



Application of a novel poly(SMAm)-Tris-Fe₃O₄ nanocomposite for selective extraction and enrichment of Cu(I) /Cu(II) from beer, soft drinks and wine samples, and speciation analysis by micro-volume UV–Vis spectrophotometry

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ABSTRACT

In this study, a novel functional nanocomposite was synthesized, characterized and selectively used in pH-controlled separation, pre-concentration and speciation analysis of Cu(I) and Cu(II) from sample matrices where extraction is assisted, facilitated and greatly enhanced by ultrasound energy. The hydrophilic composite material functionalized with tris(2-hydroxymethyl)aminomethane (Tris) and Fe₃O₄ NPs was characterized in detail by ATR-FT-IR, ¹H NMR, XRD, EDX peaks and SEM images. After optimization of the main variables influencing extraction efficiency such as pH, volumes of buffer, modified copolymer in acetone, CTAB and Triton X-114 at fixed concentrations including sonication conditions, the Cu(I) and Cu(II) were monitored against a blank at 347 nm by micro-volume UV–vis spectrophotometer. A good linearity was obtained in the range of 2–140 and 5–150 µg L⁻¹ for Cu(II) and Cu(I) with r² ≥ 0.993. The limits of detection (LODs) of 0.66 and 1.60 µg L⁻¹ for each analyte, were obtained from a pre-concentration of 70-fold. After validation, the method was applied to speciation of Cu(I), Cu(II), and total Cu in the pre-treated and diluted beverage samples before and after pre-oxidation of Cu(I) to Cu(II) due to be more sensitive of extraction process to Cu(II) at pH 6.0. The results were also compared with those obtained by FAAS analysis to ensure the reliability of the results. It was observed that there was a statistically good agreement between the results of both methods.

1. Introduction

Copper (Cu) is an essential element not only for life in mammals but also for plants, and it plays an important role in carbohydrate and lipid metabolism. It has many biological effects as an essential element as well as a toxic one [1]. In general, a daily copper intake of 1.5–2 mg is essential for adults, and Cu at nearly 40 µg L⁻¹ is required for normal metabolism of many living organisms [1,2]. In fact, Cu is obligatory for enzymes involved in aerobic metabolism, such as cytochrome c oxidase in the mitochondria, lysyl oxidase in connective tissue, dopamine monooxygenase in brain, and ceruloplasmin. As a cofactor for apo-Cu-Zn superoxide dismutase, Cu protects against free-radical damage to proteins, membrane lipids, and nucleic acids in a wide range of cells and organs. Severe Cu deficiencies, either gene defects due to mutations or low dietary Cu intakes, although relatively rare in humans, have been linked to mental retardation, anemia, hypothermia,

neutropenia, diarrhea, cardiac hypertrophy, bone fragility, impaired immune function, weak connective tissue, impaired central-nervous-system functions, peripheral neuropathy, and loss of skin, fur (in animals), or hair color [3–6]. But, Cu(II) at higher levels than 40 µg L⁻¹ is toxic, and severe oral intoxication will affect mainly the blood, kidneys, liver and brain, inducing chronic cirrhosis, Wilson and Menkes diseases related to metabolic Cu toxicity. Because of all these, the trace Cu(II) content in foods and/or beverages must be controlled on a daily basis and the European Union (EU) has fixed to the limits of 2 and 1.0 mg L⁻¹ in drinking water and wine, respectively, and the permitted maximum contaminant level of Cu(II) is set to 1.3 mg L⁻¹ in the USA similar to that in Canada (1.0 mg L⁻¹) to protect human health against exposure to its excessive levels [7–9]. It is essential to ensure that levels are below the maximum recommended of 0.5 mg L⁻¹ for Cu and 30 mg L⁻¹ for Fe and Zn that is stipulated by EU directive EC 606/2009 [10]. Therefore, monitoring and control of Cu(I) and Cu(II)

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levels as well as total Cu in beer, soft drinks and wine at low concentrations is becoming increasingly important. In this sense, there is of great importance to develop simple, easy to use, rapid, low-cost, sensitive, selective, accurate and reliable analytical methods.

Several analytical techniques have been used for determination of Cu, including flame atomic absorption spectrometry (FAAS) [11–19], electroanalytical techniques such as differential pulse anodic stripping voltammetry (DP-ASV), derivative potentiometric stripping analysis (dPSA) and stripping potentiometry (SP) [20–22], graphite furnace atomic absorption spectrometry (GFAAS) [23], electrothermal atomic absorption spectrometry (ETAAS) [24], inductive coupled plasma-optical emission spectrometry (ICP-OES) [25], inductive coupled plasma-mass spectrometry (ICP-MS) [26], and energy dispersive X-ray fluorescence spectrometry (ED-XRF) [27], including direct and derivative spectrophotometry [28,29] with and without pre-concentration at different operational modes. However, spectrophotometric methods are often preferred, as they involve simple and economic instrument (which can be available in almost any analytical research lab and easily accessible), less labor-intensive, and provide comparable sensitivity and selectivity when appropriate chromogenic reagents for analyte are available [30].

Due to meet many analytical features, analytical nanotechnology in the limits of the “nanoscale” (from 1 to 100 nm) is an important tool for pre-concentration and separation of pollutants at low levels. Nowadays, its most widely explored area is to exploit the excellent properties of nanoparticles (NPs) to improve the selectivity and sensitivity of well-established analytical methods or to develop new methods for analytes or complex sample matrices. In addition to these advantages of NPs, their use should lead to improved selectivity, sensitivity, rapidity, miniaturizability or portability of the analytical system. Nano-materials, with a new series of different physical and chemical properties superior to the traditional materials, are the basis of nanotechnology. The NPs can be used for purposes such as sample pre-treatment, instrumental separation of analytes from matrix for possible interference, or even detection. In combination with the large variety of NPs available, this provides a wide range of potential applications. Sample pre-treatment methods like separation and/or pre-concentration prior to the determination of analytes have developed rapidly due to the increasing need for accurate and precise measurements at extremely low levels of toxic organics and metal ions in diverse matrices [31,32]. In this sense, there are many studies based on the use of nanocomposites in pre-concentration step for only Cu(II) (or Cu(I)) in absence and presence of surfactants as counter-ion after modification with various chelating agents such as dithiooxamide [17], diphenylcarbazone [33], chitosan [34], nonanoic acid [35], poly-thiophene [36], chitosan-graphene quantum dots [37], polystyrene [38], and salicylic acid [39] in literature with their advantages and disadvantages. Where magnetic SPE has been recently appeared to overcome drawbacks such as clogging of cartridges, being time-consuming, necessity of pump usage, low extraction efficiency resulted from particles aggregation and impossibility treatment of large sample volumes in conventional SPE; to provide an improvement in selectivity and sensitivity of the extraction process by UA-CPE, which is in principle based on the phenomenon of phase separation with the cloud point of the non-ionic surfactant around the critical micelle concentration (CMC) as alternative approach in aqueous-micellar solution; fast, easy, efficient and versatile separation capability of mNPs was combined with hydrophilic amidic copolymer modified with tris(2-hydroxymethyl)aminomethane (Tris) as chelator for extraction, pre-concentration and speciation analysis of trace Cu(I) and Cu(II) from sample matrix at micellar interface where Cu(II) is thermodynamically stable in aqueous solution, but Cu(I) is not stable in absence of a suitable chelator due to pH dependent disproportionation.

This study proposes a novel poly(styrene-co-N-maleamide) (SMAm)-Tris-Fe₃O₄ nanocomposite synthesized from poly(styrene-co-maleic anhydride (poly(SMA))) by a chemical process, as an alternative interface in the pre-concentration and speciation of Cu(I) and Cu(II) via simple,

economic and versatile micro-volume UV-vis spectrophotometry. This hydrophilic functional nanocomposite is not only used in the pH-controlled selective Cu extraction, but also their speciation analysis in aqueous micellar media. Because of the difference in tendency of complexation of Cu(I) and Cu(II) via surface functional groups of nanocomposite at pHs of 6.0 and 8.0, respectively, it will be possible to have selective extraction of each ion. Moreover, cationic surfactant, CTAB as both sensitizer and counter-ion prevents the agglomeration of Fe₃O₄ NPs, and induces the stabilization of Cu(I) and Cu(II) against oxidation on functional copolymer via keto-enol (or amide-imidic acid) tautomerization as a function of pH in extraction and speciation analysis of soft/hard Cu(I) and Cu(II).

2. Experimental

2.1. Reagents, standard solutions and samples

Ultra-pure water (resistivity of 18.2 MΩ cm) obtained by a Labconco water purification system (Kansas City, USA) was used throughout this study. All glassware, pipettes and plastic tubes were immersed in 5.0% (v/v) HNO₃ solution, kept there for one day, thoroughly cleaned and then rinsed five times with ultrapure water just before the experiment started. The standard working solutions of Cu(I) and Cu(II) at μg L⁻¹ levels used for calibration were prepared daily by dissolving suitable amounts of solid CuCl and CuSO₄ × 5H₂O salts supplied from Merck (Darmstadt, Germany) at levels of 1000 mg L⁻¹ in 0.2 mol L⁻¹ HCl or HNO₃ solutions, and diluting with water before use. Their calibration solutions in linear working ranges, including optimization step, were obtained daily by dilution using 2.5 mL of acidic acetonitrile (0.02 mol L⁻¹ HCl). The poly (SMA) modified and magnetized with Tris and Fe₃O₄ NPs, respectively was prepared by dissolution of its suitable amount in acetone. All the ionic and nonionic surfactants, cetyltrimethylammonium bromide and sodium dodecyl sulfate (CTAB and SDS, 3.0 × 10⁻³ mol L⁻¹) and **oxyethylene glycol tert-octylphenyl ether** (Triton X-114, 5.0% (v/v)) as extractant, obtained from Sigma, were prepared by dissolving appropriate amount of each surfactant in a flask of 100 mL in water, and vortex-mixing for a homogeneous clear solution when necessary. The pH of the sample solutions in pH range of 3–10 was adjusted with universal Britton-Robinson buffer (B-R buffer, containing equal-molar concentration of phosphoric, boric acid and citric acids) (each, 0.04 mol L⁻¹) buffer solution. Home-made wine samples were collected from Cappadocia region in Nevşehir, Turkey. Beer and soft drink samples were purchased from local markets in Sivas, Turkey. For validation of the method, two certified quality control samples supplied from FAPAS were analyzed: T0783 soft drinks and T07316QC wine.

2.2. Instrumentation

A micro-volume UV-vis spectrophotometer with 1.0-cm quartz cells (Shimadzu 160A model, Kyoto, Japan) was used for all absorbance measurements. For reliability of the results, a flame atomic absorption spectrometer (FAAS) (Shimadzu, AAS-6300 model, Kyoto, Japan) equipped with D₂-background correction, a copper hollow cathode lamp and an air-acetylene flame atomizer was used for detection of copper. FT-IR spectra were taken using a Bruker (Alpha 12283105 model, Billerica, MA, Germany) spectrometer (with direct sampling at ATR mode without KBr pellet). ¹H NMR spectra (in DMSO, D₆, 400 MHz, single pulse) were recorded on a JEOL JNM-ECZ400S/L1 spectrometer (JEOL Ltd., Akishima, Tokyo, Japan) operating at 298 K with tetramethylsilane (TMS) as an internal standard. The chemical shifts (δ) are quoted in ppm and coupling constants (J) are measured in Hertz (Hz). The XRD pattern was recorded by Rigaku **Miniflex 600** X-ray diffractometer using Ni-filtered Cu Kα radiation. A scanning electron microscope (SEM) with a field emission gun using multiple detection system (SE and BSE) (MIRA 3 XMU model, Tescan, Czech Republic) is used to study the morphology

or microstructure of nanocomposite in high resolution (micro to nanoscale) under acceleration voltage of 15 kV, including elemental analysis using energy dispersive spectrometer (EDS). The pH measurements were performed using a digital pH meter (Selecta-2001 plus, Barcelano, Spain) supplied with a glass-calomel electrode. A centrifuge (Hettich universal 320 models, Darmstadt, Germany) was used to speed up the phase separation. A programmable ultrasonic bath (UCP-10 model, Seoul, Korea) was used for incubation with temperature ranging from 0 to 80 °C and ultrasound frequency of 40 kHz at power of 300 W. A vortex-mixer was used for acceleration of the mass transfer in the extraction process.

2.3. Sampling, sample preparation

The speciation analysis of Cu(I), Cu(II) and total Cu by micro-volume spectrophotometry were evaluated by analysis of beverage samples like beer, soft drinks and wine with and without alcohol. Home-made wine samples with alcohol ratio between 12 and 14% were collected from Cappadocia region in Nevşehir, Turkey. Beer (alcohol content, 3, 5 and 6%, including non-alcohol for quality control) and soft drink samples were purchased from local supermarkets in Sivas, Turkey. To prevent foaming, beer and wine samples with alcohol were spiked with 1.0 mL 1-octanol, 0.5% (v/v). After the sonication for 15 min, all the samples including orange or lemon flavored soft drinks were spiked with 0.025 g polyvinylpyrrolidone (PVP-15) to eliminate the possible interference of phenol-rich flavonol, flavone and flavonoids with the spectrometric analysis. Due to its many benefits such as shortened sample preparation times, simplicity, enhanced safety, no possibility of the extraction solution evaporating to dryness, and finally supplying possible to speciation analysis by using dilute acidic oxidant mixture when compared to other traditional methods of preparing samples such as soxhlet extraction (SE) and liquid-liquid extraction (LLE) for subsequent elemental determination [40], ultrasonic extraction was preferably adopted in this study.

At initial, the selected beverage samples (15-mL) were shaken thoroughly with vortex, degassed for 15 min in ultrasonic bath, kept away from light, and then kept in clean, dry containers. After shaking and degassing, the samples were filtered through 0.22- μm membrane filter before analysis. Aliquot of 5.0 mL of the degassed-, filtered- and homogenized-samples was transferred to another flask of 50-mL. For digestion with dilute oxidant-salt mixture, 5.0 mL of acid mixture of 0.2 mol L⁻¹ of HClO₄ and 0.02 mol L⁻¹ NaCl (4:1, v/v) were added to the samples in order to destruct sample matrix with origin of plant, avoid from internal conversion of Cu ions, stabilize Cu(I) (as CuCl₂⁻), and then completed to 50 mL with the water. The mixtures were thoroughly sonicated and extracted in optimally medium of 3.5% (v/v) ethanol (in range of 0–8.5%, v/v) and 125 mg L⁻¹ tartaric acid (in range of 0–250 mg L⁻¹) for both matrix matching and stabilization of Cu(II) in presence of excess Cu(I) (is relatively predominant in phenolic-rich acidic colored beverages such as wine, beer and orange or lemon flavored of drinks) under ultrasonic effect (300 W, 40 kHz) at 40 °C for 7 min, and then quantitatively treated with 2.0 mol L⁻¹ HNO₃ for pre-oxidation of Cu(I) to Cu(II) for 5 min at 55 °C to allow an analysis of soluble free Cu(I)/Cu(II) and total Cu, respectively. When necessary, same procedure has been repeated for additional time of 5 min until a clear solution is obtained. After cooling to room temperature, the resulting acid extracts were filtered through a membrane filter of 0.22- μm . The pH of the sample extracts was adjusted to 6.0 in presence of acidic acetonitrile (0.02 mol L⁻¹ HCl) for prevention of possible internal conversion of Cu⁺ and Cu²⁺ ions using dilute NaOH (0.5 mol L⁻¹) and completed to 50 mL with the water. Then, 5.0 mL of the pre-treated- and extracted-samples was submitted to UA-CPE procedure for UV-vis spectrophotometric monitoring of each ion at 347 nm. In a similar way, the same samples were comparably analyzed using FAAS for reliability of the results. The Cu contents of the samples were determined via spectrophotometry using matrix-matched calibration curve (so as to give a similar accuracy

degree with the three pointed-standard addition method around the method determination limit) to control the possible matrix effect. Also, at least one blank solution including suitable amounts of the certified wine and soft drink matrices was run for each sample in order to evaluate analyte contamination by reagents used. All the measurements and processing were performed at least in five times, and their mean plus standard deviations were considered.

2.4. Preparation of the magnetic nanocomposites

Synthesis of the poly (SMA) by radical polymerization, surface modification with Tris (as poly (SMAm)-Tris and poly (SMIm)-Tris) with and without thermal process at 150 °C for selective chelation of Cu(I) and Cu(II), and then their magnetization with Fe₃O₄ NPs (as poly (SMAm or SMIm)-Tris-Fe₃O₄) was sequentially performed, and the details related to synthesis were given in our first study [41]. Therefore, there is no need to speak further and explain more in detail.

2.5. UA-CPE procedure

A typical UA-CPE required the following steps: an aliquot (5.0 mL) of the pre-treated sample solutions in two separate calibration ranges of 5–150 for Cu(I) and 2–140 $\mu\text{g L}^{-1}$ for Cu(II) in optimization step, and 35-mL of a sample solution containing no less than 0.5 μg of Cu(I) or Cu(II) in pre-concentration step for the amide copolymer modified with Tris, 0.25 mL of copolymer in acetone (up to 0.1 g/100 mL), 2.0 and 1.0 mL of 5% (v/v) of Triton X-114, 0.75 and 1.5 mL of 3×10^{-3} mol L⁻¹ CTAB, for Cu(I) and Cu(II) respectively, were adjusted to pH 8.0 and 6.0 with 2.0 mL of B-R buffer solution, and then completed to 50 mL by the water. The mixture was left to stand in an ultrasonic bath at 40 °C for 10 min. After reaching to equilibria for complete complex formation, separation of the phases was achieved by centrifugation at 3000 rpm for 7 min. Due to become viscous of the surfactant-rich phase, the bulk aqueous phase was easily separated and decanted. To reduce its viscosity of the surfactant-rich phase for spectrophotometric measurements and facilitate the sample proceeding, it was diluted to a volume of 0.5 mL with acidic acetonitrile (0.02 mol L⁻¹ HCl) as diluent of both surfactant-rich phase and stabilizer of Cu(I) and Cu(II). Finally, the total Cu contents of the selected sample matrices were in parallel evaluated by using both the matrix-matched calibration curve obtained by spectrophotometry at 347 nm against a blank in extraction step, and the standard addition method in order to control the possible matrix effect around detection limits when necessary.

2.6. Analysis by micro-volume UV-vis spectrophotometer and FAAS

After pre-concentration, the maximum absorbance of the sample extracts in the cells with micro-capacity, 0.35–0.70 mL was measured at 347 nm (Cu-complexes) with a red shift of 9 nm against a blank (acidic acetonitrile). The calibration curves were plotted for the amounts of Cu(II) and Cu(I) against their relative absorbance at pH 6.0 and 8.0, respectively. These curves were used to determine the Cu contents of samples.

For comparison of the results, the Cu(I), Cu(II) and total Cu contents of sample extracts, was also determined by FAAS after conversion of Cu(I) to Cu(II). For this purpose, the most sensitive resonance wavelength, lamp current, spectral bandwidth, burner height, acetylene and air flow rates used for the sensitive determination of the Cu were: 324.8 nm, 3.0 mA, 0.5 nm, 3.0 mm, 1.8 and 8.0 L min⁻¹, respectively where the detection limit and characteristic concentration in linear working range of 0.01–0.4 mg L⁻¹ are 3.42 $\mu\text{g L}^{-1}$ and 21.3 $\mu\text{g L}^{-1}$ with sample uptake rate of 6.0 mL min⁻¹ at an optimal burner height of 3.0 mm as a result of sensitivity improvement performed in range of 1–10 mm.

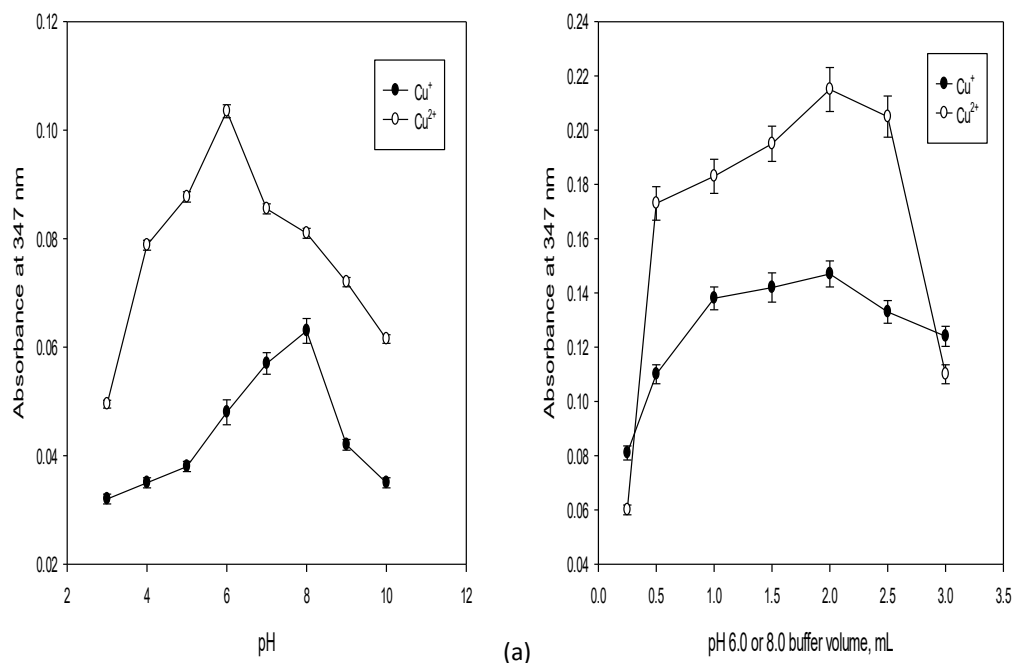


Fig. 1. (a–b) Effect of (a) pH and (b) volume of buffer of at pH 6.0 or 8.0 for detection of Cu(II) and Cu(I) at levels of $75 \mu\text{g L}^{-1}$ on the sensitivity.

3. Results and discussion

3.1. Characterization of the magnetic nanocomposites

3.1.1. FT-IR analysis

After modification with Tris, disappearance of anhydride ring bands at 1854 and 1764 cm^{-1} indicates in Fig. S1(a) that the ring has been opened, and converted into the Tris-modified amidic copolymer, TrisA-SMAm. The new peak at 1640 cm^{-1} corresponds to amide groups while the peaks at 1565 and 1405 cm^{-1} correspond to carboxylate groups. The imidation can be explained by the appearance of new bands, 1780 , 1727 and 1708 cm^{-1} . Also, the amidation/imidation can be characteristically explained by shifting bands of 1217 and 1057 cm^{-1} belong to pure SMA to 1384 , 1250 , 1163 , 1097 , 1023 and 1387 , 1170 , 1009 , 957 cm^{-1} where the bands of 1385 cm^{-1} and 1184 cm^{-1} are assigned to the (C–N) and (C–N–C) bond stretches of the tertiary aromatic amine and the maleimide. In the IR spectra of pure Fe_3O_4 NPs, the bands at 580 , 620 , 800 and 896 cm^{-1} can be attributed to bands of Fe–O. However, after magnetization, the bands at 800 and 896 cm^{-1} have gradually disappeared while the band intensity at 620 cm^{-1} increases, showing penetration of Fe_3O_4 NPs into the copolymer matrices. This is proof that Tris-modified copolymers successfully bind to Fe_3O_4 NPs [42].

3.1.2. ^1H NMR analysis

From the ^1H NMR spectra of the unmodified and modified copolymers with Tris (SMA, TrisA-SMAm, and TrisA-SMIm, as δ , ppm) in Fig. S1(b), it is clear that broad peaks overlapping between 1.0 and 2.5 , and 6.5 – 7.5 are due to methylene/methine and aromatic ring hydrogens of styrene. Methine protons of pure SMA appear between 3.5 and 4.0 as the multiplet styrene/maleic anhydride ratio of the copolymer were calculated from the integration ratio of the peaks at 7.1 – 7.3 and 3.5 – 4.0 . This ratio was about $0.95:1$. As it is expected, an alternating copolymer was produced under the conditions used in this s. After modification, it is clear that observed chemical shifts are due to the –OH groups bound to tertiary tris-N atom, forming amidic –NH and –COOH groups as well as peaks of pure SMA. Tris modified copolymers showed a singlet peak and triplet peaks due to amidic –NH at 3.5 and –OH groups at 3.0 placing in environment of different functional groups as well as typical copolymer peaks. However, peaks appeared between 6.5 and 7.5 were partly

shifted to low and high magnetic areas while their intensities clearly either decreased or completely disappeared by amidation/imidation process. The weak peaks appeared between 1.1 and 2.4 are due to methylene and methine protons of styrene units. As a result, the spectra were highly in agreement with the proposed structure.

3.1.3. XRD analysis

The powder XRD is a very powerful technique for characterizing the structure of composite materials. As can be seen from the XRD patterns of the as-prepared-only Fe_3O_4 NPs, and magnetized-TrisA-SMAm and TrisA-SMIm copolymers in Fig. S1(c), there is only a broad diffraction peak in range of 20 – 30° (2θ), which is assigned to a reflection of modification with Tris, showing their amorphous natures. The XRD pattern of pure Fe_3O_4 NPs indicates the cubic spinel structures, and the presence of sharp and intense peaks confirms the formation of crystalline NPs. The XRD pattern of the newly prepared magnetic composites shows diffraction peaks at 25 – 30 , 35 , especially 35 – 40 , 50 , 55 and 65° , which are ascribed to the known facets of the cubic spinel crystal planes of Fe_3O_4 , respectively. It is clear that after magnetization, the peaks at 25 , 55 and 65° as well as the sharp peak at 35 – 40° , remain locally unchanged while the Fe_3O_4 NPs incorporate into the structure of the composites. So, the existence of Fe_3O_4 NPs in composite matrix is confirmed with a background noise, while their reflection peaks in range of 20 – 30° are not disappeared [42]. After magnetization, it can be due to the fact that Fe_3O_4 NPs are dispersed in each copolymer matrix, so as not to exhibit an orderly structure in terms of crystallinity. Obviously, this is an indicator of that the functionalization process does not significantly affect the peak positions of the NPs.

3.1.4. SEM and SEM/EDX analysis

Additionally, scanning electron microscopy (SEM) was employed to explore the surface morphology and elemental analysis of the synthesized composite materials. Figs. S1(d–f) show the SEM images of poly (SMA), poly(SMAm)-TrisA and poly(SMAm)-TrisA- Fe_3O_4 magnetic nanocomposite (mNC), respectively. The morphology of poly(SMAm)-TrisA reveals that the amidic copolymer has more homogenous and plate-like structure than that of original poly(SMA), and average diameter of the observed particles in SEM image can be estimated under 100 nm with reasonable monotony and grainy shape (Fig. S1e). The SEM

image of poly(SMAm)-TrisA-Fe₃O₄ mNC demonstrates an aggregate structure that consists of mNC crystallites that are collected as small pseudo-spherical particles with approximate sizes in the range of 10–50 nm and are stacked with each other, which makes plate-like morphology (Fig. S1f). The successful synthesis of the mNC was qualitatively further confirmed by the chemical composition analyzed by electron dispersive X-ray (EDX). Fig. S1(g), shows the distribution of C, O, Fe and partly N elements. The disappearance of the N peak on the background in the EDX spectrum is due to the fact that the Tris-modified copolymer matrix containing a single N-atom has a relatively large molar mass. Indeed, this peak is evident in the imidic copolymer matrix (based on ring closure by condensation) obtained by thermal treatment of amidic copolymer at 150 °C. Na and Cl residual peaks are due to the separation and purification processes by filtration after the precipitation of the copolymer matrix with aqueous dilute solution of NaCl.

3.2. Optimization of UA-CPE conditions

To extent performance of the method to speciation of Cu(I) and Cu(II), their ternary complexes were extracted by UA-CPE. To achieve maximum extraction efficiency, the main variables affecting the extraction efficiency in presence of CTAB were investigated, and optimized for triplicate measurements of Cu(I)/Cu(II) (each one, 75 µg L⁻¹) by using one-variable-at-a-time method. The standard deviations of the measurements at 347 nm (n: 3) were calculated and represented as error bars. The results obtained from analysis of samples, including CRMs were statistically evaluated by using the paired *t*-test, and the calculated *t*-values were compared with the tabulated *t*-value for four and eight degrees of freedom at the 95% confidence level, respectively.

3.2.1. Effect of pH and buffer concentration

There is of highly importance to select appropriate chelating agent and metal ions to form a hydrophobic complex when metal ions are extracted by the UA-CPE. The complex is extracted to surfactant-rich phase. The extraction efficiency depends on the acidity of the solution medium as the pH has an impact on the overall charges of the analyte, thus affecting the generation of the complex between the metal ion and the surfactant active functional groups. Therefore, the different pHs on the extraction efficiency were investigated. UA-CPE of Cu(I) and Cu(II) were carried out in the pH range of 3–10. The results are shown in Fig. 1 (a). The recovery for Cu(II) and Cu(I) increased with increasing pH from 3.0 to 6.0 and/or 3.0 to 8.0, reached a maximum with pHs of 6.0 and 8.0, respectively, and sharply decreased at higher pHs, so as to give pH-controlled selective and stable metal complexes in presence of citric acid as a stabilizing component of buffer. Herein, to prevent the conversion of Cu(I) and Cu(II) into each other, it is thought that citric acid acts as a preservative with different complex formation constants in the pH range of 2.0–6.0 where citric acid forms more stable ionic chelate complex with Cu(II) than Cu(I) in form of CuL⁻ and/or Cu₂L₂²⁻ [43,44]. This pH difference in terms of stable chelate formation can be explained by hard and soft character of metal ions bound to same coordination center of tris modified chelating cyclic imidic copolymer. It is believed that this case is also fed by pH controlled-disproportionation of copper species with one electron charge transfer, depending on the nature of the chelating ligand and the reaction medium. At low pHs, the low recoveries for metal ions were observed owing to the incomplete complex formation among reagents in reaction media. The gradual decrease in analytical signal at lower pHs than 6.0 for Cu(II) and 8.0 for Cu(I), respectively, can be either due to polymeric ligand induced disproportionation in amide structure containing donor N- or NH-, -C=O and -OH functional groups or instability of Cu(I) in lower pHs than 6.0 as a result of a shift in amide-imidic acid tautomerization equilibrium in terms of chelation, depending on pH of the environment and type of each metal ion (softness and hardness) where the copper species at low concentrations are chelated and stabilized with polymeric ligand at controlled pHs. Also, it is reported in the literature [45,46] that, to

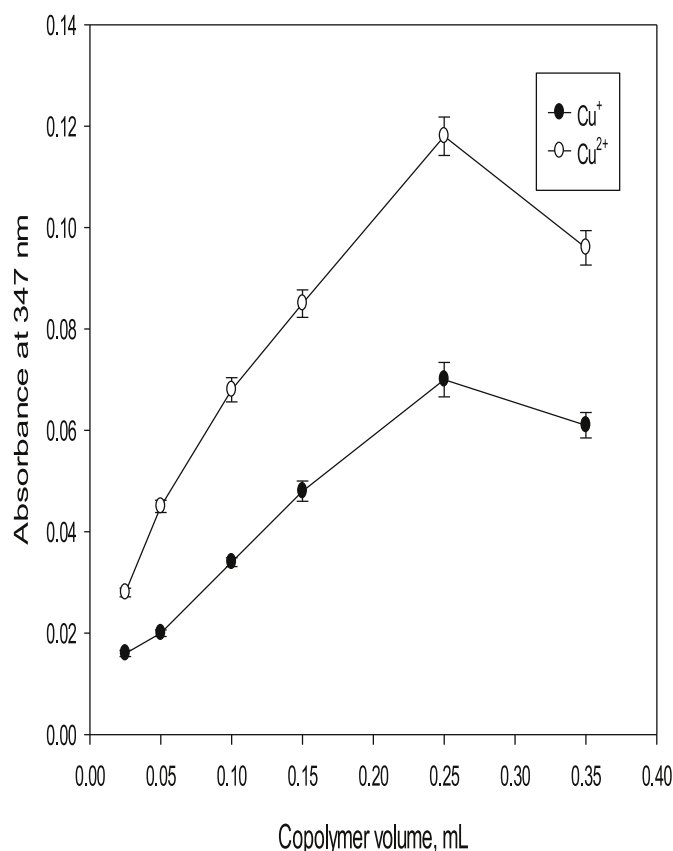
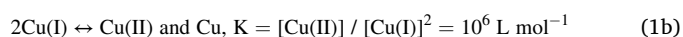


Fig. 2. Effect of copolymer volume for detection of Cu(II) and Cu(I) at pH 6.0 or 8.0 at levels of 75 µg L⁻¹ on the sensitivity.

support this situation, with acid hydrolysis constants of pK_{a1}: 2.4 and pK_{a2}: 4.0 from optical and conductivity measurements, the Cu(III) in form of Cu(OH)₃ at pH < 6.0 show a pH dependent absorption capability in the 280–350 nm wavelength range with red shift as a result of ligand induced disproportionation.



When the pH is higher than 8.0, it can be a problem for the hydrolysis of metal ions due to concentration-dependent gradual decrease or fluctuation in sensitivity. At higher pHs than 6.0 and 8.0, the low recoveries for the metal ions were observed owing to the incomplete complex formation among reagents in reaction media and further formation of pH-dependent anionic hydroxo-complexes of Cu(I) and Cu(II) like Cu(OH)₂, Cu(OH)₃²⁻ and Cu(OH)₄³⁻ as well as precipitation products like Cu(OH) or Cu₂O and Cu(OH)₂. Taking into account all these factors, the pH values of 6.0 and 8.0 as a result of participation of hydroxyl groups of on the surface of composite in complex formation in presence of CTAB as counter-ion in terms of extractable coordination saturated-complex formation of metal ions, Cu(II) and Cu(I) respectively were chosen for further studies.

The effect of buffer concentration at pH 6.0 or 8.0 on the sensitivity in Fig. 1(b) was also studied in volume range of 0.3–3.0 mL of 0.04 mol L⁻¹ B-R buffer solution. The best sensitivity was obtained at optimal volume of 2.0 mL for each ion, respectively. At lower and higher buffer volumes, the sensitivity for both Cu(I) and Cu(II) were gradually decreased. Therefore, a buffer volume of 2.0 mL for each ion was adopted for maximum sensitivity.

3.2.2. Effect of tris-modified copolymer matrix concentration

The chelating agent is one of the important factors influencing the

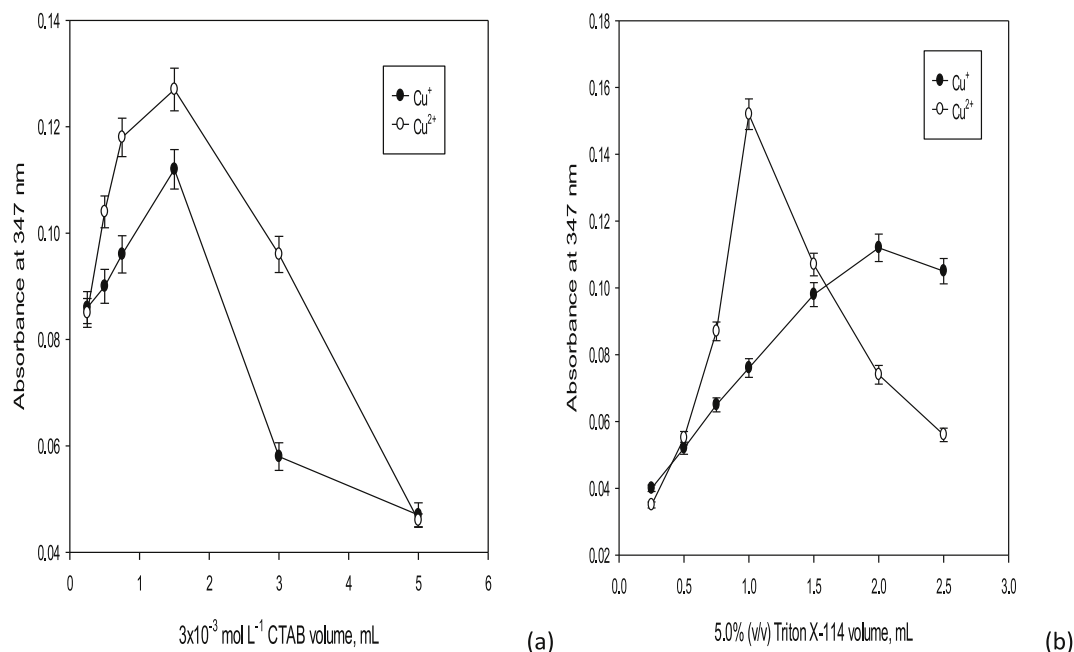


Fig. 3. (a–b) Effect of (a) 3.0×10^{-3} mol L⁻¹ CTAB and (b) 5.0% (v/v) Triton X-114 volumes for detection of Cu(II) and Cu(I) at pH 6.0 or 8.0 at levels of $75 \mu\text{g L}^{-1}$ on the sensitivity.

extraction efficiency. As can be seen from Fig. 2, the extraction of Cu(I) and Cu(II) were carried out in the Tris-modified copolymer matrix concentration ranging from 0.025 to 0.35 mL of 0.1 g/100 mL in acetone. The extraction efficiency for Cu(I) and Cu(II) linearly increased with increasing copolymer matrix volume up to a volume of 0.25 mL, reached a maximum sensitivity at a volume of 0.25 mL for each ion, respectively. However, when copolymer matrix volume in acetone is higher than 0.25 mL, the extraction efficiency gradually decreased. This decrease could be due to enter more free Tris-modified chelating copolymer matrix into the surfactant-rich phase as a result of concentration dependent interaction between molecules so as to give hydrophobic self-aggregates via hydrogen bonding, and remain more metal ions in main bulk aqueous phase without chelation. Therefore, 0.25 mL of copolymer matrix solution in acetone was concluded to be optimally enough for further experiments.

3.2.3. Effect of CTAB and Triton X-114 concentrations

The concentration of ionic and non-ionic surfactants used in the UA-CPE play key roles for maximum extraction efficiency. In presence of Triton X-114 as extractant in preconcentration of trace Cu(I) and Cu(II), at initial two ionic surfactants like CTAB and SDS in volume ranges of 0.25–5.0 mL at 3.0×10^{-3} mol L⁻¹ were used as both sensitivity enhancer and counter-ion in extraction step. The sensitivity in Fig. 3(a) linearly increased in range of 0.25–1.5 for both Cu(I) and Cu(II), reached to a maximum value at 1.5 mL, and gradually decreased at higher volumes than 1.5 mL. This decrease in sensitivity may be due to increase in analyte blank as a result of extractable ion-pair formation between CTAB and Tris-modified copolymer matrix having pH-dependent ionisable hydroxyl groups. Therefore, a CTAB volume of 1.5 mL for each ion was considered as optimal in this study. Due to its low sensitivity, except for a part signal additive at lower pHs than 5.0; SDS was not adopted in this study, and used in further studies.

Triton X-114 is one of the non-ionic surfactants widely used in UA-CPE as extractant due to its advantages such as commercial availability with high purity, low toxicity and cost as well as high density of the surfactant-rich phase thus promoting the phase separation by centrifugation, relatively low cloud point temperature (CPT: 11 °C for 0.03% in weight) and low critical micelle concentration (CMC: 0.205 mmol L⁻¹) where the presence of SDS or CTAB at levels of 1.25 mmol L⁻¹ in the

system leads to a substantial increase (about 50 °C) in its cloud point [54]. As a result, different volumes of 5.0% (v/v) Triton X-114 solution in Fig. 3(b) were investigated ranging from 0.25 to 2.5 mL for checking the extraction efficiency. The results are presented in Fig. 3(b). The recovery for Cu(I) and Cu(II) sharply increased with an increase in Triton X-114 volume up to 2.0 and 1.0 mL, respectively, so as to reach to a maximum. The sensitivity gradually decreases when the volume of Triton X-114 is higher than 2.0 or 1.0 mL. Such observations can be ascribed by an increase in volume and viscosity of the micellar phase. Thus, a volume of 2.0 and 1.0 mL at 5.0% (v/v) Triton X-114 solution for each ion was adopted as optimal for subsequent experiments in order to achieve the maximum sensitivity and thereby the highest extraction efficiency in spectrophotometric detection of metal ions at 347 nm.

3.2.4. Effect of incubation time and temperature

The largest analyte pre-concentration factor is possible when the UA-CPE process is performed with equilibration temperature well above the cloud point temperature of the micellar system. Therefore, the incubation temperatures ranging from 20 °C to 50 °C and time between 1 and 20 min in ultrasonic bath (300 W, 40 kHz) were studied. From the results, it has been observed that an equilibration time and temperature of 10 min and 40 °C is enough to reach the best sensitivity with maximum recovery for both metal ions.

3.2.5. Effect of centrifugation time

Effect of centrifugation time on the UA-CPE procedure was investigated in range of 1–20 min for fast phase separation. From the results, it has been observed that the phase separation is completed in a centrifugation time of 7 min at 3000 rpm, and found to be enough for complete UA-CPE.

3.2.6. Effect of the ionic strength

In general, the addition of salt could decrease the solubility of aqueous sample phase and lead to enhancement of the partitioning of the analyte into the surfactant rich phase by the “salting-out” phenomenon. The presence of salt can increase the incompatibility between the water structures in the hydration shells of analyte and surfactant macromolecules, which can reduce the concentration of “free water” in the surfactant-rich phase and, consequently, reduce the phase volume [47].

Table 1

The analytical features of the micellar sensitive and selective spectrophotometric method based on extraction, pre-concentration and determination of trace amounts of soluble inorganic Cu species, Cu(II) and Cu(I) at pH 6.0 and 8.0, respectively by UA-CPE.

| Amidic copolymer functionalized with Tris and Fe ₃ O ₄ | Linear working range, $\mu\text{g L}^{-1}$ | Determination coefficient, r^2 | Regression equation | *LOD, $\mu\text{g L}^{-1}$ | *LOQ, $\mu\text{g L}^{-1}$ | Precision | | Accuracy |
|--|--|----------------------------------|---|----------------------------|----------------------------|---|---|------------|
| | | | | | | Intra-day, RSD _r % (n: 5, for 25, 100 $\mu\text{g L}^{-1}$) | Inter-day, RSD _R % (n:3 \times 5, 25, 100 $\mu\text{g L}^{-1}$) | Recovery % |
| By the solvent-based calibration curves (n: 6) | | | | | | | | |
| For Cu(I) at pH 8.0 | 5–250 | 0.9929 | Abs: $(8.15 \pm 0.50) \times 10^{-4}C + (1.32 \pm 0.04) \times 10^{-2}$ | 1.60 | 5.34 | 2.5–4.7 | 4.3–7.6 | 92.5–97.0 |
| For Cu(II) at pH 6.0 | 2–140 | 0.9935 | Abs: $(1.81 \pm 0.2) \times 10^{-3}C + (1.25 \pm 0.04) \times 10^{-2}$ | 0.66 | 2.21 | 2.7–4.5 | 4.0–6.5 | 91.5–98.5 |
| By the matrix-matched calibration curves** (n: 6) | | | | | | | | |
| For Cu(I) at pH 8.0 | 7–210 | 0.9915 | Abs: $(8.30 \pm 0.50) \times 10^{-4}C + (1.40 \pm 0.06) \times 10^{-4}$ | 2.16 | 7.23 | 3.5–5.3 | 4.7–8.1 | 91.5–94.0 |
| For Cu(II) at pH 6.0 | 3–180 | 0.988 | Abs: $(1.68 \pm 0.20) \times 10^{-3}C + (1.35 \pm 0.06) \times 10^{-2}$ | 1.07 | 3.57 | 3.0–5.1 | 4.5–7.5 | 92.0–95.5 |

*The LODs and LOQs, described as $3 \times S_{\text{blank}}/m$ and $10 \times S_{\text{blank}}/m$, are based on the ratio of three and ten times of standard deviations of twelve replicate measurements of sample blanks to slopes of the calibration curves linearly ranging from 5 to 150 $\mu\text{g L}^{-1}$, respectively.

**The linear calibration curves built between concentration and analytical signal as a function of increasing copper concentration under optimal conditions, so as to fall in the range of 5–250 $\mu\text{g L}^{-1}$ before the extraction and pre-concentration of soluble Cu(I) and Cu(II) from sample matrix.

In order to investigate the effect of ionic strength on the UA-CPE performance, various experiments were performed by adding different amounts of NaCl (2–50 mmol L^{-1}). Other experimental conditions were kept constant during the analysis. The results show that ionic strength has a significant effect both on the pre-concentration factor and calibration sensitivity up to a concentration of 20 mmol L^{-1} in terms of stabilization of especially Cu^+ ions against oxidation. Thus, ionic strength was controlled at a concentration of 20 mmol L^{-1} in order to obtain reproducible and stable analytical signals in speciation analysis.

3.2.7. Effect of diluents

In order to facilitate the detectability of the sample solution by micro-volume UV–vis spectrophotometry, it was necessary to decrease the viscosity of the surfactant-rich phase. Different solvents such as acetone, acetonitrile, ethanol, methanol, and their acidified solutions (0.02 $\text{mol L}^{-1}\text{HCl}$), were tried in order to select the one producing the best results regarding sensitivity, reproducibility, and stability of the signal. The best result was obtained with acidic acetonitrile. Perhaps, this may be due to the fact that it is a solvent compatible with the sample matrix, as well as its strong chelate formation, especially with instable Cu(I) as additive. Aliquot of acidic acetonitrile (with optimum of 0.3 mL in the range of 0.1–1.5 mL) was added to the surfactant-rich phase after separation, in which the micellar phase is diluted to a volume of 0.5 mL for a pre-concentration of 70-fold from pre-concentration of optimal 35-mL sample (in the range of 5–50 mL). This amount of acidic acetonitrile was chosen to ensure a sufficient volume of the sample solution for maximum sensitivity. For smaller volumes, the reproducibility of the signals was very poor, whereas for higher volumes, there was a gradual decrease in the signal due to excess dilution.

3.3. Analytical figures of merit

Under the optimal conditions, the performance of the newly developed method was studied in detail. The method allows the detection of Cu(I) and Cu(II) by the solvent-based calibration curves (n: 6) in linear ranges of 5–150 and 2–140 $\mu\text{g L}^{-1}$ with a correlation coefficient of 0.993 while it allows the detection of metal ions by the matrix-matched calibration curves (n: 6) in linear ranges of 7–210 and 3–180 $\mu\text{g L}^{-1}$ with better determination coefficient than 0.988. In order to minimize the matrix effect and instrumental signal fluctuations, the matrix-matched standard calibrations curves, consisting of ten concentration levels (0,

5, 10, 25, 50, 100, 150, 200, and 250 $\mu\text{g L}^{-1}$ for sample extracts, were set up by spiking these different amounts of analyte into sample extracts. Blanks (samples with zero addition of the analyte) were simultaneously quantified using the standard addition method, and the analyte levels present in the samples were subtracted. To evaluate the performance of the calibration curves, all the samples were also analyzed using a standard addition method based on spiked with two levels of analyte standards (0, 25, 100 $\mu\text{g L}^{-1}$) in both solvent and sample extracts. The spiked sample extracts and blanks were run consecutively in the instrument. It has been observed that there is not a significant difference between slopes of calibration curves with a positive or negative matrix effect of +1.8% and –7.2% for Cu(I) and Cu(II), respectively, so as to cause enhancement or suppression in signal at 347 nm. From pre-concentration of 35-mL sample solution, a pre-concentration factor of 70-fold was obtained. All the remaining analytical parameters related to the pre-concentration system are extensively represented in Table 1.

3.4. The matrix effect

The interference effect of some anionic and cationic species on the pre-concentration in speciation analysis was studied. In the experiments, aqueous solutions containing Cu(I) or Cu(II) (75 $\mu\text{g L}^{-1}$) with the addition of interfering ions were treated in tolerance ratios ranging from 1:50 to 1:2000, except for possible interference of Cu(I) into detection of Cu(II), according to the recommended UA-CPE procedure under the optimal conditions, and the results are given in Table S2(a). Table S2(a) depicts the tolerance limits of the diverse ions, *i.e.* interfering-to-analyte ratios in which the relative error was less than $\pm 5.0\%$ in terms of signal variation. Only a serious interference at low tolerance ratios (in range of 50–100) has been observed from Hg(II) with a tolerance ratio of 50-fold, including sequentially the ion pairs of Sn(II)/Mn(II), As(III)/Sb(III), Fe(III)/Ag(I) with tolerance ratios of 75-, 100-, and 150-fold forming either a stable complex with the chelating ligand or oxidation of Cu(I) to Cu(II) produced as a result of disproportionation. The interference of Fe(III) and Ag(I) up to 1500-folds excess over Cu(II) were greatly suppressed using 1.0 mL of 0.02 mol L^{-1} pyridine in a medium buffered to pH 5.0. The interference of As(III) and Sb(III) were suppressed up to 1000-fold after oxidation with 0.01 mol L^{-1} H_2O_2 solution in alkaline medium. Also, the interference of Mn(II) and Sn(II) were suppressed up to 750-fold using 0.2 mL of 0.025 mol L^{-1} $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$ solutions. Finally, the serious interference of Hg(II) was suppressed up to 250-fold using 1.5

Table 2

The accuracy and/or validity of the method developed for Cu(II) at pH 6.0 after oxidation with diluted oxidant-salt mixture, 0.2 mol L⁻¹ HClO₄-0.02 mol L⁻¹ NaCl (4:1, v/v) at 40 °C in ultrasonic bath due to be more selective and sensitive as a function of ligand induced disproportionation for analysis of total Cu levels in two certified samples (n: 5).

| CRMs from FAPAS | Matrix | *Range, µg L ⁻¹ | Assigned value, µg L ⁻¹ | ^a Observed, µg L ⁻¹ | RSD% | Recovery% | ^b The z-score |
|-----------------|------------|----------------------------|------------------------------------|---|------|-----------|--------------------------|
| T07316QC | Wine | 169–384 | 277 | 285 ± 5.0 | 1.75 | 102.9 | 1.60 |
| T0783 | Soft drink | 257–553 | 405 | 410 ± 8.0 | 1.95 | 101.2 | 0.62 |

*The range for $|z| \leq 2$ is the concentration range for within the limits of ± 2 z-scores where the assigned value has been established from the proficiency teste data, and is suitable for use by many laboratories (with data points of 34) as a fit-for-purpose quality control measure.

^a The mean plus its standard deviation of five replicate measurements using the proposed method.

^b The experimental z-scores were calculated by using $z\text{-score} = (x - x_{\text{assigned}})/s$ for five replicate measurements at confidence interval of 95% where the $|z| < 2$, $2 < |z| < 3$ and $|z| > 3$ are sequentially satisfactory, questionable and unsatisfactory for reliability of the results.

Table 3a

The intra- and inter-day accuracy and precision studies of total Cu levels (as equivalent to Cu(II)) measured in the selected two quality control samples via matrix-matched calibration curve.

| Sample | Dilution ratio | Spiked, µg L ⁻¹ | Accuracy and precision | | | | | |
|---------------------------|----------------|----------------------------|--|------------|-------|--|------------|-------|
| | | | Intra-day (n: 5) | | | Inter-day (n: 3 × 5) | | |
| | | | ^a Found, µg L ⁻¹ | Recovery % | RSD % | ^a Found, µg L ⁻¹ | Recovery % | RSD % |
| Wine | 1:15 | – | 12.8 ± 0.5 | – | 3.9 | 12.4 ± 0.6 | – | 4.8 |
| | | 10 | 22.1 ± 0.8 | 93 | 3.6 | 21.5 ± 0.8 | 91 | 3.7 |
| Beer with alcohol of 3.0% | 1:10 | – | 8.5 ± 0.4 | – | 4.7 | 8.3 ± 0.4 | – | 4.8 |
| | | 10 | 17.7 ± 0.7 | 92 | 4.0 | 17.3 ± 0.7 | 90 | 4.0 |
| Beer without alcohol | 1:10 | – | 3.5 ± 0.2 | – | 5.7 | 3.3 ± 0.2 | – | 6.1 |
| | | 10 | 12.7 ± 0.5 | 92 | 3.9 | 12.3 ± 0.5 | 90 | 4.1 |

^a The mean plus its standard deviation of five replicate measurements obtained by using matrix-matched calibration approaches prepared from sample extracts, and 5.0 mL of the degassed-, pretreated- and extracted-sample solutions were independently analyzed after dilution with 0.2% (v/v) HNO₃ at ratio of 1:10 and 1:15.

mL of 0.01 mol L⁻¹ S₂O₃²⁻ solutions in a medium buffered to pH 5.0–5.5. Moreover, it is clear in Tables S2(a–b) that Cu(II), in speciation analysis performed in binary mixtures of Cu(II) and Cu(I) at ratios ranging from 1:1 to 1:30 where the recovery rates and precision changed in range of 91.0–97.7%, and 3.3–5.7%, respectively, for three replicate measurements, can be reliably able to detect up to a tolerance ratio of 1:35 in presence of excess Cu(I) at pH 6.0 without using any further masking agent. As can be seen from Table S2(a), it is clear that the UA-CPE process at the chelating hydrophilic copolymer matrix-micellar interface is relatively selective in terms of the possible major matrix components in real samples. The recoveries in the range of 92.0–105.5% with a lower RSD than 7.3% were obtained for different metal to interfering ratios for the Cu(II) studied. The results indicated that the sample matrix did not affect the absorbance signals of Cu(II) at the studied interfering-metal tolerance ratios.

3.5. The method accuracy

The accuracy was controlled and validated by analysis of two certified quality control samples, T07316QC and T0783 wine and soft drink supplied from FAPAS before extraction and spectrophotometric analysis. It can be seen that the results found by the method in Table 2 are statistically in good agreement with their assigned values using the z-score where the $|z| < 2$, $2 < |z| < 3$ and $|z| > 3$ are sequentially satisfactory, questionable and unsatisfactory for reliability of the results for 4° of freedom at confidence interval of 95%. Also, from five replicate measurement results, it is clear that accuracy and precision levels can be quantitatively accepted with a recovery rate in range of 102.9–102.2% and a RSD in range of 1.62–0.62%, respectively.

3.6. The speciation analysis of the samples

The method was applied into the speciation of Cu(I), Cu(II), and total Cu levels present in beverage samples. The results are shown in Table 3 (a-b). It was found that copper at low ppb levels observed in sample matrices studied. According to the paired t-test, the accuracy of the

method was also controlled and verified by comparison of the results obtained from replicate measurements with those of FAAS as an independent comparison method, in terms of total Cu levels after dilution at suitable ratios with and without spiking. Also, the selected sample matrices were spiked with each ion at concentration levels of 10 µg L⁻¹ including two quality control samples, before extraction and analysis. Moreover, the total Cu analysis of two quality control samples via matrix-matched calibration curves after dilution with and without spiking at 10 µg L⁻¹ were performed in Table 3(a), and it has been observed that the intra-day and inter-day accuracy and precision are quantitative with a recovery equal to or higher than 90% and a lower precision than 6.1%, respectively.

From measurement results without spiking via matrix-matched calibration curve to overcome the matrix effect in spectrophotometric analysis, it has been observed in Table 3(b) that the total Cu levels of the beverage samples after pre-oxidation and dilution in range of 1:5–1:15 folds are in range of 8.7–29.7 µg L⁻¹ while the total Cu levels obtained by FAAS are in range of 8.5–30.5 µg L⁻¹. From the comparison of results according to the paired t-test, it can be seen that there is not statistically a significant difference between the results found by the both methods with lower t-value (0.63–2.53) than critical t-value of 2.78. In a similar way, the Cu(I) and Cu(II) levels of the samples by the present method were established, and observed to in range of 6.7–25.1 and 2.3–4.8 µg L⁻¹, respectively where the speciation results found by FAAS ranged from 6.5 to 25.5, and 2.5–5.2 µg L⁻¹. It is clear that the results are compatible with each other to confirm for the detectability of Cu(I) and Cu(II). When considered aliquots of 15 mL of liquid samples and 5.0 mL of the pre-treated and extracted sample solutions, the total Cu levels obtained by the present method were in range of 37.5–89.1 µg L⁻¹ in wine while those of beer and soft drinks were in range of 26.1–54.3 and 32.1–44.4 µg L⁻¹, respectively, with a minimal value of 10.5 µg L⁻¹ for a non-alcohol beer sample preferred for quality control.

Finally, copper was detected in all the analyzed samples in concentrations below the maximum acceptable limit set by the OIV [48] (1 mg L⁻¹), which is not considered as a health risk. Also, our total Cu levels were reasonably in agreement with those found in the literature,

Table 3b

Speciation analysis of soluble inorganic copper, Cu(I) and Cu(II) in beer, soft drinks and wine samples (n: 5).

| Sample matrix | Dilution ratio | ^a By FAAS | | | ^b By the present method | | | | | | | | Total Cu, $\mu\text{g L}^{-1}$ | ^c The paired t-test, t_{cal} |
|---------------|----------------|-----------------------------|------------|-----------|------------------------------------|--------|-----------------------------|------------|---------|--------|------------|--------|--------------------------------|--|
| | | Found, $\mu\text{g L}^{-1}$ | | | Spiked, $\mu\text{g L}^{-1}$ | | Found, $\mu\text{g L}^{-1}$ | | RSD % | | Recovery % | | | |
| | | Total Cu | Cu(I) | Cu(II) | Cu (II) | Cu (I) | Cu(I) | Cu(II) | Cu (II) | Cu (I) | Cu (II) | Cu (I) | | |
| Beer1 | 1:5 | 8.5 ± 0.4 | 6.5 ± 0.3 | 2.5 ± 0.1 | – | – | 6.7 ± 0.3 | 2.3 ± 0.1 | 4.5 | 4.3 | – | – | 8.7 ± 0.4 | 0.79 |
| | | | | | 10 | – | 16.1 ± 0.5 | – | 3.1 | – | 94 | – | | |
| | | | | | – | 10 | – | 12.0 ± 0.4 | – | 3.3 | – | 97 | | |
| Beer2 | 1:5 | 10.5 ± 0.4 | 7.5 ± 0.3 | 2.5 ± 0.1 | – | – | 7.2 ± 0.3 | 2.3 ± 0.1 | 4.2 | 4.3 | – | – | 10.7 ± 0.4 | 0.79 |
| | | | | | 10 | – | 16.7 ± 0.5 | – | 3.0 | – | 95 | – | | |
| | | | | | – | 10 | – | 11.8 ± 0.4 | – | 3.4 | – | 95 | | |
| Beer3 | 1:10 | 15.5 ± 0.4 | 12.5 ± 0.3 | 2.5 ± 0.1 | – | – | 12.7 ± 0.5 | 2.3 ± 0.1 | 3.9 | 4.3 | – | – | 15.2 ± 0.4 | 1.19 |
| | | | | | 10 | – | 22.1 ± 0.7 | – | 3.2 | – | 94 | – | | |
| | | | | | – | 10 | – | 11.8 ± 0.4 | – | 3.4 | – | 95 | | |
| Beer4 | 1:10 | 18.5 ± 0.5 | 13.5 ± 0.4 | 5.1 ± 0.2 | – | – | 13.0 ± 0.4 | 5.3 ± 0.2 | 3.1 | 3.8 | – | – | 18.1 ± 0.5 | 1.26 |
| | | | | | 10 | – | 22.6 ± 0.7 | – | 3.1 | – | 96 | – | | |
| | | | | | – | 10 | – | 14.8 ± 0.5 | – | 3.4 | – | 95 | | |
| Soft drinks1 | 1:10 | 12.5 ± 0.4 | 9.8 ± 0.3 | 2.5 ± 0.1 | – | – | 9.6 ± 0.3 | 2.4 ± 0.1 | 3.1 | 4.2 | – | – | 12.7 ± 0.4 | 1.26 |
| | | | | | 10 | – | 19.2 ± 0.6 | – | 3.1 | – | 96 | – | | |
| | | | | | – | 10 | – | 12.0 ± 0.4 | – | 3.3 | – | 96 | | |
| Soft drinks2 | 1:10 | 15.5 ± 0.5 | 11.8 ± 0.4 | 3.5 ± 0.1 | – | – | 11.7 ± 0.4 | 3.3 ± 0.1 | 3.4 | 3.0 | – | – | 14.8 ± 0.5 | 2.21 |
| | | | | | 10 | – | 21.3 ± 0.6 | – | 2.8 | – | 96 | – | | |
| | | | | | – | 10 | – | 12.7 ± 0.4 | – | 3.1 | – | 94 | | |
| Soft drinks3 | 1:15 | 10.5 ± 0.4 | 8.0 ± 0.3 | 2.8 ± 0.1 | – | – | 10.1 ± 0.3 | 2.7 ± 0.1 | 3.0 | 3.7 | – | – | 10.7 ± 0.4 | 0.63 |
| | | | | | 10 | – | 19.5 ± 0.6 | – | 3.1 | – | 94 | – | | |
| | | | | | – | 10 | – | 12.3 ± 0.4 | – | 3.2 | – | 96 | | |
| White wine1 | 1:10 | 12.8 ± 0.5 | 9.5 ± 0.3 | 3.5 ± 0.1 | – | – | 9.2 ± 0.3 | 3.6 ± 0.2 | 3.3 | 5.5 | – | – | 12.5 ± 0.5 | 0.95 |
| | | | | | 10 | – | 18.7 ± 0.5 | – | 2.7 | – | 95 | – | | |
| | | | | | – | 10 | – | 13.0 ± 0.4 | – | 3.1 | – | 94 | | |
| White wine 2 | 1:10 | 15.5 ± 0.5 | 12.2 ± 0.4 | 3.0 ± 0.1 | – | – | 11.7 ± 0.4 | 2.8 ± 0.1 | 3.4 | 3.6 | – | – | 15.1 ± 0.5 | 1.27 |
| | | | | | 10 | – | 21.3 ± 0.6 | – | 2.8 | – | 96 | – | | |
| | | | | | – | 10 | – | 12.2 ± 0.4 | – | 3.3 | – | 94 | | |
| White wine 3 | 1:10 | 17.5 ± 0.5 | 13.5 ± 0.3 | 3.7 ± 0.1 | – | – | 16.8 ± 0.5 | 3.4 ± 0.2 | 3.0 | 5.9 | – | – | 17.2 ± 0.5 | 0.95 |
| | | | | | 10 | – | 26.1 ± 0.7 | – | 2.7 | – | 93 | – | | |
| | | | | | – | 10 | – | 12.8 ± 0.4 | – | 3.1 | – | 94 | | |
| Red wine1 | 1:15 | 21.5 ± 0.6 | 16.5 ± 0.5 | 4.3 ± 0.2 | – | – | 16.2 ± 0.5 | 3.9 ± 0.2 | 3.1 | 5.1 | – | – | 20.8 ± 0.6 | 2.21 |
| | | | | | 10 | – | 25.4 ± 0.7 | – | 2.8 | – | 92 | – | | |
| | | | | | – | 10 | – | 13.3 ± 0.4 | – | 3.0 | – | 94 | | |
| Red wine2 | 1:15 | 24.5 ± 0.7 | 19.5 ± 0.6 | 4.5 ± 0.2 | – | – | 19.0 ± 0.6 | 4.1 ± 0.2 | 3.2 | 4.9 | – | – | 23.7 ± 0.7 | 1.81 |
| | | | | | 10 | – | – | – | 2.8 | – | 94 | – | | |

(continued on next page)

Table 3b (continued)

| Sample matrix | Dilution ratio | ^a By FAAS | | | ^b By the present method | | | | | | ^c The paired <i>t</i> -test, <i>t</i> _{cal} | | | | |
|---------------|----------------|---------------------------|------------|-----------|------------------------------------|--------|---------------------------|------------|---------|--------|---|------------|------------|------------------------------|--|
| | | Found, µg L ⁻¹ | | | Spiked, µg L ⁻¹ | | Found, µg L ⁻¹ | | RSD % | | | Recovery % | | Total Cu, µg L ⁻¹ | |
| | | Total Cu | Cu(I) | Cu(II) | Cu (II) | Cu (I) | Cu(I) | Cu(II) | Cu (II) | Cu (I) | | Cu (II) | Cu (I) | | |
| | | | | | | | 28.4 ± 0.8 | | | | | | | | |
| | | | | | – | 10 | – | 13.5 ± 0.4 | – | 3.0 | – | 94 | | | |
| Red wine3 | 1:15 | 30.5 ± 1.0 | 25.5 ± 0.7 | 5.2 ± 0.2 | – | – | 25.1 ± 0.8 | 4.8 ± 0.2 | 3.2 | 4.2 | – | – | 29.7 ± 1.0 | 2.53 | |
| | | | | | 10 | – | 34.5 ± 0.8 | – | 3.2 | – | 94 | – | | | |
| | | | | | – | 10 | – | 14.2 ± 0.4 | – | 2.8 | – | 94 | | | |

* Beer samples were degassed, sonicated and diluted at a ratio of 1:5 and/or 1:10 fold according to copper levels of samples before analysis.

** Soft drinks and wine samples were sonicated, bleached with PVP-15 and diluted at a ratio of 1:5 and/or 1:15 fold according to copper levels of samples before analysis.

^a The mean plus its standard deviation of five replicate measurements obtained by means of FAAS chosen as a comparison method for the reliability of results after pretreatment with 5.0 mL of the dilute acid-salt mixture of 0.2 mol L⁻¹ HClO₄ and 0.02 mol L⁻¹ NaCl (4:1, v/v) in 3.5% (v/v) ethyl alcohol and 125 mg L⁻¹ tartaric acid for matrix matching under ultrasonic bath conditions in a centrifuge tube of 50-mL (for 7 min at 40 °C at amplitude of 40 kHz at 300 W) in sample extracts buffered to a pH of 6.0 in presence of acidic acetonitrile (0.02 mol L⁻¹ HCl) for prevention of possible internal conversion of Cu(I) and Cu(II), and quantitatively pre-oxidation of Cu(I) to Cu(II) with 2.0 mol L⁻¹ HNO₃ for duration of 5 min at 55 °C under similar sonication conditions in analysis step of soluble free Cu(I), Cu(II) and total Cu, respectively.

^b ^a The mean plus its standard deviation of five replicate measurements obtained by using matrix-matched calibration approaches prepared from sample extracts, and 5.0 mL of the degassed-, pretreated- and extracted-sample solutions were independently analyzed after dilution with 0.2% (v/v) HNO₃ in range of 1:5–1:15-folds.

^c The experimental *t*-values were calculated by using $t = (x_{\text{average},1} - x_{\text{average},2})/s_{\text{pooled}} \times [(N_1 + N_2)/(N_1 \times N_2)]^{1/2}$ for five replicate measurements at confidence interval of 95% in which the critical *t*-value is 2.78 for 8° of freedom at confidence interval of 95%.

indicating that the results obtained from wine and beer samples chosen from different regions of Turkey were sequentially in range of 76–126 [49] and 17.38–126.84 µg L⁻¹ [50], respectively, except that of red wines collected in Southeast Turkey with a value ranging from 1.01 to 1.94 mg L⁻¹ [51] as a result of surface contamination caused the pesticides that involve use of copper sulfate salt as a protective against pests. According to Green et al. [22], the presence of Cu in the wine can be also attributed to application of fungicides in the vineyards, by spraying products containing Cu that are used for pests and disease control as well as by the contact of the must with utensils and equipment that incorporate this metal within their components.

3.7. Comparison with other related methods

The method was compared with other detection methods that had recently been reported in the literature for extraction, pre-concentration, and determination of copper from sample matrices. The analytical performance properties of the method are given in Table S5. As can be seen from Table S5, it is clear that the method has quantitatively the reasonable linear working ranges for the magnetic composite. Moreover, the detection limits of the method, 0.66 and 1.60 µg L⁻¹ for Cu(II) and Cu(I) at pH 6.0 and 8.0 respectively, is either better than or comparable with that of other methods which even use more sensitive detection techniques such as spectrophotometry, FAAS, ICP-OES, and ED-XRF after pre-concentration with similar or different further extraction procedures [12–18,26,28,29], except for pre-concentration procedures based on selective and charge transfer sensitive reagents participating in the extraction process such as safranin T and cupferron [11,21,22,27]. In fact, the sensitive detection techniques like ED-XRF, ICP-MS, ICP-OES, ETAAS or GFAAS for trace and/or ultra-trace analysis, require expert user in his/her research area, including expensive, complicated, and time-consuming sample cleaning/preparation, and extraction procedures at different operational modes. Shortly, the method, based on sensitive and selective detection of low levels of Cu(I) and Cu(II) by micro-volume UV–vis spectrophotometry at 347 nm, can be evaluated as simple, cost-effective, eco-friendly, accurate, and reliable analytical detection tool with a pre-concentration factor of 70-fold

from pre-concentration of 35-mL sample because it uses low-volume non-toxic organic solvents and shows more favorable properties as simplicity, quickness, and relatively low cost when compared to dual CPE, SPE, and different modes of SPE using magnetic particles and composites, requiring generally longer analysis time, high aqueous sample volume, and manipulation of sample as well as possibility of contamination, loss of analyte, risk of degradation of compounds during long analysis time, and less accuracy and precision. In addition, the method gave comparably reliable results in terms of linearity, accuracy, and repeatability/reproducibility, and provided an evidence of spectrophotometry's feasibility as an alternative approach to routine quality control of low amounts of soluble free Cu ions in other complicated sample matrices.

4. Conclusions

A new UA-CPE procedure was developed for extraction/pre-concentration of Cu(I) and Cu(II) from sample matrix by using tris-modified amidic copolymer matrix as a chelating agent, Triton X-114 and CTAB as extracting and sensitivity enhancer surfactants at pH 8.0 and 6.0, respectively. The developed protocol has been successfully employed for the speciation analysis of low levels of Cu(I) and Cu(II) in beverage matrices via micro-volume UV–vis spectrophotometry at 347 nm against a blank. Five calibration solutions can be analyzed with three sample blanks in a complete analysis (20 min), including enrichment and determination steps by combination of UA-CPE with micro-volume UV–vis spectrophotometry. Briefly, a complete sample pre-concentration and determination experiment takes a duration of max. 30 min in ultrasonic bath. With respect to its achieved analytical features, the proposed method is simple, low-cost, reasonably rapid/selective, eco-friendly, low in LODs (0.66 and 1.60 µg L⁻¹), wide in linear ranges (2–140 and 5–150 µg L⁻¹) and highly reproducible (RSD < 8.1%).

Credit author statement

Hacı Bayram Zengin, Dr.: Conceptualization, Investigation, Formal

analysis, Conduction of experimental studies, Data collection, Interpretation of XRD and SEM/EDX peaks, Writing-Review and Editing. Ramazan Gürkan, Prof. Dr.: Supervision, Method development, Methodology, Validation, Writing - original draft preparation, Review and Editing

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2020.121789>.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Informed consent is not applicable to this study.

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