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Simultaneous derivatization and extraction of free cyanide in biological samples with home-made hollow fiber-protected headspace liquid-phase microextraction followed by capillary electrophoresis with UV detection

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ABSTRACT

A new method is reported for the simultaneous derivatization and extraction of free cyanide in biological samples using home-made hollow fiber-protected headspace liquid-phase microextraction (HF-HS-LPME) followed by capillary electrophoresis (CE) determination. The acceptor phase containing Ni(II)-NH_3 (as derivatization agent), sodium carbonate and ammonium pyromellitate (as internal standard) is held within a hollow fiber membrane, affixed to a syringe needle and immersed in the headspace of sample container. The extracted cyanide from the neutral samples forms a stable $Ni(CN)_4^{2-}$ complex which is determined by CE. Parameters affecting extraction efficiency were investigated and optimized. For optimum peak shapes, the capillary was coated with cetyltrimethylammonium bromide (CTAB). The calibration curve was linear for concentrations of CN^- in the range from 0.1 to 20 μmol L^{-1} (R^2 = 0.9987). The LOD (S/N = 3) was estimated to be 0.01 μmol L^{-1} of CN^- concentration. Such a detection sensitivity is high enough for free cyanide determination in common environmental and biological samples. Excellent repeatability of the extraction (RSD ≤ 5.6%, n = 5) was achieved. The feasibility of this method was demonstrated by analyzing human urine and saliva samples with spiked recoveries in the range of 92–103.4%. This work provided an efficient alternative to the present headspace microextraction techniques such as headspace solid-phase microextraction (HS-SPME) and headspace single-drop microextraction (HS-SDME).

1. Introduction

Cyanide is a very short-acting and powerful toxic agent readily absorbed by living organisms through the inhalation, oral and dermal routes of exposure. Cyanide is commonly referred to as a blood agent due to its propensity for binding to the metal centers of certain metabolic enzyme systems, e.g., cytochrome oxidase, there by stopping cellular respiration. Suicidal, homicidal and accidental deaths by inhalation of hydrogen cyanide or ingestion of cyanide salts are encountered in clinical and forensic science practice [1–3]. Most of the cyanide species are generated and released into the environment by industries involved in electroplating, electronic manufacturing, ore leaching, blast furnacing or petroleum refining, etc. Other sources of exposure to cyanide include cyanogenic glycosides occurring in digestible plants, motor vehicle exhaust fumes, and therapeutic treatment with sodium nitroprusside [4,5]. Inhalation of tobacco smoke is an important source of cyanide, blood cyanide concentrations of smoker is much higher than nonsmokers. Furthermore, in fire, hydrogen cyanide is formed from polymers that contain nitrogen, and can be the cause of death. In this new era of possible terrorist activity, cyanide has been identified as a potential chemical terrorism agent due to its easy of accessibility and acute toxicity [6,7].

Therefore, cyanide analysis in forensic analysis and their monitoring at very low levels is of great importance. Several analytical methods, including optical [8,9], electrochemical [10,11] and chromatographic [12–14], have been published for the determination of cyanide in various sample matrices. The cyanide determination in body fluids requires the separation of cyanide from sample matrix, which is usually achieved through acidification. The liberated HCN is then trapped in an alkaline acceptor solution. The separation process is prone to errors due to incomplete liberation and artefactual cyanide production [15]. In addition, most of these sample preparation techniques are time-consuming and some lack sensitivity. There are several reports of using the CE technique for the cyanide determination [12,16–19]. However, these methods are not adequate sensitive for free cyanide determination in body fluids [16,17], but only in non-biological samples [12,18,19].

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In the past decade, miniaturized modification of the traditional liquid–liquid extraction method termed liquid-phase microextraction (LPME) has up to date development [20] since introduced by Dasgupta [21] and Cantwell [22] firstly in 1996. Compared with other traditional sample pretreatment techniques, the LPME is a simple, low-cost and virtually solvent-free technique which integrates sampling, extraction and concentration in to a single step. Due to the advantage of the excellent sample clean-up effect, high extraction recovery and enrichment factors, the novel technique has been widely used in environmental, food, fragrance, flavor, pharmaceutical and biological analyses [20]. Headspace analysis has been used for volatile and semi-volatile compounds without matrix interference because there is no direct contact with the sample matrix. Lee [23] reported a headspace single-drop microextraction (HS-SDME) technique in which an organic solvent film was formed in a microsyringe barrel and used as the extraction interface. The selection of extractant solvents in HS-SDME seems to be more flexible, since without any contact with the sample, the issue of possible solvent solubility in the sample does not arise.

However, there are some problems in HS-SDME. For example, the surface area of the organic solvent is limited. And the extraction contact interface between the headspace inside the syringe channel and organic solvent film is limited. This limited interfacial contact area between sample and solvent of HS-SDME maybe the reason that the extraction efficiency for this technique is not very high. To address such problems, Lee [24] developed the hollow fiber-protected headspace liquid-phase microextraction (HF-HS-LPME) technique in which the hollow fiber protects and holds the extractant droplet. With the support of the hollow fiber, the surface area of the extraction phase in contact with the headspace is increased dramatically.

Padarauskas developed the HS-SDME technique to determine the free cyanide [25] and weak acid dissociable cyanide [26]. In the present work, we used the home-made hollow fiber-protected HS-LPME technique for the determination of the free cyanide in human urine and saliva, and compared with the Padarauskas' work. Different parameters affecting the extraction process were studied and optimized. The performance of the proposed method can provide higher stability and extraction efficiency in comparison with the HS-SDME. This work provided an efficient alternative to the present headspace microextraction techniques such as HS-SPME and HS-SDME.

2. Experimental

2.1. Reagents and materials

All electrolytes and standard solutions were prepared with doubly distilled water. Potassium cyanide (≥97%) and potassium tetracyanonickelate(II) (99.99%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Pyromellitic acid (≥98.5%) was obtained from Sinopharm (Shanghai, China). All other reagents used for experiments were purchased from Kermel (Tianjin, China) and of analytical-reagent grade.

The stock KCN solution (0.01 mol L^{-1}) was prepared in aqueous 0.01 mol L^{-1} NaOH solution and was standardized by titration with $AgNO_3$. Stock solution was stored under refrigeration at 4 °C in amber-colored glass bottle. Standard solutions of cyanide were prepared just before use by dilution of a stock KCN solution in 1 mmol L^{-1} NaOH. The stock pyromellitic acid solution (0.2 g L^{-1}) was prepared in 4 mmol L^{-1} ammonia. Carrier electrolytes were prepared by neutralization of 0.02 mol L^{-1} H_2SO_4 solution with Tris to the desired pH value.

The home-made hollow fiber membrane was made with polyethersulfone (average molecular weight ~10,000, Tianbang Membrane, Dalian, China). The method is shown in Fig. 1. First, a tip of pipette (inner diameter of outlet end is 450 μm) was fixed on a stable rack. Then the polyethersulfone was injected into the tip with an injector. A segment of capillary (outer diameter 375 μm, 30 cm) was inserted into the polyethersulfone vertically, and passed through the outlet end of the tip. Furthermore, the capillary was suspended vertically and tied a tack to its bottom. A 1 L jar filled with double distilled water was placed under the tip. When everything was ready, the capillary was released and fell into the jar. After 2 min, the capillary was taken out from the water. A membrane was formed because the surface of fallen capillary had a polyethersulfone layer which can form membrane with water immediately. The membrane was extracted from the capillary and the hollow fiber membrane had a 375 μm inner diameter, a 450 μm outer diameter, and the thickness of the wall was 75 μm, the wall pore size was 0.2 μm which measured by the scanning electron microscopy (SEM).

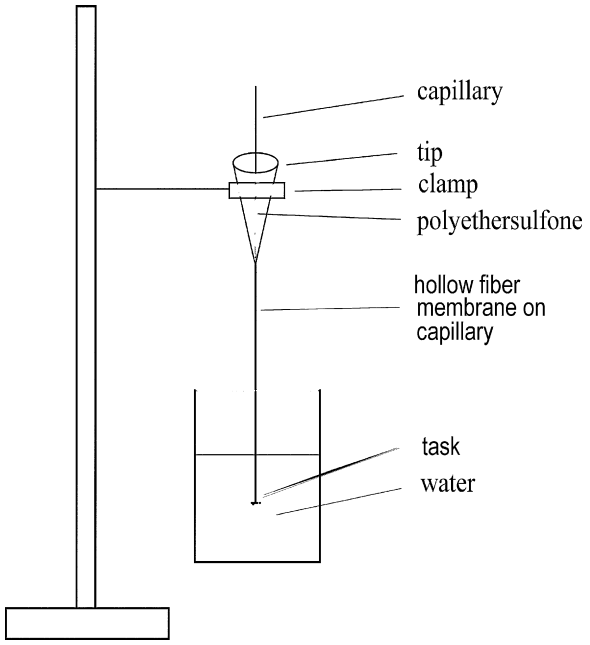

Fig. 1. Schematic of the fabrication of hollow fiber membrane.

2.2. Instrumentation

Capillary electrophoresis was performed with a MDQ instrument (Beckman, Fullerton, CA, USA) equipped with a UV detector. Separations were accomplished on a 50 μm I.D. fused-silica capillary (Reafine, Hebei, China) with an effective length of 36 cm (total length 44 cm). The capillary was coated with cetyltrimethylammonium bromide (CTAB). Detection was performed *via* on-column measurement of the UV absorptions at 254 nm. The measurements were performed at room temperature.

A 25 μL Waters microsyringe was used for extraction. HF-HS-LPME was carried out from 5 mL of sample solution placed in a 10 mL vial capped with the PTFE-faced silicone rubber septum tightly, and a magnetic bar was placed into the solution to ensure efficient stirring during the extraction process. The vial was positioned in a DF-101S constant temperature magnetic stirrer (Yuhua, Henan, China). Water bath was used to keep the sample temperature.

2.3. Extraction process

The experimental setup of HF-HS-LPME is illustrated in Fig. 2. Headspace microextraction was carried out as follows: the hollow fiber membrane was cut manually and carefully into 1.5 cm lengths.

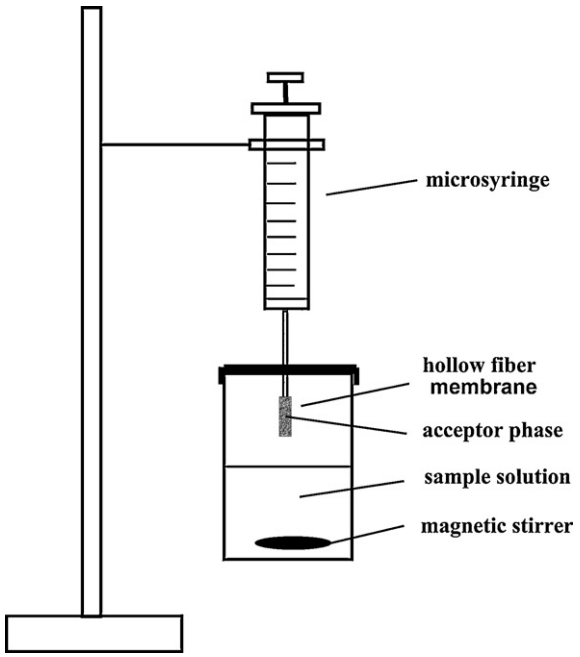

Fig. 2. Experimental setup of HF-HS-LPME.

These segments were ultrasonically cleaned in acetone aqueous solution (v/v, 2:3) and dried in air before use, then ultrasonically immersed in acceptor solution (0.5 mmol L^{-1} Ni^{2+}, 300 mmol L^{-1} NH_3, 6 mg L^{-1} pyromellitate acid and 1 mmol L^{-1} sodium carbonate) for 20 s for the impregnation of the porous wall. A 7 μL of acceptor solution followed by 7 μL of air was withdrawn into the microsyringe. The sample vial septum cap was pierced by the microsyringe. The needle tip was inserted into the hollow fiber membrane, and the air in the syringe was injected to flush the hollow fiber membrane to remove excess acceptor solution from inside the fiber. After that, the fiber together with the syringe was fixed on the retort stand. A 4.5 mL of sample solution and 0.5 mL of 1 mol L^{-1} sodium phosphate buffer (pH 6.5) saturated by Na_2SO_4 was placed in a sample vial. The vial was then placed in position and capped such that the fiber-needle assembly was in the headspace region. The syringe plunger was depressed and the inside of the hollow fiber membrane was filled with the acceptor solution. The sample was stirred at 900 rpm. After an optimized period of time (10 min), the plunger was withdrawn and the acceptor solution was retracted back into the syringe. The needle was removed from the headspace and its content (5 μL) was introduced to a CE microvial for subsequent analysis. Each piece of hollow fiber membrane was used only for a single extraction.

2.4. CE–UV analysis

All electrolyte solutions were filtered through a 0.45 μm membrane filter degassed by ultrasonication. Each new fused-silica capillary was coated with CTAB. All samples were introduced by electrokinetic injection for 20 s at −15 kV. During analysis the instrument was operated at −25 kV, generating a current level of approximately 50 μA. Between all electrophoretic separations the capillary was rinsed at 10 p.s.i. with 0.1 mol L^{-1} NaOH for 3 min followed with carrier electrolyte for 3 min.

3. Results and discussion

3.1. Optimization of CE conditions

Cyanide reacts rapidly with Ni^{2+} in ammoniacal solutions and forms relatively stable $Ni(CN)_4^{2-}$ complex which has an absorption maximum at 267 nm and a molar absorptivity of 11,300 [27]. This reaction is widely used for the derivatization of free cyanide prior spectrophotometric [27], polarographic [28] or CE [29,30] determinations. In this work, a detection wavelength of 254 nm was chosen, as the CE instrument was equipped with a fixed wavelength detector. Furthermore, at 254 nm most of the commonly existing anions do not absorb light and do not interfere. For quantitative analysis, we have selected pyromellitate anion as an internal standard [25].

The CE behavior and separation conditions of $Ni(CN)_4^{2-}$ and pyromellitate anions have been already investigated by Padarauskas' work [25]. However, during the experiments, we found that although the electrophoretic mobilities of highly charged $Ni(CN)_4^{2-}$ and pyromellitate anions were large enough to cause them to move toward anode, the peak area of $Ni(CN)_4^{2-}$ anion was small, and the peak of pyromellitate anions did not appear in 25 min. So the electroosmotic flow (EOF) velocity needs to be lowered or reversed by adding additive to the carrier electrolyte or modifying the capillary. For this purpose, initial CE experiments were performed under counter-EOF conditions. We tested to add several different concentrations (0.01 mmol L^{-1}, 0.02 mmol L^{-1}, 0.05 mmol L^{-1} and 0.1 mmol L^{-1}) of CTAB to the carrier electrolyte. But the peak efficiency and separation time were also not acceptable. Then we noticed that the CTAB was easily absorbed onto the capillary wall but difficult to be eluted. With the non-covalent CTAB-coated capillary, we can control the EOF velocity well and no need to add any additive to the carrier electrolyte. The capillary was coated with 1 mmol L^{-1} CTAB and in order to form the stable and uneasily eluted electric double layer, the flush of the capillary was important. Each new fused-silica capillary was flushed at 10 p.s.i. with 1 mol L^{-1} NaOH for 30 min, doubly distilled water for 30 min, 1 mmol L^{-1} CTAB for 30 min, again doubly distilled water for 10 min and carrier electrolyte for 30 min. Then electroconditioned with the carrier electrolyte for 30 min at −25 kV. Everyday before the CE use, the capillary needs to be flushed at 10 p.s.i. with 0.1 mol L^{-1} NaOH for 10 min, doubly distilled water for 10 min, 1 mmol L^{-1} CTAB for 10 min and carrier electrolyte for 20 min, and then electroconditioned with the carrier electrolyte for 20 min at −25 kV to baseline smoothness. At the same time, the coated capillary is not applied in the acid condition as possible as it could.

For optimum peak shapes, the mobility of the electrolyte coion 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^{-1} of HCl, H_2SO_4 or H_3PO_4 adjusted pH to 8.3 with Tris. The best separation performance in respect to peak efficiency and separation time was obtained for a Tris-sulfate electrolyte. The sample was introduced by electrokinetic injection for 20 s at −15 kV. During analysis the instrument was operated at −25 kV. The best peak efficiency and separation time of $Ni(CN)_4^{2-}$ and pyromellitate anions was obtained for the CTAB-coated capillary under the CE conditions chosen in this work, see Fig. 3. As can be observed, an excellent separation of both ions was obtained in about 3 min.

3.2. Optimization of extraction conditions

In order to obtain the best extraction performance, different parameters affecting the extraction process were studied and optimized such as the pH of the sample solution, composition of the acceptor phase, temperature, extraction time, stirring speed and ionic strength. All the LPME experiments were performed with 5 mL of sample solution and 7 μL of aqueous acceptor phase containing $NiCl_2$, NH_3 and ammonium pyromellitate as an internal standard.

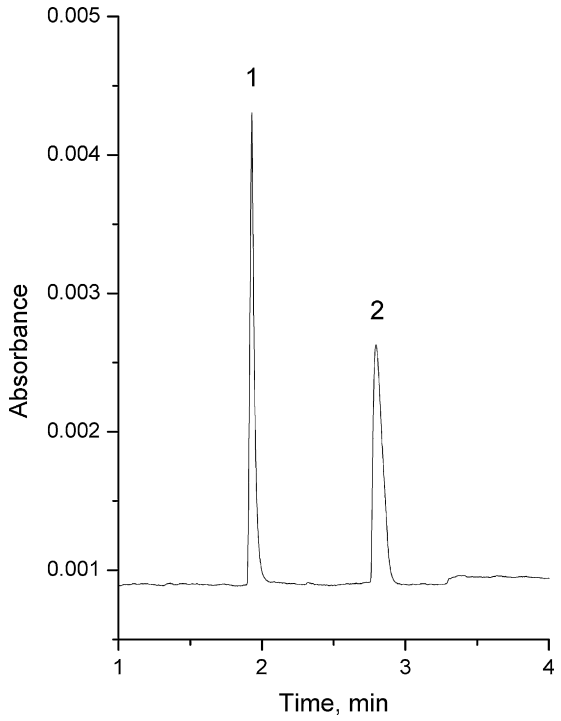

Fig. 3. Electropherogram obtained for a standard cyanide solution (6 μmol L^{-1}) extracted under optimized conditions. Electrolyte, 20 mmol L^{-1} H_2SO_4 adjusted to pH 8.3 with Tris; injection, electrokinetic −15 kV for 20 s; voltage, −25 kV; capillary, 36 cm (effective length) × 50 μm I.D.; detection, UV at 254 nm. Peaks: (1) $Ni(CN)_4^{2-}$ and (2) pyromellitate.

3.2.1. *Effect of the pH of the sample solution*

Since hydrogen cyanide is weakly acidic (p*K*a = 9.21), the pH of both the sample solution and the acceptor phase should be a very important parameter in HF-HS-LPME of cyanide. Basically, the sample solution should be acidic enough in order to promote protonation of the weakly acidic analyte, while the acceptor phase should be strongly alkaline in order to ionize the analyte and consequently reduce its volatility. In addition, to avoid the pH decrease during the extraction process due to coextraction of other acidic volatile compounds, the acceptor phase should have sufficiently high buffering capacity.

The effect of sample pH was tested in the pH range between 2.5 and 10.5 by headspace extraction at room temperature for 10 min. Cyanide standards were diluted in 0.1 mol L^{-1} H_2SO_4 electrolytes neutralized with Tris to desired pH. Three independent experiments for each pH value at two different cyanide concentrations (3 and 6 μmol L^{-1}) have been carried out. As shown in Fig. 4, the peak areas are maximum and do not depend on pH in the pH range 4.5–7.5. In order to avoid the coextraction of the acid from the sample solution which reduces the pH of the acceptor phase and, consequently, the extraction efficiency, the acidification of the sample is not necessary.

3.2.2. *Composition of the acceptor phase*

The initial acceptor phase is composed of $NiCl_2$, NH_3 and ammonium pyromellitate as an internal standard. Ammonia should be present in the acceptor phase to prevent precipitation of nickel hydroxide, so the pH of the acceptor phase was varied by increasing the amount of NH_3 in the range from 50 to 500 mmol L^{-1}. The results obtained for the sample without acidification are seen in Fig. 5. The results show that the extraction efficiency increases with the NH_3 concentration, and there was no significant difference in the extraction performance obtained for $c(NH_3) \geq 200$ mmol L^{-1}.

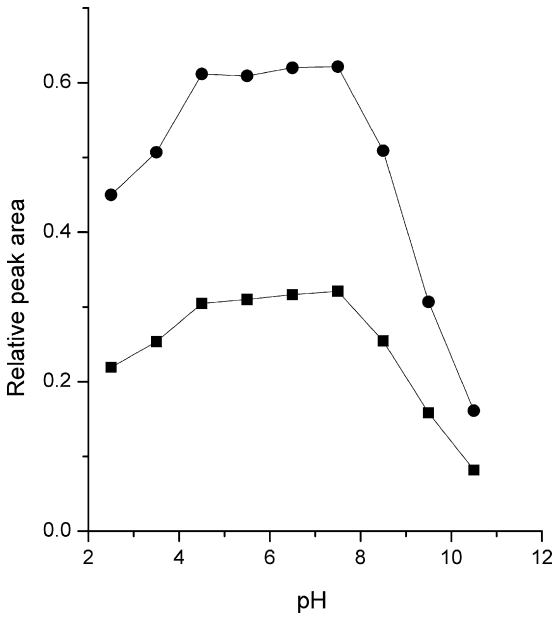

Fig. 4. Effect of sample pH on the extraction efficiency obtained for two cyanide concentrations. Extraction conditions: extraction time, 10 min; extraction temperature, 25 °C; stirring speed: 900 rpm; acceptor phase, 200 mmol L^{-1} NH_3, 0.3 mmol L^{-1} $NiCl_2$, 6 mg L^{-1} ammonium pyromellitate; CE conditions as in Fig. 3; (●) 6 μmol L^{-1}, (■) 3 μmol L^{-1}.

The concentration of Ni^{2+} in the acceptor phase is lower compared with the cyanide ions which extracted into the acceptor phase, and limited amounts of cyanide ions react with Ni^{2+} in ammoniacal solutions. Since the cyanide ions cause a complete formation of $Ni(CN)_4^{2-}$ anion with the Ni^{2+}, the extraction efficiency increases with Ni^{2+} concentration. However, the high Ni^{2+} concentration may cause the precipitation of nickel hydroxide. Increasing NH_3 concentration can prevent the precipitation of nickel hydroxide, but it was found that even high concentrations of ammonia in the acceptor phase cannot stabilize its pH during longer extractions due to the transfer of ammonia to the

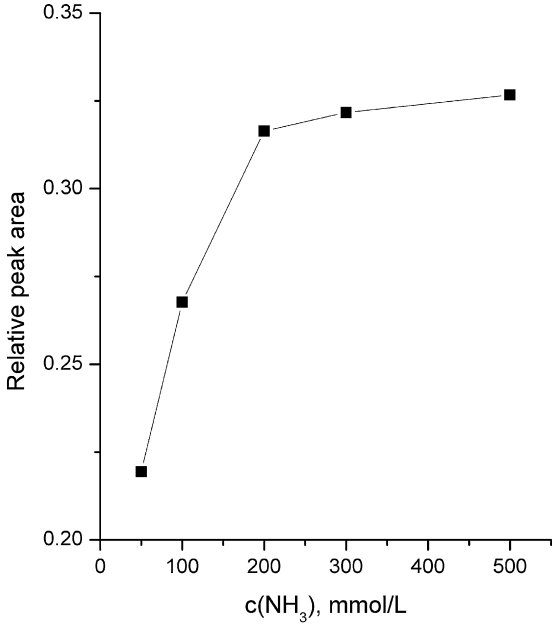

Fig. 5. Effect of NH_3 concentration in the acceptor phase on the extraction efficiency obtained for 3 μmol L^{-1} cyanide standard solution at pH 6.5. For other conditions, see Fig. 4.

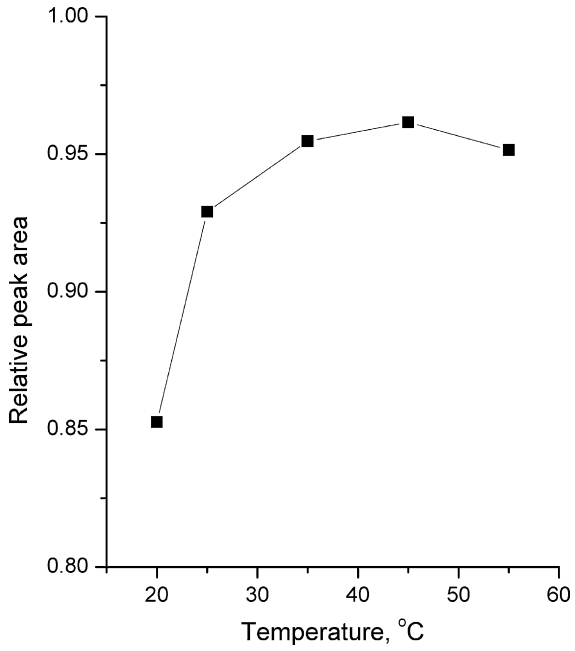

Fig. 6. Effect of temperature on the extraction efficiency obtained for 6 μmol L^{-1} cyanide standard solution at pH 6.5. Extraction conditions: extraction time, 10 min; stirring speed: 900 rpm; acceptor phase, 300 mmol L^{-1} NH_3, 0.5 mmol L^{-1} $NiCl_2$, 6 mg L^{-1} ammonium pyromellitate, 1 mmol L^{-1} sodium carbonate (pH 11); CE conditions as in Fig. 3.

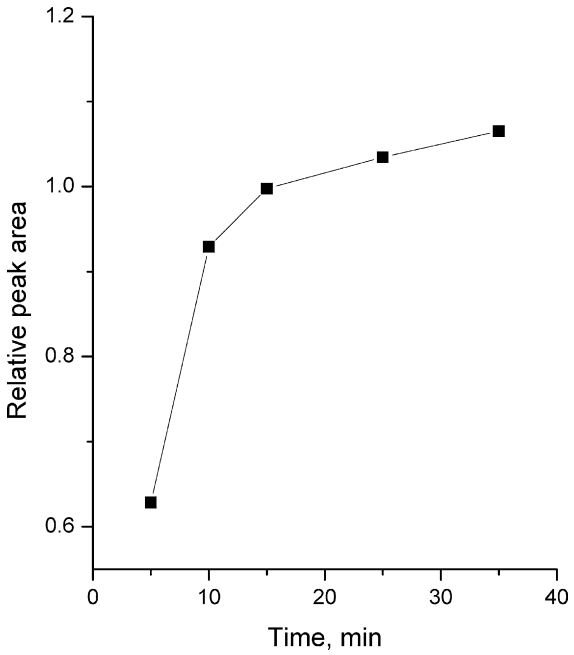

Fig. 7. Effect of extraction time on the extraction efficiency. Extraction temperature, 25 °C. For other conditions, see Fig. 6.

sample. To sum up, we selected 0.5 mmol L^{-1} Ni^{2+}, 300 mmol L^{-1} NH_3, and 6 mg L^{-1} ammonium pyromellitate. In addition, adding of 1 mmol L^{-1} sodium carbonate buffer (pH 11) to the acceptor phase which is nonvolatile and has sufficiently high buffering capacity that can stabilize the pH and prevent transfer of ammonia to the sample.

3.2.3. Effect of temperature

For headspace analysis of semi-volatile compounds, another important parameter is temperature. Temperature has a significant effect on both the kinetics and the thermodynamics of the extraction process. Higher temperatures lead to higher vapor pressure of the analyte and hence its concentration in headspace increases. On the other hand, the distribution constant of ammonia between the acceptor phase and headspace decreases with temperature. The effect of extraction temperature was evaluated from 20 to 55 °C. As seen in Fig. 6, the extraction efficiency gradually increases with increasing extraction temperature, up to 45 °C, but slight decrease with the higher temperature. It may be due to that higher extraction temperatures lead to the extraction solvent depletion or transfer of ammonia to the sample. Therefore, room temperature was adopted to ensure a stable, more reproducible and simple operation without decreasing the sensitivity of the method significantly.

3.2.4. Effect of extraction time

For HF-HS-LPME, the extraction does not attain equilibrium. It is due to the fact that it is not practicable to maintain an extraction time long enough for equilibrium to be established. Extraction time effect was studied in the range of 5–30 min at 25 °C. As seen in Fig. 7, the amount of cyanide extracted increases with sampling time. In addition, the problem of extraction solvent depletion should also be considered. The longer the extraction, the more likely the solvent will be depleted. Thus, a 10-min extraction time was deemed to be sufficient for subsequent experiments.

3.2.5. Effect of stirring speed

The penetration theory of mass of solute indicates that the aqueous phase mass transfer coefficient of solute increases with increasing stirring rate. Therefore, agitation of the sample solution improves the mass transfers in the aqueous phase and induces convection in the headspace, and consequently the equilibrium between the aqueous and vapor phase can be more rapidly attained. Fig. 8 describes the influence of stirring speed on the extraction efficiency. The extraction efficiency increased with increasing stirring rate up to 900 rpm. However, at 1000 rpm the equipment would shake lightly and there was a small decrease in extraction efficiency. This resulted in air entering the solution and the formation of air

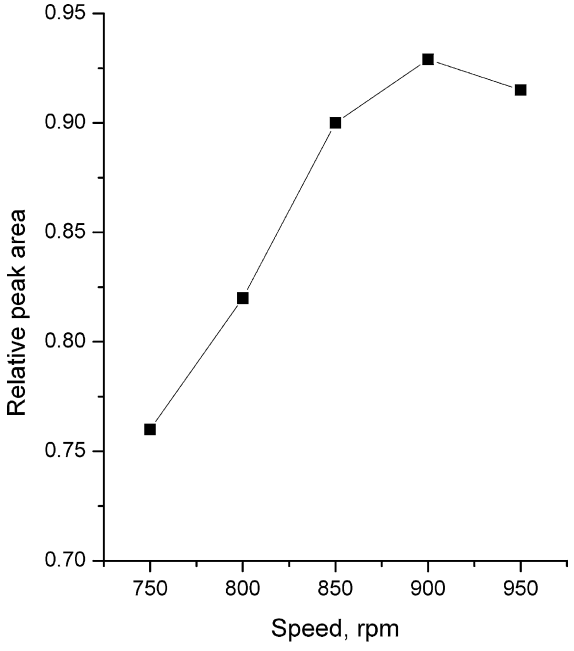

Fig. 8. Effect of stirring speed on the extraction efficiency. Extraction temperature, 25 °C. For other conditions, see Fig. 6.

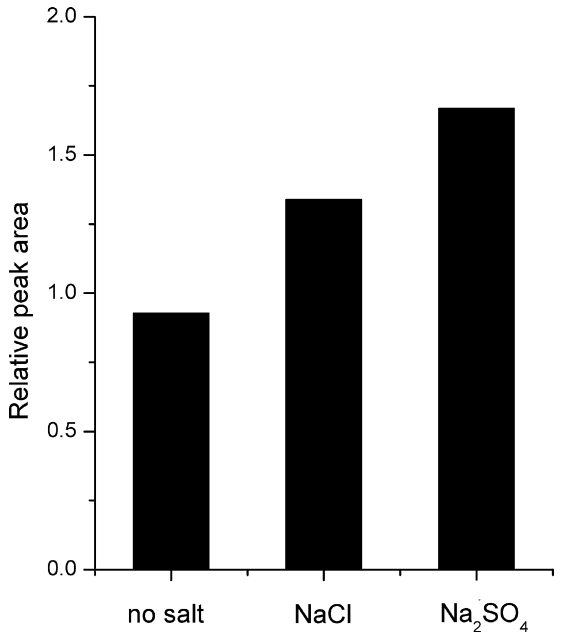

Fig. 9. Effect of ionic strength on the extraction efficiency. Extraction temperature, 25 °C. For other conditions, see Fig. 6.

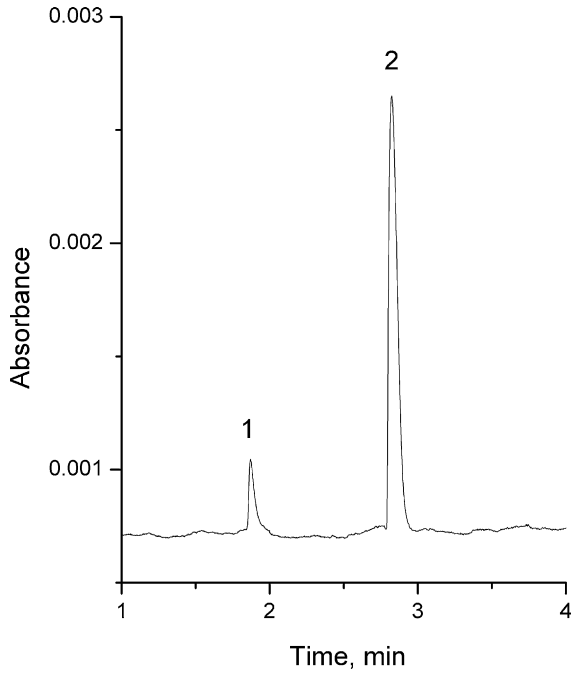

Fig. 10. Electropherogram of urine sample from a smoker. Extraction temperature, 25 °C. Peaks: (1) $Ni(CN)_4^{2-}$, (2) pyromellitate. For other conditions, see Fig. 6.

bubbles in and on the fiber that might have occupied contact sites on the fiber surface so that the amount of analytes extracted into the acceptor solution decreased. A stirring speed of 900 rpm was used for subsequent experiments.

3.2.6. Effect of ionic strength

The addition of a salt can often improve extraction recovery when conventional extraction methods are used. Sodium chloride is commonly used for this purpose. In our current work, the effect of ionic strength on extraction efficiency was studied by saturating sample solutions with NaCl or Na_2SO_4. An even greater effect was seen in samples saturated with Na_2SO_4 as shown in Fig. 9. Thus, the sample solutions were saturated by Na_2SO_4 for the best extraction efficiency.

3.3. Quantitative analysis

In order to proceed with the current evaluation of the proposed HF-HS-LPME technique, linearity, LOD, and repeatability were investigated under optimized conditions with the standard solutions of the analytes. The performance of the developed procedure is summarized in Table 1, and compared with the HS-SDME [25], HS-SPME [14]. The calibration curve was linear for concentrations of CN^- in the range from 0.1 to 20 μmol L^{-1} (R^2 = 0.9987). The LOD (S/N = 3) was estimated to be 0.01 μmol L^{-1} of CN^- concentration. Such detection of sensitivity suggests a high potential for monitoring free cyanide in common environmental and biological samples by HF-HS-LPME-CE.

The RSDs obtained after five consecutive extractions of cyanide standard at two concentration levels (6 and 10 μmol L^{-1}) were calculated to be 5.6% and 4.5%, respectively. The repeatability was acceptable and comparable with other methods reported in the literature.

3.4. Analysis of biological samples

The proposed HF-HS-LPME-CE procedure described above was applied to determine free cyanide in nonsmokers' and smokers' urine and saliva samples. For each sample, the extraction was repeated three times within 1 day. Fig. 10 shows a typical electropherogram of the smoker's urine sample derived from CE-UV analysis using optimized HF-HS-LPME parameters, and the results obtained are summarized in Table 2. Higher cyanide levels were determined in the urine and saliva samples collected from tobacco smokers compared with the nonsmokers. Testing samples are spiked with 0.5 and 1.0 μmol L^{-1} cyanide standard to the nonsmoker's saliva and urine samples for recovery testing. The recoveries were between 92% and 103.4% for both cyanide concentration levels, indicating the method accuracy.

In addition to the high cyanide preconcentration, HF-HS-LPME also accomplished a substantial sample clean-up. Macromolecules and most of the small organic and inorganic compounds are nonvolatile under extraction conditions employed and therefore remained in the sample solution. In the process of analysis, the samples were not acidified, which can avoid the interference of some anions, especially the thiocyanate which commonly in the biological samples. Because the price of each extraction hollow fiber membrane was very low (comparable with the price of a SPE or SPME column), each hollow fiber membrane was disposable, no

Table 1
Summary of the performance of the developed method, compared with the HS-SDME and HS-SPME.

Method	Linear range (μmol L^{-1})	R^2	LOD (μmol L^{-1})	RSD (%)	Recovery (%)
HF-HS-LPME	0.1–20	0.9987	0.01	<5.6	92–103.4
HS-SDME [25]	0.25–20	0.997	0.08	<6.8	91.7–105.6
HS-SPME [14]	0.77–153.5	0.9992	0.09	<8	47.8–112.3

Table 2
Results of the cyanide in biological samples (n = 3).

Subject (male)	Sample	c(CN^-) (μmol L^{-1})	RSD (%)
Nonsmoker	Urine	0.07	7.0
	Saliva	0.28	5.9
Smoker	Urine	0.39	6.0
	Saliva	0.81	5.3

carry over effects occurred. Moreover, the samples were introduced by electrokinetic injection at negative voltage. Thereby in spite of the relatively universal detection wavelength, no other peaks were present in the electropherograms.

4. Conclusions

This study has demonstrated the successful application of water-based home-made hollow fiber-supported headspace liquid-phase microextraction with simultaneous derivatization and subsequent capillary electrophoresis to the analysis of free cyanide from urine and saliva samples. The results show that the home-made HF-HS-LPME combined with CE-UV is an effective method for the qualitative and quantitative analysis of free cyanide in biological samples. HF-HS-LPME provides an alterative to HS-SDME and HS-SPME for the analysis of volatile and semi-volatile compounds. HF-HS-LPME can tolerate a relatively larger amount of extraction solvent than drop-based HS-LPME. The extraction interface that facilitated more efficient mass transfer is increased by the supporting hollow fiber membrane.

However, the problem of extraction solvent depletion due to the large the surface area should be considered. In order to decrease the extraction solvent loss, some groups introduced the dynamic HF-LPME [24] in which the extraction solvent was withdrawn into the syringe barrel with the automated movement of the plunger during extraction, or solvent cooling assisted dynamic HF-LPME [31] in which the syringe was mounted in a cooling system. Further studies are still in progress.

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