybiškas silpnai surišto cianido suardymas (cianido išgavos siekia =96%) pasiektas tik panaudojus etilendiamino ir ditizono mišini. Cianido ekstrakcijai trukdo tik didesni nei 0,1 mmol/L sulfido kiekiai meginyje, taciau sulfido itaka lengvai pašalinama prieš ekstrakcija nusodinus ji švino karbonatu.

Maksimalus amonio mikroekstrakcijos efektyvumas pasiekiamas ekstrahuojant ji iš pašarmintu meginiu (pH=11) akceptorine faze, kurios pH 3-7. Amonio sukoncentravimo laipsnis beveik 4 kartus mažesnis už cianido, kadangi cianidas papildomai derivatizuojamas nikeliu akceptorineje fazeje.

Naujos mikroekstrakcines sistemos pritaikytos cianidui ir amoniui nustatyti sudetingos matricos meginiuose (kraujas, šlapimas, seiles, pienas). Lyginant su standartiniais metodais, siulomas metodas yra greitesnis ir paprastesnis, visiškai eliminuojama meginio matricos itaka, nereikalingos jokios drastiškos (deproteinizacija, veikimas rugštimis/šarmais, distiliacija ir pan.) manipuliacijos su meginiu, todel išvengiama analiciu praradimo/padidejimo del pašaliniu reakciju. Mikroekstrakcija iš viršerdves vandens lašu praplecia mikroekstrakcijos metodu taikymo sritis: atveriama galimybe labai poliniu organiniu ir neorganiniu junginiu mikroekstrakcijai bei galimybe mikroekstrakcija tiesiogiai apjungti su kapiliarine elektroforeze bei jai gimininga is analizes metodais.