Spectrophotometric Determination of Copper and Nickel in Marine Brown Algae after Perconcentration with Surfactant Assisted Dispersive Liquid-Liquid Microextraction

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ABSTRACT: *A simple, fast and effective pre-concentration method for the determination of Cu (copper) and Ni (nickel) was developed using a Surfactant Assisted Dispersive Liquid-Liquid Microextraction (SA-DLLME) coupled by UV-Vis spectrophotometry in marine brown algae. The complexing agent was 1- (2-pyridylazo) – 2 - naphthol (PAN). The effects of various experimental parameters in extraction step including type and volume of extraction solvent, type and amount of surfactant, ionic strength, extraction time, pH, amount of ligand and centrifugation time and rate were investigated. Under optimal experimental conditions, good linearity was observed in the range of 0.1-100.0 µg/L for analytes by limits of detection of 0.031 and 0.033 µg/L for Ni and Cu, respectively. The proposed procedure was used for the analysis Ni and Cu in marine brown algae. Good recoveries for spiked samples in the range of 100-118 % were obtained. Using this technique, metals content in algea from chabahar Bay (Southeast Iran) were detected in the range of 12.80- 39.46 µg/L.*

KEYWORDS *Copper; Nickel; Algea; Surfactant Assisted Dispersive Liquid-Liquid MicroExtraction (SA-DLLME); Chabahar bay.*

INTRODUCTION

Toxic pollutants, such as heavy metals originating from human activities, smelting processes, fuel combustion and industrialization are continuously introduced to water environment via rivers, waste dumping and Aeolian processes. Then they can be deposited in marine sediments. One of the largest problems associated to the persistence of heavy metals and their potential for bioaccumulation and bio magnification that resulted in potential long-term implications on human health and ecosystem [1-3].

Excessive Cu intake can cause nausea, vomiting, kidney failure blood cell damage, and central nervous system inhibition [4]. Ni is primarily accumulated in the spinal cord, brain and organs due to its mutability, carcinogenicity and is also a category one carcinogen [4]. Due to the toxicity, pollution degree, transportation, ability to bio accumulate of heavy metal (HMs) such as copper and nickel in water ecosystems [3,5-7], it is necessary to investigate the determination of heavy metal (e.g. Cu and Ni) in different real samples such as algea.

Owing to the complexity of sample matrix and very low concentration of these metals in algea, a pre-concentration step is often necessary before introduction of the samples into analytical instrument [8]. Numerous techniques have been employed to analyze of heavy metal, such as Flame Atomic Absorption Spectrometry (FAAS) [9,10], inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) [11], Graphite Furnace Atomic Absorption Spectrometry (GFAAS) [12, 13], chromatography [14], electrophoresis [15], voltammetry [16], spectrophotometry [17] and spectrofluorimetry [18].

Among these analytical methods, spectrophotometry is a relatively inexpensive and easy handling method with by good precision and accuracy of analysis, offers the practical and economic advantages over the other technique but it lacks the required selectivity and sensitivity for the determination of low level of analytes in the complex matrices [19-21]. However, a selective separation and pre-concentration step prior to determination of the compound with spectrophotometry is often required [22-25].

A number of methods were recently used for Cu^{+2} and Ni^{+2} pre-concentration, including dispersive liquid-liquid microextraction [26], ionic liquid based dispersive liquid- liquid microextraction [27,28], Solid Phase Extraction (SPE) [27], molecularly imprinted polymer coated stir bar [27], magnetic nanoparticles [29] and Cloud Point Extraction (CPE) [27,28].

Dispersive Liquid-Liquid MicroExtraction (DLLME) is a pre-concentration technique that employs a ternary system of solvents. This was reported for the first time in a technique for the determination of organophorus pesticides in water sample [30]. So, this method has also been widely applied for the pre-concentration of organic and inorganic analytes [31]. DLLME is based on the formation of the fine droplets of an extracting solvent in a sample solution when a water-miscible extractant (extraction solvent) dissolved in a water-miscible organic dispersive solvent rapidly injected into a sample solution. The compounds in the water sample are micro extracted into the fine droplets, which are further separated with centrifugation, and the concentrated analytes in the sedimented phase are determined by UV-Vis instrument. The advantages of the DLLME method are fast, simplicity of operation, low cost, green extraction procedure (the consumption of organic solvent is reduced to the

microliter) and high enrichment factor [32]. A surfactant-assisted DLLME (SA-DLLME) apply a surfactant to assist the dispersion of the extraction solvent. This method takes advantage of the particular properties of surfactants, mass-transfer and achieving better emulsification than traditional organic solvents, by the subsequent increase in recovery and reduction in extraction time [33]. SA-DLLME coupled with spectrophotometry has been used for determination of metal including nickel [34]. The aim and novelty of this paper is to develop a simple, rapid and sensitive surfactant assisted dispersive liquid-liquid microextraction (SA-DLLME) method coupled with UV-Vis for the determination of Cu and Ni in marine brown algae of Chabahar Bay. The effect of various parameters affecting the extraction step such as type and volume of extraction and surfactant, ionic strength, extraction time, pH, amount of ligand and centrifugation time and rate were also investigated.

EXPERIMENTAL SECTION
Materials

All chemicals in this research were of analytical grade and were applied without further purification. Nickel (II) nitrate hexahydrate, copper (II) acetate, sodium chloride (NaCl), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), carbon tetrachloride (CCl_4), trichloroethylene ($C_2H Cl_3$), tetrachloroethylene ($C_2 Cl_4$) were purchased from Merck KGaA (Darmstadt, Germany). Triton X-100, sodium dodecyl sulfate (SDS) and cetyl trimethyl ammonium bromide (CTAB) were obtained from sigma Aldrich. A standard solution of Cu and Ni (1000 mg/L) was prepared separately by dilution of stock solution by ultrapure water to get required concentration is the range 0.1-100.0 µg/L. A 0.20 mol/L PAN stock solution was prepared with diluting 0.5 mL of commercially available PAN solution to 25 mL by purified water. All the glassware were previously washed by acetone and finally dried in an oven at 100 °C.

Instrumental

Spectrophotometry was carried out on UNICO S2100 UV-Vis spectrophotometer (China) equipped by a 400 µL quartz microcell (model Q-01701, Starna Company, UK). A Centurion scientific K3 Series K241R centrifuge was applied to accelerate phase separation. A TPS WP-80 digital pH meter was used for pH adjustments (TPS Pty Ltd,
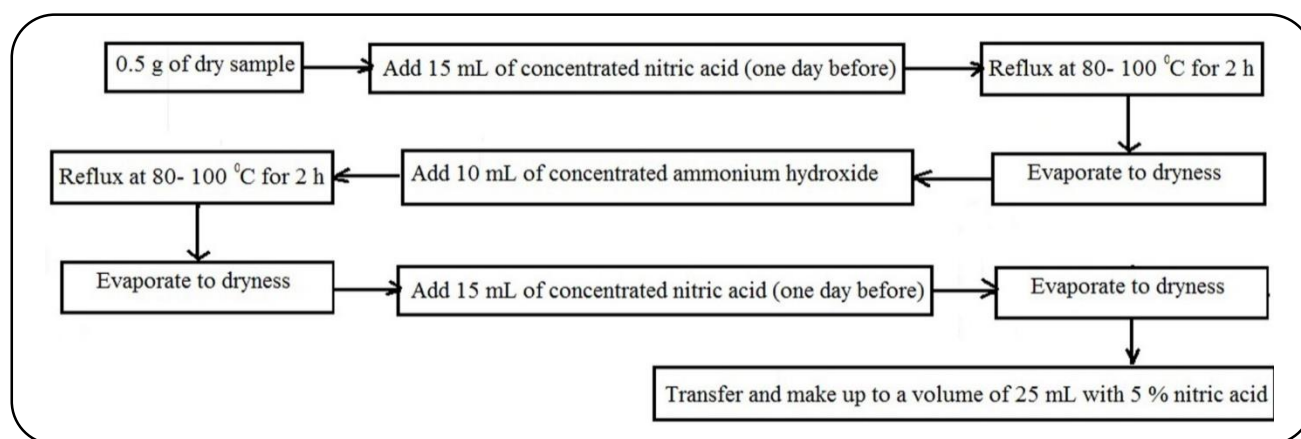
Fig. 1: Diagram of method proposed in the research, for extraction of metals in algal tissue.

Australia) and a 100 µL Hamilton syringe (Hamilton Company, NA, USA) was applied for phase separation of collected sediments. A model PARSONIC 15S Ultrasonic (Iran) was applied for shaken of solution.

Procedure

8.5 mL of water sample containing proper concentration of Cu and Ni was placed separately in 25 mL conic tube. Then, the mixture of 5 mg/L of surfactant (Triton X-100) and 200 µL of dichloromethane (as extraction solvent) was rapidly injected into the sample solution with syringe and make up to a volume of 10 mL by purified water. The mixture solution was gently shaken with ultrasonicated for 2 min for form a cloudy solution.

Then, the sedimentation of the solvent droplets in the bottom of the conical test tube was performed by centrifugation times of 10 min for Ni and 15 min for Cu and at rate of 4000 rpm for both analytes. Finally, 100 µL of the organic phase was removed with a micro syringe and transferred to a micro-cell for the determination by an UV-Vis spectrophotometer. Determination of the concentration of Cu and Ni were done spectrophotometrically against the blank in 460 and 580 nm. The blank was obtained in the same way but without the analytes. Fig. 1 is showed flow diagram of method proposed in the research, for extraction of metals in algal tissue [35].

RESULTS AND DISCUSSION
Absorption spectra of complex

In order to find the wavelength of maximum signals of the metal complexes, i.e. Cu- PAN and Ni- PAN,

the absorption spectra of each was determined in the wavelength range of 400 to 800 nm against the reagent blank. As can be seen in Fig. 2, maximum absorption wavelength for complexes of Cu- PAN and Ni- PAN were found to be 460 and 580 nm respectively. Hence, these wavelengths were selected as the absorption wavelengths for further experiments. During all of the following experiment, the blank absorbance of all reagents was corrected.

Optimization of the extraction parameters

The factors affecting on the extraction efficiency such as type and volume of the extracting solvent and surfactant, pH of the sample, amount of ligand, extraction time, ionic strength, centrifugation time and rate were investigated.

These parameters were studied carefully, and the optimal amounts were determined as are indicated in the following sections and used to the extraction of analytes.

Selection of extracting solvent

To establish a SA-DLLME method, it is necessary to select a proper extraction organic solvent. The selection of extracting solvent needs the following consideration such as lower/ higher density than water, low solubility in water, good affinity for target analytes and also it must be miscible in the dispersive solvents.

Chloroform, dichloromethane, carbon tetrachloride, trichloroethylene and tetrachloroethylene were examined as extraction solvents. A sample solution (the mixture of 500 µL of surfactant and 200 µL of extraction solvent) was applied for optimization of extracting solvent. The best
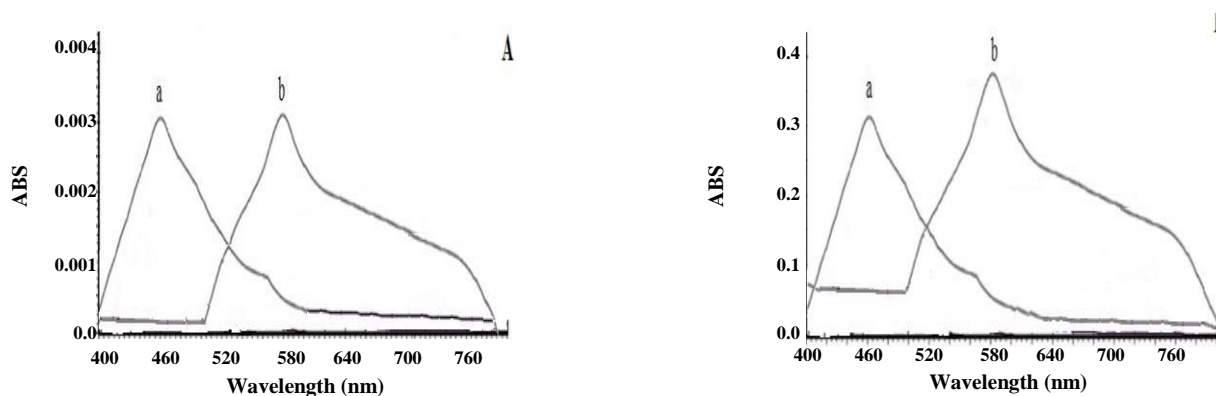
Fig. 2: Absorbance spectra for complexes of Cu- PAN (a) and Ni- PAN (b) before (A) after (B) extraction. Conditions: sample solution, 8.5 mL; extraction solvent, 200 µL; surfactant concentration, 5 mg/L; extraction time, 10 min for Cu and 5 min for Ni; centrifugation times of 10 min for Ni and 15 min for Cu and at rate of 4000 rpm; metal concentration, 100 µg/L; PAN concentration 200 µg/L; NaCl concentration, 12 mg/L for Cu and 15 mg/L for Ni and pH = 6.

absorbance was obtained with using dichloromethane as extracting solvent (Fig. 3). Because the PAN complexes of Zn and Ni are insoluble in water, good affinity of dichloromethane as non- polar solvent and low solubility in water for target analytes is characterized. So, dichloromethane was selected as extracting solvent for next experiments.

Selection of surfactant

For this technique, the surfactant should be miscible with the extraction solvent as well as the aqueous phase. Appropriate surfactant can disperse the extraction solvent to fine droplets in water sample and increases the absorbance for transferring the analyte compounds from sample matrix to extraction solvent. Several surfactants such as SDS, CTAB and triton X-100 were used to investigate their influence on the extraction efficiency. The experimental results showed that, among the surfactants tested, only triton X-100 had higher absorbance (Fig. 4). Therefore, it was selected as the surfactant.

Effect of extracting solvent volume

To investigate the effect of the volume of extracting solvent on the extraction efficiency, the solution containing different volumes of dichloromethane (100, 150, 200, 250 and 300 µL) were subjected to the SA-DLLME method. By increasing the volume of dichloromethane, the recovery was first increased to 200 µL for Cu and Ni. At higher volumes of dichloromethane due to increasing of sedimented phase volume and dilution of the HMs, efficiency extractions of the analytes

were decreased (Fig. 5). So, 200 µL for Cu and Ni of dichloromethane were applied in subsequent experiments.

Effect of surfactant concentration

The concentration of surfactant is also an important parameter that has significant effect on the recovery of the HMs. To determine the optimal concentration of triton X-100, additional experiments were performed by 200 µL of dichloromethane containing various concentration of triton X-100 (i.e. 1-6 mg/L). With increasing the surfactant concentration from 1 to 5 mg/L, the extraction efficiency increased, but when the concentration of triton X-100 exceeded from 5 mg/L for Cu and Ni, the recovery decreased. Thereby, the concentration of 5 mg/L of triton X-100 was chosen as the optimum volume of surfactant for further work (Fig. 6).

Effect of pH

A critical parameter for effective formation of the metal- PAN complexes in this research is pH. Because when the pH is low, the pridyl structure of PAN is protonated and while is high, the hydroxyl ions can compete by PAN for interacting by metal ions [36]. According to Fig. 7, the absorbance intensity is affected with the variation of the solution pH in the range 2.0-8.0. Therefore, it is clear that both sensitivity and selectivity depend on the established pH value. The results show that the best pHs for extraction of the metal ions are 6.0. So, pH= 6.0 were selected as the optimum value for further experiments.
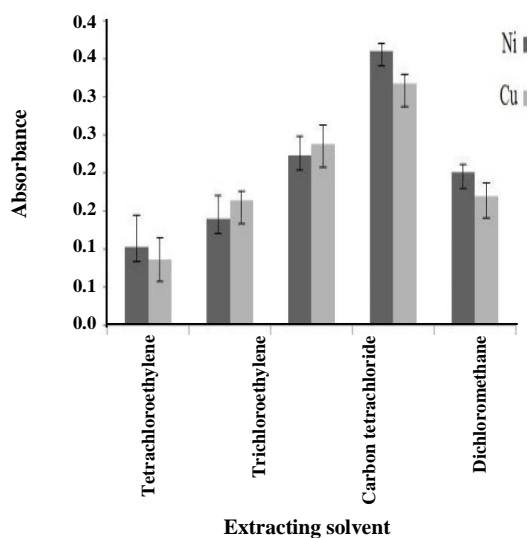
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***Fig. 3: Effects of extracting solvent on the metal extraction.
Conditions: sample solution, 8.5 mL; extraction solvent, 200 μL;
surfactant concentration, 5 mg/L; extraction time, 10 min
for Cu and 5 min for Ni; centrifugation times of 10 min for Ni
and 15 min for Cu and at rate of 4000 rpm; metal
concentration, 100 μg/L; PAN concentration 200 μg/L; NaCl
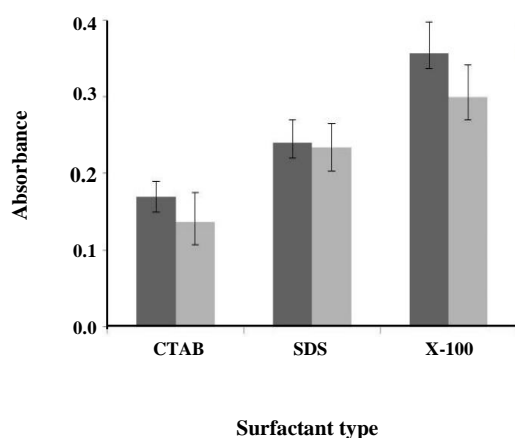concentration, 12 mg/L for Cu and 15 mg/L for Ni and pH = 6.***

Fig. 4: Effects of surfactant type on the metal extraction.

Effect of amount of PAN

In order to study the optimum amount of ligand (PAN)
on the quantitative extraction of Cu and Ni with
SA-DLLME, ion extraction was conducted with varying
the [ligand]/ [metal] mole ratio from 1 to 3. The extraction
of analytes was found to be quantitative with [ligand]/ [metal]
mole ratios greater than 2. So, subsequent experiments
were performed by [PAN]/ [metal] ratio of 2.

Effect of extraction time

The effect of the extraction time upon extraction
efficiency was investigated. The results demonstrate that
the absorbance intensity was constant after 10 min for Cu
and 5 min for Ni. Hence, 10 min for Cu and 5 min for Ni
were selected for extraction time.

Effect of ionic strength

The effect of ionic strength on the absorbance intensity
was studied with adding different concentrations of NaCl
(2-20 mg/L). The results indicated that the extraction
process was improved due to salting out effect at 12 mg/L
of NaCl for Cu and 15 mg/L for Ni and then decreased
at higher concentration. So, 12 mg/L for Cu and 15 mg/L
for Ni of NaCl concentration were used for the subsequent
experimental work.

Effect of centrifugation time and rate

The effect of centrifugation rate and time on the
absorbance was investigated in the range of 1000-5000 rpm
and 5-20 min, respectively. A centrifugation time of
10 min for Ni and 15 min for Cu and 4000 rpm
as centrifugation rate were selected for the subsequent
experimental work; since complete phase separation
occurred at the end of this period, while at lower or higher
centrifuge time and rate, the recovery were both lower.

Analytical performance
*Linear range, limit of detection, limit of quantification,
enrichment factors*

Under the selected optimum experimental condition, the
calibration curve was linear over a concentration range of
0.1-100.0 μg/L for analytes. The least square
equation over the dynamic linear range is showed in Table 1.
The Limit of Detection (LOD) and Limit of Quantification (LOQ)
obtained, respectively, from $3\ S_d$ (blank) and $10\ S_d$ (blank)
(where S_d is the standard deviation of 7 consecutive
measurements of the blank) [37]. The LOD were found 0.031
for Ni and 0.033 for Cu and the LOQ were 0.1 for Ni and 0.11
for Cu. The enrichment factors (EFs) and recoveries
were obtained with Eqs. (1) and (2), respectively [38].

$$EF = C_{org}/ C_{aq} \qquad (1)$$

$$R = (C_{org} \times V_{org}) / (C_{aq} \times V_{aq}) \times 100\% = \qquad (2)$$
$$EF \times (V_{org} / V_{aq}) \times 100\%$$

Table 1: Analytical figure of merit for DLLME coupled by UV for the determination of Cu^{+2} and Ni^{+2}.

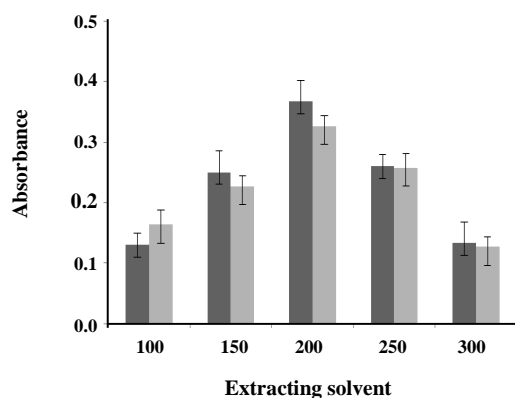| Metal	Linear range (µg/L)	Equation of calibration curve[a]	R^2	LOD (µg/L)	LOQ (µg/L)	EF
Ni (II)	0.1-100.0	Y= 0.0033x + 0.048	0.9993	0.031	0.10	119
Cu (II)	0.1-100.0	Y= 0.003x + 0.03916	0.9976	0.033	0.11	104

Fig. 5: Effects of extracting solvent volume on the metal extraction. Conditions: sample solution, 8.5 mL; triton X-100 concentration, 5 mg/L; extraction time, 10 min for Cu and 5 min for Ni; centrifugation times of 10 min for Ni and 15 min for Cu and at rate of 4000 rpm; metal concentration, 100 µg/L; PAN concentration 200 µg/L; NaCl concentration, 12 mg/L for Cu and 15 mg/L for Ni and pH = 6.
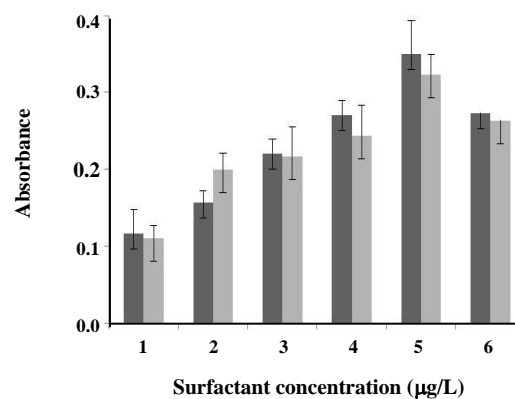

Fig. 6: Effects of surfactant concentration on the metal extraction. Conditions: sample solution, 8.5 mL; extraction solvent, 200 µL; extraction time, 10 min for Cu and 5 min for Ni; centrifugation times of 10 min for Ni and 15 min for Cu and at rate of 4000 rpm; metal concentration, 100 µg/L; PAN concentration 200 µg/L; NaCl concentration, 12 mg/L for Cu and 15 mg/L for Ni and pH = 6.

Where EF, R, C_{aq}, C_{org}, V_{org} and V_{aq} are the enrichment factor, recovery, initial concentration of analyte in the sample, sediment phase (extraction solvent volume) and water sample volume, respectively. As shown in Table 1, the enrichment factors were calculated 104- fold for Cu, 119-fold for Ni.

In Table 2, the proposed research compared with other extraction methods reported in the literatures for metal determination.

Interference study

The potential interference in the present research was studied. The interference was due to the competition of other heavy metal ions for the chelating agent and their subsequent co- extraction by Cu^{+2} and Ni^{+2}. In these experiments, solutions containing 100 µg/L of Cu^{+2} and Ni^{+2} separately, and the interfering ions such as Pb^{+2} and Zn^{+2} were treated according to the recommended method. The limits of tolerance of the co- existing ions, defined as the largest amount making efficiency extraction of Cu^{+2}

and Ni^{+2} less than 90 %. The results indicated that there was no significant interference.

Determination of metal in algae

In order to assess the applicability of the proposed technique in real samples, it was applied to the extraction and separation of Cu^{+2} and Ni^{+2} from 10 mL of different samples taken from algae that were also spiked separately at the concentration level of 100 µg/L with HMs. The recoveries for algae are also presented in Table 3. The recoveries were obtained from 90 to 118. These results indicate that the developed method can be successfully used for the determination of HMs in very complicated matrices such as algae. During all of the following experiment, the blank absorbance of all reagents was corrected. A reagent blank spectrum was subtracted separately from spectra of these solutions of Ni $(PAN)_2$ and Cu $(PAN)_2$. The spectra were then normalized to a concentration of 50 µg/ L for each metal ion in real sample.

Table 2: Comparative data of analytical characteristics of present DLLME-UV-Vis for Cu^{+2} and Ni^{+2} with other preconcentration methods.

metal	metods	Dynamic range (µg/L)	LOD (µg/L)	techniqe	Ref.
Cu^{+2}	Magnetic nanoparticles	10-200	0.90	ICP-OES	27
	DLLME	20-90	5	UV-Vis	28
	DLLME	50-2000	3	FAAS	39
	SA-DLLME	0.1-100.0	0.033	UV-Vis	This work
Ni^{+2}	SPE	10-100	4.6	FAAS	29
	CPE	10-65	0.92	FAAS	40
	DLLME	10-50	0.04	ETAAS	41
	SA-DLLME	0.1-100.0	0.031	UV-Vis	This work

Table 3: Recovery results for determination of HMs in various algae.

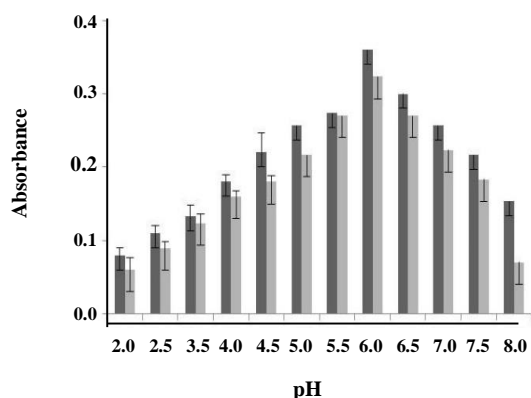| alga	metal	HMs added (µg/L)	Found (µg/L)	Recovery
Nizamuddinia zanardini	Cu(II)	-	39.46	
		100	142.80	103
	Ni (II)	-	35.24	
		100	138.27	100
Padina gymnospora	Cu(II)	-	12.80	
		100	129.46	117
	Ni(II)	-	21.21	
		100	124.24	103
Ulva rigida	Cu(II)	-	22.80	
		100	126.13	103
	Ni(II)	-	24.24	
		100	142.42	118

Fig. 7: Effects of pH on the metal extraction. Conditions: sample solution, 8.5 mL; extraction solvent, 200 µL; surfactant, 5 mg/L; extraction time, 10 min for Cu and 5 min for Ni; centrifugation times of 10 min for Ni and 15 min for Cu and at rate of 4000 rpm; metal concentration, 100 µg/L; PAN concentration 200 µg/L; and NaCl concentration, 12 mg/L for Cu and 15 mg/L for Ni.

CONCLUSIONS

In this work, a surfactant assisted dispersive liquid-liquid microextraction (SA-DLLME) coupled by UV-Vis spectrophotometry for the detection of HMs in algae has been evaluated. The linear ranges had a wide concentration and SA-DLLME could use selectively for determination of HMs even at the low concentration of 100 µg/L. This technique is sensitive, inexpensive, simple and fast and requires only to a small volume of organic solvents. This can be applied for very complicated matrices as algae.

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