Journal of Chromatography B, 917–918 (2013) 5–10

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Development of ultrasound-assisted emulsification microextraction for determination of thiocynate ion in human urine and saliva samples

Mahdi Hashemi [a], Seyed Mosayeb Daryanavard [a,*], Sana Abdolhosseini [b]

[a] Faculty of Chemistry, Bu-Ali Sina university, Hamedan, Iran
[b] Department of Chemistry, Payame Noor University (PNU), Hamedan, Iran

ARTICLE INFO

Article history:
Received 11 September 2012
Received in revised form
27 December 2012
Accepted 31 December 2012
Available online 8 January 2013

Keywords:
Ultrasound-assisted emulsification microextraction
Thiocynate ion
Rhodamine B
UV–vis spectrophotometry
Urine
Saliva

ABSTRACT

Ultrasound-assisted emulsification microextraction (USAE-ME) procedure coupled with UV–vis spectrophotometric measurement has been developed for determination of thiocyanate ion (SCN^-) in water and biological fluids samples. The method is based on protonation of SCN^- ions in acidic medium and extraction of thiocyanic acid into fine droplets of chloroform as an extraction solvent contains rhodamine B (RhB). The RhB was protonated in presence of thiocynanic acid to form highly colored ion-pair complex of [thiocynate][$RhBH^+$] in chloroform, which used for subsequent spectrophotometric determination of SCN^- ions. Experimental parameters for both spectrophotometric reaction and USAE-ME procedure have been optimized. Under optimized conditions the calibration curve for SCN^- showed good linearity in the range of 38.0–870.0 ng mL^{-1} (R^2 = 0.9967). The limit of detection (S/N = 3) and preconcentration factor were 5.0 ng mL^{-1} and 40, respectively. Relative standard deviation for determination of 200 ng mL^{-1} of SCN^- was 2.8% (n = 5). The proposed method has been successfully applied for determination of SCN^- ion in tap water, mineral bottled water and human saliva and urine samples with an average recovery of 99.2%.

1. Introduction

Thiocyanate ion (SCN^-) usually exists in the industrial wastewaters, pesticides residues and organism metabolites [1]. Low levels of SCN^- that normally present in human body fluids (e.g. serum, saliva, urine) are produced during digestion of some vegetables (cabbage, turnip, kale) [2] or by intake of thiocyanate-containing foods such as milk and cheese [3]. Higher concentrations of SCN^-, which is a major metabolite of cyanide, in physiological fluids arise from exposure to cyanide, inhalation of fires or tobacco smoke [4]. The presence of SCN^- in body fluids may indicate cyanide exposure. Since SCN^- is an end product of detoxification of hydrogen cyanide included in cigarette smoke, its excretion in urine and saliva can provide a useful marker of exposure in smokers and non-smokers [1]. Although the toxicity of SCN^- is significantly less than that of cyanide, chronically elevated levels of SCN^- can inhibit the uptake of iodine by the thyroid gland, thereby, reducing the formation of thyroxine [5]. High concentration of SCN^- in the human body will give rise to vertigo or unconsciousness [6]. Therefore, precise knowledge of SCN^- concentration in food, biological fluids and water samples is mandatory. Many methods have been developed for determination of SCN^- ions in various samples, for example, spectrophotometric [7–9] and flow injection [10] methods based on the reaction with Fe^{3+} ion or on the König reaction, atomic absorption spectrophotometry [11], electrochemical methods (with ion selective electrodes) [12–14], gas chromatography with electron capture [15] or mass spectrometric detection [16], capillary electrophoresis [17,18] and micellar electrokinetic capillary chromatography [19]. Many of these methods suffer from poor reproducibility, sensitivity and selectivity. They are complicated, laborious to perform and require unpleasant or toxic reagents. During the last two decades perhaps the most common analytical technique used for SCN^- determination was ion chromatography (IC). For a long time IC with conductivity detection [20] had been the most popular method for the determination of inorganic anions. However, this detector is not selective and therefore not suitable for the determination of SCN^- in real samples containing large amounts of other anions (Cl^-, SO_4^{2-}, etc.). On the other hand, SCN^- is electroactive and absorbs in the UV region, therefore selectivity can be significantly improved by using amperometric or photometric detectors [21,22]. However, even these selective detectors often do not provide accurate determination of SCN^- in complex anion mixtures due to a relatively low efficiency of the conventional anionexchange stationary phases.

Since the matrices of biological samples are often complex, sample preparation plays an important role in the determination of these species. In last decade, liquid phase microextraction (LPME)

* Corresponding author. Tel.: +98 811 8282807; fax: +98 811 8282807.
E-mail addresses: s.m.daryanavard@gmail.com, daryanavard@basu.ac.ir (S.M. Daryanavard).

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http://dx.doi.org/10.1016/j.jchromb.2012.12.030

has been introduced as an efficient alternative to traditional methods for sample preparation and extraction of organic and inorganic compounds. LPME is a single-step extraction method that has a very high sample to solvent ratio which leads to a higher enrichment factor of target analytes. LPME is fast, simple, inexpensive and since very little solvent is used, there is minimal exposure to toxic organic solvent.

Recently, the use of emulsions generated by ultrasonic radiation has found interest in LPME. In this way, a microextraction technique for aqueous samples, known as ultrasound-assisted emulsification–microextraction (USAEME) has been proposed [23]. This approach is based on the emulsification of microvolume of organic extractant solvent in an aqueous sample by ultrasonic radiation and further separation of both liquid phases by centrifugation. The application of ultrasonic radiation accelerate the mass-transfer process between two immiscible phases, which with the large surface of contact between both phases leads to an increment in the extraction efficiency in minimum time. Thus, USAEME can be employed as a simple and efficient extraction and preconcentration procedure. Up to now, this method has been successfully applied for determination of organic and inorganic species using proper detection methods such as gas chromatography [24], high performance liquid chromatography [25], electrothermal atomic absorption spectrometry [26], graphite furnace atomic absorption spectrometry [27] and flame atomic absorption spectrometry [28].

Compared with the expensive instrumentation, the application of spectrophotometry is mainly limited by its poor sensitivity. Recently, some research works focused on the combination of miniaturized extraction methods with micro volume UV–vis spectrophotometry [29] and fiber optic-linear array detection spectrophotometry [30]. Cost of conventional spectrophotometry and its simplicity makes it a widely used detection method. Therefore, hyphenation of advanced microextraction methods and ordinary spectrophotometry should be meaningful and important, which could improve its sensitivity and expand its applications.

One of the multiple options for the extraction spectrophotometry of ions is based on the formation of ion pairs followed by their extraction in organic solvents. In this way, quaternary ammonium salts (e.g. tetrabutylammonium ion, Bu_4N^+; trioctyl methyl ammonium ion, $MeOc_3N^+$), organometallic cations (e.g. tetraphenylarsonium ion, Ph_4As^+; tetraphenylphosphonium ion, Ph_4P^+; triphenyltin ion, Ph_3Sn^+) or cationic colorants (e.g. rhodamine B) have been applied with this aim. These cationic species form non-solvated ion pairs with anionic species such as $SnCl_6^{-2}$, $AuCl_4^-$, MnO_4^-, $SbCl_6^-$ and SCN^- [31]. Several approaches involving the formation of ion pairs with SCN^- and subsequent solvent extraction have been reported in the literature [32]. Guerrero et al. showed that various acids, H_nX extracted in benzene and those which are strong enough react with RhB, forming ion association complexes with the general formula $(RhBH^+)X^{n-}$ [33].

In continuation of our previous research work on application of USAE-ME for determination of some organic compound using GC [24], the present paper describes the successful application of USAE-ME procedure for extractive-spectrophotometric determination of SCN^- using RhB reagent. To the best of our knowledge, this study is the first report describing the application of USAE-ME for the spectrophotometric determination of an inorganic species. The method is based on the ion-pair formation between SCN^- with rodamine B cation and subsequent USAE-ME of ion-pair complex for spectrophotometric determination of SCN^-. The main parameters influencing extraction and determination were investigated in details. The characteristics and performance parameters of the proposed method are described below.

2. Experimental

2.1. Chemicals and standards

All chemicals were of analytical high grade. Carbon tetrachloride, chloroform, nitrobenzene and chlorobenzene, as extraction solvent, RhB as a cationic dye, potassium thiocynate, sodium chloride, sulfuric acid (98%) and nitric acid (65%) purchased from Merck (Darmstadt, Germany, http://www.merck-chemicals.com). Doubly distilled deionized water was used throughout. SCN^- working standard solutions prepared daily by stepwise dilution from standard stock solution (1000 mg L^{-1}) in double distilled water. Solution of the RhB dissolved in chloroform was prepared daily. All test tubes cleaned with 0.1 M nitric acid, deionized water and acetone.

2.2. Instrumentation

A UV-Vis Spectrophotometer Model T80 (PG Instruments Ltd., Korea) and 100 μL micro-quartz cell (Fisher Co., Germany) was used for the spectrophotometric determination. A 40 kHz ultrasonic water bath Model Parsonic 2600s (Parsnahand Co, Iran) was applied for emulsification process and phase separation of USAE-ME was achieved via a centrifuge Model 16105 (Farayand Co., Iran) in 10 mL calibrated conical glass tubes (Isolab Co., Germany). Vortex mixer Model L46 (LABIN Co., Netherlands) was used for better combining and accelerating reaction between reagents.

2.3. USAE-ME procedure

A 4.5 mL aliquot of the SCN^- sample solution was placed in a 10 mL screw cap glass test tube with conical bottom. 0.5 mL of 1.0×10^{-3} M H_2SO_4 solution was added to glass tube and solution mixed by using vortex mixer. The tube was immersed into ultrasonic bath in such a way that the levels of both liquids (in bath and sample tube) were the same. Then, 180 μL of chloroform (extraction solvent) containing RhB (1.5×10^{-4} mol L^{-1}) was injected rapidly into the sample solution using a 250 μL syringe. Emulsification and extraction was performed at 40 kHz of ultrasonic frequency for 20 s at 25 ± 1°C. As a result, oil-in-water (O/W) emulsions of chloroform (dispersed phase) in water (continuous phase) were formed. After equilibrium time (1 min), emulsion disrupted by centrifugation at 3500 rpm for 5 min, which resulted in the sedimentation of colored organic phase at the bottom of the conical test tube. 100 μL of the settled down phase was quantitatively transferred to quartz microcell using a syringe for the spectrophotometric analysis.

3. Results and discussion

3.1. Optimization for USAE-ME

In this work, UV–vis spectrophotometry has been applied to hyphenate with USAE-ME for determination of SCN^-. Presented method is based on extracting an acidic solution of SCN^- in chloroform and subsequent protonation of RhB dissolved in chloroform to form a highly colored ion-pair complex which is readily soluble in organic solvents. The absorption of ion-pair complex was measured in the range of 400 nm to 700 nm. The ion-pair shows maximum absorbance at 560 nm (Fig. 1) which can be used as the wavelength for the analytical determination. The reagent blank at this wavelength shows a low absorption. The remarkable color difference after USAE-ME procedure between sample solution containing SCN^- (pink color) and blank solution (colorless) is the key factor contributed to the high sensitivity of method for SCN^- determination.

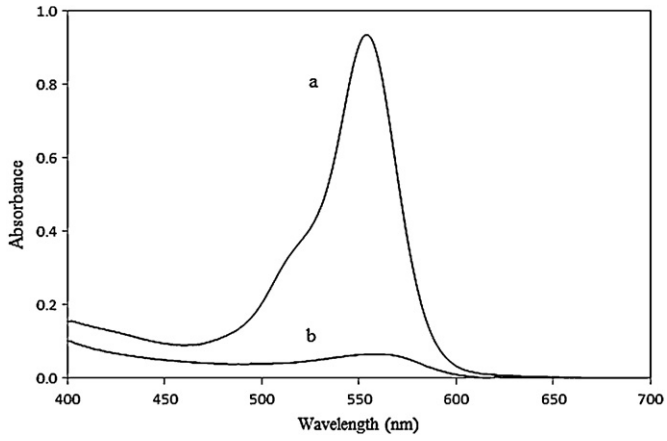

Fig. 1. Absorption spectra for the ion-pair of $[SCN^-][RhBH^+]$ (a) and blank solution (b) after USAE-ME: conditions; sample volume: 5.0 mL, SCN^-: 0.5 μg mL^{-1}, RhB: 1.5×10^{-4} mol L^{-1}, H_2SO_4: 1.0×10^{-4} mol L^{-1}, extractant: 180 μL chlorobenzene, sonication time: 20 s, equilibrium time: 1 min, centrifuging time: 5 min at 3500 rpm.

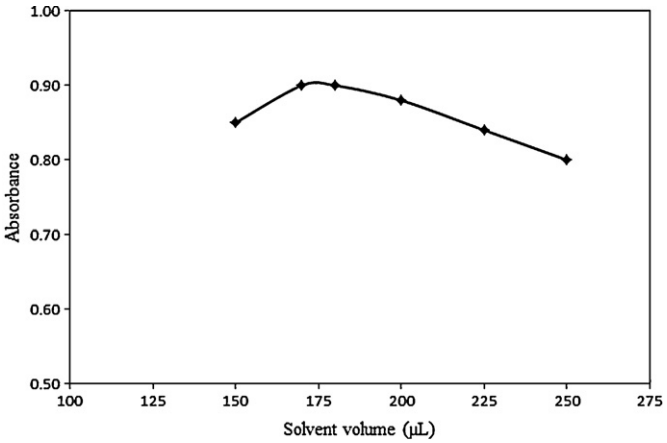

Fig. 2. Effects of different extraction solvent volumes on ion-pair complex absorption. Conditions; Sample volume: 5.0 mL, SCN^-: 0.5 μg mL^{-1}, RhB: 1×10^{-4} mol L^{-1}, H_2SO_4: 1.0×10^{-3} mol L^{-1}, Sonication time: 30 s, Equilibrium time: 2 min, Centrifuging time: 5 min at 3500 rpm.

In order to find the appropriate conditions for USAE-ME, experimental parameters have been studied. There are several factors that can affect the spectrophotometric reaction and extraction process, like the kind and the volume of extraction solvent, H_2SO_4 and RhB concentration, salt addition effect, sonication, equilibrium and centrifugation time. The optimization was carried out on working solutions of 0.5 μg mL^{-1} for SCN^-. All the experiments performed in triplicate and the means of the results used for optimization.

3.1.1. Selection of type and volume of the extraction solvent

Selection of appropriate extraction solvent is critical to the UASEME process since its physicochemical properties not only affect the emulsification phenomenon but also the extraction efficiency. The extraction solvent should meet the following requirements: it should have higher density than water, low solubility in water, high extraction capability for the formed ion-pair, least tendency to the reagent blank and good emulsification efficiency. On the basis of these considerations, carbon tetrachloride, chloroform, nitrobenzene and chlorobenzene selected as potential extraction solvents for the study. 5.00 mL of SCN^- sample solution and 200 μL of each solvent sonicated for 30 s. After centrifugation, phase separation was observed in all cases. Among studied solvents, chloroform exhibit high extraction efficiency, considerably lower absorbance for reagent blank and forms a well stable cloudy solution, therefore, chloroform was chosen as extraction solvent for the subsequent studies. In order to examine the effect of extraction solvent volume, and according to volume of quartz microcell (100 μL), solutions containing different volumes of chloroform (150, 170, 180, 200, 225 and 250 μL) subjected to the same USAE-ME procedures. The volumes smaller than 150 μL were avoided due to dissolution of organic phase in aqueous phase and because of the difficulty of sample manipulation which led to a reduction in precision. The sensitivity increased by increasing the volume of chloroform from 150 to 170 μL (Fig. 2). As can be seen, the sensitivity decreased with increasing the volume of extracting solvent from 180 to 250 μL due to dilution effects. This behavior can also be explained by considering the enrichment factor equation:

$$E = \left(\frac{C^o}{C_0^{aq}}\right) = \frac{K_t}{1 + K_t(V^o/V^{aq})}$$

Where C^O and C_0^{aq} are the analyte concentration in organic phase and its initial concentration in the aqueous phase, respectively. V^o/V^{aq} is the volume ratio of organic phase to the aqueous phase and K_t is the distribution coefficient at time of t. This equation shows that for a constant aqueous volume, the enrichment factor has a reverse relationship with the volume of organic phase. At volumes lower than 150 μL of the extraction solvent, collection and transfer of settled down phase to quartz microcell not quantitative. Therefore, the gain in sensitivity was achieved using 180 μL of chloroform.

3.1.2. Effect of sulfuric acid and rhodamine B concentration

The sulfuric acid concentration is a key parameter on the formation of the HSCN acid and its effective extraction into chloroform droplets. Thus, the influence of the H_2SO_4 concentration in the range of 1.0×10^{-6} to 1.0×10^{-2} mol L^{-1} was studied on the extraction efficiency. According to the results, sensitivity was nearly constant by increasing concentration of H_2SO_4 up to 1.0×10^{-3} mol L^{-1}, and decreased gradually in higher concentration. Therefore, 1.0×10^{-4} mol L^{-1} H_2SO_4 was selected as optimum amount of acid for further studies.

RhB as cationic dye was chosen due to its ability to form an ion-pair with the SCN^- ion in acidic medium, which can be extracted into an organic solvent [32,33]. The effect of the RhB concentration was studied in the range 1.0×10^{-5} to 5.0×10^{-4} mol L^{-1}. As shown in Fig. 3, increase in the extraction efficiency of the ion-pair occurred with increasing the RhB concentration up to 1.5×10^{-4} mol L^{-1} and then decreased gradually upon further increasing in RhB concentration. Thereby, 1.5×10^{-4} mol L^{-1} concentration of RhB was selected for subsequent experiments.

3.1.3. Effect of salt addition

To evaluate the possibility of salting out effect, the salt effect on extraction of $[SCN^-][RhBH^+]$ ion-pair complex was investigated by adding different amount of NaCl in the range of 0–3% w/v. The results (Fig. 4) showed that salt addition decrease the extraction efficiency and sensitivity of SCN^- determination. Although, the addition of salt could decrease the solubility of analytes in the aqueous phase and promote the transfer of the analytes towards the organic phase, however, it could also increase the viscosity of the solution. The viscosity of sample solution plays an important role in the USAE-ME procedure since ultrasonic waves can be absorbed by the viscous resistance of the solution and dispersed as heating energy. As a consequence, the organic phase was not able to be dispersed in so fine droplets and therefore, the efficiency of emulsion formation can be drastically reduced and the extraction efficiency decreased with the addition of NaCl. On the other hand, by increasing the ionic strength, the solubility of the extraction solvent in the

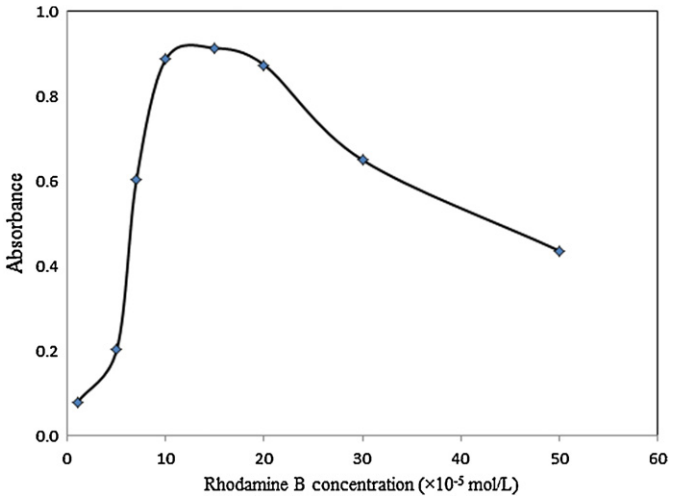

Fig. 3. Effects of Rhodamine B concentration on ion-pair complex absorption. Conditions: sample volume: 5.0 mL, SCN^-: 0.5 μg mL^{-1}, H_2SO_4: 1.0×10^{-4} mol L^{-1}, sonication time: 30 s, equilibrium time: 2 min, centrifuging time: 5 min at 3500 rpm.

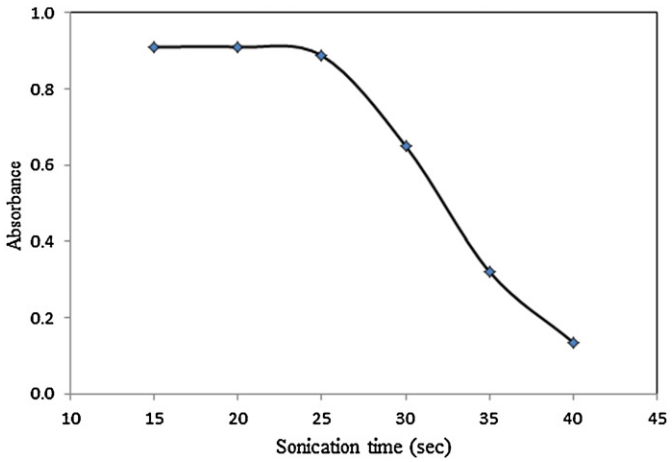

Fig. 5. Effects of sonication time on the ion-pair complex absorption. Conditions: sample volume: 5.0 mL, SCN^-: 0.5 μg mL^{-1}, H_2SO_4: 1.0×10^{-4} mol L^{-1}, RhB: 1.5×10^{-4} mol L^{-1}, equilibrium time: 2 min, centrifuging time: 5 min at 3500 rpm.

aqueous phase diminishes. As a result, the volume of the settled down phase increases, which decreases the extraction efficiency [29]. According to the results, no addition of salts was chosen in the subsequent studies.

3.1.4. Effect of sonication time

Sonication time plays an important role in the emulsification and mass transfer phenomena. As the sonication time increases, the fraction of dispersed phase increases. This can lead to a greater surface contact between two phases and therefore provide efficient mass transfer and better extraction efficiency. However, long sonication time may result in the increasing of the solubility of formed ion-pair and organic solvent. These can reduce the extraction efficiency. The effect of sonication time on the extraction efficiency was studied in the range of 15–40 s under constant ultrasonic power. The results are shown in Fig. 5. As can be seen, by increasing of sonication time, the absorption of the formed ion-pair complex remind nearly constant up to 25 s and decreased gradually up to 40 s. Therefore, the sonication time of 20 s was selected for further studies. This sonication time was sufficient to ensure that effective emulsification was occurred without any possible analyte loss due to increased solubility.

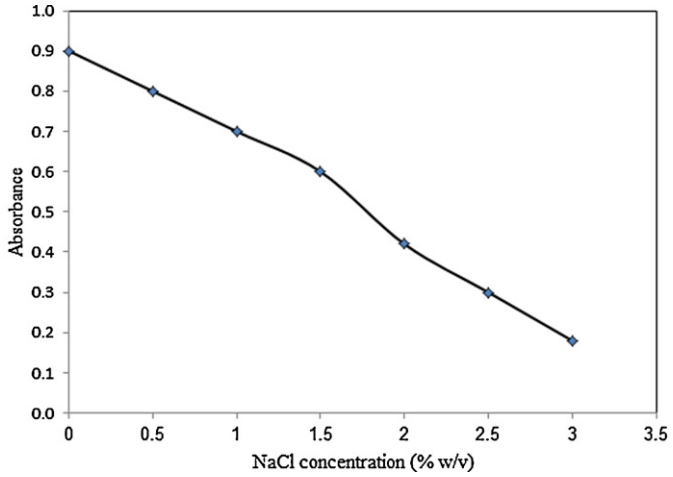

Fig. 4. Salt addition effect on ion-pair complex absorption. Conditions: sample volume: 5.0 mL, SCN^-: 0.5 μg mL^{-1}, RhB: 1.5×10^{-4} mol L^{-1}, H_2SO_4: 1.0×10^{-4} mol L^{-1}, extractant: 180 μL chlorobenzene, sonication time: 30 s, equilibrium time: 2 min, centrifuging time: 5 min at 3500 rpm.

3.1.5. Effects of equilibrium and centrifugation time

Equilibrium time is usually an important factor in the most of microextraction procedures. In this work, equilibrium time is defined as interval time from the occurrence of the cloudy state and just before centrifugation. The effect of the equilibrium time was investigated in the range of 0.5–5 min. The results showed that the variations of complex absorbance versus extraction time are not remarkable. In fact, the surface area between microdrops of organic phase and aqueous sample solution is infinitely large and consequently, the mass transfer from sample solution to extracting solvent is very fast. Therefore, the equilibrium state is achieved quickly and extraction time is very short. This is the most important advantage of this method. Thus the time of 1 min was selected as equilibrium time for subsequent experiments.

Centrifugation was required to break down the emulsion and accelerate the phase separation process. The effect of centrifugation time at 3500 rpm was examined in the range of 2–10 min. The results showed that the best extraction efficiency was achieved with a centrifuging time of 5 min. At shorter time the emulsion state was not well broken and the complete phase separation was not achieved, thereby, the extraction recovery decreased. Also long centrifuging time resulted in the heat generation which led to the increasing of the solubility of chloroform and complex in aqueous phase and loss of sensitivity. Therefore, 5 min was adopted for further use.

3.2. Interference study

The effect of diverse ions on the determination of 100 ng mL^{-1} of SCN^- was studied according to the above-described procedure. The tolerable amount of each interferent was taken as SCN^-: interferent ratio that resulted in error not exceeding ±5%. As could be seen in Table 1, alkaline and alkaline earth ions and common anions such as Na^+, Mg^{2+}, Ca^{2+}, Cl^-, F^-,$SO_4{}^{2-}$ did not interfere with the determination of SCN^- at more than 1000-fold excess. Also, presented method was remarkably free from transition metal interferences. However, some ions such as Mn^{2+}, Cr^{6+}, I^-, $NO_2{}^-$ showed nearly significant interference during SCN^- determination.

Table 1
Tolerance ratios of diverse ions on the determination of 100 ng mL^{-1} of SCN$^-$.

Ion	Tolerance limit (w/w)
F^-, Cl^-, Br^-, NO_3^-, CO_3^{2-}, CH_3COO^-, SO_4^{2-}, PO_4^{3-}	1000
Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Co^{2+},Ni^{2+}, Cu^{2+} Zn^{2+}, Fe^{2+}, Fe^{3+}, Sb^{3+}, As^{3+}	1000
Mn^{2+}, Cr^{6+}, I^-, NO_2^-	50

3.3. Performance of the analytical procedure

Analytical characters of the presented method evaluated under optimized conditions. The results are presented in Table 2. A linear calibration graph was obtained over the range 38.0–870.0 ng mL^{-1} of SCN$^-$ with the linear regression equation $A = 0.0016C + 0.0066$ (C, ng mL^{-1} SCN$^-$) and correlation coefficient (R^2) 0.9967 (number of calibration points, $n = 12$). The limit of detection (LOD), based on signal to noise ratio of 3 was 5.0 ng mL^{-1}. The precision of the method, expressed as relative standard deviation (RSD %), for five replicate determination of 200 ng mL^{-1} of SCN$^-$ was found to be 2.8%. The pre-concentration factor (PF), which was defined as the ratio between the volumes of settled down phase (V_{sed}) and the sample volume (V_o), was used to evaluate the extraction efficiency under different experimental conditions, was found to be 40 for the system. These results indicated the present method had high sensitivity and stability and high potential to be a powerful and suitable preconcentration method for trace analysis. A compression of the analytical features achieved by the proposed method and other methods for SCN$^-$ determination are presented in Table 2. The presented method has distinct advantages in terms of low limit of detection, wide linear range and simplicity of instrumentation.

3.4. Application to real water samples

Since there is no interference from major consistent of water sample and transition metal ions, the method is especially suitable for water analysis by simple standard calibration. To evaluate the applicability of the proposed method, the extraction and determination of the SCN$^-$ in different water samples were performed. As a result (Table 3), no residues of the SCN$^-$ were found in the samples. To test the accuracy of the method, these water samples were spiked with the standards of the SCN$^-$ at the concentrations of 60.0, 120.0 and 240.0 ng mL^{-1}, respectively. For each concentration level, three replicate experiments for whole analysis process as described in the experimental section were made. The recoveries of the method were expressed as the mean percentage between the amounts found and the ones added. The results are given in Table 3.

Table 2
Analytical features of the proposed method.

Regression equation ($n = 14$)	$A = 0.0016\,C^a + 0.0066$, $R^2 = 0.9967$
Linear range (ng mL^{-1})	38.0–870.0
Limit of detection ($3S_B$/m blank, ng mL^{-1}) ($n = 5$)	5.0
Preconcentration factor[b]	40
Relative standard deviation (for 200 ng mL^{-1} of SCN$^-$, $n = 5$)	2.8%

[a] Concentration in ng mL^{-1}.
[b] The ratio between the volume of settled down phase (V_{sed}) and the sample volume (V_0).

Table 3
Determination of SCN$^-$ in the spiked water samples by the proposed method.

Sample	Added (ng mL^{-1})	Found (ng mL^{-1})[a]	Recovery (%)
Tap water Intra-day ($n = 3$)	–	nd[b]	–
	60.0	63.0 ± 2.1	105.0
	120.0	117.2 ± 2.0	97.6
	240.0	248.1 ± 2.6	103.3
Inter-day ($n = 3$)	–	nd	–
	60.0	64.8 ± 2.1	108.0
	120.0	118.3 ± 1.9	98.6
	240.0	250.6 ± 3.7	104.4
Mineral bottled water Intra-day ($n = 3$)	–	nd	–
	60.0	59.3 ± 2.4	98.8
	120.0	122.1 ± 2.2	101.7
	240.0	252.3 ± 3.8	105.1
Inter-day ($n = 3$)	–	nd	–
	60.0	57.9 ± 2.3	96.5
	120.0	124.0 ± 2.7	103.3
	240.0	255.1 ± 4.2	106.3

[a] Mean ± SD ($n = 3$).
[b] Not detected.

Table 4
Determination of thiocyanate in the spiked human biological fluids samples.

Sample	SCN$^-$ added (μg mL^{-1})	SCN$^-$ found[a] (μg mL^{-1})	Recovery (%)
Saliva (non-smoker)[b]	–	94.2 ± 2.1	–
	60.0	156.6 ± 3.4	101.5
	120.0	209.0 ± 2.2	97.5
	240.0	332.3 ± 5.4	99.4
Saliva (smoker)	–	226.7 ± 4.1	–
	60.0	281.5 ± 3.1	98.1
	120.0	355.9 ± 6.7	102.6
	240.0	465.0 ± 8.2	99.6
Urine (non-smoker)	–	100.9 ± 3.5	–
	60.0	159.1 ± 2.4	98.3
	120.0	221.4 ± 3.4	100.2
	240.0	340.0 ± 3.3	99.7
Urine (smoker)	–	302.0 ± 3.6	–
	60.0	374.0 ± 3.6	103.3
	120.0	416.5 ± 4.8	98.6
	240.0	530.2 ± 7.6	97.8

[a] Mean ± SD ($n = 3$)
[b] All saliva and urine samples are diluted 20 times by deionized water.

3.5. Human saliva and urine samples analysis

To evaluate the proposed method for physiological samples, it was applied for determination of SCN$^-$ in nonsmoker and smoker saliva and urine samples. Saliva and urine samples from smokers

Table 5
Comparison of USAE-ME with some reported procedures.

Method	LOD[a] (ng mL^{-1})	LR[b] (ng mL^{-1})	Ref.
Capillary zone electrophoresis	41.0	147–2900	[1]
Ion selective electrode – modified by nanoparticles	2.3	30–2300	[34]
Head space–gas chromatography	2.9	Up to 1700	[35]
Partial least squares regression	25.0	50–1200	[36]
Flow injection analysis	29.0	250–5000	[37]
Ultrasound-assisted emulsification microextraction	5.0	38–870	This work

[a] Limit of detection.
[b] Linear range.

(five man, mean age = 28 years) and non-smokers (five man, mean age = 24 years) were collected and centrifuged for 3 min with a rate of 3000 rpm. After appropriate dilution, the samples analyzed according to the procedure described above. The recoveries of SCN^- have been determined by adding known concentrations to saliva and urine samples. As is shown in Table 4, the recoveries of SCN^- from human saliva and urine (smoker and nonsmoker) samples were 97.5–102.6% and 97.8–103.3%, respectively. These results suggest that the matrix components do not interfere with the determination of SCN^-

4. Conclusions

This study presents the successful development and application of USAE-ME procedure coupled with UV–vis spectrophotometry for the determination of SCN^- in water and biological fluids samples. The results indicate that this extraction procedure has outstanding advantages such as simplicity, high enrichment factor and minimizes the sample preparation time and the consumption of organic solvents. Also, analyses of real samples showed that sample matrices had no adverse effect on the efficiency of USAE-ME procedure and SCN^- concentrations in saliva and urine samples determined rapidly and easily by the proposed method without any special sample pretreatment, except centrifugation and appropriate dilution. Compared with other conventional sample preparation methods applied for SCN^- determination (Table 5), the analytical technique offers advantages such as ease of operation, low cost, short analysis time, environmentally friendly and corresponds to the requirements of green analytical chemistry. The results of this study show that hyphenation of USAE-ME procedure with ordinary UV–vis spectrophotometer equipped with 0.1 mL quartz cells can significantly improve the sensitivity of measurements. The enrichment factor of 40 achieved allows determining SCN^- in natural waters, human saliva and urine samples at ultra trace levels. As a consequence, the developed USAE-ME method has been demonstrated to be viable, rapid and easy to use for the qualitative and quantitative analysis of SCN^- in different water and biological samples.

References

[1] Z. Glatz, S. Novakova, H. Sterbova, J. Chromatogr. A 916 (2001) 273.
[2] W. Weuffen, C. Franzke, B. Türkow, Die Nahrung 28 (1984) 341.
[3] R.E. Bliss, K.A. O'Connell, Health Psychol. 3 (1984) 563.
[4] Y. Michigami, K. Fujii, K. Ueda, Y. Yamamoto, Analyst 117 (1992) 1855.
[5] Risk Information Web Server, Toxicity summary for cyanide, http://www.Risk.lsd.ornl.gov/tox/profiles/cyanide
[6] R.B.H. Gradwohl, Clinical Laboratory Methods and Diagnosis, Moshy GV, St. Louis, MO, USA, 1956.
[7] A.R. Pettigrew, G.S. Fell, Clin. Chem. 18 (1972) 996.
[8] P. Lundquist, J. Mårtensson, B. Sörboand, S. Öhman, Clin. Chem. 25 (1979) 678.
[9] W.C. Butts, M. Kuehneman, G.M. Widdowson, Clin.Chem. 20 (1974) 1344.
[10] C.J. Vesey, C.J.C. Kirk, Clin. Chem. 31 (1985) 2704.
[11] A.M. Westley, J. Westley, Anal. Biochem. 181 (1989) 190.
[12] A. Abbaspour, M.A. Kamyabi, A.R. Esmaeilbeig, R. Kia, Talanta 57 (2002) 859.
[13] M.K. Amini, S. Shahrokhian, S. Tangestaninejad, Anal. Chim. Acta 402 (1999) 137.
[14] Z.Q. Li, A.Y. Wu, R. Yuan, M. Ying, G.L. Shen, R.Q. Yu, Electrochim. Acta 44 (1999) 2543.
[15] S. Kage, T. Nakata, K. Kudo, J. Chromatogr. B 675 (1996) 27.
[16] J.S. Torano, H.J. van Kan, Analyst 128 (2003) 838.
[17] L.G. Song, Q.Y. Ou, W.L. Yu, L.C. Fang, Y.B. Jin, J. Chromatogr. A 715 (1995) 376.
[18] Y. Tanaka, N. Naruishi, H. Fukuya, J. Sakata, K. Saito, S. Wakida, J. Chromatogr. A 1051 (2004) 193.
[19] C. Bjergegaard, P. Moller, H. Sorensen, J. Chromatogr. A 717 (1995) 409.
[20] M.G. Valdes, M.E. Diaz-Garcia, Crit. Rev. Anal. Chem. 34 (2004) 9.
[21] O. Shpigun, Yu. A. Zolotov, Ion Chromatography in Water Analysis, John Wiley and Sons, New York, US, 1988.
[22] S. Chinaka, N. Takayama, Y. Michigami, K. Ueda, J. Chromatogr. B 713 (1998) 353.
[23] J. Regueiro, M. Llompart, C. Garcia-Jares, J.C. Garcia-Monteagudo, R. Cela, J. Chromatogr. A 1190 (2008) 27.
[24] M. Hashemi, N. Jahanshahi, A. Habibi, Desalination 288 (2012) 93.
[25] W. Chunxia, L. Ning, W. Qiuhua, W. Chun, Z. Wang, Anal. Chim. Acta 679 (2010) 56.
[26] P. Berton, E. Martinis, L. Martinez, R.G. Wuilloud, Anal. Chim. Acta 713 (2012) 56.
[27] F. Fathirad, D. Afzali, A. Mostafavi, M. Ghanbarian, Talanta 88 (2012) 759.
[28] J.M. Jing, X. Du, J.W. Zhang, J.C. Li, L.Z. Wang, Talanta 80 (2009) 980.
[29] N. Sharma, A. Pillai, N. Pathak, A. Jain, K. Verma, Anal. Chim. Acta 648 (2009) 183.
[30] N. Shokoufi, F. Shemirani, Y. Assadi, Anal. Chim. Acta 597 (2007) 349.
[31] M. Valcárcel, M. Silva, Teoría y práctica de la extracción líquido–liquid, Madrid, Alhambra, 1984.
[32] A.M. Roig, A.H. Guerrero, Determination of thiocyanate and iodide by means of rhodamine, XX IUPAC Congress, Moscow, 1965.
[33] A.H. Guerrero, Antonio M. Roig, Anal. Chem. 45 (1973) 1943.
[34] G.F. Wang, M.G. Li, Y.C. Gao, B. Fang, Sensors 4 (2004) 147.
[35] Kouichiro Tsuge, Mieko Kataoka, Yasuo Seto, J. Health Sci. 46 (2000) 343.
[36] A. Afkhami, N. Sarlak, A.R. Zarei, Talanta 71 (2007) 893.
[37] G. Demetrius Themelis, D. Paraskevas Tzanavaras, Anal. Chim. Acta 452 (2002) 295.