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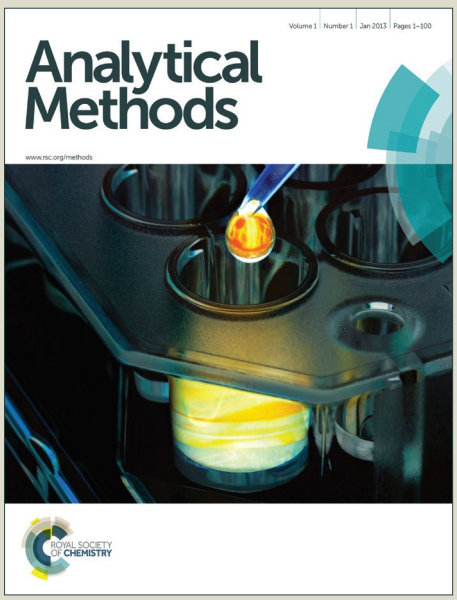

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Ionic liquid-based microextraction method for highly selective and sensitive trace determination of nickel in environmental and biological samples

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A simple and rapid dispersive liquid–liquid microextraction procedure (IL-DLLME) based on an ionic liquid was developed for selective determination of nickel with spectrophotometric detection. Nickel was initially complexed with 5-(2-benzothiazolylazo)-8-hydroxyquinolene [BTAHQ] reagent at pH 4.0. The IL-DLLME procedure was then performed by using a few microliters of the room temperature ionic liquid (RTIL) 1-hexyl-3-methylimidazolium hexafluorophosphate $[C_6mim][PF_6]$ as extractant while methanol was the disperser solvent. After microextraction procedure, the Ni-enriched RTIL phase was solubilized in methanol and directly measured the absorbance at λ_{max} 682 against a reagent blank similarly prepared. The effect of several variables on Ni–BTAHQ complex formation, extraction with the dispersed RTIL phase, and analyte detection with spectrophotometry, was carefully studied. An enrichment factor of 200 was obtained with only 10 mL of sample solution and under optimal experimental conditions. The resultant limit of detection (LOD) was 9.8 ng L^{-1}, while the relative standard deviation (RSD) was 1.47% (at 1.0 μg L^{-1} Ni level and n = 10. The accuracy of the proposed method was tested by analysis of a certified reference material. The method was successfully applied for the determination of nickel in environmental standard reference materials and biological samples.

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Introduction

To determine trace metals in aquatic environments by instrumental analysis, a separation and preconcentration technique is frequently required, because of low concentration of trace metal ions and presence of interferences.[1–3] Many sample pretreatment methods including solvent extraction, cloud point extraction, solid-phase extraction, membrane filtration, electrodeposition, flotation, coprecipitation and ion exchange have been developed for preconcentration of trace metals from natural waters.[2–8] The traditional methods such as liquid–liquid extraction and coprecipitation often require large amounts of high purity organic solvents, some of which are harmful to health and cause environmental problems.

Nickel is a toxic trace element of widespread distribution in the environment. It, usually, enters waters from waste disposals of different industrial processes such as electroplating, batteries, pigments for paints and ceramics, surgical and dental prostheses, magnetic tapes and computer components, catalysts and also it is emitted to the atmosphere from volcanoes and windblown dusts.[9] Long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation. High levels of Ni in the diet may be associated with an increased risk of thyroid problems, cancer, and heart disease.[10] Epidemiological studies showed that the majority of the factors leading to the development of tumor in humans have arisen from environmental factors and 65–70% of all cancers in humans are associated with the environment, including the work environment, 30–40% with nutritional habits and only 2.0 % with consequences of genetic predispositions.[11] Therefore, the development of novel and sensitive methods to determine the nickel content of environmental, biological and food samples is necessary and important.[12]

Numerous separation and preconcentration techniques for Ni determination in water and biological samples have been proposed, including solid phase extraction (SPE),[13–15] Conventional LLE with regular organic solvents is widely employed for sample preparation due to its simplicity and flexibility.[13] Even though this procedure can effectively decrease detection limits and eliminate matrix interference, it also requires large amounts of high purity organic solvents for the extraction, resulting in environmental and safety concern due to high volatility, toxicity and flammability.[13] On the other hand, many of the problems linked with regular organic solvents as well as loss of solvent by evaporation can be significantly avoided using ionic liquids (ILs) as alternative solvents, since they have no detectable vapor pressure and are relatively thermal stable even at elevated temperatures.[14] Extractions of metal ions using room temperature ionic liquids (RTILs) combined with suitable complexing agents have been recently developed in analytical chemistry, thus allowing extraction of low polar compounds from aqueous solution.[15] Since miniaturization of sample pretreatment protocols is of special importance when expensive sample and reagents are employed, or only very limited amount of these are available,[16] RTILs based on 1-alkyl-3-methylimidazolium hexafluorophosphates ([C_6mim] [PF_6], n = 4, 6, 8) have been used in single drop microextraction (SDME) technique in both direct immersion (DI-SDME) and headspace (HS-SDME) modes.[13] However, both methods are time-consuming, have limited reproducibility and presents some practical drawbacks such as emulsion formation and the fact that the drop is broken up and air bubbles are formed when increasing agitation rate or when dealing with some dirty samples.[14] Classical dispersive liquid–liquid microextraction based on ILs as extractant phase (IL-DLLME), with organic solvents as dispersing agents,[17] and temperature-controlled IL dispersive liquid phase microextraction (TILDLME)[18] have both been proposed as novel homogeneous LLME techniques for metal extraction, thus avoiding many of the problems observed in earlier methods.

The determination of nickel in environment and biological samples has been carried out by various instrumental techniques such as neutron activation analysis (NAA),[19] inductively coupled plasma optic emission spectrometry (ICP-OES)[20], inductively coupled plasma mass spectrometry (ICP-MS),[21] X-ray fluorescence spectroscopy[22] and chromatography.[23,24] Despite the sensitivity and selectivity of analytical techniques such as flame atomic absorption spectrometry (FAAS), there is a great necessity for preconcentration of metal prior to its determination, basically due to its low concentration or the effects of matrix in aqueous samples.[25]

Recently, the application of RTILs in LLME procedures has been reported for nickel determination.[26,27] In fact, pyridylazo-type reagents in combination with ILs have been used for determination of several metal ions. However, it has to be noticed that, despite the favorable stability constants of their complexes, this class of reagents shows limited selectivity towards metal complexation. Thus, extraction procedures based on these reagents could be prone to suffer from matrix interferences occurring in real complex samples. On the other hand, BTAHQ forms stable complexes with numerous metal ions[28–33] and it can selectively react with nickel under specific conditions. Moreover, BTAHQ has been employed for spectrophotometric determination of nickel in the past,[28] but no report has been so far published regarding its use and combination with RTILs, for development of LLME procedures.

In this work, a highly selective separation and preconcentration method for nickel determination at trace levels is proposed. Nickel was initially complexed with BTAHQ reagent, followed by application of IL-DLLME technique based on the RTIL 1-hexyl-3-methylimidazolium hexafluorophosphate ($[C_6mim][PF_6]$). The proposed method was successfully applied for the microdetermination of nickel at trace levels in environmental and biological samples.

Experimental

Instrumentation

A Perkin-Elmer Lambda 12 UV–vis spectrophotometer (Waltham, MA, USA) with a 1.0 mm quartz cell was used for all spectral measurements. A funnel tipped glass tube (60 mm × 6 mm) was used as a column for preconcentration. The laboratory glassware (Superior, Germany) and column was kept overnight in a 5.0 % nitric acid solution. A Perkin Elmer model 5300 DV; ICP-AES (Waltham, MA, USA) was used for all ICP-AES measurements. An Orion research model 601 A/digital ionalyzer pH meter (Tokyo, Japan) was used for checking the pH of solutions.

A centrifuge (Luguimac, Buenos Aires, Argentina) model LC-15 was used to accelerate the phase separation process. A thermostated bath (Vicking, Buenos Aires, Argentina) model Masson Digital, maintained at the desired temperature, was used for heating. A vortex model Bio Vortex V1 (Boeco, Hamburg, Germany) was used for mixing the reagents. UV-photolysis of urine samples was performed with a 15W/G15T8 UV-C lamp (Philips, Holland).

Reagents

Standard stock solutions of Ni(II), was prepared by dissolving an appropriate weigh amount of $Ni(NO_3)_2.6H_2O$, Aldrich (Milkwaukee, WI, USA) in a small volume water and diluted to 1.0 L with distilled water. The stock solution was then standardized gravimetrically using dimethylglyoxime.[34] More dilute standards were prepared daily by dilution of these solutions. BTAHQ was synthesized according to the method described previously.[28] Stock solutions of 5.0×10^{-4} M BTAHQ were prepared by dissolving an appropriate weight of pure reagent in least amount of ethanol (15 mL) and then diluted to the mark in a 100-mL calibrated flask with ethanol.

A 2.0 M acetic acid–acetate solution, Merck (Darmstadt, Germany) adjusted to pH 4.0 by dissolution of sodium hydroxide, Merck was employed as buffer solution. Individual surfactant solutions containing 2.0×10^{-2} M Triton X-114, Merck or 1.5×10^{-2} M Triton X-100, Merck were evaluated as anti-sticking agents. A 50% (w/v) sodium nitrate solution was prepared by dissolving 5.0 g of $NaNO_3$, in 10 mL of ultra pure water.

$[C_6mim][PF_6]$ was synthesized according to a method proposed by Huddleston et al.[35,44] and stored in contact with ultra pure water to equilibrate the water content in the RTIL phase.[36] The ionic liquid was synthesized in two steps. The first step involved synthesis of the organic cation and the second step, addition of the anion. The ionic liquids was characterized by ^{1}H-NMR spectroscopy. The ionic liquid was dried under vacuum in order to remove excess water content. The water content was measured by a Karl Fischer coulometer, did not exceed 50 ppm Qualitative analysis of synthesized IL was performed by comparison of infrared spectra with commercially available $[C_6mim][PF_6]$, Solvent Innovation GmbH (Kۏln, Germany).

Ultra pure water (18 MΩ cm) was obtained from a Millipore Continental Water System (Bedford, MA, USA). All glassware was washed with a 5.0 M HNO_3 solution at least for 24 h and thoroughly rinsed 5.0 times with ultra pure water before use.

Sample collection and conditioning

Water samples

For tap water samples collection, domestic water was allowed to run for 20 min and approximately a volume of 1000 mL was collected in a beaker. River water samples were collected in cleaned bottles rinsed three times with water sample prior to collection. A sample volume of 1000 mL was collected at a depth of 5.0 cm below the surface. Tap water samples were analyzed immediately after sampling. River

water samples were filtered through 0.45 μm pore size membrane filters, Millipore Corporation (Bedford, MA, USA) immediately after sampling. All samples were acidified to pH 1.0 with concentrated HNO_3 and stored at 4 ^oC in bottles; Nalgene Nalge (Rochester, NY, USA). The samples were analyzed as soon as possible.

Biological samples

Urine and saliva samples were collected from men and women volunteers, aged from 25 to 35 years, living in Benha (Egypt), without having eaten breakfast. Informed consent was obtained from all participants, and the procedures were approved by Benha University Hospital. In order to minimize the possibility of contamination with food debris or cigarette and airborne particles, the subjects were asked to thoroughly rinse their mouths three times with ultrapure water. Human saliva samples were collected between 8 and 9 h to reduce possible circadian contributions, into Co-free polystyrene test tubes.[37] The samples (7.0 mL) were acidified with HNO_3 to pH 2.0 and then placed in a graduated centrifuge tube and centrifuged for 20 min at 1500 rpm (377.2 g). Five milliliters of the supernatant were diluted to 25 mL with bi-distilled water and Ni was determined by the proposed method. Dilution prior to analysis is practical since collection of large volumes may be tedious and uncomfortable to the donor. Blanks were prepared with the same reagents, without the samples, undergoing an identical process.

Urine samples were digested by UV-photolysis as described by Husakova et al.[38] Briefly, 5.0 mL of sample was placed in a decomposition glass beaker, added with 200 μL of 30% (w/w) H_2O_2, and the mixture was then irradiated for 45 min. Then, another 200 μL aliquot of 30% (w/w) H_2O_2 was added and irradiation process was continued for 45 min. Finally, 10 mL of H_2O was added and the irradiation process was repeated for another 120 min. After completion of the irradiation procedure the volume of the digested sample was set to 25 mL.

Certified reference materials samples

About 0.1 g of each oven-dried (110 ^{o}C) alloy sample was dissolved in 15 mL aqua-regia. The solution was heated to near dryness and the nitrate was expelled from the residue using 5.0 mL of concentrated hydrochloric acid. Each residue was then extracted in double-distilled water and made up to 500 mL. An appropriate aliquot was analyzed for nickel(II) by there commended general procedure.

Dispersive liquid–liquid microextraction procedure based on ionic liquid

A mixture of 10 mL of the pre-treated sample or a 1.0 μg L^{-1} Ni(II) standard solution (for method optimization), 200 μL of 5 × 10^{-4} M BTAHQ solution, 50 μL of 2.0 M (pH 4.0) acetate/acetic acid buffer, 300 μL of 50% (w/v) sodium nitrate solution and 30 μL of 2.0 × 10^{-2} M Triton X-114, was heated in a thermostated bath at 50 ^{o}C for 10 min. After formation of Ni– BTAHQ complex, the tube was placed in an ice bath for 5.0 min to diminish the temperature, and pH 2.0 was adjusted by adding HCl (1.0 M). An amount of 50 mg of [C_6mim][PF_6] (extraction solvent) and 500 μL of methanol (disperser solvent) were then added to the sample solution. A cloudy solution was immediately formed, by dispersion of the immiscible RTIL into the aqueous sample, thus greatly enlarging the contact area between the two phases. Consequently, the Ni – BTAHQ complex was extracted into the dispersed RTIL phase. After 5.0 min of extraction time, centrifugation at 1500 rpm (377.2 g) for 10 min allowed the formation of two well-defined phases. The upper aqueous phase was then manually removed with a syringe and the RTIL phase dissolved with 50 μL of methanol, followed by measuring absorbance at 1.0 mm cell at λ_{max} 682 for Ni determination against reagent blank similarly prepared. Calibration was performed against aqueous standards and blank solutions.

Results and discussion

Absorption spectra.

The absorption spectra of Ni(III) - BTAHQ before and after IL-DLLME are shown in Fig. 1. The absorption bands are located at 635 nm [28] and 682 nm for Ni(III) - BTAHQ complex before and after IL-DLLME, respectively.

Spectrophotometric conditions for Ni determination in RTIL phase

Initial studies were focused on obtaining high accuracy and precision for spectrophotometric measurements of Ni in the presence of the RTIL matrix. Direct measurements carries some drawbacks due to the high viscosity of the resulting phase. Therefore, in order to achieve reproducible of the RTIL, dissolution in an appropriated solvent was studied. Acetone, dioxin, ethanol and methanol were assayed in this work. Although dilution of the RTIL phase in solvents was feasible, the best performance was achieved with methanol as diluent. Total dissolution of the RTIL phase was observed for 50 μL methanol, while lower volumes turned out into a deterioration of analytical sensitivity. Thereby, 50 μL of methanol was employed for further experiments.

Selection of RTIL and disperser solvent

The selection of a suitable RTIL was performed based on specific properties, such as low solubility in water, good extraction ability, and higher density than water. Thus, we focus on hydrophobic and relatively inexpensive imidazolium-ILs containing $[PF6]^-$ as counter anion. For the most used within that class, i.e. $[C_4mim][PF_6]$, $[C_6mim][PF_6]$ and $[C_8mim][PF_6]$, the solubility in water diminishes following: 18.8, 7.5, to 2.08 g L^{-1}, respectively.[39] On the contrary, viscosity of these RTILs increases as follows: 450, 585, to 710 mPas, respectively.[39] Both parameters have to be considered, since a lower solubility allows minimal RTIL consumption, while a high viscosity could lead to practical drawbacks during the microextraction procedure. Thus, $[C_6mim][PF_6]$ was chosen as the extractant phase considering its relatively

high hydrophobicity, lower solubility as compared with $[C_4mim][PF_6]$ while showing an acceptable viscosity to work with the DLLME approach. Since both extraction efficiency and analyte detection in spectrophotometry can be remarkably affected by RTIL amount, it was critical to establish the minimal amount of RTIL yielding total Ni extraction while achieving the best analytical sensitivity. Recovery of Ni upon RTIL amount was examined within the range of 40–60 mg and using 0.5 mL methanol as disperser solvent. The results revealed that 50 mg was the lowest amount of $[C_6mim][PF_6]$ required to achieve 100% recovery. Higher amounts of the RTIL did not improve extraction efficiency, while could lead to increase background signals. Therefore, 50 mg was used for subsequent experiments in this work.

The choice of a disperser solvent was done considering the immiscibility between IL phase and aqueous sample. Thus, acetone, dioxin, ethanol and methanol were particularly evaluated. Recovery efficiency was evaluated using 50 μL of each disperser solvent and 50 mg $[C_6mim][PF_6]$. Methanol yielded the highest recovery for Ni, and thereby this solvent was selected as the disperser for our studies. This higher recovery can be attributed to the better dispersion obtained in methanol.[40] On the other hand, the volume of disperser directly affects RTIL solubility in aqueous phase, significantly determining the volume of the final phase, and thus influencing the efficiency of the microextraction technique. Thus, methanol volumes ranging within 20–100 μL were assayed. It was observed that the extraction efficiency increased by increasing the methanol volume up to 50 μL. A higher volume of methanol slightly reduced the preconcentration factor. Finally, 50 μL was chosen as the optimum volume of disperser solvent.

Influence of sample volume on extraction efficiency

Since $[C_6mim][PF_6]$ solubility has been reported to be 7.5 g L^{-1},[39] the final volume of the RTIL phase and its effect on Ni recovery were evaluated upon sample volume increase. Ni recovery remained constant up to 10 mL of sample. Despite a higher

volume of the RTIL sedimented phase was achieved for lower sample volumes, it was more difficult to obtain reproducible absorbance due to background deterioration originated from insufficient pyrolysis treatment during Ni measurements. Thus, the best absorbance-to-background ratio was obtained when 10 mL-aliquots of sample were chosen.

Complex formation conditions and selectivity of Ni extraction

The pH plays an important role, not only on metal-complex formation but also on DLLME performance, as it defines the charge of the complex and its affinity for the RTIL phase. The effect of pH on the formation of Ni– BTAHQ was studied in the range of 1–8 (Fig. 2). The optimum pH was observed in the interval of 3.5–4.5, confirming that the complex requires a weakly acidic solution for quantitative formation.[41] Therefore, samples and standards were adjusted at pH 4.0 before IL-DLLME procedure. In order to maintain a constant working pH that allows formation and stability of the complex, an acetic/acetate buffer solution was selected. The possible influence of buffer concentration on Ni extraction efficiency was studied in the range of $0–4\times10^{-2}$ M. It was observed that Ni extraction increased by increasing the buffer concentration up to 2×10^{-2} M. This improvement on the system performance could be explained due to major stability of Ni in solution at low pH when acetic acid is present.[42] A buffer concentration of 2×10^{-2} M was chosen for subsequent experiments.

The high stability of the Ni– BTAHQ complex, at different pH values after formation, has been already reported.[28] Therefore, the effect of pH on complex formation and the performance of ILDLLME procedure could be individually study in this work. After complex formation, the effect of pH on the extraction performance was studied within the range of 2.0–6.0 by adding appropriate volumes of HCl or NaOH solution (Fig. 2). No changes on the extraction efficiency were observed within this pH interval. Thus, in order to significantly increase the selectivity of Ni complexation with BTAHQ reagent and determination, solutions

with low pH are preferred due to high instability of others metal– BTAHQ complexes. Consequently, after the Ni– BTAHQ complex was formed at pH 4.0, IL-DLLME procedure was performed at pH 2.0 by adding HCl (1.0 M).

Due to the polarity of Ni ions, their extraction efficiency by the sole application of $[C_6mim][PF_6]$ could be too low.[43] In order to increase the extraction efficiency of metal ions it is necessary to improve their affinity for the RTIL phase by complexing with a suitable reagent such as BTAHQ. Moreover, imidazolium-based ILs present a high chemical affinity to substances with one or more aromatic rings in their structures. The effect of BTAHQ concentration on the complexation was evaluated (Fig. 2). A maximum Ni extraction was observed using 200 μL of 5×10^{-4} M BTAHQ. Since the formation of the Ni– BTAHQ complex is a slow process that can be speeded up by heating the solution, the effect of temperature on reaction kinetic and final Ni extraction was studied. A 0.0–60 min time window was chosen to investigate the formation of the complex both, at room temperature and at 50 ^{o}C in a thermostated bath. It was observed that extraction recovery reached the highest value for 10 min in a thermostated bath at 50 ^{o}C before developing the IL-DLLME procedure. Furthermore, it has been demonstrated that Ni– BTAHQ is a stable complex over a 24-h period.[28]

Surfactant and salt as additives

The Ni–BTAHQ complex precipitates in aqueous medium due to its low polarity, negatively affecting the extraction efficiency of the technique. A non-ionic surfactant not only can avoid this problem, but also reduce the adherence of the RTIL on the wall of the centrifuge tube. The effect of different concentrations (0.0–5.6×10^{-4} M) of two non-ionic surfactants (Triton X-100 and Triton X-114) was studied and compared. It was observed that both the complexing agent and the metallic complex remained in solution within the range studied. For Triton X-100, it was observed that extraction efficiency decreased by increasing surfactant concentration. On the other hand, when using Triton X-114, Ni extraction improved

using 30 μL of 2.0×10^{-2} M Triton X-114. Thus, Triton X-114 was chosen as anti-sticking agent.

Generally, the addition of salt in traditional L–L extraction using conventional organic solvents increases the extraction performance due to salting out effect. This effect was investigated over a $NaNO_3$ concentration range of 0–6% (w/v). As shown in Fig. 4, the extraction efficiency increased as a result of salting out effect in the range of 0–2% (w/v) $NaNO_3$, while it decreased at concentrations higher than 1.5 % (w/v) $NaNO_3$ due to solubilization of the RTIL phase into aqueous phase. Thus, a concentration of 1.5% (w/v) $NaNO_3$ was selected for subsequent experiments.

Evaluation of minimal extraction and centrifugation time

Extraction is a time-dependant process involving transferring of analytes from aqueous into RTIL phase. The extraction time, defined as the interval between addition of the mixture of methanol and RTIL and the moment the centrifugation process started, was evaluated in the range of 0.0–20 min. The recovery–time study showed that the highest extraction efficiency could be attained since 5.0 min and longer extraction times did not significantly improve Ni extraction. These results show that IL-DLLME is a very fast extraction process, as right after the cloudy solution was formed; the surface area between the RTIL droplet and the aqueous phase was very large, thus improving the diffusion of Ni– BTAHQ into the extractant. In order to achieve the highest extraction efficiency in the shortest time, extraction was performed during 5.0 min.

The effect of centrifugation time on Ni recovery was studied in the range of 5.0–25 min at 1500 rpm (377.2 g). The volume of the sedimented IL phase, and consequently recoveries, increased as the centrifugation time was extended up to 10 min. The analyte recovery remained constant for longer times, indicating total definition of RTIL phase at the bottom of centrifuge tube. A centrifugation time of 10 min was then selected.

Study on potential interfering species

In view of the high selectivity achieved for Ni–BTAHQ complex formation at pH 4.0, followed by extraction at pH 2.0, interference effects for our method could be mainly considered during the extraction/preconcentration step. Therefore, the selectivity of the proposed method was assayed by evaluating the individual effect of possible concomitant ions at the levels usually found in water and biological samples. The procedure was performed with 10 mL of 1.0 μg L^{-1} Ni solutions individually containing different concentrations of such ions. As shown in Table 1, quantitative separation and determination of Ni were obtained even when foreign ions were at higher concentrations than those normally found in the samples under study. Additionally, their contribution to the ionic strength of the system is insignificant and does not affect the extraction efficiency. Although cobalt react with BTAHQ,[44] it has no effect on Ni(II) determination under the optimum conditions of the proposed method.

Analytical performance

In order to evaluate the performance of the proposed method, three main parameters were employed, namely: extraction recovery, enrichment factor and consumptive index. Extraction recovery (ER) was defined as the percentage of total analyte which was extracted into the IL phase:

$$ER = mIL_{phase} / m_{aq} = CIL_{phase} \times VIL_{phase} / C_{aq} \times V_{aq} \times 100$$

where mIL_{phase} and m_{aq} are the mass of analyte in the final IL phase and the initial concentration in the sample solution, respectively. CIL_{phase} and C_{aq} are the concentration of the analyte in the IL phase and in the sample phase, respectively. VIL_{phase} and V_{aq} are the volumes of the phases involved.[45] Therefore, an extraction recovery of about 99.8 % was achieved when the procedure was developed under optimal experimental conditions (Table 1).

Likewise, the enrichment factor (EF) is defined as the ratio of the calibration curve slopes for Ni before and after the preconcentration step.[46] The obtained enrichment factor (EF) for a sample volume of 10 mL and a resulting RTIL phase in methanol volume of 50 μL was 200. The relative standard deviation (RSD) was 1.47% (Ni concentration: 1.0 μg L^{-1}, n = 10). The calibration graph was linear between 0.03 and 1.5 μg L^{-1}, with a correlation coefficient of 0.9992 (Table 2). The limit of detection (LOD), calculated based on the absorbance at intercept and three times the standard deviation about regression of the calibration curve[47], was 9.8 ng L^{-1} for the proposed methodology. Finally, the consumptive index (CI) can be defined for practical purposes as:

$$CI = V_s / EF$$

where V_s is the volume of sample (in milliliters) consumed to achieve the EF value.[48] The CI obtained for the proposed method was 0.05. Regarding the frequency of analysis, although the whole preconcentration procedure (metal complexation, extraction into the dispersed IL phase, and centrifugation) could take about 30 min, it is possible to simultaneously treat as many samples as can be placed in the centrifugation equipment. For our work, the frequency of analysis was at least 30 samples per hour.

Finally, a comparative study on analytical performance allows us to show the strengths of our method with respect to others reported in the literature. Our method presents a linear range and more sensitive that is comparable to, or better than other methodologies developed for Ni determination in biological and environmental samples (Table 3). A high enrichment factor was obtained with a reduced sample volume, yielding a low CI. Thus, CI reflects the efficiency of sample utilization, and it is useful tool for selecting a preconcentration method when sample amount is limited, such as the case of body fluid analysis.[48] All in all, the results indicate that the proposed method is a simple, fast, interference-free, selective and environment-

friendly analytical approach for trace Ni determination in biological and water samples. However, the major degree of sophistication, high cost, and limited frequency of analysis originated from its combination with SDME technique, could be prohibitive for application in routine analytical laboratories. On the contrary, IL-DLLME technique combined with spectrophotometric detection, presents high frequency of analysis, comparable and good limit of detection, with the advantage of using low cost and widely spread instrumentation.

Analytical characteristics

The relative standard deviation (RSD) and relative error for six replicate measurements of 1.0 μg L^{-1} of Ni was 1.47% and 2.14% and for 1.5 μg L^{-1} of Ni was 1.21% and 1.85%, respectively.

Determination of Ni in environmental, biological and standard reference materials samples

Nickel is commonly used in dental cast alloys, orthodontic wires and implantable orthopedic devices, releasing it into human tissue due to corrosion.[58] Since saliva is an easy-to-collect low-cost sample which is very useful for screening large populations,[59] it can be used for monitoring Ni released from orthopedic devices. However, a major challenge for detection of chemical contaminants in saliva is that concentrations are often 1 or 2 orders of magnitude lower than in blood.[60] On the other hand, blood and urine are proposed as biomarker of recent exposure to soluble Ni species.[61] However, urine is preferred for heavy metals monitoring due to non-invasive sampling and easier collection.[59] To best of our knowledge, there have been no reports demonstrating the viability of performing a RTIL-based microextraction technique for metal extraction from non-invasive biological samples such as saliva and urine. Only Xia et al.[62] applied an IL-LLME technique for metal extraction in human serum samples. Therefore, the results obtained after urine and saliva analysis

are summarized in Table 4. Furthermore, analyte recovery in the presence of biological matrix was studied. The proposed method was applied to six portions of both saliva and urine matrices and the average concentrations of Ni were taken as base values. Then, 1.0 μg L^{-1} Ni was added to samples and the same procedure was followed. The results obtained with the proposed method were in good agreement with those previously reported for urine samples,[63] while Ni recoveries were highly satisfactory for all cases.

The proposed method was applied to the determination of soluble Ni in tap and river water samples (Table 4). The recovery of Ni was between 98.8 and 102.5%. The Ni concentrations in river water samples were in the range of 0.45–0.57 μg L^{-1} and in tap water were in the range of 0.53–0.69 μg L^{-1}. Results were not significantly different to those previously reported in river and tap water samples.[64] Additionally, the accuracy of the proposed methodology was evaluated by analyzing a certified reference material (CRM) of natural water NIST SRM 1643e, with a Ni content of 27.06 ± 0.32 μg L^{-1}. This CRM contains several ions commonly present in natural water samples. Since the certified concentration value in the CRM was higher than the upper limit of the linear range achieved by this method, a dilution by a factor of 15 had to be implemented for analysis. Using the method developed in this work, the Ni content found in the CRM was 27.26 ± 0.83 μg L^{-1} (95% confidence interval; n = 6).

Aiming to demonstrate the usefulness of the proposed system a set of standard reference materials samples was analyzed. The system was run using the optimized parameters. The results of analysis are shown in Table 5. Accuracy was assessed by comparing results with these obtained using inductive coupled plasma optical emission spectrometry (ICP–AES). Applying the paired t-test no significant difference at 95% confidence level was observed.

Conclusions

A highly selective and rapid microextraction method based on [C_6mim][PF_6] RTIL for Ni determination was developed. The great potential that IL-based microextraction has for trace Ni determination, with the help of BTAHQ as a selective chelating reagent was demonstrated. The variation of pH is an effective way to eliminate possible interfering species that on other hand could form stable complexes with the organic reagent and would be co-extracted with the analyte. Thus, BTAHQ showed good tolerance to possible interferences caused by other co-existing metal ions, due to the high stability of Ni– BTAHQ complex at pH 2.0. the high selectivity of the proposed method was assayed by evaluating the individual effect of possible concomitant ions at the levels usually found in water and biological samples. This study indicates that IL-DLLME technique using [C_6mim][PF_6] and BTAHQ complexing reagent is a highly efficient (~100%) and green extraction technique for Ni separation and preconcentration, even from complex matrices like biological ones. An enrichment factor of 200 was obtained with only 10 mL of sample solution and under optimal experimental conditions. The resultant limit of detection (LOD) was 9.8 ng L^{-1}, while the relative standard deviation (RSD) was 1.47% (at 1.0 μg L^{-1} Ni level and n = 10. The accuracy of the proposed method was tested by analysis of a certified reference material. In fact, the preconcentration method was successfully applied for Ni determination in water, urine and saliva samples, with good accuracy and good reproducibility.

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Table 1 Effect of foreign ions on the recovery of Ni [a].

Ion	Added as	Concentration (mg L^{-1})	Ni recovery (%)
Ca^{2+}	$Ca(NO_3)_2$	4000	102
K^+	KNO_3	3600	102
Na^+	$NaNO_3$	3250	101
Mg^{2+}	$Mg(NO_3)_2$	2500	99.2
Mn^{2+}	$MnSO_4$	1700	101
Li^+	LiCl	1400	98.5
Al^{3+}	$AlCl_3$	1200	98.2
Co^{2+}	$Co(NO_3)_2$	1000	103
Hg^{2+}	$HgCl_2$	800	101
Cd^{2+}	$Cd(NO_3)_2$	650	101
Fe^{3+}	$FeCl_3$	500	98.7
Cu^{2+}	$Cu(NO_3)_2$	350	98.3
Zn^{2+}	$Zn(NO_3)_2$	200	104
NO_3^-	$NaNO_3$	4500	101
Cl^-	KCl	3000	100
PO_4^{3-}	NaH_2PO_4	2000	101
CH_3COO	CH_3COONa	4000	99.9
SO_4^{2-}	$MnSO_4$	1700	98.3

[a] This study was performed using 10 mL of 1.0 μg L^{-1} Ni standard

Table 2 Analytical figures of the proposed method

Parameters	IL-DLLME method	Before IL-DLLME
Amount of methanol	50 μL	---
Amount pH 4.0	50 μL	2.5 mL
Optimum [BTAHQ] 5×10^{-4} M	200 μL	2.0 mL
Reaction time (min)	10	5.0
Stirring time (min)	10	----
Beer's range (μg $L^{-1)}$	0.03 – 1.5	5.0 -100
Ringbom range (ng $L^{-1)}$	0.05 – 1.4	8.0 - 95
Molar absorptivity (L mol^{-1} cm^{-1})	3.47×10^7	3.56×10^3
Sandell sensitivity (ng cm^{-2})	0.0016	1.517
Regression equation[a]		
Intercept		
Slope (μg L^{-1})	6.25	0.66
intercept	0.004	- 0.011
Correlation coefficient (*r*)	0.9992	0.9978
RSD [a] (%)	1.47	2.65
Detection limits (ng L^{-1})	9.8	5000
Quantification limits (ng L^{-1})	31	15533
Enrichment factor	200	

[a] A = a + bC, where C is the concentration of nickel in μg L^{-1}.

Table 3 Comparison of the present method with other spectrophotometric methods

Reagent	pH	λ_{max} (nm)	ε ($\times 10^4$)	Beer's μg mL^{-1}	Remarks	Ref.
Biacetylbis (4-phenyl)-3-thiosemicarbazone	2.5	460	0.228	0.2–2.0	Very poor sensitivity	49
Salicylaldehyde thiosemi-carbazone	6.5–7.0	370	0.98	0.4–4.0	λ_{max} near UV and several metal ions interfere	50
2-oximinodimedone dithiosemicarbazone	5.8	440	0.72	1.0–6.0	Less sensitive	51
2,4dihydroxyacetopheneone thiosmicarbazone	7.0–8.0	385	0.82	1.0–8.0	λ_{max} near UV and very poor sensitivity	52
Phthalimidedithiosemi carbazone	10.0	440	1.13	0.1–3.2	Less sensitive	53
Pyrodoxal-4-phenyl-3-thiosemicarbazone	4.0–6.0	430	1.92	0.5–5.0	poor sensitive and selective	54
7-Methyl-2-chloro quinoline-3-carbaldehyde thiosemicarbazone	6.0	410	0.0167	4.0	Ce(III), Al(III), Ga(III) interfere and very poor sensitivity	55
2-Hydroxy-4-isopropoxy acetophenone thiosemicarbazone	9.0	400	0.084	16.44	Very poor sensitivity	56
Acenaphthanequinone mono thiosemicarbazone	6.3–8.4	420	0.285	14.7	Less sensitive	57
5-(2-benzothiazolylazo)-8-hydroxyquinolene	4.0	682	3472	0.03 1.5 μg L^{-1}	Highly sensitive and selective	This work

Table 4 Determination of nickel in water and biological samples by the proposed method

Sample	Boron (μg L^{-1})		T- test [b]	F- value [b]	ICP-AES (μg L^{-1})
	Added	Found [a]			
Potable water	--	1.40 ± 0.08		- -	1.4 ± 0.12
	0.5	1.94 ± 0.09	1. 83		
	1.0	2.37 ± 0.10		2. 67	
Well water -		3.30 ± 0.06			3.25 ± 0.15
	0.4	3.72 ± 0.04		3.32	
	0.8	4.12 ± 0.08	1.36		
River Nile water	--	2.55 ± 0.04			2.42 ± 0.20
	0.5	3.04 ± 0.10	1.73		
	1.0	3.56 ± 0.05		2.97	
Rain water	--	0.70 ± 0.03			0.71 ± 0.21
	0.5	1.18 ± 0.09		3.21	
	1.0	1.76 ± 0.08	1.25		
Mineral water	-	0.90 ± 0.11			34.5 ± 0.37
	0.4	1.31 ± 0.07	1.74		
	0.8	1.68 ± 2.61		3.33	
Tap water	--	1.50 ± 0.06			90.4 ± 0.21
	0.5	2.04 ± 0.09	1. 27		
	1.0	2.52 ± 0.12		2.48	
Sea water	--	17.8 ± 0.07			17.85 ± 0.18
	1.25	19.1 ± 0.09		2.47	
	2.50	20.3 ± 0.11	1. 43		
Saliva	--	0.22 ± 0.08			0.20 ± 0.30
	0.5	0.70 ± 0.10	1.30		
	1.0	1.25 ± 0.09		2.79	
Urine	--	0.55 ± 0.11			0.53 ± 0.25
	0.5	1.10 ± 0.05		2.43	
	1.0	1.52 ± 0.08	1.22		

[a] Average of four determinations ± standard deviation

[b] Theoretical values for t and F at 95% confidence limit (n = 5) were 2.57 and 5.05, respectively.

Table 5 Determination of nickel(II) in standard and certified reference materials[a]

Alloy sample	Composition (%)	Ni(II)[b] found %		RSD(%)		T-test[b]	F-value[b]
		PM	ICP-AES	PM	ICP-AES		
Alloy steel (BCS 233)	Ni, 11.22; [c]Co, 23.4; Sn, 7.95; Mn, 0.235; [c]Cu, 5.09	11.20	11.18	0.56	0.87	1.33	
Nickel base super alloy (CM 247 LC)	Ni, 61.91; Cr,8.1; Mo, 0.5; Al, 5.6; Ta, 3.2; Zr,0.015; C, 0.06; [c]Co, 9.0; W, 9.5; Ti, 0.7; Hf, 1.4; B, 0.025	61.90	61.88	0.57	0.89		2.95
Alloy steel (BCS 266)	Ni, 1.33; Al, 7.95; [c]Co, 23.4; [c]Cu, 3.33	13.28	13.260	0.46	0.80	1.18	
Nickel base super alloy (IN 718)	Ni, 54.9; Cr, 18; Mo, 3; [c]Fe, 19; [c]Co, 5.1	54.88	54.86	0.64	0.98		3.17
Low alloy steel (BCS 251)	Ni, 5.15;Mo,0.185; Mn, 0.165; [c]Co, 0.007; [c]Cu, 0.090	5.13	5.12	0.57	0.92	1.28	
Low alloy steel (BCS 253)	Ni, 2.92; Mo, 0.94; Cr, 0.34; V, 0.220; [c]Cu, 0.495	2.90	2.89	0.52	0.94		3.43

[a] No statistically significant differences were found between Ni(II) concentrations measured by ICP-AES method and the present method
[b] Average of six determinations.
[c]Masked with EDTA.
PM: Proposed method

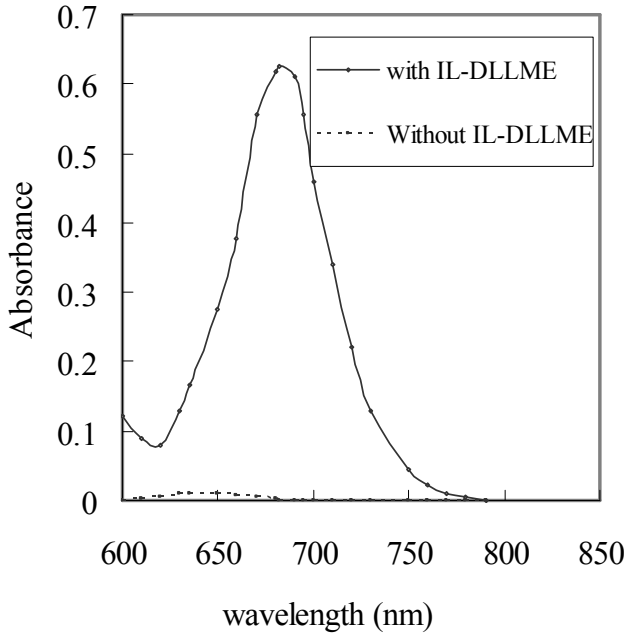

Fig. 1. Absorbtion spectra for 1.0 μg $L^{-1}$of Ni(II) complexed with 200 μL 5×10^{-4} M BTAHQ at the optimum conditions.

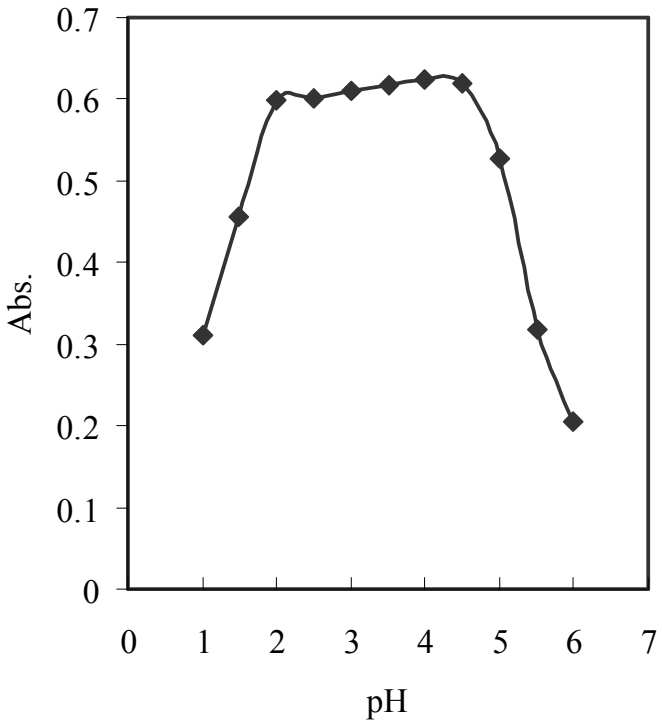

Fig. 2 Effect of pH on the complexation of 1.0 μg L^{-1} Ni(II) with 200 μL 5×10^{-4} M BTAHQ at the optimum conditions.

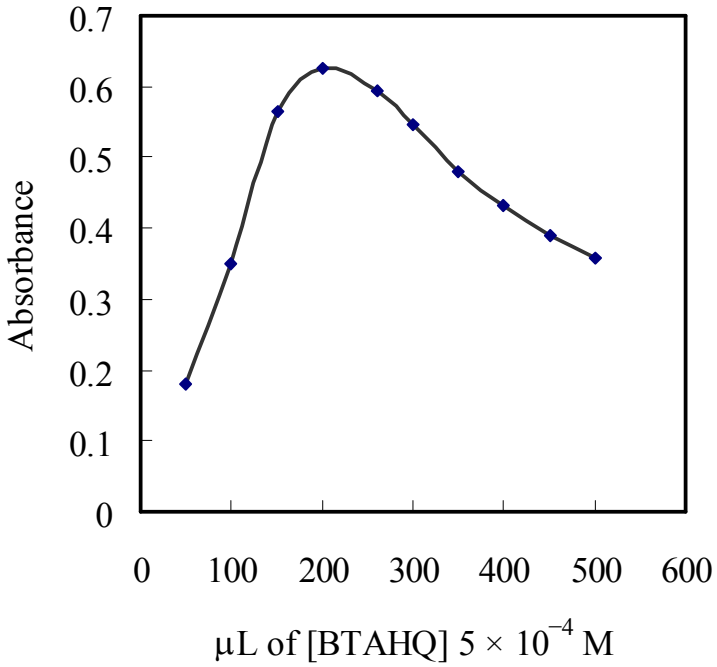

Fig. 3 Effect of [BTAHQ] on the complexation of 1.0 μg L^{-1} of Ni(II) using the optimum conditions

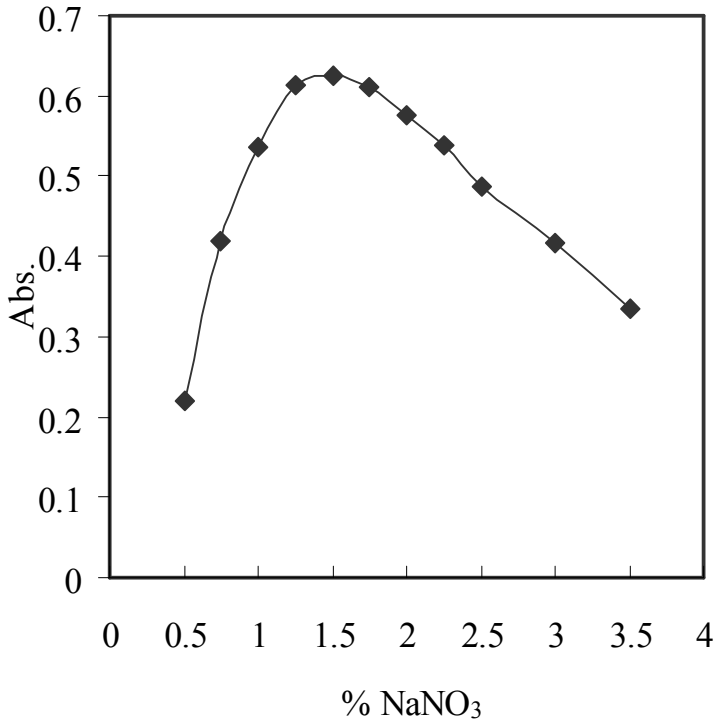

Fig. 4 Effect of $NaNO_3$ on the complexation of 1.0 µg L^{-1} Ni(II) with 200 µL 5×10^{-4} M BTAHQ at the optimum conditions.

Nickel was complexed with BTAHQ at pH 4.0. The IL-DLLME procedure was performed by using a few mL of $[C_6mim][PF_6]$ as extractant while methanol was the disperser solvent. The Ni-enriched RTIL phase was solubilized in methanol and directly measured the absorbance at λ_{max} 682 against a reagent blank similarly prepared.