ORIGINAL PAPER

A magnetic nanocomposite based on amine-functionalized pH-sensitive functional poly(styrene-co-maleic anhydride) copolymer for selective extraction, pre-concentration and determination of sub-trace Ag$^+$ and Cu^{2+} ions from edible vegetable oils by a combination of spectrophotometry and ultrasound-assisted cloud point extraction

H. B. Zengin[1] · R. Gürkan[1]

Abstract

In this study, a new magnetic nanocomposite based on amine-functionalized pH-sensitive poly(styrene-co-maleic anhydride) copolymer derived from the tris(2-hydroxymethyl)aminomethane and Fe_3O_4 nanoparticles was designed, characterized and used in selective extraction and pre-concentration of sub-trace Ag^+/Cu^{2+} ions from aqueous solutions by ultrasound-assisted cloud point extraction. The method is based on the formation of charge transfer complexes among Ag^+/Cu^{2+} ions and weak basic amidic copolymer matrix at pH 4.0/5.0, respectively, in the presence of pH-sensitive anionic surfactant, and their detection by spectrophotometry at 346 nm. The variables affecting extraction efficiency were optimized in detail. Under the optimized conditions, the good linear relationships were obtained in ranges of 0.8–110/1.0–120 μg L^{-1} for Ag$^+$ and 1–125/2.0–140 μg L^{-1} for Cu^{2+} by two calibration curves with a better determination coefficient than 0.992. The limits of detection, repeatability/intermediate precision (as RSDs%, 5, 25 and 100 μg L^{-1}, n: 5) and the percent recoveries were 0.25/0.41 μg L^{-1}, 3.0–7.1% and 90.4–98.5%, respectively. From pre-concentration of 25 mL sample solution, a pre-concentration factor was found to be of 62.5 with sensitivity enhancements of 35.2- and 28.3-fold in calibration for each ion. A matrix effect was not observed (n: 3, for 50 μg L^{-1} of each ion). The accuracy was validated by analysis of the two certified samples. The standard addition method after two sample pre-treatment procedures was successfully applied to determination of total Ag/Cu contents of edible vegetable oils, and the quantitative recoveries were obtained in the ranges of 87–94% with lower RSD than 6.8% after spiking with 10 μg L^{-1}.

Keywords Cloud point extraction · Tris-modification · Copolymer · UV–Vis spectrophotometry · Ag$^+$ and Cu^{2+} · Edible vegetable oils

Introduction

Copper 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 [2, 3]. But, Cu(II) at higher levels is toxic and severe oral intoxication will affect mainly the blood and kidneys. Because of these, the trace Cu(II) content in foods must be controlled on a daily basis and the European Commission has fixed the limit of 2 mg L^{-1} for Cu(II) in drinking water and the allowed limit of Cu(II) is set to 1.3 mg L^{-1} in the USA similar to that in Canada (1.0 mg L^{-1}) [4, 5].

Silver as soluble Ag compounds may be used as external antiseptic agents (15–50 μg L^{-1}), as bacteriostatic agents (up to 100 μg L^{-1}) and as disinfectants (> 150 μg L^{-1})

✉ R. Gürkan
rgurkan@cumhuriyet.edu.tr; rgurkan95@gmail.com

1   Faculty of Sciences, Department of Chemistry, University of Cumhuriyet, TR-58140 Sivas, Turkey

(WHO, 2003). Most foods contain traces of silver in the 10–100 µg kg^{-1} range [6]. Although this oligo-dynamic metal is usually present in foods and beverages at trace/ultra-trace levels, long-term exposure can have negative impacts on human health. Therefore, there is an increasing need to monitor Ag and Cu levels in edible vegetable oils at ever decreasing concentrations. From the analytical chemistry point of view, there is of great importance to develop simple, low-cost, rapid, sensitive, selective, accurate and reliable separation, pre-concentration and determination methods.

Trace amounts of essential metal ions, such as Fe and Cu, are known to reduce the oxidation stability of edible vegetable oils which has deleterious effects on tastes, odors and flavors [7, 8]. Therefore, the International Olive Council has established as quality criteria a maximum residue level (MRL) or secondary maximum contamination level (SMCL) for their contents in both olive oils and olive–pomace oils: 0.1 µg g^{-1} for Cu and Ag (including As and Pb) [9]. At trace/sub-trace levels, Cu^{2+} and Ag^+ ions, due to their redox and bactericidal activities, can act as a catalyst on the decomposition of hydroperoxides, aldehydes, ketones, acids and epoxides present in edible oils. Moreover, these metal ions can attack olefinic bonds of unsaturated fat acids by forming stable or metastable d$_{II}$ complexes in redox cycle and thus decrease quality of liquid oil. Thus, accurate and reliable determination of the trace amounts of Ag and Cu is of great importance for the assessment of oil quality with regard to freshness, stability and storage. The presence of these oligo-metals in edible vegetable oils was quantitatively measured by different detection techniques such as inductively coupled plasma mass spectrometry (ICP-MS) [10], electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS) [11], inductively coupled plasma optical emission spectrometry (ICP-OES) [8, 12], graphite furnace atomic absorption spectrometry (GFAAS) [13], flame atomic absorption spectrometry (FAAS) [14–16], derivative potentiometric stripping analyses (dPSA) [17], adsorptive stripping square wave voltammetry (Ad-SSWV) [18], stripping chronopotentiometry (SCP) [19], UV–Vis spectrophotometry [20] and ion chromatography with variable wavelength UV detection (IC-UV detection) [21]. Most of these detection techniques require sample pre-treatments involving the addition of one or more organic reagents, extraction with acids or acidic mixtures, wet digestion or dry ashing for eliminating the matrix effect. These sample pre-treatment procedures are time-consuming, are prone to contamination and involve potential risks such as analyte losses, impoverishment of the overall detection limit as a result of dilution of sample and the use of dangerous acid or acid mixtures. GFAAS [13] has been directly used to analyze edible oils requiring no sample preparation. However, such expensive instrumentation is not commonly available in the underdeveloped research laboratories.

Among these detection techniques, the use of UV–Vis spectroscopic techniques can be considered as direct, fast, simple, easy-to-use, less expensive and versatile alternatives. Also, the sensitivity and selectivity of spectrophotometry can greatly be improved when it is used with a suitable chromogenic or fluorogenic reagent based on charge transfer for detection of the analytes in the UV–Vis region. Two important limitations arise in determining different sample matrices: (i) when the analyte level is lower than the method detection limit, and (ii) there is a matrix effect that systematically causes a suppression and enhancement of the signal. To overcome these problems, a separation/pre-concentration procedure is usually necessary prior to determination of analyte(s) by spectrophotometry. There is still need to pre-concentration techniques combined with spectrophotometry. The authors have reported many techniques in the literature for the separation and extraction of Ag^+ and Cu^{2+} alone or in combination, or multiple metal ions from different sample matrices and their pre-concentration in another phase such as supported liquid membrane technology [22], triple-phase single-drop microextraction (TP-SDME) [23], ultrasound-assisted temperature-controlled ionic liquid microextraction (UA-TC-ILME) [24], ultrasound-assisted emulsification microextraction (UA-EME) [25], rapidly synergistic cloud point extraction (RS-CPE) [26], solvent-assisted dispersive solid-phase extraction (SA-dSPE) [27], and dispersive liquid–liquid microextraction based on solidification of floating organic drop (DLLM-SFOD) [28], including reversed-phase ultrasonic-assisted liquid–liquid microextraction (RP-UA-LLME) [29], with their self-advantages and self-disadvantages.

Among these, CPE is an eco-friendly alternative separation/pre-concentration procedure, which is based on the phase behavior of nonionic and zwitterionic surfactants in aqueous solutions which exhibit phase separation after an increase in temperature or the addition of a salting-out agent [30]. The procedure enables us to avoid hazardous organic solvents and allows to achieve a much higher concentration of analyte than in the case of conventional LLE, because the micellar phase volume is about 10–100-fold less than that of an aqueous phase. The effectiveness of the CPE is due to its high selectivity and the possibility of obtaining high pre-concentration factor while analyzing small sample volumes. In order to reduce the time and steps needed, as well as selectivity, and thus greatly improve extraction efficiency, different ways such as vortex-inducing, ultrasound and microwave energies which help and facilitate sample preparation have been efficiently used in CPE. Moreover, the dilute reagents in CPE assisted by ultrasound energy are normally used, decreasing blank values (thus improving detection limit) and reducing both reagent and time consumption unlike traditional CPE [30].

Recently, magnetic NPs with and without modification have wide application in separation of heavy metals. Recently, the magnetic nanocomposite based on $Fe_3O_4@$ styrene–maleic anhydride copolymer [31] has successfully been used in extraction and pre-concentration of Ag^+ ions and Ag-NPs. Also, the chitosan, which is a natural biopolymer with a pK_a of 6.5 at pH 5.0 [32], and a cross-linked chitosan modified with histidine moiety at pH 6.0 [33] containing imine, amine, amine/imidazole and carboxylic surface functional groups for chelation of Ag^+ ions were selectively used in analysis step in real-time samples by means of detection tools, which are sensitive, but expensive, complex and requiring expert user in his/her area such as ET-AAS, ICP-MS and ICP-OES. To provide a significant enhancement in selectivity and sensitivity of the extraction process and enough to judge the novelty of the method, spectrophotometer as a low-cost, easy-to-use, inexpensive and versatile detection tool was successfully combined with UA-CPE based on hydrophilic pH-sensitive poly(styrene-co-maleic anhydride) modified with tris(2-hydroxymethyl)aminomethane (Tris) as a chelator and magnetized with Fe_3O_4 NPs, poly(SMAm and/or SMIm)/Fe_3O_4 NPs for selective extraction and pre-concentration of both trace Ag^+ ions in linear ranges of 4–160 and 10–350 μg L^{-1} with a detection limit of 1.21/4.28 μg L^{-1} at pH 10 [34], and speciation analysis of Cu^+ and Cu^{2+} ions in linear ranges of 0.3–150 and 10–350 μg L^{-1} with detection limits of 0.095/3.03 μg L^{-1} at pH 7.0 and 5.0 [35], respectively, from the selected sample matrices in the presence of ionic surfactant, cetyltrimethylammonium bromide (CTAB) ion our previous studies.

In this work, for the first time, UA-CPE based on amine-functionalized pH-sensitive poly(SMA) was used as a new sample preparation method for selective extraction and pre-concentration of Ag^+ and Cu^{2+} ions. The proposed method was applied for their determination in edible vegetable oils at trace levels using micro-volume UV–Vis spectrophotometer. As in our first two studies, the poly(SMAm)/Fe_3O_4 NPs were synthesized by radical polymerization, surface modification with Tris, and then magnetization with Fe_3O_4 NPs, including pure Fe_3O_4 NPs, as the selective binding ligand of Ag^+ and Cu^{2+} ions in the presence of ionic surfactant and sodium dodecyl sulfate (SDS) at pH 4.0 and 5.0, respectively, where the proton exchange rate between amide and imidic acid (or iminol) tautomers is selectively controlled with changing pH. To evaluate and optimize the influence of the main factors on extraction step, one-variable-at-a-time method was used. To examine the applicability of the method, UA-CPE was applied for pre-concentration and trace monitoring of Ag^+ and Cu^{2+} ions in edible vegetable oil matrices.

Experimental

Reagents, standard solutions and samples

Ultra-pure water (resistivity of 18.2 MΩ cm^{-1}) obtained by a Labconco Water Purification System (Kansas City, USA) was used throughout this study. All glass wares, pipettes and plastic tubes were cleaned by soaking in 5.0% (v/v) HNO_3 solution for 1 day and later rinsed five times with ultra-pure water before starting experiment. The standard working solutions of Ag^+ and Cu^{2+} ions at μg L^{-1} levels used in optimization step were prepared daily by diluting 1000 mg L^{-1} metal stock solutions purchased from Merck (Darmstadt, Germany) with 0.2 mol L^{-1} HNO_3 solution immediately before use. Due to being different of the extraction conditions for each metal ion, two working solutions for calibration were used: one for Ag^+ and another for Cu^{2+}. The modified and magnetized amide copolymer matrix with Tris and magnetite (Fe_3O_4), respectively (as 0.1 g/100 mL), was prepared by dissolution of its suitable amounts in tetrahydrofuran (THF). The ionic and nonionic surfactants, SDS (4.0% (w/v)), polyethylene glycol *tert*-octylphenyl ethers (Triton X-114, 5.0% (v/v)) as mixed extractant, obtained from Sigma-Aldrich (St. Louis, MO, USA), were prepared by dissolving appropriate amounts of surfactant in 100-mL volumetric flasks and vortex-mixing for a homogeneous clear solution when necessary. The pH of the sample solutions was adjusted by using McIlvaine buffer (each one, 0.2 mol L^{-1} Na_2HPO_4 and 0.1 mol L^{-1} citric acid, for adjusting to pH to 4.0 for Ag^+ and 5.0 for Cu^{2+}). Edible oil samples were supplied from local markets (Sivas, Turkey) to evaluate the applicability of the method. For accuracy of the method, two certified samples supplied from National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA) and SCP Science, Canada, were analyzed: SRM 1643a Trace elements in natural water and CRM EnviroMAT™ HU-1 Used Oil.

Instrumentation

All the absorbance measurements were taken with a double-beam spectrophotometer with micro-cell sample holder (Shimadzu UV-1800 model, Kyoto, Japan). The maximum absorbance of the ion pair complex based on CT in a cell of 400 μL was measured at 346 nm against sample blank (in THF as diluent) without analyte for background correction. FTIR spectra were taken using a Bruker (Alpha 12,283,105 model, Billerica, MA, Germany) spectrometer (with direct sampling at ATR mode without KBr pellet) in the range of 4000–400 cm^{-1} at 4 cm^{-1} resolutions. ^{1}H-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. 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 with energy dispersion of X ray (SEM and/or SEM/EDX) (TESCAN MIRA3 model, Czech Republic) was used to study the morphological characteristics of the magnetic nanocomposite. The pH measurements were taken using a digital pH meter (Selecta-2001 plus, Barcelona, Spain) supplied with a glass–calomel electrode. A centrifuge (Hettich Universal 320 model, 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 a power of 300 W. An ultrasound agitator was used for acceleration of the mass transfer in the extraction process.

Preparation of the magnetic nanocomposite, poly(SMAm)-Tris-Fe$_3$O$_4$ NPs

Synthesis of the poly(SMA) by radical polymerization, surface modification with Tris (as poly(SMAm) and/or poly(SMIm)) by chemical process for selective chelation of Ag$^+$ and Cu^{2+} ions, and then its magnetization with Fe$_3$O$_4$ NPs (as poly(SMAm)-Tris-Fe$_3$O$_4$ NPs), including pure Fe$_3$O$_4$ NPs, was sequentially performed, and the details related to this synthesis were given and discussed in our first two studies spectrophotometrically prior to detecting Ag$^+$ in linear ranges of 4–160 and 10–350 µg L^{-1} with detection limits of 1.21 and 4.28 µg L^{-1} in the presence of CTAB at pH 10 [34], and also in speciation analysis of Cu$^+$ and Cu^{2+} ions in linear ranges of 0.3–150 and 10–350 µg L^{-1} with detection limits of 0.095 and 3.03 µg L^{-1} at pH 7.0 and 5.0, respectively [35]. Therefore, it has been considered that there is no need to speak further and explain more in detail, except for use of SDS as ion pair in selective extraction of Ag$^+$ and Cu^{2+} ions with poly(SMAm) based on charge transfer (CT) where the proton exchange rate between amide and imidic acid (or iminol) tautomers is selectively controlled with changing pH in the pH range of 2.0–6.0.

Collection and preparation of samples to analysis

The detection and analysis of Ag$^+$ ions as total Ag content by spectrophotometry were evaluated by analysis of the selected edible vegetable oil samples. All the samples were supplied from a local supermarket in Sivas, Turkey. To obtain accurate and reproducible results, in this study the two sample preparation procedures systematically optimized

in the literature were adopted and independently used with a slight modification [36, 37]. The results were comparably evaluated by checking whether or not there is statistically a significant difference between the results found via multiple standard addition method after two sample preparation procedures by considering the amounts of the un-treated and pre-treated sample solutions and multiplying by the dilution factor and the selected samples, according to total Ag or Cu contents of CRMs. However, the value of the dilution factor may change depending on the weighed amounts of edible oil samples and CRM. Since the element concentrations of the CRM (EnviroMAT HU-1 Used oil) are low or high for Ag and Cu, respectively, a dilution of 1000- and/or 2500-fold was made prior to analysis by spectrophotometry.

Before analysis by multiple standard addition method, all sample solutions were independently subjected to two extraction processes as follows:

(i) Shortly, 3.0 g of edible oil (for Ag and Cu) was weighted accurately into a 15-mL polypropylene centrifuge tube and 2.5 mL of 0.01 mol L^{-1} EDTA solution adjusted to pH 8.0 was added to the tube. The tube was capped and vigorously shaken for a few seconds and then thoroughly mixed by vortex. Then, it was sonicated for 20 min at 35 °C in ultrasonic bath (300 W, 40 kHz), prior to the EDTA extract being separated by centrifugation at 3000 rpm for 7 min. After the separation of the oil phase from upper, aqueous part in the tubes, the metal concentrations in the EDTA solutions were measured against similarly prepared sample blanks, using micro-volume UV–Vis spectrophotometer with and without spiking after dilution of 1:5-fold with dilute, 2.0% (v/v) HNO$_3$. Calibration standards in solvent and sample extracts for matrix matching were prepared daily by serial dilution of the stock solutions (1000 mg L^{-1}) using 0.01 mol L^{-1} EDTA solution prepared in pH 8.0 buffer and by adding increasing volumes of standard solution of each analyte.

(ii) The extraction of Ag$^+$ and Cu^{2+} from the edible vegetable oils was performed through the emulsion breaking using optimized conditions. The procedure is based on formation of stable water-in-oil emulsions, which are obtained by vigorous mixing of 3.0 mL of fish oil (approx. 3.0 g with standard deviation of $\pm$ 0.05 g) with 5.0 mL of a mixture of 4.0% (m/v) Triton X-114 and 1.5 mol L^{-1} HNO$_3$ (2:3, v/v) in a capped polyethylene flask of 15.0 mL. The final concentration of Triton X-114 and HNO$_3$ was found to be 0.53% (m/v) and 0.3 mol L^{-1}, respectively. Afterward, the flasks containing the emulsions were transferred to ultrasonic bath at 80 °C and kept until breaking of the emulsion, for approxi-

mately 12 min. After the emulsion breaking, three well-separated phases were obtained: (i) the upper phase, which is an organic phase containing only the edible oil, (ii) an acidic aqueous phase containing the extracted metal ions, and (iii) the lower phase, which is a surfactant-rich phase. The metal ions were pre-concentrated in the aqueous phase, as a result of their acidic extraction from edible oil. Finally, the acidic aqueous phase containing metal ions was collected with the aid of a micropipette and diluted at a ratio of 1:5 with dilute, 2.0% (v/v) HNO_3 before pre-concentration by UA-CPE. The sample solutions with and without spiking were then submitted to the micro-volume UV–Vis spectrophotometer using THF as diluent, and the absorbance at 346 nm was taken against similarly prepared sample blanks as the analytical response.

In a similar way, sample blanks, including suitable amounts of two CRMs, were prepared without analyte and submitted to the same extraction procedures in order to evaluate analyte contamination by reagents used. All the measurements and processing were performed at least five times, and their mean values plus standard deviations were considered.

Finally, 5.0 mL of the pre-treated and extracted samples was in parallel submitted to UA-CPE procedure after pre-treatment with two sample preparation procedures. The total Ag and Cu contents of the samples were determined at 346 nm via spectrophotometry using the multiple standard addition method around the LOQs against sample blanks to suppress the matrix effect.

UA-CPE procedure

A typical UA-CPE requires the following steps: An aliquot (5.0 mL) of the pre-treated sample solutions in calibration ranges of 0.8–110 µg L^{-1} for Ag$^+$ ions and 1–125 µg L^{-1} for Cu^{2+} ions in optimization step, and 25 mL of a sample solution containing no more than 0.25 µg of Ag$^+$ and Cu^{2+} in pre-concentration step for 200, 250 µL of poly(SMAm) copolymer in THF (up to 0.1 g/100 mL), 150, 200 µL of 4.0% (w/v) SDS, 750 µL of 5.0% (v/v) of Triton X-114, and 350 µL of 0.2 mol L^{-1} KNO$_3$, were sequentially mixed in a centrifuge tube of 50 mL, adjusted to pH 4.0 and 5.0 with 200, 250 µL of 0.1 mol L^{-1} citrate–phosphate buffer solution for Ag$^+$ and Cu^{2+} ions (each one, 50 µg L^{-1}), respectively, and then completed to 50 mL by the water. The mixture was sonicated for 5 min at 40 °C. After reaching equilibria for fast, efficient and complete ion pairing CT complex formation, separation of the phases was achieved by centrifugation at 3000 rpm for 7 min. Due to becoming viscous of the surfactant-rich phase, the bulk aqueous phase was

easily separated and decanted. To reduce the viscosity of the surfactant-rich phase for spectrophotometric measurements and facilitate the sample proceeding, it was diluted to a volume of 0.4 mL with THF as diluent of both micellar phase and poly(SMAm) copolymer matrix. Finally, after extraction with two sample preparation procedures, the total Ag and Cu contents of the edible vegetable oils were in parallel determined by spectrophotometry at 346 nm against sample blank using the multiple standard addition method around the method quantification limits, so as to fall in calibration ranges in order to control the possible matrix effect.

Statistical analysis

The average and standard deviation of the analyte concentrations were calculated for analysis of each sample matrix. The obtained results for two SRMs and samples were statistically evaluated by employing the paired Student's t test for the multiple standard addition method after two sample preparation procedures, and the calculated t values were compared with the tabulated t value for four and eight degrees of freedom at the 95% confidence level.

Results and discussion

Analysis by FTIR, ^{1}H-NMR, XRD and SEM/EDX

Surface functional groups on the copolymer structures before and after modification were comparatively evaluated and characterized by using ATR-FTIR and ^{1}H-MR spectra of solids converted to film form. From spectral data of reaction products that progress with anhydride ring opening, amidation process was verified by disappearance of anhydride ring bands at 1854 and 1764 cm^{-1} and appearance of a new peak at 1640 cm^{-1} corresponding to amide group as well as peaks at 1565 and 1405 cm^{-1} corresponding to carboxylate groups. After Tris-modification, the intensity of absorption band at 620 cm^{-1} gradually increased as a result of incorporation of Fe$_3$O$_4$ NPs into the copolymer matrix, while the only two absorption bands at 800 and 896 cm^{-1} from the absorption bands of 580, 620, 800 and 896 cm^{-1} that appeared in IR spectrum of pure Fe$_3$O$_4$ NPs disappeared as shown in Fig. 1a. This suggests the successful binding and thorough dispersion of Fe$_3$O$_4$ NPs to polymer matrix. Other remaining FTIR, ^{1}H-NMR and XRD spectral/diffraction peak details related to surface functional groups and crystal (or amorphous) structure of magnetic copolymer where the Fe$_3$O$_4$ NPs exhibits partly a homogenous dispersion in copolymer matrix before and after modification were represented and discussed in detail in our preliminary two studies [34, 35].

Additionally, scanning electron microscopy (SEM) was employed to explore the surface morphology and elemental
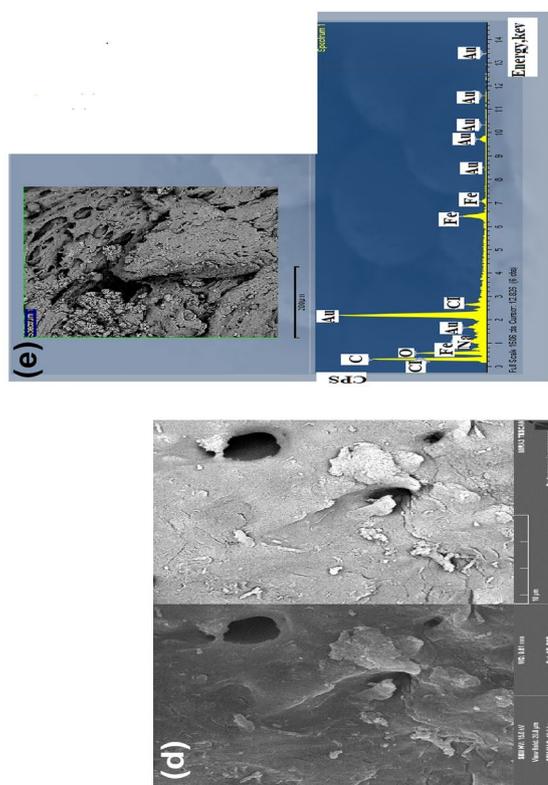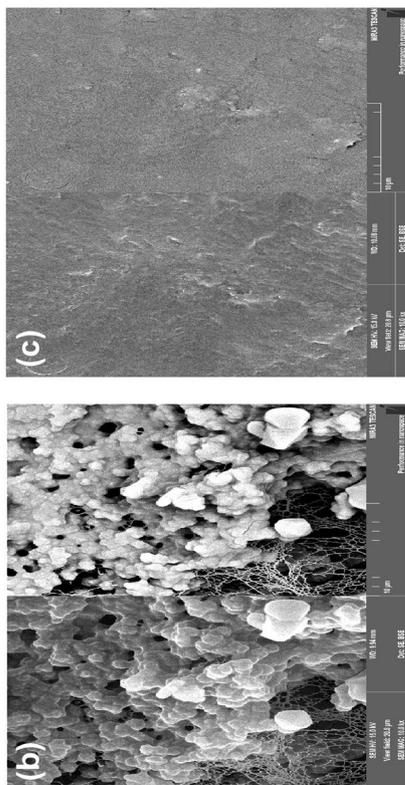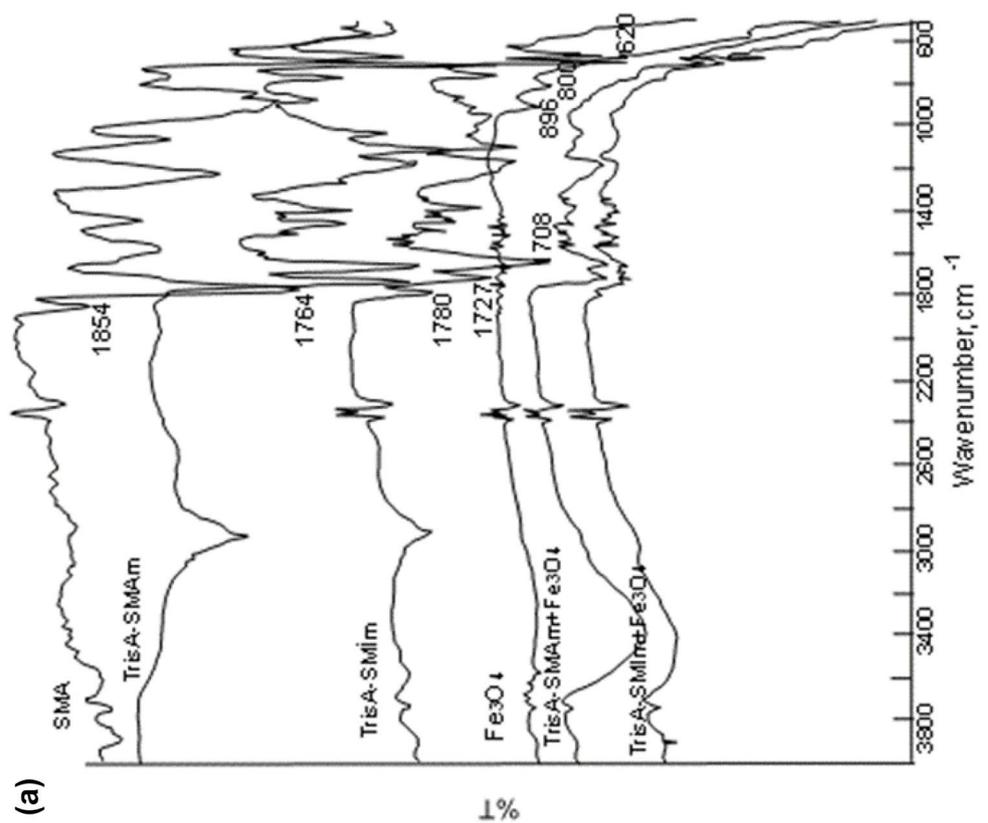
Fig. 1 **a** FTIR spectra of the poly(SMA), poly(SMAm) and poly(SMIm) copolymers after modification with Tris and Fe3O4 NPs, respectively. **b-e** SEM images of **b** poly(SMA) **c** poly(SMAm)-Tris after modification with Tris **d** poly(SMAm)-Tris-Fe3O4 NPs functionalized with Tris and Fe3O4 NPs, respectively, and **e** SEM image/EDX spectra of poly(SMAm)-Tris-Fe3O4 nanocomposite

analysis of the synthesized composite materials. Figure 1b-d shows the SEM images of poly(SMA), poly(SMAm)/Tris and poly(SMAm)/Tris/Fe$_3$O$_4$ magnetic nanocomposite (mNC), respectively. The morphology of poly(SMAm) modified with Tris reveals that the amidic copolymer has more homogenous and platelike structure than that of original poly(SMA) in Fig. 1b, and average diameter of the observed particles in SEM image can be estimated under 100 nm with reasonable monotony and grainy shape in Fig. 1c. The SEM image of poly(SMAm)/Tris/Fe$_3$O$_4$ 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 platelike morphology in Fig. 1d. The successful synthesis of the mNC was qualitatively further confirmed by the chemical composition analyzed by energy-dispersive X-ray (EDX). Figure 1e shows the dispersion of C, O, Fe and partly N elements. The disappearance or appearance in background fluctuation of the N peak on the background of 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 thermal 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.

The mechanism for selective extraction of trace Ag$^+$ and Cu^{2+} ions

For selection of a wavelength being specific to each ion, and showing a linear relationship with its increasing concentration, prior studies were carried out in wavelength ranges, 180–380 and 380–780 nm. From the results, the sample blanks without silver or copper have given the characteristic peaks at 328 and 351 nm, respectively, for amidic copolymer in the absence and presence of SDS as pH and synergistic auxiliary ligand causing an increase in basicity of amidic copolymer by providing a facilitation in chelation of Ag$^+$ and Cu^{2+} ions, while it is observed at 340 nm in the case of nanocomposite. By a redshift and/or blueshift of 16/19 nm after addition of sub-trace amounts of Ag$^+$ and Cu^{2+} ions, there was a linear relationship between the absorbance and the increasing concentrations of Ag$^+$ and Cu^{2+} ions in the presence of SDS at 346 nm. It is clear that this wavelength shift shows the formation of a pH and CT sensitive-extractable complex among Ag$^+$, Cu^{2+} ions and amine-functionalized copolymer phase in the presence of SDS where for significant enhancement in the selectivity and sensitivity of the method, the soft acid–soft base compatible hydrophilic

chelating ligands such as biopolymer chitosan with and without histidine moiety for Ag$^+$ ions were successfully used in the literature due to high affinity to Ag$^+$ ions [32, 33]. Therefore, it was adopted a wavelength of 346 nm as the measurement wavelength for further optimization steps. Based on these observations, an UA-CPE method for extraction and pre-concentration of trace Ag$^+$ and Cu^{2+} ions from aqueous solution was designed before analysis by micro-volume UV–Vis spectrophotometer, and important parameters influencing extraction and determination steps were optimized.

It is believed that extraction mechanism proceeds by proton-coupled CT among reagents participating into the extraction process at pH 4.0 and 5.0 for pre-concentration/determination of trace Ag$^+$ and Cu^{2+} ions by the present method as follows:

(1) $SDS + H_2O \rightarrow HSDS + OH^-$, hydrolysis of SDS as a function of pH at pH values equal to 5.0 and lower, $HL^+ + OH^- \rightarrow L + H_2O$, increase in basicity of chelating ligand for efficient chelation of Ag$^+$ and Cu^{2+} ions

(2) L, tautomer form of amide, $-(C=O)-NH- \leftrightarrow L$, tautomer form of imidic acid or iminol, $-(C-OH)=N-$, tautomerization equilibrium in the presence of SDS micelles as a function of pH and temperature

(3) $Ag^+ + HL^+ \rightarrow AgL^+ + H^+$, proton transfer and pH-dependent complex formation where L is Tris-modified acid-compatible poly(SMAm) with -COOH, C=O, and $-NH-C(CH_2OH)_3$ groups

(4a) $2AgL^+ + H_2O \rightarrow Ag(OH)L^+ + H^+ + Ag$, ligand-induced disproportionation in the presence of SDS.

(4b) $2AgOHL^+ + H_2O \rightarrow Ag(OH)_3 + AgL^+ + H^+$, further fast disproportionation due to metastable of Ag^{2+} ions as a function of pH at 195 and 343 nm with isosbestic point at 310 nm where amide-imidic acid tautomer forms by displacement of proton on molecular structure were controlled with changing pH in acidic region.

In fact, it is implied in the literature [38] that instable or metastable of Ag^{2+} ions (pK$_{a1,2}$ values of 5.35 and 8.35) is spontaneously disproportioned where CT process is about four times faster at a pH of 5.5.

In a similar way, it is reported in the literature [38, 39] that to support this situation with acid hydrolysis constants of pK$_{a1}$: 2.4 and pK$_{a2}$: 4.0 from optical and conductivity measurements, the Cu^{3+} ions in the form of Cu(OH)$_3$ at pH < 6.0 show a pH-dependent absorption capability in the 280–350 nm wavelength range with redshift as a result of ligand-induced disproportionation where the copper species at low concentrations are chelated and stabilized with polymeric ligand at controlled pHs.

(5a) $2CuL^{2+} + 2H_2O \leftrightarrow Cu(OH)_2^+ + 2H^+ + CuL^+$, ligand-induced disproportionation.

(5b) $2CuL^+ \leftrightarrow CuL^{2+} + Cu$, further fast disproportionation due to thermodynamically metastable of Cu^+ ions in aqueous micellar media where the disproportionation equilibrium constant is $K = [Cu^{2+}]/[Cu^+]^2 = 10^6 \ M^{-1}$ in the absence of co-polymeric ligand. Finally, the ion pair formation based on CT selectively proceeds by extracting into the core and/or surface of the mixed nonionic surfactant micelles in the presence of SDS as both stabilizer and counter-ion acting like a concentration-dependent auxiliary ligand at pH 5.0 as follows:

(6) AgL^+ or CuL^+ + ionic surfactant, SDS → [AgL(SDS) or CuL(SDS)] ion pair formation, before pH-controlled detection at 346 nm by micro-volume UV–Vis spectrophotometer

Optimization of the main variables affecting extraction process

During the use of amine-functionalized poly(SMA) copolymer matrix in UA-CPE process for accurate/reliable analysis of sub-trace Ag^+ and Cu^{2+} ions, it is needed to standardize it. There is a need to the CRMs being compatible with the sample matrix for standardization of nanostructure, especially when having selective surface functional groups for each ion. At this condition, in case of using the newly synthesized functional co-polymeric structure at hydrophilic character as selective and sensitive probe for the analytical determination purposes, it is recommended (i) to optimize the effective parameters to reach the highest sensitivity and reproducibility, (ii) statistically evaluate the reliability of the method in terms of accuracy and precision, and (iii) comparatively analyze the real-time samples by matrix-matched calibration or standard addition method. In this study, details of the optimization process for triplicate measurements of 50 μg L^{-1} Ag^+ and Cu^{2+} with error bars by using one-variable-at-a-time method, analytical figures of merit and some statistical evaluations are as follows:

Effect of pH and buffer concentration

It is of highly importance to select appropriate chelating agent for the selected metal ions to form hydrophobic complexes when metal ions are extracted by the UA-CPE. The complex is extracted to surfactant phase. The extraction efficiency depends on the acidity of the solution as the pH has an impact on the overall charges of the analyte, thus affecting the generation of the complex between the metal and the surface active functional groups.

The complex formation and its chemical stability are the two important factors involved in the separation and pre-concentration of metal ions by UA-CPE. They need to present sufficient hydrophobicity to be extracted into the small volume of the surfactant-rich phase. The pH plays a critical role on complex formation and subsequent extraction and has been a significant parameter for UA-CPE. Thus, extraction efficiency depends on the pH at which complex formation is investigated. The UA-CPE of Ag^+ and Cu^{2+} ions as a function of pH and calibration sensitivity at concentration levels of 50 μg L^{-1} were carried out in the pH range of 2.0–8.0. The results are shown in Fig. 2a. The recovery for Ag^+ and Cu^{2+} ions sharply increased with increasing pH from 2.0 to 5.0 and reached a maximum with pH at 4.0 and 5.0, respectively. At low pHs, the low recoveries for Ag^+ and Cu^{2+} ions were observed owing to the incomplete complex formation among reagents in reaction media. When the pH is in a range of 5.0–8.0, it could be a change in stability of tautomer forms with increasing pH as well as their hydrolysis for efficient chelation of Ag^+ and Cu^{2+} ions, so as to cause a sharp decrease in sensitivity. At higher pHs than 4.0 or 5.0, another cause can be a decrease in synergistic capability of SDS micelles with increasing pH where amide copolymer acts as a base, so as to cause a modification in complexation based on CT among metal ions and chelating co-polymer. Taking into account all these factors, a pH value of 4.0 and 5.0 as a result of participation of free carboxyl, nucleophilic amide/imidic acid groups on Tris-modified copolymer into the complexation in the presence of SDS for an extractable complex formation of Ag^+ and Cu^{2+} ions was adopted to be optimal for further studies.

The effect of buffer concentration at pH 4.0 and/or 5.0 on the sensitivity in Fig. 2b was also studied in the volume range of 25–250 μL of citrate–phosphate buffer solution at 0.1 mol L^{-1}. The best sensitivity was obtained at volumes of 200 and 250 μL for Ag^+ and Cu^{2+} ions, respectively. At lower and higher buffer volumes, the sensitivity for each ion was gradually decreased. Perhaps, this may be due to the concentration-dependent reducing nature of citric acid as a component of the buffer and its competitive nature with tautomer forms of the complexing amide copolymer available in equilibrium. Therefore, buffer volumes of 200 and 250 μL for selective extraction of Ag^+ and Cu^{2+} ions were adopted as optimal for further studies.

Effect of amine-functionalized poly(SMA) copolymer and ion-pairing, SDS concentrations

Chelating agent concentration is one of the important factors influencing the extraction efficiency. As can be seen in Fig. 3a, the extraction of Ag^+ and Cu^{2+} ions at pH 4.0 and 5.0, respectively, was carried out in the amine-functionalized copolymer concentration ranging from 25 to 500 μL of 0.1 g/100 mL copolymer in THF. The sensitivity as a measure of quantitative extraction of Ag^+ and Cu^{2+} ions sharply increased with increasing chelating copolymer
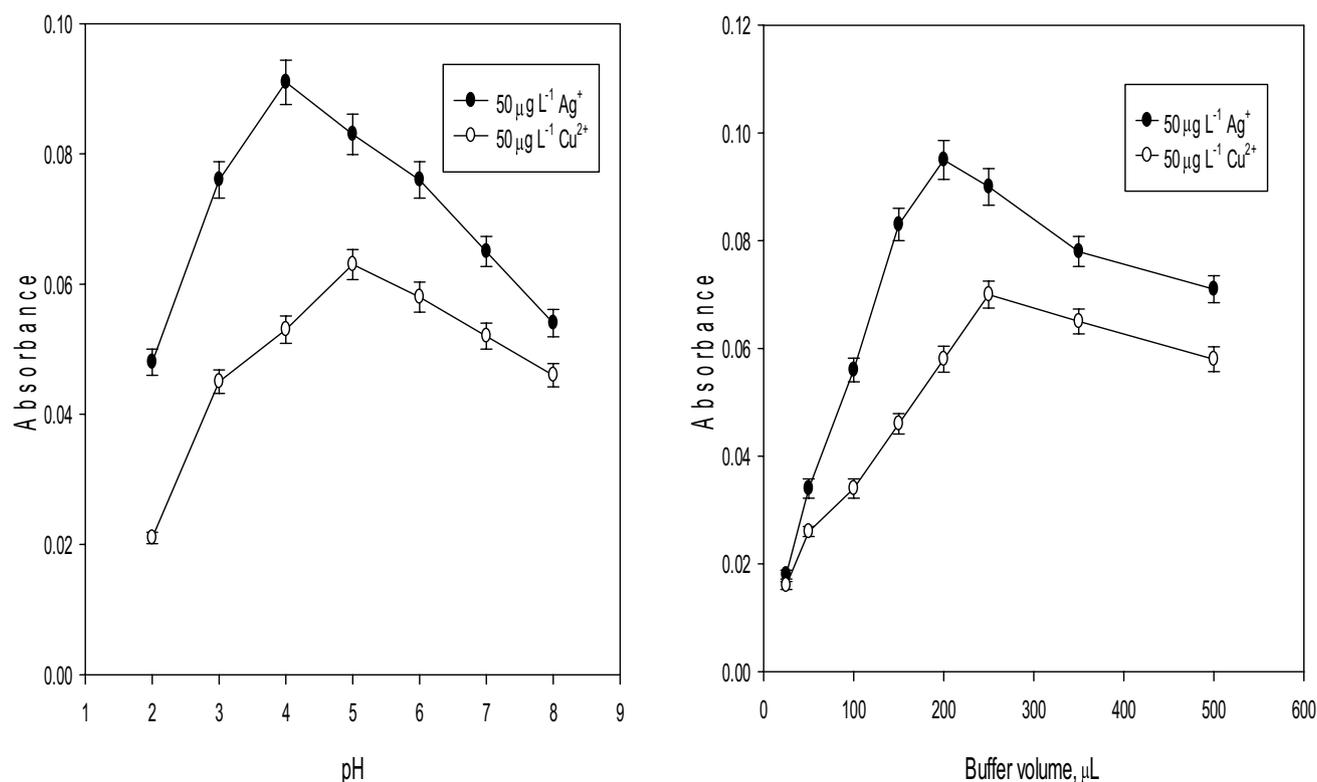
Fig. 2 The effect of **a** pH **b** buffer volume, μL at pH 4.0, 5.0 on the calibration sensitivity as a measure of quantitative extraction efficiency of Ag$^+$ and Cu^{2+} ions at levels of 50 μg L^{-1}

matrix concentration in the range of 25–200 μL of its solution and reached a maximum at volumes of 150 for Ag$^+$ and 200 μL for Cu^{2+}. However, when copolymer solution volume in THF is higher than 150 or 200 μL, the sensitivity gradually decreased. This decrease in sensitivity could be due to formation of extractable ion associate by intermolecular interactions (like weak hydrogen bonding and $_{II}$⁻$_{II}$ stacking) among excess amine-functionalized amidic chelating copolymer (in fact, containing pH- and concentration-dependent functional groups such as amide/imidic acid C(=O)–NH–/C–OH)=N–, cyclic tertiary-N and –OH, including phenyl ring) and other reagents in the absence of Ag$^+$ and Cu^{2+} ions at pH 4.0 and 5.0, respectively, so as to lead to an increase in sample blank. Therefore, the optimal volume for amine-functionalized amidic copolymer was considered to be 150 and 200 μL in THF for further studies.

The concentration of ionic surfactants used in the UA-CPE plays key roles in extraction process. In the presence of nonionic surfactant, Triton X-114 as extractant in preconcentration of sub-trace Ag$^+$ and Cu^{2+} ions, at initial the ionic surfactant, SDS in volume ranges of 25–500 μL at 4.0% (w/v) as ion pairing auxiliary ligand was adopted as a synergistic auxiliary ligand in the extraction step. The sensitivity in Fig. 3(b) sharply increased in the range of 25–150 or 25–200 μL for amine-functionalized amidic copolymer,

poly(SMAm), reached a maximum at 150 and 200 μL for Ag$^+$ and Cu^{2+} ions, and gradually decreased at higher volumes than 150 or 200 μL. At low SDS concentrations, this sharp increase in sensitivity may be due to synergistic relationship between pH and SDS concentration in microheterogeneous environment, so as to facilitate complexation of Ag$^+$ and Cu^{2+} ions with chelating amidic copolymer as a result of increasing basicity of ligating copolymer [40–42]. In the presence of SDS around its CMC, it is clear that the ratio of amidic to imidic acid tautomers of the amidic copolymer as weak Lewis base is predominantly changed as a function of pH for quantitative extraction of Ag$^+$ and Cu^{2+} ions. In fact, it is believed that this is resulted from a sharp decrease in CMC of SDS with an equilibrium concentration of 0.417 and 0.556 mmol L^{-1} in the presence of 0.2 mol L^{-1} KNO$_3$ as salting-out agent at pH 4.0 and 5.0 where the CMC of SDS ranged from 8.08 to 1.99 in unbuffered (in water) and buffered media (50 mmol L^{-1} phosphate buffer, pH 7.0), respectively [41, 42]. At low pHs (below pH 5.0–5.5), it is also implied in the literature[52] that the CMC of SDS decreases, whereas at higher pHs it remains constant. However, the decrease in sensitivity at higher volumes than 150 or 200 μL may be due to an increase in analyte blank as a result of extractable ion pair formation between SDS and protonated poly(SMAm) ligand via pH-sensitive amide or imide
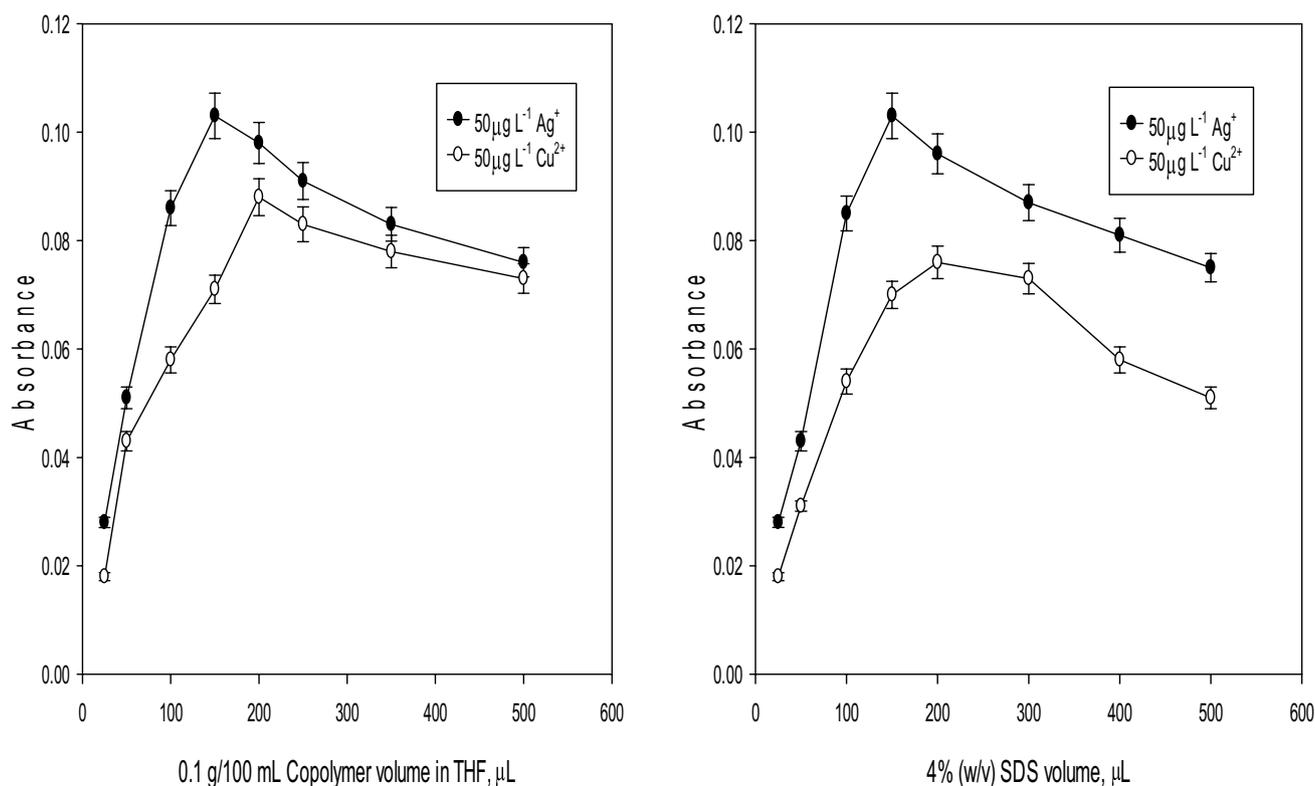
Fig. 3 The effect of **a** 0.1 g/100 mL of modified copolymer volume in THF, μL, **b** 4.0%(w/v) SDS volume, μL, on the calibration sensitivity as a measure of quantitative extraction efficiency of Ag^+ and Cu^{2+} ions at levels of 50 μg L^{-1}

groups in the absence of Ag^+ and Cu^{2+} ions. Therefore, SDS volumes of 150 and 200 μL (equivalent to a concentration of 0.417 and 0.556 mmol L^{-1}) for Ag^+ and Cu^{2+} ions were considered to be enough for further studies.

Effect of Triton X-114 and KNO$_3$ concentrations

Triton X-114 is one of the nonionic surfactants extensively 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 and low CMC in combination with SDS. In order to monitor very low levels of silver and copper in food matrices such as edible vegetable oils, such a combination was conceived and implemented to achieve low analyte blank and high calibration sensitivity. As a result, different volumes of Triton X-114 in Fig. 4a were investigated ranging from 150 to 1500 μL at a concentration of 5.0% v/v for checking the extraction efficiency. As can be seen in Fig. 4a, the sensitivity for 50 μg L^{-1} Ag^+ and Cu^{2+} ions linearly increased with an increase in surfactant volume from 150 to 750 μL for poly(SMAm) and reached a maximum at a volume of 750 μL for both ions. The sensitivity gradually decreases when the volume of 5.0%(v/v)

Triton X-114 is higher than 750 μL. Such observation can be ascribed an increase in volume and viscosity of the micellar phase, so as to lead to a decrease in pre-concentration factor. Thus, a volume of 750 μL of the nonionic surfactant at 5.0% (v/v) was adopted to be optimal for further studies in order to achieve the highest sensitivity in spectrophotometric detection of Ag^+ and Cu^{2+} ions at 346 nm.

In general, the addition of salt could decrease the solubility of aqueous sample phase and lead to enhancement in the dispersion of the analyte into the surfactant-rich phase by the "salting-out effect" phenomenon. The presence of salt can increase the incompatibility between the water structures in the hydration shells of analytes and surfactant macromolecules, which can reduce the concentration of "free water" in the surfactant-rich phase and, consequently, reduce the volume of the phase [30]. In order to investigate the effect of ionic strength on the extraction efficiency, various experiments in Fig. 4(b) were performed by adding different volumes of 0.2 mol L^{-1} KNO$_3$ (in range of 25–750 μL) in a centrifugation tube of 50 mL. Other experimental conditions were kept constant during the analysis. The results show that ionic strength has linearly a significant effect on the sensitivity up to a volume of 350 μL (equivalent to a concentration of 1.4 mmol L^{-1}) for each ion. At higher volumes than 350 μL, there is not a significant increase in sensitivity.
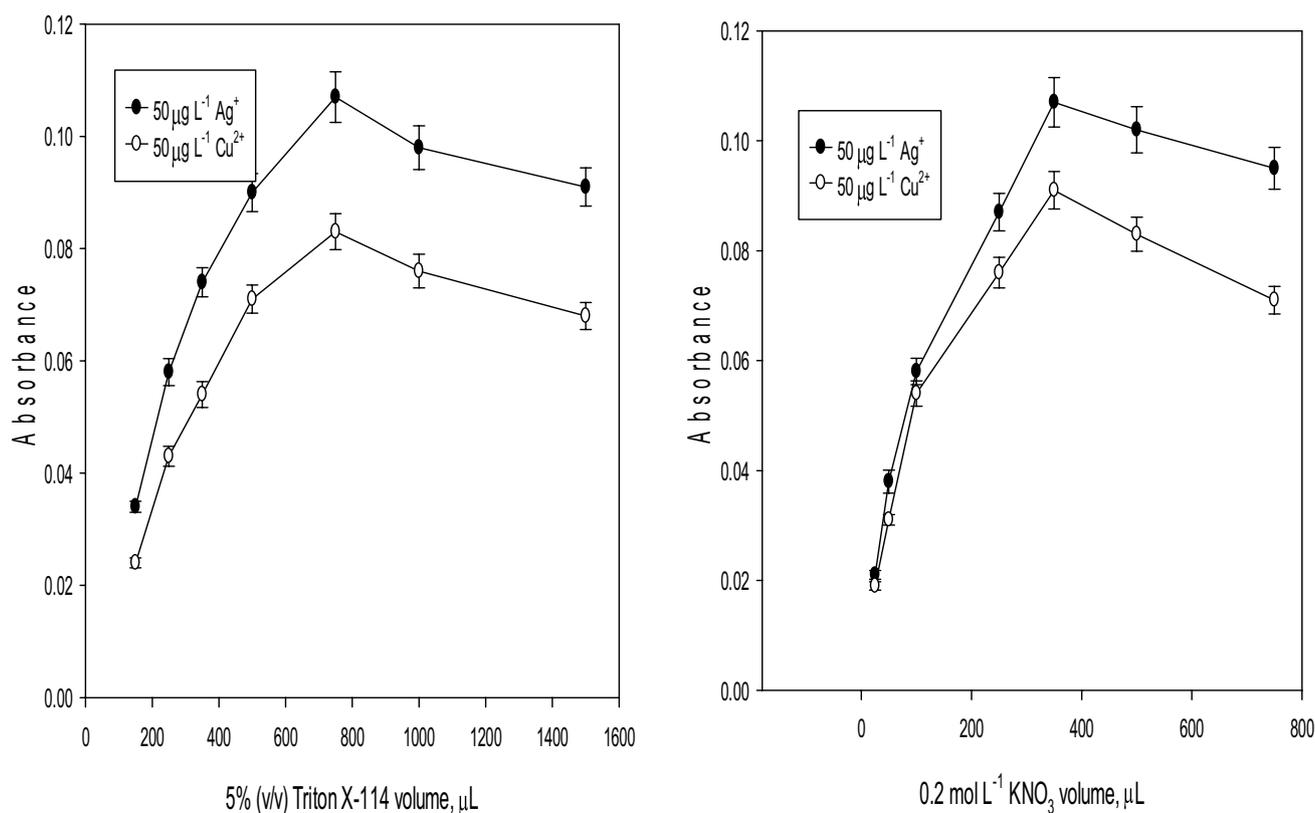
Fig. 4 The effect of **a** 5.0% (v/v) Triton X-114 volume, μL, **b** 0.2 mol L^{-1} KNO$_3$ volume, μL, on the calibration sensitivity as a measure of quantitative extraction efficiency of Ag$^+$ and Cu^{2+} ions at levels of 50 μg L^{-1}

Thus, ionic strength was kept constant at a salt concentration of 1.4 mmol L^{-1} in order to obtain reproducible and stable analytical signals.

Effect of incubation temperature and time

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 mixed micellar system. Therefore, the incubation temperatures ranging from 25 °C to 55 °C and time between 1 and 15 min in ultrasonic bath (300 W, 40 kHz) were studied. From the results, it has been observed that an equilibration time of 5 min and temperature of 40 °C are enough to reach the best sensitivity with maximum recovery for amine-functionalized poly(SMA) copolymer.

Effect of centrifugation rate and time

To obtain a good phase separation in pre-concentration by UA-CPE, resulting in high sensitivity of metal ions in a short time, the effects of centrifugation rate and time were evaluated in the range of 1000–4000 rpm and 1–15 min, respectively. For complete phase separation, a centrifugation rate

of 3000 rpm and centrifugation time of 7 min were found to be optimum.

Effect of diluents

In order to facilitate the detectability of the sample solution by spectrophotometry, it was necessary to decrease the viscosity of the surfactant-rich phase. Different solvents, such as acetone, acetonitrile, ethanol, methanol, THF and solutions of ethanol and methanol acidified with 0.2 mol L^{-1} HNO$_3$, 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 THF. An aliquot of THF (0.2 mL) was added to the surfactant-rich phase after phase separation, in which the micellar phase (approx. 0.2 mL) is diluted to a volume of 0.4 mL for a pre-concentration factor of 62.5 from pre-concentration of optimal 25-mL sample (in the range of 5–35 mL). This amount of THF was chosen to ensure a sufficient volume of the sample for maximum sensitivity. For smaller volumes, the reproducibility of the signals was very poor, whereas for higher volumes, there was a decrease in the signal due to dilution.

Validation of the analytical method

Under the established optimal conditions, the analytical performance of the method was studied in detail. To check the linearity of the method, six standard solutions of Ag^+ and Cu^{2+} in ranges of 1–125 or 2.5–150 µg L^{-1} were sequentially applied to the developed method. The absorbance at 346 nm was subjected to linear regression analysis. It has been observed that the analysis was highly linear across the six standards with better R^2 values than 0.992 or 0.993 for each analyte. The method allows low detection limits of 0.25 and 0.41 µg L^{-1} by the solvent-based calibration curves (n: 6) in linear ranges of 0.8–110 and 1.0–125 µg L^{-1} for Ag^+ and Cu^{2+} ions, while it allows detection limits of 0.40 and 0.72 µg L^{-1} by the matrix-matched calibration curves (n: 6) in the linear range of 1.0–120 and 2–140 µg L^{-1}. In order to minimize the possible matrix effect and instrumental signal fluctuations, the matrix-matched standard calibrations curves, consisting of six concentration levels (0, 2.5, 10, 25, 75, 100 and 150 µg L^{-1}) for sample extracts, were set up by spiking these different amounts of each metal ion into sample extracts. Blanks (samples with zero addition of each analyte) were simultaneously quantified using the standard addition, and the levels of Ag^+ and Cu^{2+} ions 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 three levels of analyte standards (0, 5, 25, 100 µg 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 matrix effect of -6.9 and -6.5%, so as to cause suppression in signal at 346 nm. Also, the paired Student's t test was also performed to see if there was a matrix effect. For this purpose, from calibration data (each one, for n: 6) the two average slopes were compared using the formula, $t_{cal} = m_1\text{-}m_2 / s_{pooled} \times (n_1 + n_2 / n_1 \times n_2)^{1/2}$ for degrees of freedom of $(n_1 + n_2)$-2 and 95% confidence level. From the results, it was observed that the calculated t values of 1.66 and 1.41 for Ag^+ and Cu^{2+} ions are lower than the critical t value of 2.23. This indicates that there is no significant matrix effect. Similarly, the confidence intervals of slopes of $(1.88 \pm 0.42) \times 10^{-3}$ and $(1.75 \pm 0.28) \times 10^{-3}$ for Ag^+ and $(1.39 \pm 0.34) \times 10^{-3}$ and $(1.30 \pm 0.28) \times 10^{-3}$ for Cu^{2+} have been determined at 95% from the calibration curves where $t_{(n-2)}$ is 2.78 for degrees of freedom of 4, and it has been observed that the confidence intervals are coincided with recovery efficiencies of 93.1 and 93.5%. The confidence interval was described as $m \pm t_{(n-2)} \times s_m$ where m is the slope and s_m is the standard deviation of slope for each calibration curve. The corresponding regression equations for Ag^+ and Cu^{2+} were found to be Abs: $(1.88 \pm 0.15) \times 10^{-3}C + (3.40 \pm 0.14) \times 10^{-3}$ and Abs: $(1.39 \pm 0.12) \times 10^{-3}C + (4.60 \pm 0.17) \times 10^{-3}$ for calibration in solvent, while it was Abs: $(1.75 \pm 0.12) \times 10^{-3}C + (3.25 \pm 0.21) \times 10^{-3}$ and Abs: $(1.30 \pm 0.1) \times 10^{-3}C + (4.35 \pm 0.28) \times 10^{-3}$ for matrix-matched calibration in sample extracts where Abs is the absorbance and C is the concentrations of silver and copper, respectively. The limits of detection and quantification (LODs and LOQs, 3.3σ/m and 10σ/m) calculated by using the expressions at $3.3 \times s_{blank}/m$ and $10 \times s_{blank}/m$, respectively, where s_{blank} is the standard deviation of twelve replicate measurements of blanks and m is the slope of the calibration curves, according to ICH [43] were 0.25/0.77 and 0.41/1.23 µg·L^{-1} for the solvent-based calibration curves for Ag^+ and Cu^{2+} where σ is the standard deviation of twelve replicate measurements of the blank and m is the slope of the calibration curve, while these values were in the range of 0.40/0.72 and 1.20/2.17–2.15 µg L^{-1} for the matrix-matched calibration curves. The repeatability and intermediate precision of RSDs of the extraction process for both calibration approaches were found to be in ranges of 3.0–5.7% and 3.8–7.1% for both ion (5, 25 and 100 µg·L^{-1}, n = 5, 3 × 5 for same day and three consecutive days) with a recovery rate in the range of 90.7–98.5%. In fact, regarding repeatability, the RSDs are lower than the maximum allowed RSD of 21% in the studied concentration range, according to AOAC [44]. In a similar way, regarding intermediate precision in the literature [45], the RSDs below 10% are recommended when analyzing elements. As an indicator of the method accuracy, the percent recoveries (92.1–98.5% and 91.5–96.7% for two calibration curves) are also within the acceptable limit values of 60–115% established by AOAC [43] for the studied concentration ranges. From pre-concentration of 25 mL sample solution, a pre-concentration factor of 62.5 was obtained with a sensitivity enhancement of 35.2- and 28.3-fold. Herein, it can be defined as the ratio of the original aqueous matrix sample volume to the surfactant-rich phase volume, $CF = V_{Matrix}/V_{SRP}$. However, if the mass transfer of metal ions into the micellar phase is not quantitative, it is better represented by the enrichment factor, EF. Thus, EF, which is also described as sensitivity improvement factor, can be calculated by the ratio of the slopes of the calibration curves with and without pre-concentration [30]. All the analytical parameters related to the pre-concentration system are represented in detail in Table 1.

The selectivity study

The effect of potential interference of some anionic and cationic species on the pre-concentration and determination of each ion was studied. In these experiments, aqueous solutions containing Ag^+ and Cu^{2+} ions (50 µg L^{-1}) with the addition of interfering ions were treated in tolerance ratios ranging from 1:35 to 1:2000, according to the recommended UA-CPE procedure under the established optimal conditions, and the

Table 1 Analytical figures of merit of the methods developed for detection of Ag^+ and Cu^{2+} ions by amine-functionalized poly(SMA) copolymer at pH 4.0 and 5.0, respectively

Analytes	Calibration type	From regression analysis at 346 nm				[a]LOD, µg L^{-1}	[a]LOQ, µg L^{-1}	Precision		Accuracy
		Linear range, µg L^{-1}	Slope ± SD	Intercept ± SD	Determination coefficient, r^2			Repeatability (as RSD% for same day, 5, 25 and 100 µg L^{-1}, n: 5)	Intermediate precision (as RSD% for three consecutive days, 5, 25 and 100 µg L^{-1}, n: 5)	Recovery%
For Ag^+ ions at pH 4.0	By calibration curve in solvent	0.8–110	$(1.88\pm0.15)\times10^{-3}$	$(3.40\pm0.14)\times10^{-3}$	0.992	0.25	0.77	3.5–4.8	4.0–6.2	91.3–96.5
	By matrix-matched calibration curve prepared from sample extracts	1.0–120	$(1.75\pm0.12)\times10^{-3}$	$(3.25\pm0.21)\times10^{-3}$	0.993	0.40	1.20	3.8–5.7	4.3–7.1	90.4–95.1
For Cu^{2+} ions at pH 5.0	By calibration curve in solvent	1.0–125	$(1.39\pm0.12)\times10^{-3}$	$(4.60\pm0.17)\times10^{-3}$	0.993	0.41	1.23	3.0–4.5	3.8–5.7	92.1–98.5
	By matrix-matched calibration curve prepared from sample extracts	2.0–140	$(1.30\pm0.10)\times10^{-3}$	$(4.35\pm0.28)\times10^{-3}$	0.995	0.72	2.17	3.8–5.7	4.5–7.1	90.7–94.5
[b]Matrix effect, %						-6.9%, -6.5%				
[c]Sensitivity enhancement factor (EF)						35.2, 28.3				
[d]Pre-concentration factor (PF)						62.5				

[a]Limits of detection and quantification (LODs, LOQs) for Ag^+ and Cu^{2+} ions were determined according to the expressions at $3.3\times s_{blank}/m$ and $10\times s_{blank}/m$, respectively (where s_{blank} is the standard deviation of twelve consecutive blanks (n: 12) and m is the slope of calibration curves)

[b]Based on comparison of slopes of two calibration curves established in solvent and sample extracts by using formula, $ME\% = (1-m_{solvent}/m_{matrix-matched})\times100$

[c]Ratio of slopes of calibration curves established with and without pre-concentration

[d]Ratio of the bulk aqueous sample solution volume to volume of the surfactant-rich phase

results are given in Table 2. Table 2 depicts the tolerance limits of the diverse ions, *i.e.*, interferent-to-analyte ratios in which the relative error was less than ± 5.0% in terms of a variation in signal. Only an interference at low tolerance ratios (in the range of 25–200) has been observed from Hg^{2+}, Fe^{3+}, Mn^{2+}/Sn^{2+}, As^{3+}/Sb^{3+} ions and bisulfite either forming a stable complex with main ligand, amine-functionalized poly(SMA) copolymer matrix or causing fluctuation in oxidation step of Ag and Cu at pH 4.0 and 5.0, respectively. The serious interfering effect of Hg^{2+} ions up to 250-fold excess over silver and copper was greatly suppressed and improved using 1.5 mL of 0.01 mol L^{-1} iminodiacetic acid (IDA) solution. The interference of Sn^{2+} and Mn^{2+} ions was suppressed up to 500-fold using 0.2 mL of 0.025 mol L^{-1} $Na_2H_2P_2O_7$ solution. The interference of As^{3+} and Sb^{3+} ions was suppressed up to 500-fold after pre-oxidation of As^{3+} and Sb^{3+} to As^{5+} and Sb^{5+} with 0.01 mol L^{-1} H_2O_2 solution in alkaline medium. Due to pH-dependent stable addition product, the interference of formaldehyde was suppressed up to 750-fold after pre-treatment with 1.0 mL of 0.02 mol L^{-1} metabisulfite around pH 5.0 before pre-concentration by UA-CPE. As can be seen from Table 2, it is clear that the developed UA-CPE method is relatively selective in terms of major species present in real samples. The recoveries in the range of 92.0–103.5% with a RSD ranging from 2.0% to 4.1% were obtained for different tolerance ratios for Ag^+ and Cu^{2+} ions. The results indicated that the selected matrix components did not affect the absorbance of each ion at 50 μg L^{-1} at any of the studied tolerance ratios.

Accuracy of the method

The accuracy of the method for each ion was controlled by analysis of two certified samples, CRM EnviroMAT™ HU-1 Used Oil and SRM 1643a Trace elements, in natural water under the established optimal conditions by taking suitable aliquots of the sample solutions pre-treated and diluted at 1000- or 2500-fold. It can be seen that the results found by the present method in Table 3 are statistically in good agreement with their certified values where the experimental t values ranging from 0.99 to 2.28 are lower than the critical t value of 2.31 for four degrees of freedom at confidence interval of 95%. Clearly, it can be seen that there is no significant difference between the results found in the present method and the certified values when considered the lower experimental t values than 2.31, so as to imply the method accuracy. Also, after spiking, it is clear that recovery and precision levels can be quantitatively accepted with RSDs in the range of 3.7–8.5% and recovery rates of 93.4–101.6% for five replicate measurements. The percent recoveries (*R*) of spike standards were calculated as follows:

$$R(\%) = (C_m - C_o)/C_m \times 100 \qquad (1)$$

where *Cm* is the value of metal in a spiked sample, C_o is the value of metal in a sample, and *m* is the amount of metals spiked.

Table 2 The possible matrix effect on the extraction of 50 μg L^{-1} Ag^+ and/or Cu^{2+} ions at pH 4.0 and 5.0 by UA-CPE prior to analysis by spectrophotometry (*n*: 3)

Co-existing ions	Tolerance ratio, [Interferent]/[analyte ion]	Recovery %	RSD %
NH_4^+, K^+, Na^+	2000:1	98.0–102.5	2.0
Ca^{2+}, Mg^{2+}, Sr^{2+}, NO_3^-, F^-	1500:1	97.5–99.5	2.5
Ba^{2+}, Cl^-, SO_4^{2-}, HPO_4^{2-}, $C_2O_4^{2-}$	1250:1	96.0–101.5	2.3–3.5
Al^{3+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Ni^{2+}	1000:1	97.0–102.0	2.0–3.5
HCO_3^-, Br^-, tartaric acid, citric acid	750:1	95.0–98.5	2.5–3.7
Zn^{2+}, EDTA, ascorbic acid	500:1	92.5–95.5	3.0–3.8
Cd^{2+}, MoO_2^{2+}, VO^{2+}, VO_2^+, urea	400:1	94.0–97.0	2.1–3.5
Pb^{2+}, Bi^{3+}, NO_2^-, I^-	350:1	95.0–97.5	3.0–3.7
As^{5+}, Sb^{5+}	300:1	93.2–97.5	3.1–3.5
$Fe^{3+, a}$	200:1 (1500)	101.5–103.5	3.5
$(As^{3+}, Sb^{3+})^b$	150:1 (1000)	93.0–96.0	2.5–4.0
$(Formaldehyde)^c$	100:1 (750)	92.0–95.0	2.5
$(Sn^{2+}, Mn^{2+})^d$	50:1 (500)	93.0–95.5	3.0–3.7
$Hg^{2+, e}$	35:1 (250)	101.2–105.3	4.1

[a]By using 1.0 mL of 0.02 mol L^{-1} NH_4F solution in medium buffered to pH 4.0 or 5.0

[b]After pre-oxidation of As^{3+}/Sb^{3+} to As^{5+}/Sb^{5+} with 0.01 mol L^{-1} H_2O_2 solution in alkaline medium

[c]After pre-treatment of 0.25 mL of 0.02 mol L^{-1} Na^-metabisulfite, $Na_2S_2O_5$ around pH 5.0

[d]By using 0.2 mL of 0.025 mol L^{-1} $Na_2H_2P_2O_7$ solution

[e]By using selectively 1.5 mL of 0.01 mol L^{-1} iminodiacetic acid (IDA) chelation solution in acetic acid medium buffered to pH 4.0–5.0

Table 3 The validation of the method developed for Ag^+ and Cu^{2+} ions at pH 4.0 and 5.0 with a significant sensitivity difference for analysis of total Ag and Cu levels in two certified samples (n: 5)

CRMs	Certified, µg kg^{-1} or µg L^{-1}		[a]Observed, µg kg^{-1} or µg L^{-1}		RSD %	Recovery %	The one paired t test [b], t_{exp}
	Ag^+	Cu^{2+}	Ag^+	Cu^{2+}			
CRM EnviroMAT™ HU-1 Used Oil from SCP Science, Quebec, Canada	*13	3132 ± 226	12.3 ± 0.7	2925 ± 250	5.7, 8.5	94.6, 93.4	2.24, 1.85
SRM 1643a Trace elements in natural water from NIST, Gaithersburg, Canada	8.017 ± 0.042	85.07 ± 0.48	8.15 ± 0.30	81.5 ± 3.5	3.7, 4.3	101.6, 95.8	0.99, 2.28

*Not certificated, and it is reported as only mean information value

[a]The mean plus SD of five replicate measurements obtained by using the proposed method for aliquots (3.0 and 0.3 g) of original sample, 5.0 mL of the sample solutions pre-treated and diluted at ratios of 1000- and 2500-fold dilution for Ag^+ and Cu^{2+} ions, respectively

[b]The experimental t values calculated by using $t = N^{1/2} (\mu - x_{average})/s$ for five replicate measurements at confidence interval of 95% in which the critical t value is 2.78 for four degrees of freedom at confidence interval of 95%

Analytical applications of the method

The proposed method was applied for the determination of low levels of Ag^+ and Cu^{2+} ions (as total Ag/total Cu) in selected food matrices. The results are shown in Tables 4 and 5. It was found that total Ag and total Cu at sub-ppb levels were observed in edible oil samples studied. The accuracy of the method was verified in terms of statistical evaluation by means of Student's t test of experimental data based on multiple standard addition method for two quality control samples after two sample preparation procedures for selected beer and wine samples, and recovery rates obtained from replicate measurements with and without spiking. The selected food samples were spiked with the target analytes at concentration levels of 10 µg L^{-1} of each ion, including two quality control samples, before extraction and analysis. Extractions were carried out under the optimized reagent conditions, and intra-day and inter-day accuracy and precision measurement results (as the percent recoveries and RSDs, n: 5) are summarized in Tables 4 and 5. Accuracy (percentage recovery values) from multiple

standard addition method was in the range of 87–94% with RSDs lower than 6.4 and 6.8% for two sample preparation procedures. From the measurement results with and without spiking via standard addition method to compensate for the matrix effect in spectrophotometric analysis, it has been observed that the total Ag levels are in the range of 0.80–6.50 and 0.74–6.20 µg L^{-1} in edible vegetable oils for both sample preparation procedures after fivefold dilution with dilute, 2.0% (v/v) HNO$_3$ to overcome the matrix effect, while the total Cu levels are in the range of 8.2–55.1 and 8.5–54.7 µg L^{-1} after fivefold dilution with dilute HNO$_3$. A paired Student's t test showed that the mean values ($t_{exp} < t_{crit}$; 0.79–2.64 < 2.78, $n_1 + n_2 = 8$, 95% CI for silver and 0.35–1.58 < 2.1, $n_1 + n_2 = 8$, 95% CI, for copper) do not significantly differ. Taking into account these results, no significant differences between the two sample preparation procedures were observed, which strongly indicates an absence of systematic errors [46]. When aliquots of sequentially 3.0 g and 5.0 mL of the original sample, pre-treated and extracted sample solutions are considered, the total Ag levels have been in the range of 6.7–54.2

Table 4 The accuracy and precision studies of total Ag and Cu levels measured in the selected two quality control samples via the matrix-matched calibration curves

Sample	Added, µg L^{-1}	Accuracy/precision					
		On the same day (n: 5)			On three consecutive days (n: 3×5)		
		[a]Found, µg L^{-1}	Recovery %	RSD %	[a]Found, µg L^{-1}	Recovery %	RSD %
Fish oil	–	$0.8 \pm 0.05, 2.3 \pm 0.12$	–	6.2, 5.2	$0.77 \pm 0.05, 2.1 \pm 0.12$	–	6.5, 5.7
	10, 10	$9.8 \pm 0.5, 11.5 \pm 0.5$	90, 92	5.1, 4.3	$9.5 \pm 0.5, 11.2 \pm 0.5$	87, 91	5.3, 4.5
Fish flour	–	$1.5 \pm 0.08, 3.2 \pm 0.15$	–	5.3, 4.7,	$1.3 \pm 0.08, 3.0 \pm 0.15$	–	6.2, 5.0
	10, 10	$10.7 \pm 0.5, 12.5 \pm 0.5$	92, 93	4.7, 4.0	$10.4 \pm 0.5, 12.2 \pm 0.5$	91, 92	4.8, 4.1

[a]The mean plus its standard deviation of five replicate measurements obtained by using matrix-matched calibration approach where 5.0 mL of the pre-treated and extracted sample solutions was independently analyzed with and without spiking with 5, 15 and 30 µg L^{-1} after dilution of 1:5-fold with dilute, 2.0% (v/v) HNO$_3$

Table 5 The analysis results of total Ag and Cu levels in edible vegetable oils by the developed spectrophotometric method (n: 5)

| Sample | Added, µg L⁻¹ | By multiple standard addition method | | | | | | **The paired Student's t test[b], t_{exp} |
| | | *After ultrasonic extraction for 20 min at 35 °C with 2.5 mL of 0.01 mol L⁻¹ EDTA adjusted to pH 8.0 | | | *After extraction induced by emulsion breaking using 5.0 mL of 4.0% (w/v) Triton X-114 and 1.5 mol L⁻¹ HNO₃ (2:3, v/v) mixture for 12 min at 55 °C in ultrasonic bath | | | |
		[a]Found, µg L⁻¹	Recovery %	RSD %	[a]Found, µg L⁻¹	Recovery %	RSD %	
Natural olive oil	–	1.20 ± 0.06, 15.5 ± 0.6	–	5.0, 3.9	1.10 ± 0.06, 15.2 ± 0.5	–	5.4, 3.3	2.64 (1.21)
	10, 10	10.0 ± 0.4, 24.5 ± 0.8	88, 90	3.8, 3.3	9.9 ± 0.4, 24.1 ± 0.7	88, 89	4.0, 2.9	–
Riviera olive oil	–	1.8 ± 0.08, 12.8 ± 0.6	–	4.4, 4.7	1.7 ± 0.08, 12.5 ± 0.5	–	4.7, 4.0	1.98 (1.21)
	10, 10	10.5 ± 0.4, 22.0 ± 0.8	87, 92	3.8, 3.6	10.3 ± 0.4, 21.7 ± 0.7	87, 92	3.9, 3.2	–
Corn oil	–	3.8 ± 0.2, 10.2 ± 0.4	–	5.3, 3.9	3.5 ± 0.2, 9.8 ± 0.4	–	5.7, 4.1	2.37 (1.58)
	10, 10	13.1 ± 0.5, 19.3 ± 0.6	93, 91	3.8, 3.1	12.8 ± 0.5, 19.0 ± 0.6	93, 93	3.9, 3.2	–
Sunflower oil	–	3.1 ± 0.2, 15.8 ± 0.6	–	6.4, 3.8	3.0 ± 0.2, 15.5 ± 0.6	–	6.7, 3.9	0.79 (0.79)
	10, 10	12.3 ± 0.5, 24.8 ± 0.8	92, 90	4.1, 3.2	12.1 ± 0.5, 24.5 ± 0.7	91, 90	4.1, 2.9	–
Almond oil	–	6.1 ± 0.3, 55.1 ± 1.8	–	4.9, 3.3	5.8 ± 0.3, 54.7 ± 1.8	–	5.2, 3.3	1.58 (0.35)
	10, 10	15.3 ± 0.6, 64.5 ± 2.0	92, 94	3.9, 3.1	14.8 ± 0.6, 64.2 ± 2.0	90, 95	4.1, 3.1	–
Soybean oil	–	4.0 ± 0.2, 23.5 ± 1.0	–	5.0, 4.2	3.7 ± 0.2, 23.2 ± 1.0	–	5.4, 4.3	2.37 (0.47)
	10, 10	13.3 ± 0.5, 32.6 ± 1.2	93, 91	3.8, 3.7	12.7 ± 0.5, 32.3 ± 1.2	90, 91	3.9, 3.7	–
Hazelnut oil	–	6.5 ± 0.3, 13.5 ± 0.6	–	4.6, 4.4	6.2 ± 0.3, 13.3 ± 0.5	–	4.8, 3.8	1.58 (0.81)
	10, 10	15.7 ± 0.6, 22.5 ± 0.8	92, 90	3.8, 3.6	15.3 ± 0.6, 22.6 ± 0.8	91, 93	3.9, 3.5	–
Sesame oil	–	0.80 ± 0.05, 8.2 ± 0.3	–	6.2, 3.7	0.74 ± 0.05, 8.5 ± 0.3	–	6.8, 3.5	1.90 (1.58)
	10, 10	9.8 ± 0.4, 17.3 ± 0.6	90, 91	4.1, 3.5	9.5 ± 0.5, 17.6 ± 0.6	87.6, 91	5.2, 3.4	–

*The mean plus its standard deviation of five replicate measurements obtained by using multipoint standard addition method chosen for determination of total Ag and Cu levels around the method quantification limits after (i) ultrasonic extraction for 20 min at 35 °C with 2.5 mL of 0.01 mol L⁻¹ EDTA extraction adjusted to pH 8.0, and (ii) extraction induced by emulsion breaking using 5.0 mL of 4.0% (w/v) Triton X-114 and 1.5 mol L-1 HNO₃ (2:3, v/v) mixture for 12 min at 55 °C in ultrasonic bath to ensure reliability of the results against possible matrix effect

**The accuracy and precision results based on statistical comparison of the results obtained by two different sample preparation procedures for Ag⁺ and Cu²⁺ ions at pH 4.0 and 5.0, respectively

[a]The mean plus its standard deviation of five replicate measurements obtained by using standard addition method in which aliquot (5.0 mL) of the pre-treated, extracted and diluted sample solutions was independently analyzed by multiple standard addition method after dilution at ratios of 1:5 with dilute, 2.0% (v/v) HNO₃ to overcome the matrix effect

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

and 6.2–51.7 µg kg⁻¹ for both sample preparation procedures, while the total Cu levels have been in the range of 68.3–459.2 and 70.8–455.8 µg kg⁻¹. It can be seen that total Cu levels in the edible vegetable oils are highly in agreement with the results found by selective, sensitive, but complex and expensive spectroscopic analysis techniques such as ETV-ICP-MS, ICP-OES, GFAAS, FAAS [8, 11–15] and electroanalytical detection techniques such as dPSA and SCP [16, 19]. Also, that for Cu was either lower or higher than MRL established as quality criterion by the International Olive Oil Council: 100 µg kg⁻¹ [9]. When considered reactivity of Cu²⁺ and Ag⁺ ions, it is clear that higher copper and silver concentrations than MRL can lead to a decrease in oxidative stability of edible oils. Finally, the results clearly show that the presented method can be successfully applied to accurate and reliable determination of total Ag and Cu

levels in edible oils, which further indicates the capability of the method in the analysis of real-time samples containing different matrix components under the optimal conditions.

Comparison of the method with other reported methods

The method was compared with a variety of detection methods that had recently been reported in the literature for extraction, pre-concentration, and determination of Ag⁺ and Cu²⁺ ions from sample matrices. The analytical performance features of the method are given in Table 6. As can be seen from Table 6, it is evident that the method has quantitatively wide linear working ranges for pre-concentration of Ag⁺ and Cu²⁺ ions by the amine-functionalized poly(SMA) copolymer matrix. Moreover, the LODs/LOQs of the

Table 6 The extraction methods used for single and/or multiple metals determination in edible vegetable oils and comparison of their performance properties

Extraction method	Chelating agent	Single or multiple metals determined	Detection technique	Linear working range	Detection limit	Accuracy and precision		Average Cu, Ag contents of samples	References
						Recovery%	RSD%		
Online emulsion formation with 2.0% (v/v) Triton X-100	–	Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, Mg, Mn, Ni, Pb, Tl and Zn	ICP-OES	0.0–1.0 mg L^{-1}	–	95–103, 95–99	3–5, 3–6	57 ± 10 µg kg^{-1} for Cu	[8]
Online emulsion formation with 2.0% (v/v) Triton X-100	–	Ba, Bi, Cd, Co, Cu, Mn, Ni, Pb, Sn, Al and V	FI-ICP-MS	0–60 µg L^{-1}	1.09–3.31 µg kg^{-1}	78.2–122	4.3–10.4	1.7–4.6 µg kg^{-1} for Cu	[10]
By direct injection of emulsions containing 5% m/v vegetable oil, 1.5% v/v Triton X-100 and 50 µg mL^{-1} ascorbic acid	Pd-NPs as matrix modifier	Cu, As, Hg, Pb	ETV-ICP-MS	0–20 µg L^{-1}	0.02 µg L^{-1} (or 0.4 µg kg^{-1} after dilution of 50-fold)	98.6–99.2	<10	93.4, 143, 188;94.1, 144, 183; 5.87, 12.9, 9.23 µg kg^{-1} with three methods	[11]
Extraction with 10% HNO$_3$ (50 Hz, 60 s) for 2 h at 50 °C in a shaker	–	Cu, Fe, Mn, Co, Cr, Pb, Ni, Cd, Zn	ICP-OES	1–100 µg L^{-1}	–	85–90	<10	8.20–85.0 µg kg^{-1}	[12]
After dilution with 2% lecithin as emulsifier-cyclohexane, furnace program for 3.5 min	NH$_4$H$_2$PO$_4$ as matrix modifier	Cu	GFAAS	0–25 µg L^{-1}	2.0 µg L^{-1}	85.5–93	2.1–3.5	–	[13]
Extraction with CCl$_4$ and 2 mol L^{-1} HNO$_3$ after pre-treatment with conc. HNO$_3$ (1:1, v/v; ultrasonic bath, for 10 min at 30 °C)	–	Fe, Cu, Zn, Ni	FAAS	0–0.25 mg L^{-1}	–	96.5–97.1	<2.2	0.028–0.076 mg kg^{-1}	[14]

Table 6 (continued)

Extraction method	Chelating agent	Single or multiple metals determined	Detection technique	Linear working range	Detection limit	Recovery%	RSD%	Average Cu, Ag contents of samples	References
Extraction with mixture of CCl_4 and 2 mol L^{-1} HNO_3 (1:1, v/v, ultrasonic intensification) for 10 min at 30 °C	–	Fe, Cu, Ni, Zn	FAAS	0–0.25 mg L^{-1}	–	96.4–97.1	<2.2	0.41–3.40 µg g-1	[15]
Extraction by 88% (v/v) ethanol–water for 60 min at 30 °C	N,N′-bis(salicylidene)-2,2′-dimethyl-1,3-propanediamine (LDM)	Cu, Fe	FAAS/Spectrophotometry, 363 nm at pH 4.0	–	2.0 mg kg^{-1}	100.2	5.6	–	[16]
Extraction with 35% H_2O_2 and 36% HCl (1:5, v/v; 30 min, 90 °C)	–	Cd, Cu, Pb, Zn	dPSA	0–1000 µg kg^{-1}	0.4–0.9 µg kg^{-1}	93.5–97.0	<2.1	53.80 to 674.45 µg kg^{-1}	[17]
Extraction with conc. HCl for 5 min 50 °C	5,5-dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone, DCDT	Cu	Ad-SSWV	0–35 µg L^{-1}	0.49 µg L^{-1}	104.4	<9.5	4.66–29.56 µg kg^{-1}	[18]
Ultrasound treatment with mixture of conc. HCl and H_2O_2 (1:1, v/v) for 60 min	–	Cu, Pb	SCP	0–0.3 mg L^{-1}	13 µg kg^{-1}	82–107	<7.0	0.013–2.67 mg kg^{-1}	[19]
Extraction with conc. HNO_3 and 6% H_2O_2 (1:1, v/v) (i) Ultrasonic extraction with 2.5 mL of 0.01 mol L^{-1} EDTA adjusted to pH 8.0 for 20 min at 35 °C	1-phenyl-1,2-propanedione-2-oxime thiosemicarbazone	Cu, Ni	UV–Vis spectrophotometry, 463 nm	0.38–7.63, 0.94–2.82 mg L^{-1}	–	89.6–119.5	4.6	0.632–11.463 mg kg^{-1}	[20]

Table 6 (continued)

Extraction method	Chelating agent	Single or multiple metals determined	Detection technique	Linear working range	Detection limit	Accuracy and precision		Average Cu, Ag contents of samples	References
						Recovery%	RSD%		
(ii) Ultrasonic extraction induced by emulsion breaking using 5.0 mL of 4.0% (w/v) Triton X-114 and 1.5 mol L⁻¹ HNO₃ (2:3, v/v) mixture for 12 min at 55 °C	Amine-functionalized poly(SMA) copolymer/SDS at pH 4.0, 5.0	Cu, Ag	Micro-volume UV–vis spectrophotometry, 346 nm	0.8–110, 1–125 µg L⁻¹	0.23, 0.37 µg L⁻¹	90.4–96.5, 92.1–98.5	3.5–6.2, 3.8–5.7	0.8–6.5, 0.74–6.2 for Ag; 8.2–55.1, 8.5–54.7 µg L⁻¹ for Cu	This study

dPSA: derivative potentiometric stripping analysis; Ad-SSWV: adsorptive stripping square wave voltammetry; SCP: stripping chronopotentiometry; FAAS: flame atomic absorption spectrometry; GFAAS: graphite furnace atomic absorption spectrometry; ICP-OES: inductively coupled plasma-optical emission spectrometry; FI-ICP-MS: flow injection inductively coupled plasma-mass spectrometry; ETV-ICP-MS: electrothermal vaporization inductively coupled plasma-mass spectrometry

method, 0.23/0.76 and 0.36/1.20 µg L⁻¹ for Ag^+ and Cu^{2+} ions, are better than that of other methods which use detection techniques such as UV–Vis spectrophotometry, FAAS, direct GFAAS, SCP and dPSA, including ICP-AES, after extraction with either acidic oxidant mixture or oil in water emulsion breaking with and without sonication [8, 11, 12, 14, 15, 19]. In fact, the GFAAS requires expert user in his/her area, including expensive, complicated, time-consuming furnace programs and use of matrix modifiers. The more sensitive plasma techniques such as ICP-AES and ICP-MS with FI and ETV [10, 11], with high-cost equipment, operating under Ar gas and air-conditioning laboratory setup, require multiple high-purity gases and high level of staff expertise, and interferences need to be controlled (generally requiring the use of an internal standard and the appropriate reaction mode in plasma), including memory effect. Shortly, the method, based on sensitive/selective detection of sub-ppb levels of Ag^+ and Cu^{2+} ions by spectrophotometry at 346 nm, can be evaluated as simple, cost-effective, safe, eco-friendly, accurate and reliable analytical detection tool with a pre-concentration factor of 62.5-fold from pre-concentration of 25 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 SPE 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 accurate and reliable results in terms of linearity, LODs, LOQs, accuracy, repeatability/intermediate precision and matrix effect and provided evidence of spectrophotometry's feasibility as an alternative approach to routine quality control of low amounts of silver and copper in other sample matrices.

Conclusions

In the current work, a new amine-functionalized pH-sensitive poly(SMA) copolymer matrix was designed, characterized and efficiently used in selective extraction/pre-concentration of Ag^+ and Cu^{2+} ions at pH 4.0 and 5.0, respectively, by means of UA-CPE. By providing improvement in phase separation and mass transfer between aqueous and micellar phases in the presence of pH-sensitive SDS, the UA-CPE procedure has resulted in a significant enhancement in the sensitivity, selectivity, and LODs in the popularity of micro-volume UV–Vis spectrophotometry besides the non-toxic solvent extraction of heavy metals from complex matrices. The developed protocol has been successfully employed for the determination of total Ag and Cu in edible oil samples after two sample pre-treatment procedures via

micro-volume UV–Vis spectrophotometry at 346 nm. With respect to its achieved analytical parameters, the proposed method is simple, reasonably rapid, cost-effective, low in LODs (0.25/0.40, 0.41/0.72 μg L^{-1}), wide in linear working ranges (0.8–110/1.0–120 for Ag$^+$ and 1–125/2–140 μg L^{-1} for Cu^{2+}) and highly reproducible (RSD < 7.1%) by two calibration curves.

Also, the use of cells with micro-capacity in the analysis step results in low consumption of samples and reagents, and so negligible generation of wastes. The method provides the possibility of simple, cost-effective, accurate and reliable determination of trace Ag$^+$ and Cu^{2+} ions with better and/or comparable results than those of other spectrophotometric, FAAS, direct GFAAS and electroanalytical (SCP, dPSA and Ad-SSWV) techniques, and it can be considered as an alternative economic tool to the more sensitive, but expensive and complex atomic spectrometric detection techniques such as ICP-OES, FI-ICP-MS and ETV-ICP-MS.

Declarations

Conflict of interest The authors strongly declare that no scientific and/or financial conflicts of interest exists with other people or institutions.

Ethical approval This article does not contain any studies with human or animal subjects.

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