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Determination of trace labile copper in environmental waters by magnetic nanoparticle solid phase extraction and high-performance chelation ion chromatography

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ABSTRACT

Cobalt magnetic nanoparticles surface functionalised with iminodiacetic acid were evaluated as a nanoparticulate solid phase extraction absorbent for copper ions (Cu^{2+}) from environmental water samples. Using an external magnetic field, the collector nanoparticles could be separated from the aqueous phase, and adsorbed ions simply decomplexed using dilute HNO_3. Effects of pH, buffer concentration, sample and sorbent volume, extraction equilibrium time, and interfering ion concentration on extraction efficiency were investigated. Optimal conditions were then applied to the extraction of Cu^{2+} ions from natural water samples, prior to their quantitation using high-performance chelation ion chromatography. The limits of detection (LOD) of the combined extraction and chromatographic method were ~0.1 ng ml^{-1}, based upon a 100-fold preconcentration factor (chromatographic performance; LOD=9.2 ng ml^{-1} Cu^{2+}), analytical linear range from 20 to 5000 ng mL^{-1}, and relative standard deviations=4.9% (c=1000 ng ml^{-1}, n=7). Accuracy and precision of the combined approach was verified using a certified reference standard estuarine water sample (SLEW-2) and comparison of sample determinations with sector field inductively coupled plasma mass spectrometry. Recoveries from the addition of Cu^{2+} to impacted estuarine and rain water samples were 103.5% and 108.5%, respectively. Coastal seawater samples, both with and without prior UV irradiation and dissolved organic matter removal were also investigated using the new methodology. The effect of DOM concentration on copper availability was demonstrated.

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1. Introduction

Copper (Cu) is an essential micronutrient required for a wide variety of physiological processes [1]. However, like many such essential elements, Cu is also considered potentially toxic when present at elevated concentrations [2]. In natural waters excess Cu may prove toxic to sensitive aquatic organisms, or if present in potable waters, have an impact not only on taste, but also human health. Copper overabundance can negatively affect plant chloroplast function, leading to the formation of reactive oxygen species, which can result in reduced biomass and altered nutrient content [3–5]. It has also been reported, for *Lemna minor*, a common aquatic plant species, that elevated Cu can negatively affect the uptake of other essential metals, growth, and pigmentation [6]. In addition, a wealth of studies have demonstrated that environmentally relevant concentrations of Cu can be toxic to fresh water snails and other aquatic organisms [7]. Furthermore, excessive ingestion of inorganic Cu from drinking water and Zn deficiency have recently been suggested as contributing factors in cognitive loss associated with Alzheimer's disease [8].

Consequently, there remains an interest in reliable analytical methods for Cu (and indeed other such transition metal ions) in natural and potable water samples, for reasons of both environmental control and public health. Atomic absorption spectroscopy (AAS), and inductively coupled plasma based methods (optical emission spectroscopy (ICP-OES), and mass spectrometry (ICP-MS)), are common instrumental approaches currently applied to elemental analysis. However, excluding high resolution instruments, most atomic spectroscopic methods are prone to significant interferences when dealing with complex sample matrices, particularly samples of high ionic strength, and those with excess concentrations of alkali and alkaline earth salts (e.g. estuarine waters) [9]. Sample dilution is often required, or a process of matrix removal/elimination employed prior to ICP analysis [10].

As an alternative to atomic spectroscopic methods, high-performance chelation ion chromatography (HPCIC) was specifically

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developed to analyse high ionic strength samples for trace levels of transition and heavy metal cations [11–12]. The chelating ion-exchange phases employed within HPCIC exhibit cation selectivity resultant from the formation and dissociation of complexes between metals ions and an immobilised chelating functional group on the surface of the stationary phase. In most cases alkali metal cations are very weakly retained, and alkaline earth metals moderately so, under acidic conditions [13,14]. Divalent and trivalent transition metal ions are strongly retained and so can be selectively separated. However, despite attractive selectivity, the sensitivity (and detection limits) of most published HPCIC methods are insufficient for the direct determination of most transition metals in non-polluted natural waters. For example, recently, a HPCIC method for the direct determination of trace transition metals in fuel ethanol was developed, and the LOD for Cu^{2+} was quoted as 7.4 ng ml^{-1} [15]. Although this LOD for Cu^{2+} is relatively low, it is still higher than dissolved Cu concentrations typically found in clean water bodies, which are generally <5 nM [16].

Thus, for most of the above mentioned analytical approaches, some preliminary solute preconcentration and matrix elimination is often required. Solid-phase extraction (SPE) is commonly used, typically applying chelating resins for trace metals [17,18]. However, standard SPE can be rather time consuming and demand high volumes of both sample and eluents. More recently, functionalised magnetic nanoparticles have been employed for selective extraction, in what has now been termed magnetic solid-phase extraction (MSPE) [19,20]. These magnetic extraction phases take various forms, and have been successfully applied to the preconcentration of both inorganic and organic target solutes [21–22]. The process itself sees the addition of the 'collector phase' of functionalised nanoparticles directly to the sample solution, and then uses the application of an external magnetic field to physically concentrate the magnetic nanoparticles, providing a means for the efficient and rapid separation of sorbed solutes (e.g. metal ions) from the matrix, without additional centrifugation or filtration steps. To-date MSPE, using nanoparticles functionalised with metal ion chelating groups, has been successfully applied to the preconcentration of trace transition and heavy metal ions from biological and environmental samples prior to their determination by AAS [23], ICP-AES [24], and ICP-MS [25]. However, a disadvantage of this procedure is that the magnetic nanoparticle itself, typically based upon functionalised iron oxide nanoparticles, can also contaminate the sample (e.g. with relatively high concentrations of Fe^{3+}), particularly as strong acid eluents are used to liberate complexed target metal cations, thus causing partial dissolution of the core nanoparticle. To-date this issue has restricted the application of iron oxide based magnetic chelating nanoparticles in combination with HPCIC, as the excess Fe^{3+} in the concentrated extract is strongly adsorbed upon the HPCIC chelating column, rapidly fouling the column and requiring strong acid washes to elute the Fe^{3+} between sample assays [26].

Recently a new chelating magnetic nanoparticle has become available, based upon iminodiacetic acid (IDA) functionalised cobalt nanoparticles. These magnetic cobalt nanoparticles are coated with a thin ($\sim$2 nm) layer of graphitic carbon, upon which the IDA groups are covalently attached. These cobalt based particles exhibit greater compatibility with cation exchange and chelating ion-exchange chromatography columns, such as those used within HPCIC, as Co^{2+} contamination emanating from nanoparticles during extraction can be more easily pre-eluted from the analytical column under less acidic conditions (than is the case for Fe^{3+}). Therefore in the current study, magnetic cobalt nanoparticles functionalised with IDA were investigated as a magnetic solid phase extraction absorbent for the concentration of Cu^{2+} ions from environmental waters prior to their quantitation using HPCIC. The effects of sample pH, the volume and pH of desorption solution used, the amounts of magnetic sorbent and sample volume, adsorption equilibrium time, and the concentration of potentially interfering ions, and dissolved organic matter on the extraction efficiency, have each been investigated in detail. The method was then successfully applied to the determination of Cu^{2+} in natural waters, including coastal seawater, and method recovery and accuracy was confirmed using sector field inductively coupled plasma-mass spectrometry (ICP-MS).

2. Experimental

2.1. Instrumentation

The chelation ion chromatography system used for sample quantitation was based upon a modular Dionex ion chromatograph, comprised of an AS25 absorbance detector, IP25 pump, AS50 autosampler, and AS50 column heater (Dionex, Thermo Scientific, Sunnyvale, USA). Post-column reaction detection was achieved using 4-(2-pyridylazo) resorcinol (PAR) as the post-column reagent (PCR), delivered by peristaltic post-column pump. The detection wavelength employed was 510 nm. Chromeleon software (Dionex, Thermo Fisher Scientific, Sunnyvale, CA, USA) was used for data acquisition and processing of chromatograms. A PEEK 150 mm length and 4.0 mm I.D. column packed with 5 μm spherical IDA bonded silica particles was purchased from JPP Chromatography (Plymouth, UK).

A sector field inductively coupled plasma mass spectrometer (ICP-MS, Thermo Fisher Element 2, Bremen, Germany), equipped with a CETAC autosampler (ASX-500, Ohmaha, USA) was employed for the determination of total Cu and other matrix elements in sample extracts. Multiple spectral resolutions were used to help separate signals of interest from potentially overlapping polyatomic interferences [9,27]. The method of external calibration was used for quantification, using a series of standards prepared from commercially available premixed solutions (QCD Analysts, Environmental Science Solutions, Spring Lake, USA). Indium (High Purity Standards, Charleston, USA) was added as an internal standard to all samples and standards at a final concentration of 100 μg l^{-1}. Calibration accuracy was verified via the analysis of independent NIST 1640 "Trace Elements in Natural Water" SRM (Gaithersburg, MD, USA).

2.2. Reagents and solutions

Stock solutions of Cu^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, and Zn^{2+} (1 mg ml^{-1}) were prepared from the following salts; $Cu(NO_3)_2 \cdot 2H_2O$ (Ajax chemicals Ltd., Sydney, Australia), $Cd(NO_3)_2 \cdot 4H_2O$, $Pb(NO_3)_2$, $MnCl_2 \cdot 4H_2O$ (BDH, Poole, UK), and $Zn(NO_3)_2 \cdot 6H_2O$ (May and Baker, London, UK), prepared in diluted HNO_3. Calibration and test solutions were prepared by stepwise dilution of their stock solutions. Magnetic cobalt nanoparticles functionalised with IDA were purchased from Turbobeads (Zurich, Switzerland), marketed as Turbobeads Complexon. The magnetic nanoparticles exhibited diameters of below 50 nm, with the surface of the particles covalently functionalised with IDA (>0.1 mmol/g, immobilised IDA).

The magnetic nanoparticles were purified and regenerated by the following procedure: The nanoparticles were first washed with 4 M HNO_3 to remove adsorbed metal ions, then with several deionised water washes, followed by 50 mM NaOH, and further deionised water, until the pH value of the suspension was in range 7–8. All other chemicals were of analytical reagent or higher grade. Deionised water from a Milli-Q water supply (Millipore, Bedford, MA, USA) was used throughout this work.

Eluents were prepared from KNO_3 (BDH, Poole, UK), and 2-pyridinecarboxylic acid (picolinic acid) (Sigma, St. Louis, USA), and acidified using dilute HNO_3. The post-column reagent was 0.15 mM PAR (Kodak, Rochester, NY, USA), prepared in 0.4 M ammonia, and

adjusted to pH 10.5 with HNO_3. All eluents were filtered before use through a 0.22 μm nylon membrane filter and degassed through sonication.

2.3. Magnetic solid phase extraction procedure

Test solutions containing 1 μg ml^{-1} Cu^{2+} were adjusted to the desired pH value using sodium acetate/nitric acid (pH 2–3) and sodium acetate/acetic acid solutions (pH 4–8). 30 mg of the IDA functionalised nanoparticles were added into test solutions and the mixtures dispersed by ultrasonication. The magnetic nanoparticles were then magnetically separated and transferred to the dilute HNO_3 solution for release of the surface complexed metal ions. After desorption, the magnetic cobalt nanoparticles were again magnetically separated. The pH of the extract was adjusted to between 2 and 3 using dilute NaOH, (added gravimetrically to provide an accurate dilution factor), and the extract then analysed using HPCIC. All sample extracts were filtered with 0.22 μm syringe filters.

2.4. Environmental samples and sample preparation

Rain and estuarine river water samples were collected from the Derwent River estuary, Sandy Bay, Hobart, Tasmania. Industrial activity has severely impacted the quality of Derwent River waters and sediments over the past 100 years [27–30]. Samples were collected following recommended sampling procedures [31]. Immediately after sampling, the water samples were filtered with 0.22 μm syringe filters and stored in acid washed polypropylene bottles in a refrigerator at 4 °C. Under final optimised conditions, 200 ml aliquots of water samples were used for extraction experiments. Deionised water was employed as the blank solution and subjected to the exact procedures as the environmental waters. The accuracy of the developed method was assessed via the analysis of an estuarine water certified reference material for trace metals, SLEW-2 (National Research Council of Canada (NRCC)). The CRM sample was adjusted to the desired pH prior to analysis. Water samples were also analysed directly by ICP-MS to further verify accuracy and recovery of the combined MSPE–HPCIC method.

Coastal seawater samples were collected from the Tasman Peninsula, Tasmania, acidified, filtered and stored according to established procedures [31]. Prior to analysis samples were UV irradiated using a UV Spectrolinker XL-1500 (Spectronics, Westbury, NY) for 30 min at 254 nm. The UV light intensity was 3 mW cm^{-2}. The depth of sample irradiated was ~1 cm.

Dissolved organic matter (DOM) was isolated from samples as described by Dittmar et al. (2008) [32]. Briefly, the water was filtered through Nucleopore (Agilent, Mulgrave, VIC, Australia) polycarbonate filter cartridges (3 μm, 1 μm and 0.20 μm pore sizes, sequentially) and prewashed glass microfiber Whatman GF/F filters (0.20 μm pore size, Agilent, Mulgrave, VIC, Australia) and acidified with 32% HCl to pH 2. The water was then passed through Varian Bond Elut PPL poly(styrene divinylbenzene) (PS-DVB) surface functionalised (proprietary non-polar) solid phase extraction cartridges (1 g, 60 mL) (Agilent, Mulgrave, VIC, Australia).

Artificial estuarine water samples were prepared using ultrapure water and 8.5 g l^{-1} NaCl, with DOM added either 5, 15 or 30 mg l^{-1} concentrations (Suwannee River Natural Organic Matter, International Humic Substances Society).

3. Results and discussion

3.1. Purification and regeneration of IDA nanoparticles

The stability of the IDA functionalised cobalt nanoparticles under acidic conditions required for release of complexed metal ions is of crucial importance. To assess this stability and capacity to regenerate the nanoparticle sorbent, 1 ml volumes of HNO_3 solutions, ranging in concentration from 5 to 500 mM, were each used as wash solutions, with 30 mg of the IDA nanoparticles, for 4 complete blank extraction–desorption cycles. The dissolution/release of Co^{2+} from the nanoparticles is shown in Fig. 1(A). Predictably, within the first cycle, the Co^{2+} concentrations in the final extract increased with acidity of the desorption solution. When 500 mM HNO_3 was used as the desorption solution, the concentrations of Co^{2+} determined in the extract were 461, 26.0, 4.9 and 5.0 μg ml^{-1}, for the first, second, third and fourth cycles, respectively. However, for the lower strength desorption solutions, namely 5, 10, and 20 mM HNO_3, the reduction in Co^{2+} released between the first and subsequent cycles was less dramatic, and significant Co^{2+} was still observed following the fourth cycle. Using 50 and 500 mM HNO_3 solutions, similar concentrations of Co^{2+} in the extracts were obtained after the fourth and third adsorption-desorption wash cycle, respectively.

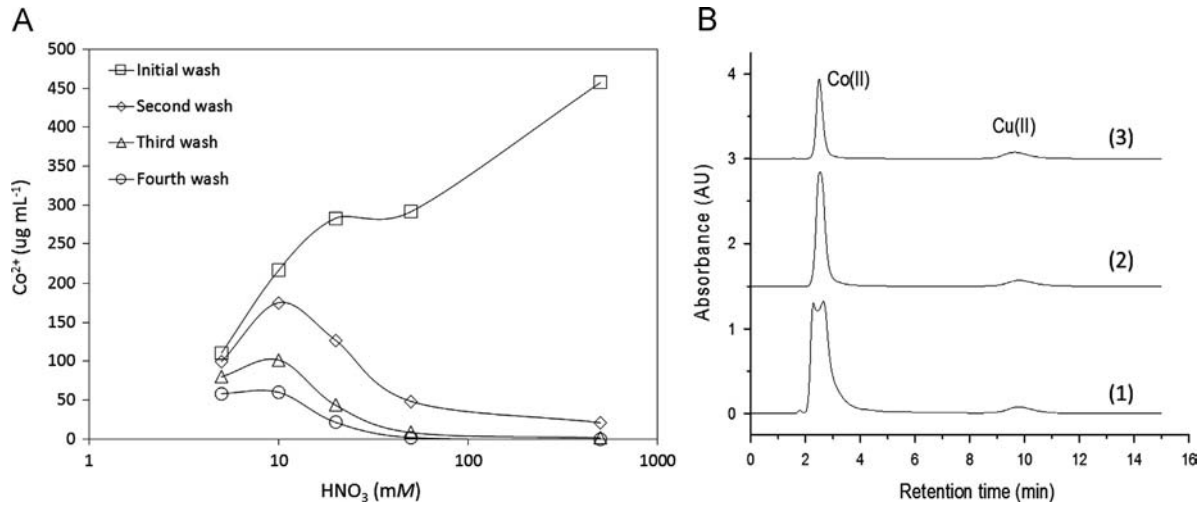

Fig. 1. (A) Release of Co^{2+} ions from cobalt-IDA magnetic nanoparticles during adsorption-desorption cycles using dilute HNO_3 solutions. (B) HPCIC chromatograms of Co^{2+} and Cu^{2+} (concentration = 5 μg ml^{-1}) in extracts using cobalt–IDA magnetic nanoparticles (1) without pre-purification, (2) after single purification procedure, and (3) after 20 purification (regeneration) and extraction cycles.

These results highlight the necessity to adequately prepare the IDA-nanoparticles prior to their application to real samples, to avoid excessive contamination by released Co^{2+}. However, it was also necessary to determine that the nanoparticles still maintained complexing capacity after 500 mM HNO_3 preparation and regeneration steps. Therefore, the magnetic IDA nanoparticles were applied to the extraction and desorption process (under optimal extraction conditions – see following sections) of a standard Cu^{2+} solution (concentration = 5 μg ml^{-1}), firstly without any prior purification cycle, then following a single wash and regeneration cycle using 500 mM HNO_3, and finally following 20 repeat combined extraction, acid desorption and regeneration cycles. Fig. 1(B) shows the resultant HPCIC chromatograms of the final extracts in each case. The calculated recovery of Cu^{2+} was found to be 93.2%, 94.6%, and 102.2%, respectively, demonstrating not only stability of the magnetic sorbent after numerous acid wash and regeneration cycles, but that recovery for target metal ions actually improved as the concentration of released Co^{2+} was reduced.

3.2. Optimisation of extraction conditions

To investigate the effect of pH on the retention of Cu^{2+} on the IDA functionalised nanoparticles, 10 ml aliquots of sample solution containing 1 μg ml^{-1} Cu^{2+} were adjusted to various pH values between 2 and 8, and then subjected to the general extraction protocol described within Section 2. The results of these scoping experiments are shown in Fig. 2(A). The extraction efficiency (expressed as percentage adsorption) for Cu^{2+} increased with pH from 2.0 to 7.0, with full quantitative extraction observed within the pH range of 7–8. These results are typical for IDA functionalised sorbents, as the pKa^1, pKa^2, and pKa^3 values for IDA are 1.77, 2.62, and 9.34, respectively [33]. When the pH value is lower than 1.77, IDA exists as the fully protonated cationic $NH_2^+(CH_2COOH)_2$ form. In the pH range of 1.77–2.62, IDA is present as its zwitterionic form, $NH_2^+(CH_2COOH)CH_2COO^-$, and in the pH range of 2.62–9.34, the $NH_2^+(CH_2COO^-)_2$ IDA anion dominates. These dissociation constants are responsible for the typical pH effects observed. Accordingly, pH 7.5 was selected for use in subsequent experiments and for real sample analysis.

The complete recovery of complexed Cu^{2+} was investigated using HNO_3 desorption solutions over the range of 0.001–4.0 mol l^{-1}. These results are shown in Fig. 2(B). Significantly it was observed that desorption using 4.0 mol l^{-1} HNO_3 was required for quantitative recovery of complexed Cu^{2+} from the magnetic IDA nanoparticles.

The effect of elution volume on desorption of Cu^{2+} was also investigated (Fig. 2(C)). The complexed Cu^{2+} was released with different volumes (0.5–3 mL) of 4.0 mol l^{-1} HNO_3, and it was observed that as little as 0.5 ml of 4.0 mol l^{-1} HNO_3 was sufficient to obtain quantitative recovery. Thus, in all subsequent experiments 1 ml of 4.0 mol l^{-1} HNO_3 was applied as the desorption solution.

The amount of absorbent required for each extraction cycle was also determined. Extractions using between 20 and 100 mg of the IDA nanoparticles were carried out, each with 10 ml aliquots of a standard solution containing 1 μg ml^{-1} Cu^{2+} (pH 7.5) and recovered as above. It was found that near quantitative extraction and recovery of Cu^{2+} could be achieved using as little as 30 mg of the absorbent (Fig. 2(D)). Thus in subsequent extractions, 30 mg of nanoparticles were used.

As ultimate method detection limits are dependent upon the preconcentration factor achieved, the effect of sample volume on the recovery of Cu^{2+} was also investigated, using standard sample volumes of 5, 10, 20, 50, 100, 150, 200, and 400 ml, each containing 10 μg of Cu^{2+} (see Fig. 2(E)). The experimental results highlight no significant reduction in recovery, over all volumes investigated, up to 400 ml, suggesting 30 mg of IDA nanoparticles provided sufficient adsorption capacity over this range. Therefore, a sample volume of 200 ml was selected for subsequent real sample analysis. With a desorption solution volume of 1 ml, and subsequent extract pH adjustment, preconcentration factors of between 100 and 200 fold were obtained for real sample analysis.

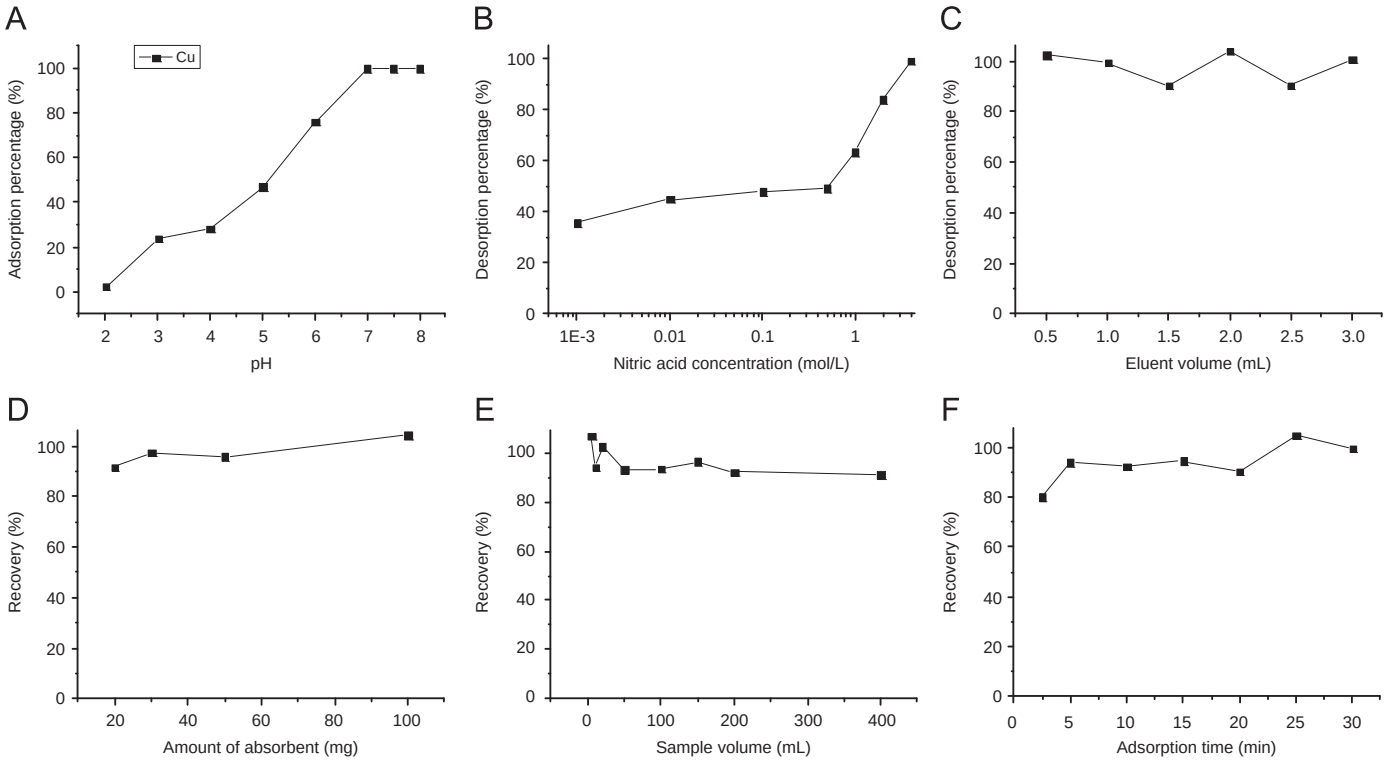

Fig. 2. Effect of experimental conditions on extraction and recovery of Cu^{2+} on IDA functionalised magnetic nanoparticles. (A) Sample pH, (B) desorption solution HNO_3 conc., (C) desorption solution volume, (D) mass of adsorbent, (E) sample volume, and (F) adsorption time.

Finally, the effect of dispersion/ultrasonication time on recovery for adsorption and desorption experiments was evaluated. The effect of dispersion/ultrasonication time on the adsorption of Cu^{2+} was studied in the time range of 2.5–30 min (Fig. 2(F)). It was found that Cu^{2+} could be quantitatively adsorbed with an ultrasonication/dispersion time greater than 5 min. The effect of time on desorption of Cu^{2+} was also studied over the range 1–15 min. It was found that Cu^{2+} could be desorbed quantitatively when desorption time exceeded 2.5 min. Consequently, ultrasonication for 10 min was selected for adsorption and 3 min for desorption in subsequent experiments.

3.3. Analytical performance and figures of merit

The operating conditions of the HPCIC method used in this work are similar to those reported previously [34], with minor modifications. In the present work, an eluent consisting of 0.5 M KNO_3, with 4 mM picolinic acid, and 10 mM HNO_3, was applied. The eluent flow rate was 1 ml min^{-1}. The peristaltic pump for delivery of PCR was also set to 1 ml min^{-1}. Column temperature was set to 25 °C. Fig. 3 shows a typical separation of common transition metal ions, plus Cd^{2+} and Pb^{2+}, obtained using the above conditions. The separation achieved provided excellent selectivity for Cu^{2+}, clearly resolved from other metals, in an acceptable run time.

Using optimised chromatographic conditions, the detection limit for Cu^{2+} (calculated by 3σ of the blank value, $n=7$) for the HPCIC method without sample preconcentration was 9.2 ng ml^{-1}, and the relative standard deviation for peak area precision found to be 4.9% ($c=1$ μg ml^{-1}, $n=7$). The calibration curves for Cu^{2+} were equally linear for both peak height and area over the range 20–5000 ng ml^{-1} [$y=66.76x-0.003$ for peak height ($R^2=0.999$), or $y=52.28x+0.014$ using peak area ($R^2=0.999$)], however, for all quantitative work peak area data was used. The detection limit and the linear range for HPCIC alone were similar to those reported previously by Dias et al. [15]. Assuming quantitative recovery of Cu^{2+} following the IDA nanoparticle extraction procedure (see Section 3.5), and applying preconcentration factors of ~100 under optimal conditions, potential detection limits for the combined MSPE–HPCIC method for Cu^{2+} were therefore in the vicinity of 100 ng l^{-1}. This level of sensitivity for the combined method compares very well with alternative advanced spectroscopic methods, according to data summarised within a recent review by Brown and Milton on trace element analysis [35]. The

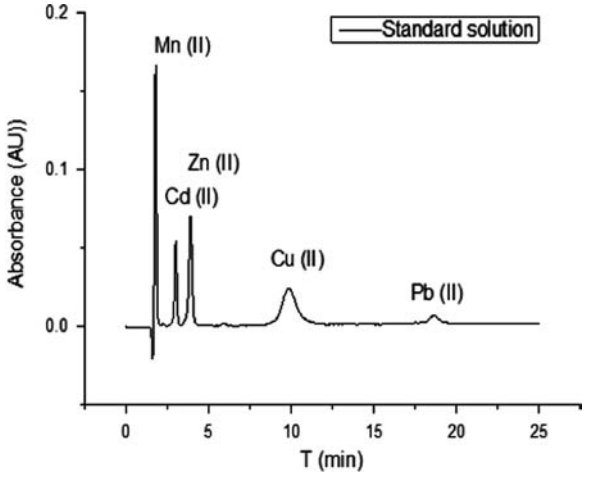

Fig. 3. Isocratic HPCIC separation of 1.5 mg/l Mn^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, and Pb^{2+}, on IDA modified silica column. Eluent: 0.5 M KNO_3, 4 mM picolinic acid, and 10 mM HNO_3. Detection: spectrophotometric at 510 nm after PCR with 0.15 mM PAR, pH 10.5.

MSPE–HPCIC method can provide lower LODs (for Cu^{2+}) than commonly reported with standard (direct) ICP-OES and flame AAS, and is comparable with those reported using electrothermal or graphite furnace-AAS. However, of course none of these alternative methods on their own can provide an insight into Cu speciation (see Section 3.5).

Environmental waters, such as seawater and estuarine waters, contain many matrix cations and anions at concentrations far in excess of Cu. These ions may potentially interfere in the quantitation of Cu^{2+}. To investigate this, solutions containing 1 μg ml^{-1} of Cu^{2+}, with added amounts of potentially interfering ions were processed according to the complete analytical protocol described above, and results were compared to solutions containing Cu^{2+} only. The tolerance for interfering ions was defined as the largest concentration affecting a reduction in Cu^{2+} recovery of less than 10%. The analytical results obtained (although not definitive) showed individual tolerances for 10 mg ml^{-1} Na^+, 1 mg ml^{-1} Mg^{2+}, 0.5 mg ml^{-1} K^+ and Ca^{2+}, 20 mg ml^{-1} Cl^-, 3 mg ml^{-1} SO_4^{2-}, 1 mg ml^{-1} NO_3^-, 0.2 mg ml^{-1} HCO_3^{2-}, 5 ug ml^{-1} Mn^{2+}, Cd^{2+}, Zn^{2+}, and Pb^{2+}. The concentrations of these ions are all higher than the major ion composition of seawater [36], which indicates that this developed analytical method is suitable for the analysis of samples from coastal or estuarine seawater locations.

3.4. Analysis of rain and estuarine water samples

The developed method was applied to the analysis of impacted estuarine and rain water samples collected from and near the Derwent Estuary, Tasmania, Australia. Contamination by metals from industrial activity is well known for samples from the Derwent Estuary [27–30]. The resultant chromatograms are shown in Fig. 4(A) and (B), respectively. There are 4 main peaks in each chromatogram. These peaks are the unretained alkaline earth ions coeluting with Mn^{2+}, the large peak associated with Co^{2+}, originating from the adsorbent itself, and the peaks of Zn^{2+} and Cu^{2+}, both extracted from each sample. In the rain water chromatogram (Fig. 4(B)), a small peak for Pb^{2+}, eluting at about 18 min, can also be observed.

The analytical results for each of the environmental water samples, including recoveries for spiked samples (standard addition calibration used for quantitation) are given in Table 1. Data reported is the average of three separate analyses. The recoveries for the addition of copper to water samples were 103.5% and 108.5%, respectively. To further evaluate the accuracy of the developed method, these samples were also analysed by ICP-MS for total Cu, and the analytical results are similarly shown in Table 1. As can be seen, the MSPE–HPCIC values are in excellent agreement with those obtained using ICP-MS. Obviously ICP-MS provides a 'total' value for copper within the two samples, whereas the MSPE–HPCIC values could be regarded as the labile Cu^{2+} concentration. The MSPE–HPCIC results range from 2% to 7% below those obtained using ICP-MS, which may well be a reflection of the non-labile (extractable) Cu content in the samples (see speciation study below). The CRM 'SLEW-2' was also analysed by the developed MSPE–HPCIC method, and the analytical results are also shown. Good agreement between the determined value and the certified value was observed. Table 2 provides the certified trace element composition of the SLEW-2 CRM. The SLEW-2 values are also certified for total Cu, and this again may be reflected in the 6% difference between the two average results. Total dissolved Cu concentrations have been investigated for the Derwent River in a number of previous studies. For example, in two such studies, the range of Cu^{2+} was reported as being between <0.5 and 21 μg l^{-1} [29], and between 1.8 and 232 nM [30]. The Cu^{2+} concentration observed in this work using the combined MSPE–HPCIC method falls within the ranges previously reported. These results indicate that this method is a reliable

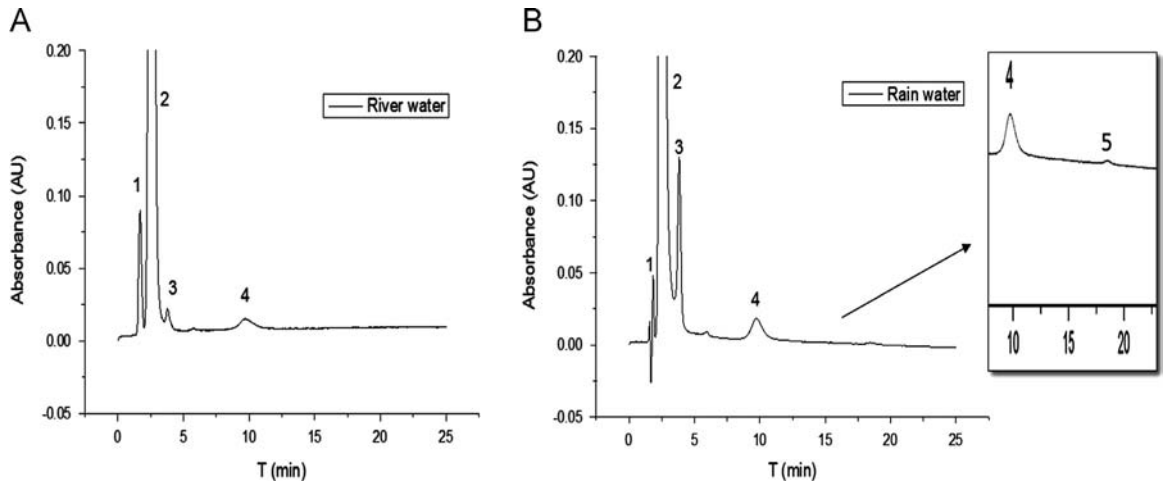

Fig. 4. HPCIC chromatograms of extracts obtained using IDA functionalised magnetic nanoparticles. Chromatogram (A) metal ions in Derwent River estuarine water, and chromatogram (B) rain water sample. Peaks=alkaline earth ions and Mn^{2+}, 2=Co^{2+}, 3=Zn^{2+}, 4=Cu^{2+}, 5=Pb^{2+}. Conditions are same as those in Fig. 3.

Table 1
Analytical results of Cu determinations in environmental water samples using MSPE–HPCIC and ICP-MS ($n=3$).

Samples	MSPE–HPCIC (ng/mL)			ICP-MS (ng/mL)
	Added	Found	Recovery*	
Estuarine water	0	2.03 ± 0.03	–	2.07 ± 0.29
	4	6.17 ± 0.12	103.5%	
Rain water	0	4.24 ± 0.07	–	4.58 ± 0.11
	4	8.58 ± 0.22	108.5%	
	Measured	Certified		
SLEW-2	1.52 ± 0.06	1.62 ± 0.11		

* Calculated using $\%R=[(F-l)/A] \times 100$, where F and l are the determined concentrations of spiked and unspiked samples, and A is the concentration of analyte added to spiked sample.

Table 2
SLEW-2 CRM certified trace element concentrations.

Elements	Concentrations (ng ml^{-1})
Co	0.055 ± 0.008
Ni	0.709 ± 0.054
Cu	1.62 ± 0.11
Zn	1.10 ± 0.14
Cd	0.019 ± 0.002
Pb	0.027 ± 0.005
V	1.35
Mn	17.1 ± 1.1
Fe	2.37 ± 0.37
As	0.792 ± 0.082
Mo	3.7
Ba	16.9
U	1.2

approach for the determination of Cu^{2+} in water samples. Table 3 shows ICP-MS results for the major ions present within both the Derwent River estuarine and rain water samples. From Table 3, it can be observed that, as expected, the concentrations of alkali and alkaline earth salts were much higher in Derwent River estuarine water than in rain water. Table 3 also confirms the matrix tolerance of the developed MSPE–HPCIC method.

3.5. Copper complexation

Coastal seawater samples were collected to investigate the effect of UV irradiation of the sample prior to MSPE using the IDA functionalised magnetic nanoparticles. Previous studies using IDA based adsorbents for transition metal extraction from seawater have reported significant increases in dissolved trace metal concentrations following sample pretreatment with UV irradiation due to photo-induced decomposition of complexing organic matter within the sample [37]. However, reported results vary widely due to lack of standard power and irradiation times across the various studies. Herein, a 254 nm 3 mW cm^{-2} lamp was applied for a period of 30 min., and samples analysed using the MSPE–HPCIC method before and after such treatment. The results for Cu^{2+} were found to vary only marginally after this treatment, increasing by only a few per cent, which was below the standard deviation of replicate analyses (1.68 ± 0.12 ng ml^{-1}, increasing to 1.70 ± 0.06 ng ml^{-1}), perhaps suggesting insufficient power and exposure time.

Therefore, an alternative approach was undertaken, which eliminates the above procedural uncertainties. The extraction of dissolved organic matter (DOM) from seawater is achieved following well established procedures. Here, the collected coastal seawater sample was once again analysed before and after pretreatment, in this case involving the extraction of DOM using the technique of Dittmar et al. [32]. Although not quantitative for all organic matter, extraction using the non-polar PS-DVB SPE cartridges is known to remove the majority of the larger organic fraction, typical of complex acids responsible for the so-called 'non-labile' metal ion concentration. Fig. 5(A) shows the resultant HPCIC chromatograms for the two coastal seawater samples, achieved with and without DOM removal. Following quantitation via standard addition, the concentration of Cu^{2+} in the extract of the seawater sample with reduced DOM was found to be 1.21 ± 0.06 ng ml^{-1}, equivalent to a 28% reduction.

Table 3
Matrix composition of Derwent River estuarine water and rain water determined using sector field ICP-MS ($n=3$).

Element	Derwent River estuarine water [a]		Rain water [b]	
	Concentration (ng l^{-1})	% RSD	Concentration (ng l^{-1})	% RSD
Na	8.49×10^6	4.9	1.15×10^3	1.9
K	3.49×10^5	3.4	476	1.8
Mg	8.81×10^5	2.8	289	1.0
Ca	3.79×10^5	3.3	651	2.1
Sr	6.98×10^3	4.3	4.80	0.5
Ba	9.22	4.5	2.31	0.7
P	9.33	7.9	5.48	3.0
S	7.43×10^5	3.9	414	1.1

[a] Analysed after dilution 10 ×.
[b] Analysed as collected.

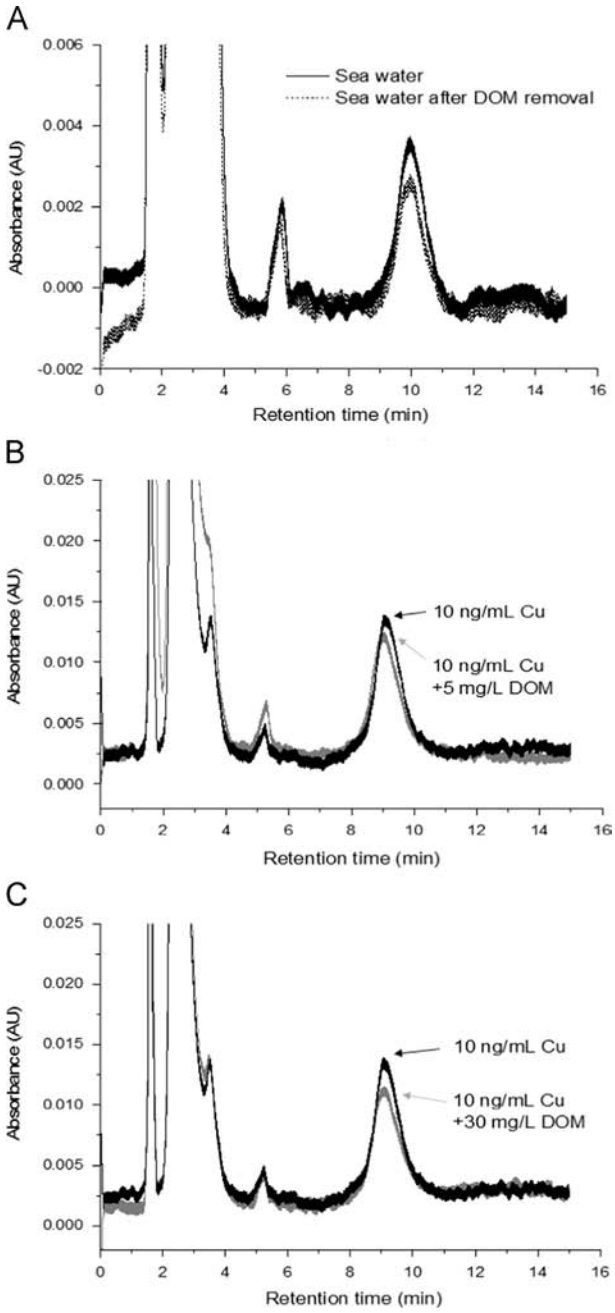

Fig. 5. (A) HPCIC chromatogram of coastal sea water sample extracts (100 fold concentration using MSPE) before and after removal of dissolved organic matter. (B) HPCIC chromatograms for 10 ng ml^{-1} Cu^{2+} spiked synthetic estuarine water samples with added DOM at 0 and 5 mg l^{-1} levels. (C) HPCIC chromatograms for 10 ng ml^{-1} Cu^{2+} spiked synthetic estuarine water samples with added DOM at 0 and 30 mg l^{-1} levels.

Table 4
Effect of DOM on Cu recovery in artificial sea water samples with MSPE–HPCIC analysis ($n=3$).

DOM added (mg l^{-1})	Cu added (ng ml^{-1})	Cu found (ng ml^{-1})	Cu recovery
0	10	9.71 ± 0.39	97.1%
5	10	8.51 ± 1.00	85.1%
15	10	8.64 ± 0.72	86.4%
30	10	7.44 ± 0.31	74.4%

Given the smaller 2–7% variations seen between the MSPE–HPCIC (dissolved labile Cu^{2+}) and ICP-MS data (total copper), these results would indicate a significant portion of DOM bound Cu^{2+} is relatively labile and amenable to extraction using the IDA functionalised magnetic nanoparticles. To further investigate this, a series of artificial estuarine water samples were prepared (8.5 g l^{-1} NaCl), and increasing amounts (0, 5, 15 and 30 mg l^{-1}) of standard DOM (Swuannee River) added. These artificial estuarine samples were then spiked with 10 ng ml^{-1} Cu^{2+} and left to equilibrate under gentle agitation. These prepared samples were then analysed using the MSPE–HPCIC method to confirm the effect upon recovery of DOM-bound Cu^{2+}. The results from these extractions are shown in Table 4, with chromatograms obtained for the spiked samples with 5 and 30 mg l^{-1} DOM shown as Fig. 5(B) and (C). As can be seen, at typical natural DOM concentrations, between 5 and 15 mg l^{-1}, the recovery of Cu^{2+} is reduced by between 10% and 12%, which supports the above conclusion regarding the 2–7% variation between MSPE–HPCIC and ICP-MS data. Combined with the observation on the 28% reduction of Cu^{2+} following complete DOM removal prior to extraction, these results suggest that although ~30% of Cu^{2+} is indeed bound within DOM, that the majority of this (> 75%) is extractable using the IDA functionalised nanoparticles.

These experimental observations are supported by stability constant data (log K) for Cu^{2+} and isolated fractions of Suwannee River DOM, as recently reported by Yan et al. [38]. The log K data reported ranged between 4.9 and 6.1, whereas log K data for IDA–Cu^{2+} complexes are ~10.6 [33].

4. Conclusion

Magnetic cobalt nanoparticles functionalised with IDA were employed as a magnetic solid phase extraction absorbent for preconcentrating dissolved labile Cu^{2+}, from environmental water samples prior to their quantitation using high-performance chelation ion chromatography. The developed magnetic nanoparticle extraction process is relatively fast, reproducible and robust, and the functionalised nanoparticles are capable of extracting greater than 75% of bound Cu^{2+} fraction within DOM found within natural waters. The combined MSPE–HPCIC method provides sub-ug l^{-1} detection limits, with excellent precision, based upon comparative methods and CRM analysis.

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