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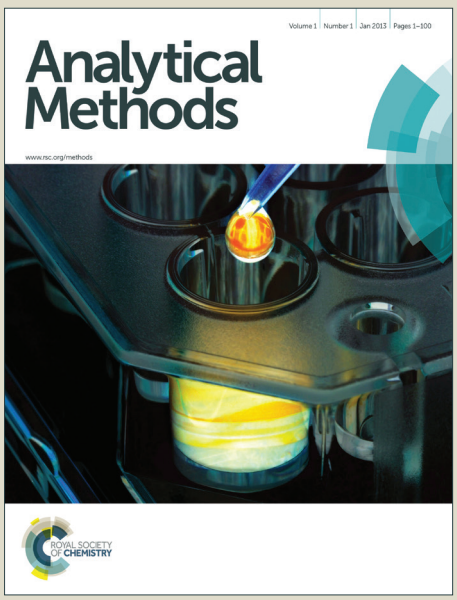

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Dispersive micro-solid phase extraction method using newly prepared poly(methyl methacrylate) grafted agarose combined with ICP-MS for simultaneous determination of Cd, Ni, Cu and Zn in vegetable and natural water samples

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Abstract:

Poly(methyl methacrylate) grafted agarose (Agarose-g-PMMA) was prepared as new adsorbent for simultaneous separation and preconcentration of trace amounts of cadmium (II), nickel (II), copper (II) and zinc (II) in vegetable and natural water samples with a simple and facile dispersive micro-solid phase extraction (D-μ-SPE) prior to ICP-MS detection. Agarose-g-PMMA was prepared by microwave-assisted free radical polymerization and the materials obtained under optimum conditions in high percentage of grafting were characterized by FTIR, FE-SEM, DSC and TGA. The effective parameters of the extraction process such as mass of adsorbent, pH of sample solution, adsorption time, type of eluent, concentrations and volume of

eluent and desorption time were optimized. A preconcentration factor (PF) of 100 was obtained for trace metals with an elution time of 120 s. Under the optimum conditions, the limits of detection (LODs) for Cd, Ni, Cu and Zn were 1.8, 0.9, 0.6 and 1.5 ng L^{-1} with relative standard deviations (RSDs) of 2.1%, 3.5%, 4.9% and 3.8% respectively at analyte concentration of 10 ng L^{-1}, n = 7. The proposed method was successfully applied for the determination of heavy metals in vegetable and natural water samples with good relative recoveries in the range of 92.0-104.0%.

Keywords: Dispersive micro-solid phase extraction, ICP-MS, Trace metals, microwave-assisted method.

1 Introduction

Heavy metals are naturally found on the earth's crust. As the rapid development of industry, a lot of the metals were used for different scientific and industrial purposes. They are non-biodegradable and can be accumulated in living tissues, causing various diseases and disorders. The discharge of these metals into the environment can pose serious problems that threaten our future.[1] Cadmium (II), nickel (II), copper (II) and zinc (II) are well-known toxic heavy metals whose main sources are industrial activity and farm work. Cadmium is commonly used in batteries, alloys, pigments, as a PVC stabilizer and electroplating but it is extremely toxic even at low concentrations.[2] Cadmium has been classified by the International Agency for Research on Cancer (IARC) as a human carcinogen and it has a biological half-life of 10–35 years in the human body.[3] Copper (Cu) plays important roles in metabolism because it is a biologically essential element. It is also an important trace element in seawater for the growth of

phytoplankton in the ocean.[4, 5] Zinc play a role as a co-factor in more than 200 enzymes and it has essential role in the biological processes of plants and animals, so deficiency of zinc is a world nutritional problem.[6] However, excess of copper and zinc can also play a role in the progression of damages in human body, including disturbances in energy metabolism or increasing oxidative stress.[7, 8] The WHO International Standards has zinc concentration of < 10 μg L^{-1} for natural surface waters and < 5 mg kg^{-1} for grains, vegetables, and fruits. Meanwhile, the guideline values for Cu, Ni and Cd are 2 mg L^{-1}, 0.03 mg L^{-1} and 0.003 mg L^{-1}, respectively. [9] In perspective of such poisonous impacts of cadmium (II), nickel (II), copper (II) and zinc (II), its detection and determination in environmental specimens is of utmost importance.

Biosorbents are inexpensive compared to other conventional sorbents and a good option for removing toxic metals as several functional groups on biosorbents like hydroxyls in agarose could potentially attract metals ion. They are biodegradable, readily available, and a non-toxic feed stock for synthesizing high performance macromolecular materials. They can be easily modified and are highly stable. However, agarose is relatively inert because the hydroxyl groups, which act as active groups, are involved in inter- and intramolecular hydrogen bonding. Therefore, functionalization approaches have been employed to improve the surface, reactivity and stability of this biopolymer. Agarose can be improved by graft copolymerization of vinyl monomers onto backbones for thermal, chemical and solvent resistance. Graft copolymerization is a powerful modification approach in which monomers with functional groups are covalently attached to a main chain of a polymer backbone to form branched copolymer.[36,37]

Various organic polymer grafted materials such as PMMA grafted psyllium [10], ethylacrylate grafted xanthan gum [11], acrylamide grafted gellan [12] and poly(alkyl methacrylate) and poly(acrylamide) grafted chitosan [13-15] etc have been snyntheized and find wide variey of

applications in removal of heavy metal ions and anionic azo dyes from aqueous solutions and drug delivery. However the use of organic polymer grafted materials have been poorly reported in the field of microextration coupled with ICP-MS, GC-MS and HPLC.

Various sample preparation methods including liquid–liquid extraction (LLE),[16] solid phase extraction (SPE),[17,18] solid phase microextraction (SPME),[19,20] solid-phase membrane tip extraction (SPMTE),[21] micro solid phase extraction, [22] single-drop microextraction,[23,24] dispersive liquid–liquid microextraction (DLLME)[25,26] and dispersive micro-solid phase extraction (D-μ-SPE).

Among the microextraction methods, D-μ-SPE has received considerable interest from researchers as this technique requires only small amounts of solvent (μLs) and sorbent (milligrams) for the extraction.[27] Compared to traditional SPE, this approach enables the analytes to interact sufficiently with all the adsorbent particles, achieving greater capacity per amount of adsorbent, as well as avoiding channeling or blocking of the cartridges or discs.

Litrature survey revaeals that, agarose is a linear biopolymer consisting of (1–3)-β-D-glactopyranose-(1–4)-3,6-anhydro-α-L-galactopyranose units. [28] However, agarose is relatively inert because the hydroxyl groups, which act as active groups, are involved in inter- and intramolecular hydrogen bonding. Therefore, functionalization approaches have been employed to improve the surface, reactivity and stability of this biopolymer.[28] Thus, it is interesting to prepare poly(methyl methacrylate) grafted agarose and use it as new sorbent for dispersive micro-solid phase extraction in pre-concentration and determination of heavy metal ions. To the best of our knowledge, this is the first attempt to prepare new poly(methyl methacrylate) grafted agarose by economical and ecofriendly route and the material was used as new sorbent for determination of heavy metals with dispersive micro-solid phase extraction method.

2 Experimental

2.1 Reagent

Agarose was obtained from Promega (Madison, USA). Methyl methacrylate monomer (MMA) and ceric ammonium nitrate (CAN) were purchased from Sigma-Aldrich (St. Louis, USA). Tetrahydrofuran (THF), acetone and ethanol were from QRëc (Selangor, Malaysia). A solution containing 4 ng mL^{-1} cadmium (Cd), nickel (Ni), copper (Cu) and zinc (Zn) in 1% HNO_3 was prepared from single-element 1000 µg mL^{-1} with the purity higher than 99% and HNO_3 (65%, extra pure) were purchased from Merck Chemicals (Darmstadt, Germany) by stepwise diluting the stock solution, and was used to optimize ICP-MS parameters before each analytical run. The solution pH values were adjusted with NaOH and/or HNO_3 (all from Sigma–Aldrich). High-purity doubly deionizes water (DDW) obtained from a Milli-Q water system (Millipore, Bedford, MA, USA) was used during the experiments. The glassware used in the experiments was soaked in 10% (v/v) HNO_3 solution overnight and they were rinsed several times before use with DDW.

2.2 Instrumentation

The FTIR spectra were recorded using Perkin Elmer (Spectrum™ 400 FTIR Spectrometer, USA) instrument. The surface morphology was analyzed with the help of JEOL (Model: JSM-6701F) field emission scanning electron microscope (FE-SEM). To determine the glass transition of the Agarose-g-PMMA, differential scan calorimetry was employed on a Thermal Advantage Instrument DSC822 Mettler-Toledo DSC instrument (Schwerzenbach, Switzerland). Thermogravimetric analyzer SDTA851 Mettler-Toledo TGA Instrument (Schwerzenbach,

Switzerland) was used to determine the thermal stability of the Agarose and Agarose-g-PMMA. The detection system used for the determination of heavy metal ions was a Perkin-Elmer ELAN 6100 ICP mass spectrometer (Perking Elmer, Germany). All the operating parameters were those recommended by the manufacturer. The optimum operating conditions and measurement parameters for ICP-MS are listed in Table 1. The pH values were controlled with a microprocessor pH meter (HANNA Instruments, Romania). A 3510E-DTH model Ultrasonic cleaner (Branconic, USA) was used during the experiment. Vortex (REAX 2000, Heidolph, Germany) was used to disperse the materials in solution and a 2420 KUBOTA centrifuge (Tokyo, Japan) was used to accelerate the phase separation.

2.3 Adsorbent preparation and purification

Graft copolymerization was carried out in a 1000-mL borosilicate beaker. Agarose (1.0 g) was dissolved in 40 mL of distilled water. Different amounts of freshly distilled methyl methacrylate (by vacuum distillation) in 10 mL of distilled water was added to the agarose solution, followed by addition of catalytic amount of ceric ammonium nitrate (CAN). The reaction mixture was irradiated under microwave at 700 W for the desired length of time ranging from 30 to 90 s. The reaction vessel was always cooled during the reaction (< 75 °C) purposely to prevent any formation of homopolymer of poly(methyl methacrylate) and also the reaction vapor. The reaction mixture turned milky white after 1 min and the solution was cooled and the product was precipitated with excess acetone. The solid was filtered using sintered glass funnel and dried under vacuum at 40 °C. The copolymerization process of agarose and methyl methacrylate was carried out using different parameters namely amount of monomer, amount of initiator concentration, and of microwave radiation time.

Prepared graft copolymer product was washed with ethanol-water (1:1 v/v) using Soxhlet extraction for 24 h to remove any homopolymer formed during the polymerization [29]. The extracts were filtered using sintered funnel. The resulting precipitate of graft copolymer was collected, dried in a vacuum oven and then ground prior to characterization. The proposed mechanism of microwave-assisted grafting is depicted in Scheme 1.

2.4 Optimization of method

20 – 120 mg of agarose-g-PMMA added to 50 mL of multielement solutions (Cd, Ni, Cu and Zn) with 4 μg L^{-1} as concentration, the pH was varied from 2 to 9. Then, the mixture was agitated 1 – 5 min with vortex. After that, the adsorbed analytes were desorbed from the adsorbent with 0.5 mL of (0.5 – 2 mol L^{-1}) HNO_3 by ultrasonication for 1 – 5 min. Finally, the eluate was separated by syringe filter and introduced into ICP-MS for the determination of metal ions.

2.5 Dispersive micro-solid phase extraction (D-μ-SPE)

In order to develop the ICP-MS method for the detection of Cd, Ni, Cu and Zn, commercially available calibration standards solutions of the four elements were prepared by diluting the appropriate volume of a 1000 μg mL^{-1} element working standard with blank solution, to final concentrations of 1, 10, 20, 30, and 50 ng mL^{-1}. The equations and coefficients of determination of the calibration curves for Cd, Ni, Cu and Zn were y_{Cd} = 2051.2x - 428.93 (R^2 = 0.9996), y_{Ni} = 3871.5x - 4403.4 (R^2 = 0.9992), y_{Cu} = 2951.2x - 1573.6 (R^2 = 0.9993), y_{Zn} = 1308.3x - 562.99 (R^2 = 0.9997), respectively. These calibration standards were used to assess the linear calibration

range of the instrument. It was found that the response of the ICP-MS was linear at least in the range of 1.0 and 50 ng mL^{-1}.

In the procedure, 80 mg of agarose-g-PMMA was added into a 50-mL centrifuge tube containing 50 mL of standard or samples solution containing Cd, Ni, Cu and Zn. The pH was adjusted to 6.0 with HNO_3 or NaOH solutions. Then, the mixture of sorbent and sample was agitated using a vortex (2,500 rpm) for 4 min to facilitate the dispersion of agarose-g-PMMA in the sample. After extraction, the mixture was then centrifuged for 2 min to form separate layers of aqueous (sample solution) and solid (agarose-g-PMMA sorbent) phases. The aqueous phase was discarded and only agarose-g-PMMA sorbent remained in the centrifuge tube. In order to desorb the analytes, 0.5 mL HNO_3 (1 mol L^{-1}) was added to the centrifuge tube containing agarose-g-PMMA, and the tube was ultrasonicated for 2 min. The solution was filtered through a 0.2-μm nylon syringe filter (Membrane Solutions, China) prior to analysis by ICP-MS.

2.6 Real sample pretreatment and analysis

The proposed method was applied to the determination of Cd, Ni, Cu and Zn in different water samples namely tap, river, and lake water samples. Tap water was collected from our lab. The samples were immediately filtered through a cellulose filter membrane (pore size 0.45 μm), and were acidified to pH 2 for storage. Before determination, the samples were adjusted to pH 6 according to optimized experimental conditions. Three types of vegetables were chosen for analysis. These were spinach, cabbage and potato, which were collected from the local supermarket and were cleaned with DDW. 5.0 mL of HNO_3 and 1 mL of H_2O_2 were added to 1 g of sample in 100-mL beaker. The mixture was covered with a watch glass and left overnight at room temperature. The mixture was heated on a hot plate. The temperature was maintained until

the volume of acid solution in the beaker was about 1.0 mL. The digested sample was diluted with DDW to 50.0 mL and the pH was adjusted to 6.0. The preconcentration procedure described above was applied to the samples.

3 Results and discussion

3.1 Characterization of the adsorbent

Poly(methyl methacrylate) grafted to agarose was synthesized by microwave-assisted method using ceric ammonium nitrate as chemical free radical initiator. The effect of different conditions of microwave-assisted synthesis on % grafting, % grafting efficiency and % conversion of grafted agarose were investigated with following equation.

$$\%G = \frac{\text{Wt. of the grafted product} - \text{Wt. of polysaccharide taken}}{\text{Wt. of polysaccharide taken}} \times 100$$

$$\%GE = \frac{\text{Wt. of the grafted product} - \text{Wt. of polysaccharide taken}}{\text{Wt. of monomer}} \times 100$$

$$\%C = \frac{\text{Wt. of the grafted product}}{\text{Wt. of monomer}} \times 100$$

where G is grafting, GE is grafting efficiency and C is conversion of grafted agarose. For the optimization of the synthetic conditions, amount of CAN, methyl methacrylate (monomer) concentration and microwave irradiation time were taken as independent variables by keeping the other parameters constant. It was found that the grafting was optimized at methyl methacrylate concentration of 9 g and CAN concentration of 0.4 g in a reaction mixture of ~50 mL in 60 s, with the microwave power maintained at 700 W. The results are shown in Table 2.

From the FTIR spectrum of agarose (Fig. 1), it was observed that a broad peak at 3407.38 cm^{-1} is due to the stretching vibrations of O–H, a smaller peak at 2929.59 cm^{-1} is attributed to the C–H stretching vibrations. The band at 1077.59 cm^{-1} is assigned to C–O–C stretching

vibrations. In the case of agarose-g-PMMA (Fig. 1), the spectra have additional peaks due to the grafted PMMA chains. The peak at 1733.18 cm^{-1} is due to C=O stretching vibrations of PMMA. The peak at 1149.14 cm^{-1} is due to coupling of C–O stretching and O–H 'in plane' bending vibrations. Thus, these extra peaks in case of agarose-g-PMMA are well explained by the presence of grafted PMMA chains and are confirmations of the grafting.

Fig. 2 shows field emission scanning electron micrographs of agarose and grafted agarose. The grafting of PMMA chains onto agarose showed morphological change from flaky structure in nature agarose to texture, porosity and fibrillar structure of modified agarose. This increase in texture and porosity of the modified agarose could increase its adsorption capacity by trapping the analytes molecules easily via diffusion into the pores during the adsorption process.

The results of DSC analysis for native agarose and optimized copolymer show the presence of Tg (Fig. 3). DSC thermogram of agarose exhibited a broad endothermic peak at around 90 °C. Similar endothermic peaks are reported for agarose in the literature.[30-31] The Tg value of copolymer was around 118 °C; it was higher than that of normal PMMA, which is at about 105 °C. Thermo gravimetric analysis (TGA) curves of agarose and the grafted copolymer are shown in Fig. 4. In the case of agarose there are two stages of mass loss. First stage (13.24 %) was from 32-135 °C. This is due to the traces of moisture present. The second zone, a 69.55 % mass loss between 250- 400 °C is due to degradation of the agar backbone. For agarose-g-PMMA, in addition to the above zones of weight loss, extra zone of weight loss (96.48%) was observed in the temperature range of 290- 410 °C, due to the grafted PMMA chains.

3.2 Optimization of dispersive micro-solid phase extraction (D-μ-SPE)

D-μ-SPE was used to extract and preconcentrate Cd, Ni, Cu and Zn from samples. In order to optimize the system, several experimental variables affecting the preconcentration system such as mass of adsorbent, the pH, extraction time, type of eluent, concentrations and volume of eluent and finally desorption time were evaluated and optimized. A 4 μg L^{-1} multielement solution was used for all the measurements and five independent experiments were carried out for each optimized variable. The ICP-MS was used to assess the extraction efficiency under different condition.

3.2.1 Optimization of the mass of adsorbent

To evaluate the effect of adsorbent mass on the recovery of metal ions, different amounts of adsorbent (20 – 120 mg), were investigated in D-μ-SPE method, following the preconcentration procedure. As shown in Fig. 5, the recoveries of metal ions increased with increasing amounts of adsorbent in the range 20 – 80 mg. Quantitative recoveries of target ions were obtained in the range 80–100 mg. Therefore, 80 mg of agarose-g-PMMA was the amount selected for further experiments.

3.2.2 pH of the sample solution

The pH of sample solution is an important factor for the retention of metal ions on the agarose-g-PMMA according to the general procedure. Solutions (50 mL) with target metals concentration of 4 μg L^{-1} were prepared separately and the pH values of sample solutions were adjusted to a range of 2–9. Agarose-g-PMMA (80 mg) was then added into the above solutions and the mixtures were dispersed by vortex. After separation with centrifuge and desorption with nitric acid, the analytes were measured by ICP-MS as described in the recommended procedure.

Fig. 6 shows the effect of pH on the adsorption percentage of Cd(II), Ni(II), Cu(II) and Zn(II). This figure shows high recoveries for all analytes in the pH range of 6.0–8.0, while strong protonation of the agarose-g-PMMA at pH < 6.0 probably accounted for the decreases in the recoveries of metal ions. These ions were quantitatively recovered in the pH 6. However, at pH of above 8, the recoveries of metal ions decreased because of the potential hydrolysis. Scheme 2 illustrates the working principle of the agarose-g-PMMA as adsorbent for the enrichment of trace metals at pH 4-6.

3.2.3 Adsorption time (time of extraction)

The effect of extraction time was investigated by agitating the sample solution using vortex at maximum speed of 2,500 rpm for 1, 2, 3, 4 and 5 min. It was found that the percentage of recovery increased to a maximum as the extraction time was increased to 4 min, and the recovery of analytes slightly decreased when the extraction time was further increased to 5 min (Fig. 7).

3.2.4 Evaluation of the type, concentration and volume of eluent

In order to examine the desorption of metal ions from agarose-g-PMMA, a series of eluents namely HNO_3, HCl and CH_3COOH were employed to extract the trace metals. As shown in Fig. 8, it was found that HNO_3 (0.5 mol L^{-1}) with the elution volume and elution time fixed as 1 mL and 4 min, respectively gave higher analyte recoveries in the ICP-MS analysis.

Additionally, considering that HNO_3 is the mostly recommended solvent in ICP-MS measurement, HNO_3 was selected as the eluent and various concentrations of HNO_3 were studied for elution of metal ions from agarose-g-PMMA. It was found that all metal ions were eluted

quantitatively within the whole tested range of 0.5 – 2 mol L^{-1} HNO_3. In the following experiments, 1 mol L^{-1} HNO_3 was employed (Table 2). The effects of elution volume for quantitative elution of Cd(II), Ni(II), Cu(II) and Zn(II) were investigated using 0.5, 1, 1.5 and 2 mL of 1 mol L^{-1} HNO_3. It was found that 0.5 mL of 1 mol L^{-1} HNO_3 was sufficient to recover all the studied metals quantitatively.

3.2.5 Elution time

The influence of elution time on the recovery of the trace heavy metals was also investigated by varying the elution time from 1 to 5 min. The experimental results indicated that all the target metals could be recovered quantitatively in 2 min. Consequently, the elution time of 2 min was selected for quantitative recovery of target analytes in the subsequent experiments. Finally, 0.5 mL of 1 mol L^{-1} HNO_3 with elution time of 2 min was employed as the optimum elution conditions (Table 3).

3.2.6 Effect co-existing substances

To assess the possibility of analytical applications for real samples, the effect of co-existing ions on the recovery of Cd(II), Ni(II), Cu(II) and Zn(II) was investigated. In order to evaluate the effect of interference in the determination of analytes, the effect of other commonly co-existing substances in water samples, including Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Cl^- (1000 mg L^{-1}), Al^{3+}, Br^- (20 mg L^{-1}) and target metal ions Cd^{2+}, Ni^{2+}, Zn^{2+} and Cu^{2+} were tested by enrichment of a standard solution of multielements (4 µg L^{-1}) according to the procedure described in the experimental section. The results showed no significant interferences in the preconcentration and determination of the analyses.

3.2.7 Effect Adsorption capacity

The adsorption capacity is an important factor to determine how much adsorbent is suitable to quantitatively adsorb a specific amount of metals ions from the solution. 80 mg of adsorbent was dispersed in 50 mL of different concentration of multielement sample solution. After separating solution from solid parts with centrifuge, the sample was determined by ICP-MS. The capacity of agarose-g-PMMA, obtained for cadmium (II), nickel (II), copper (II) and zinc (II), were found to be 31.8, 42.5, 48.3 and 34.3 mg g^{-1}, respectively. The results of adsorption capacity were compared with reported results (Table 3).

3.2.8 Method validation

Under the optimized experimental conditions, the analytical performance of the method was evaluated. Good linearities were obtained for the target ions in the concentrations range of 10-500 ng L^{-1} with good coefficients of determination (R^2 > 0.9993). In accordance with the IUPAC definition, the limits of detection (LODs) of the method for target ions were calculated as three times the standard deviation of blank (3σ), and they were found to be 1.8, 0.9, 0.6 and 1.5 ng L^{-1} for Cd(II), Ni(II), Cu(II) and Zn(II), respectively. The precision (RSDs) for 7 replicate determinations of 10 µg L^{-1} of Cd(II), Ni(II), Cu(II) and Zn(II) were 2.1%, 3.5%, 4.9% and 3.8%, respectively. Table 4 shows the comparison of LODs of this work with other literature works on the determination of the same elements. As can be seen, the LODs obtained by this work are the loweer than those previously reported. Reusability was carried out for 15 cycles of adsorption and desorption by using filter paper and 3 times washing with 0.5 mol L^{-1} nitric acid and water, after 10 repeated cycles, the average recovery was 95.3 ± 3.1%.

3.2.9 Analysis of real samples

The developed method was used for the determination of Cd(II), Ni(II), Cu(II) and Zn(II) in tap water, river water, lake water samples, spinach, cabbage and potato. The accuracy of the method was verified by the analysis of samples spiked with known amounts of the analytes. The relative recovery (RR) was obtained by using the following equation:

$$RR = \frac{C_{found} - C_{real}}{C_{added}}$$

where C_{found}, C_{real}, and C_{added} are the concentration of analyte in the final solution after addition of a known amount of a standard into the real sample, the concentration of analyte in the real sample and the concentration of a known amount of the standard which was spiked into the real sample, respectively. The results (Tables 5 and 6) showed that the recoveries of the target ions ranged from 92.0% to 104.0%. Some of the recoveries were more than 100% probably due experimental errors and sample matrix effects.

4. Conclusions

In the present work, poly(methyl methacrylate) grafted agarose was first prepared and used as an adsorbent for preconcentration of Cd(II), Ni(II), Cu(II) and Zn(II) in selected samples, namely tap water, river water, lake water, spinach, cabbage and potato with dispersive micro-solid phase extraction method. The grafting of methyl methacrylate onto agarose was confirmed by FTIR, FE-SEM, DSC and TGA technique. The results showed that the D-μ-SPE method is very simple, fast and has excellent precision and good sensitivity. Parameters that affected the extraction efficiency, including mass of adsorbent, sample pH, extraction time, type of eluent, concentrations and volume of eluent, desorption time, effect of co-existing ions and adsorption

capacity were examined in the study. Agarose-g-PMMA-D-μ-SPE is an alternative "green" microextraction method for the determination of trace metal ions in environmental samples as it requires only small amounts of the adsorbent and little amounts of the acid.

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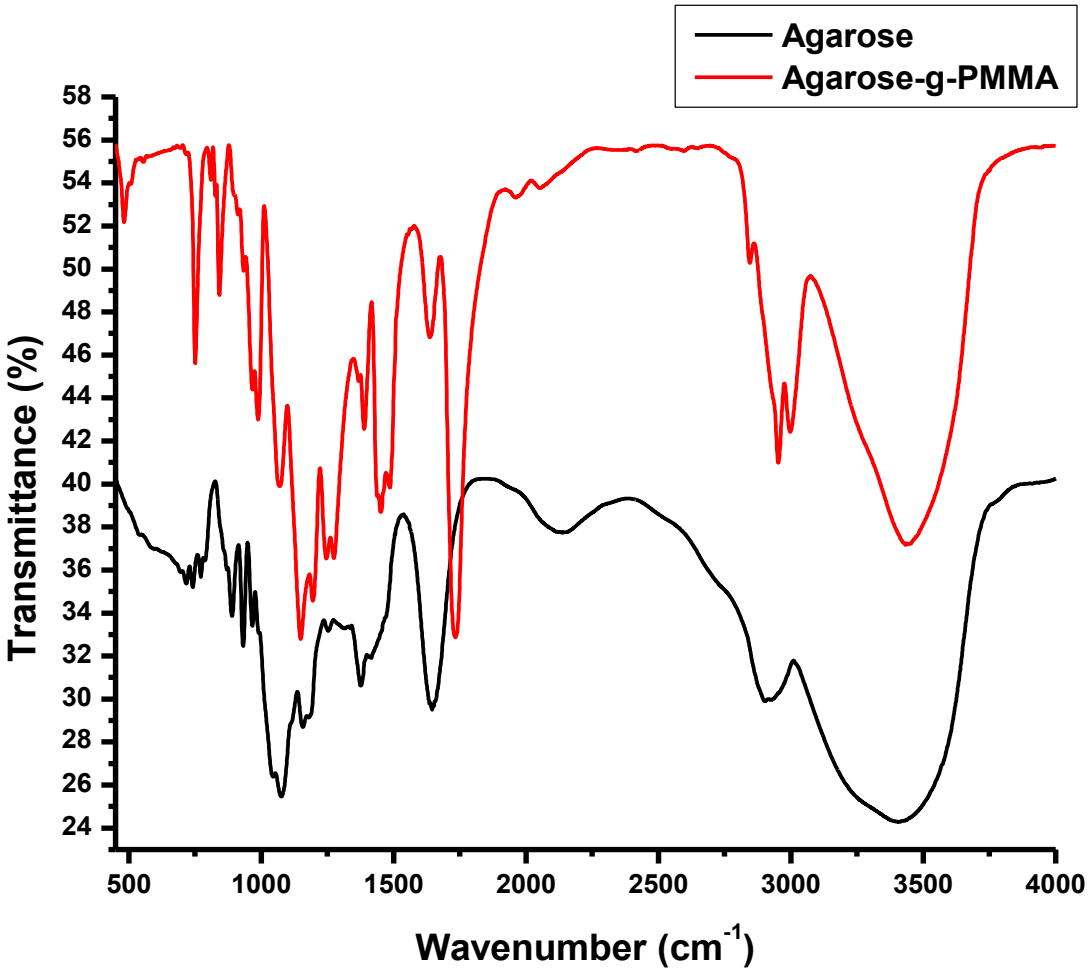


Fig. 1 FTIR spectra of Agarose and Agarose-g-PMMA.

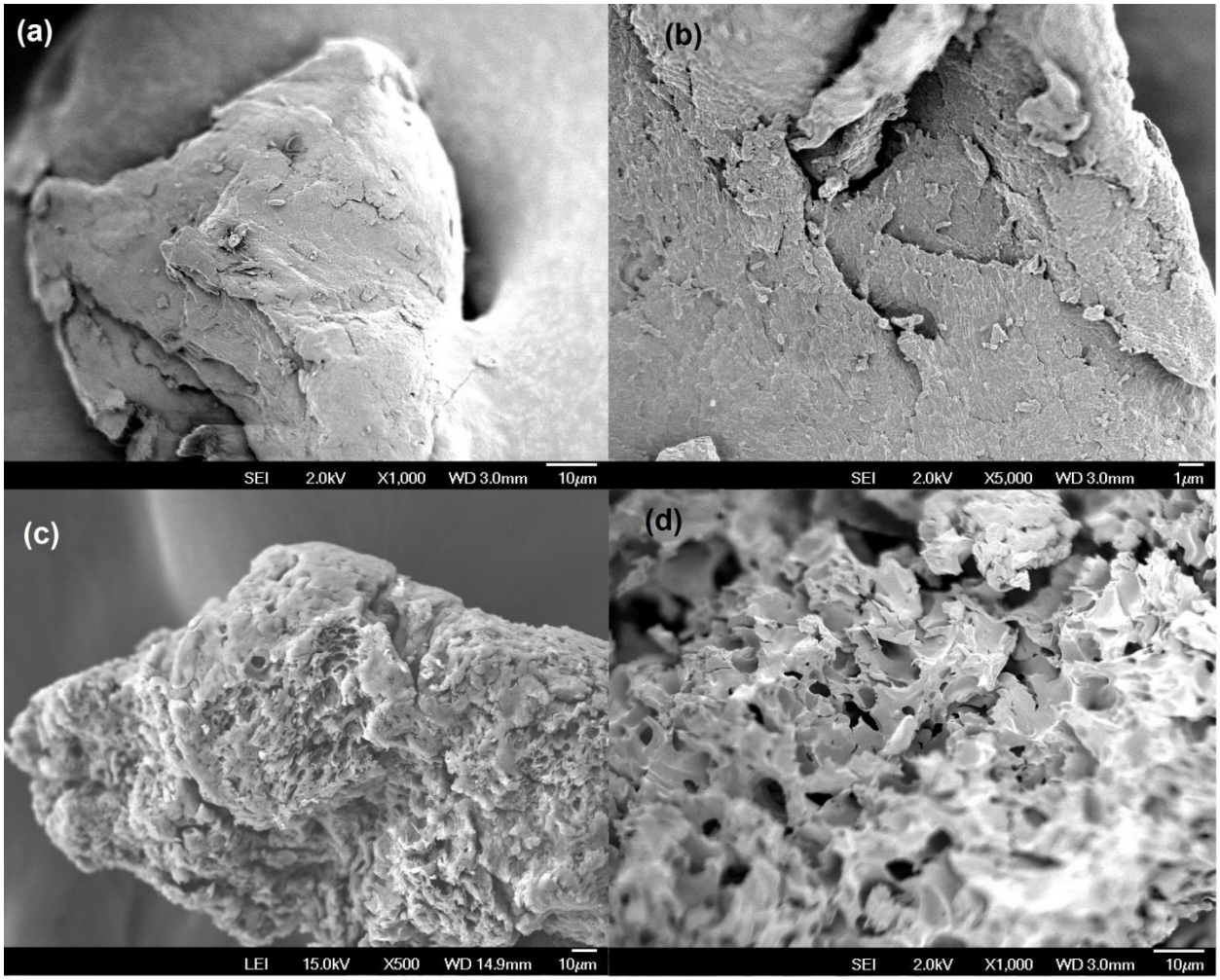


Fig. 2 FE-SEM micrographs of Agarose (a-b) and Agarose-g-PMMA (c-d).

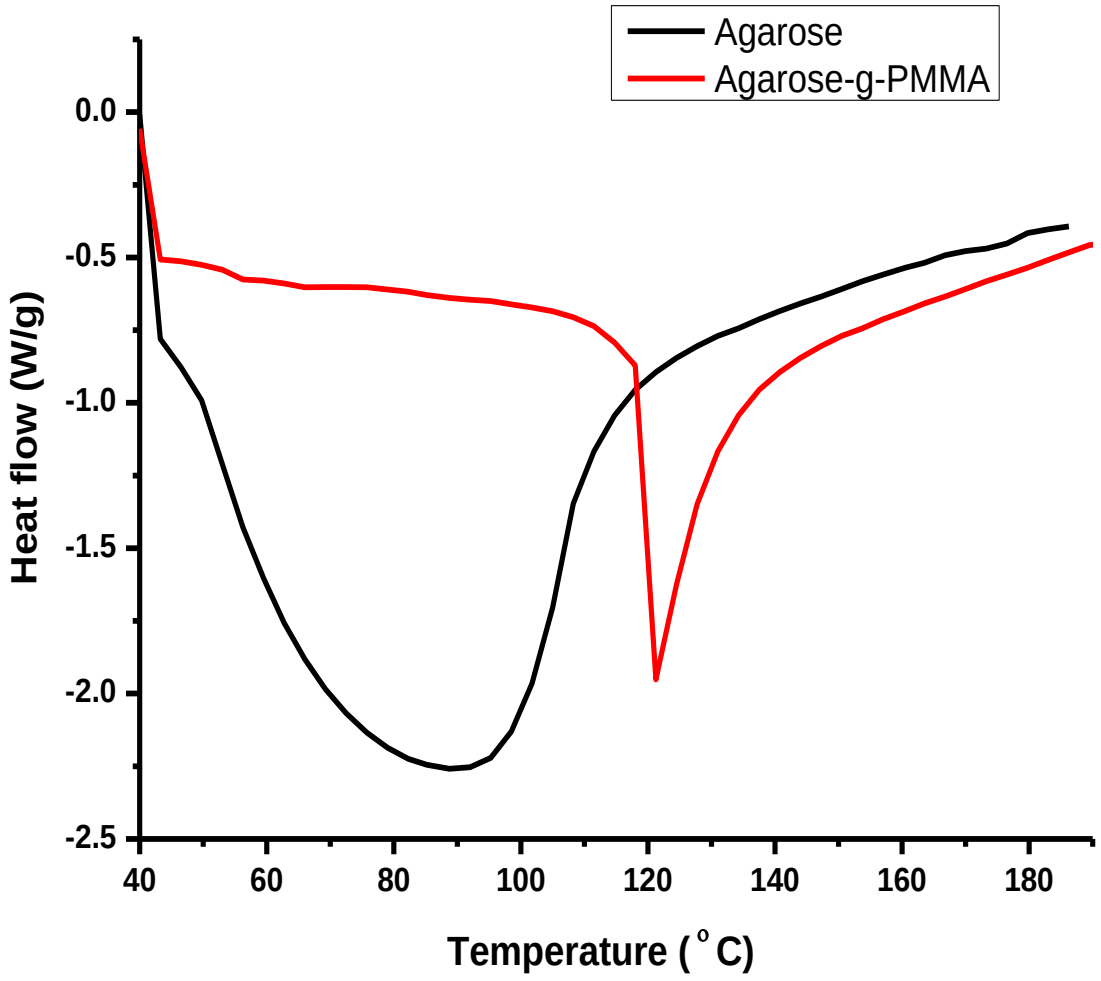


Fig. 3 DSC thermograms of Agarose and Agarose-g-PMMA.

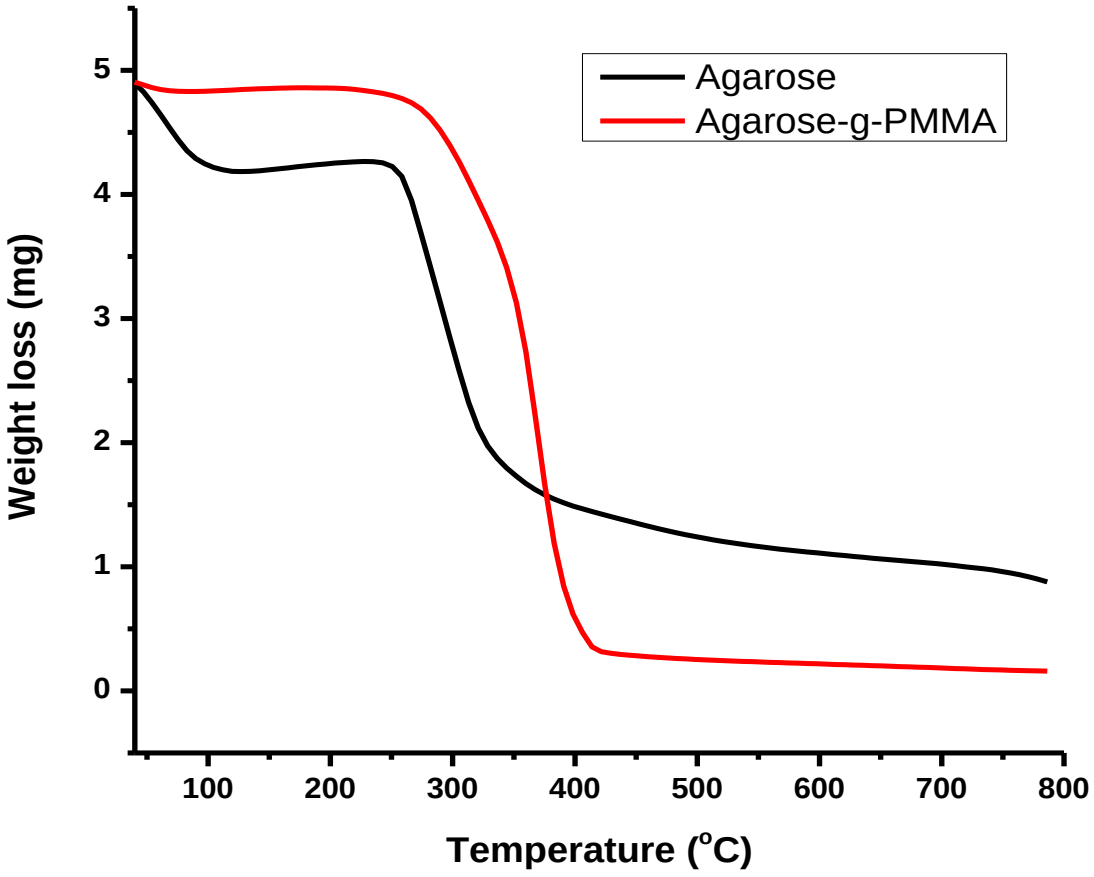


Fig. 4 TGA thermograms of Agarose and Agarose-g-PMMA.

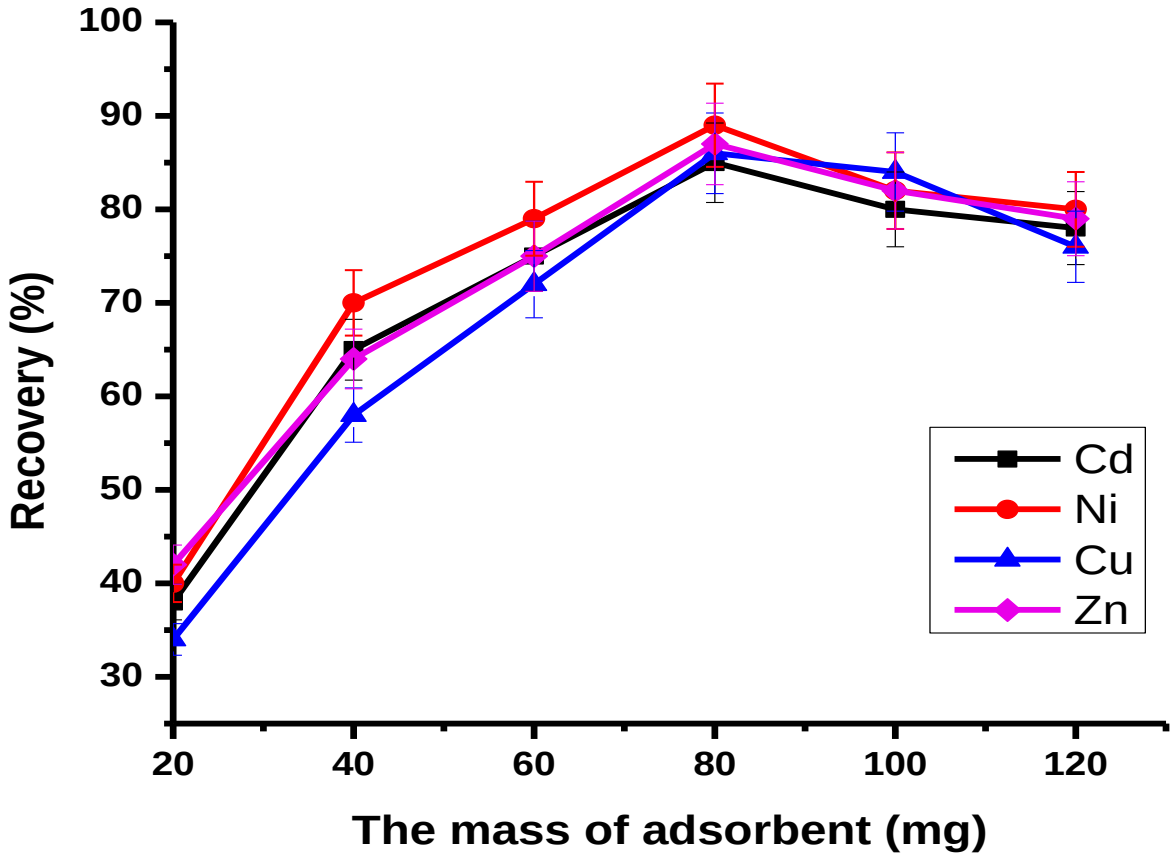


Fig. 5 Effects of adsorbent mass on the preconcentration of analytes (other conditions: sample volume, 50.0 mL; eluent, 0.5 mol L^{-1} nitric acid; pH 7; n = 5).

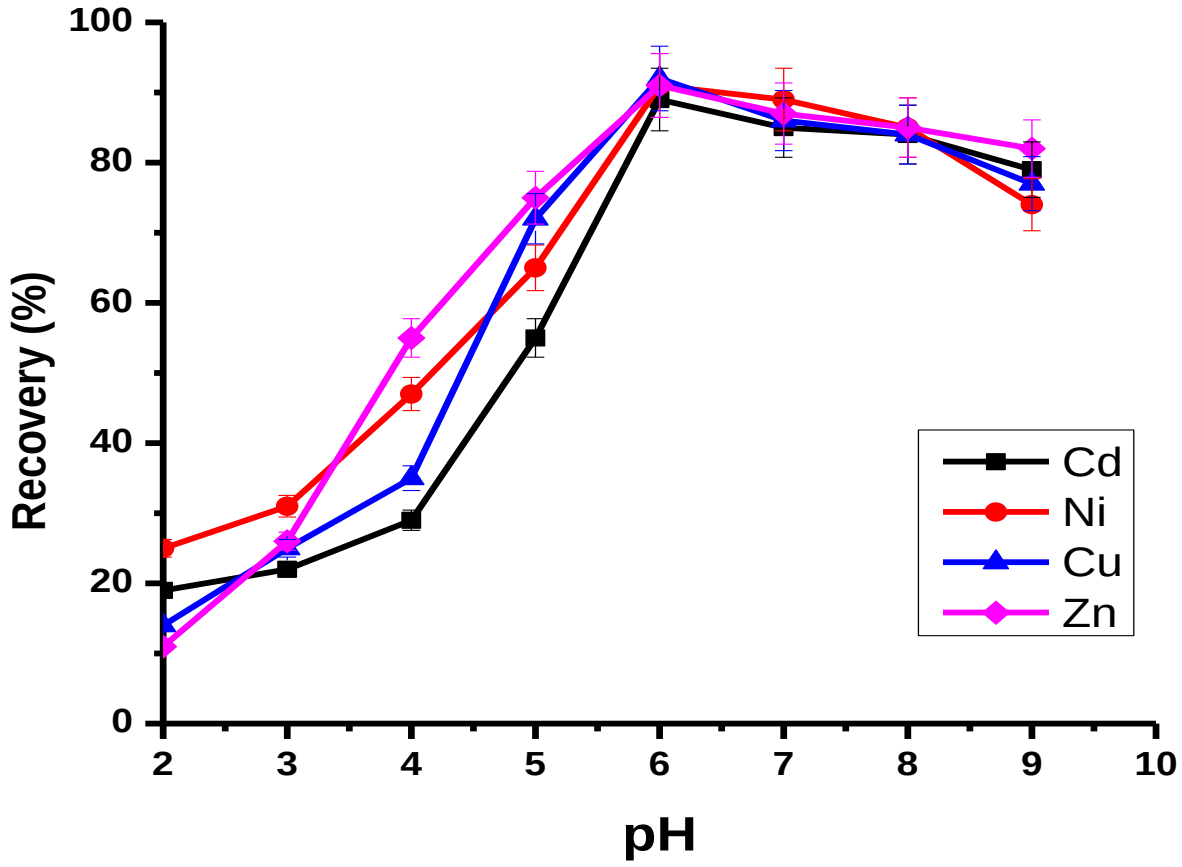


Fig. 6. Effects of pH on the recovery of analytes (conditions: sample volume, 50.0 mL; eluent, 1 mL of 0.5 mol L^{-1} nitric acid; n = 5).

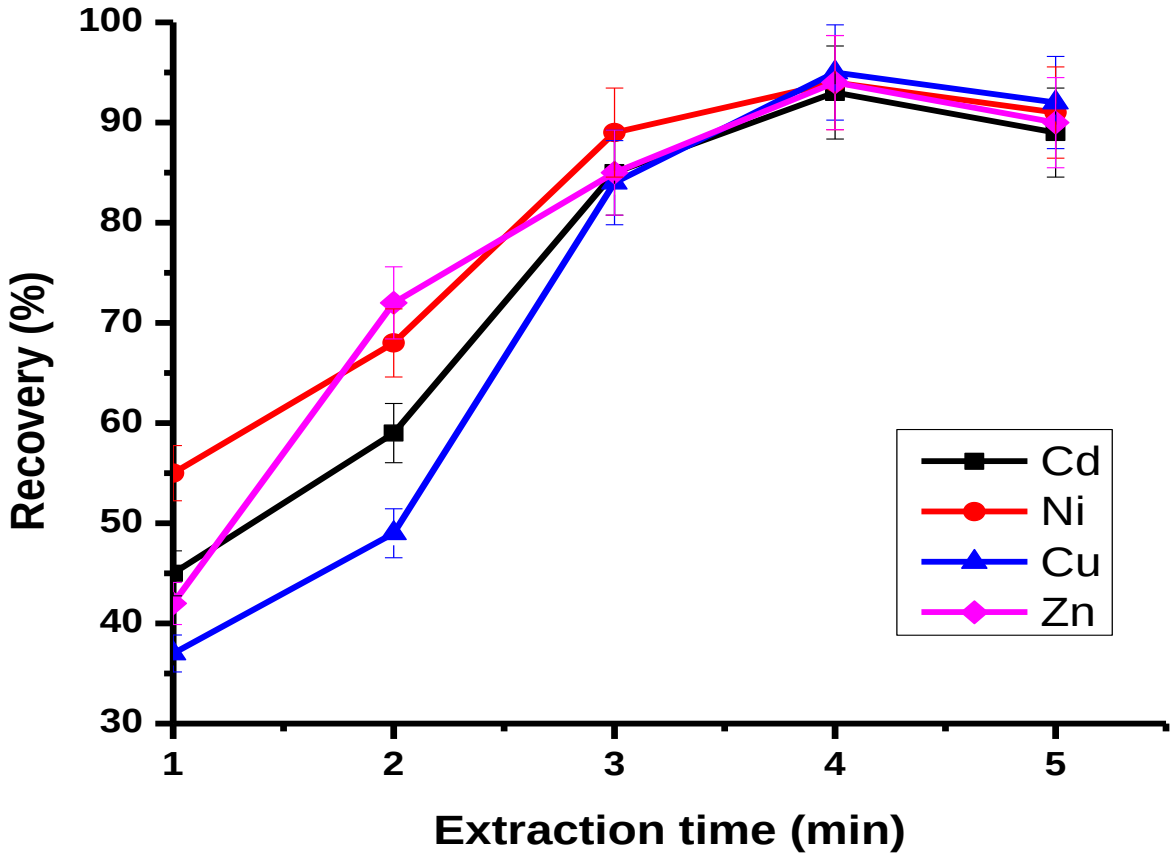


Fig. 7 Effects of extraction time on the recovery of analytes (conditions: sample volume, 50.0 mL; pH 6; eluent, 1 mL of 0.5 mol L^{-1} nitric acid; n = 5).

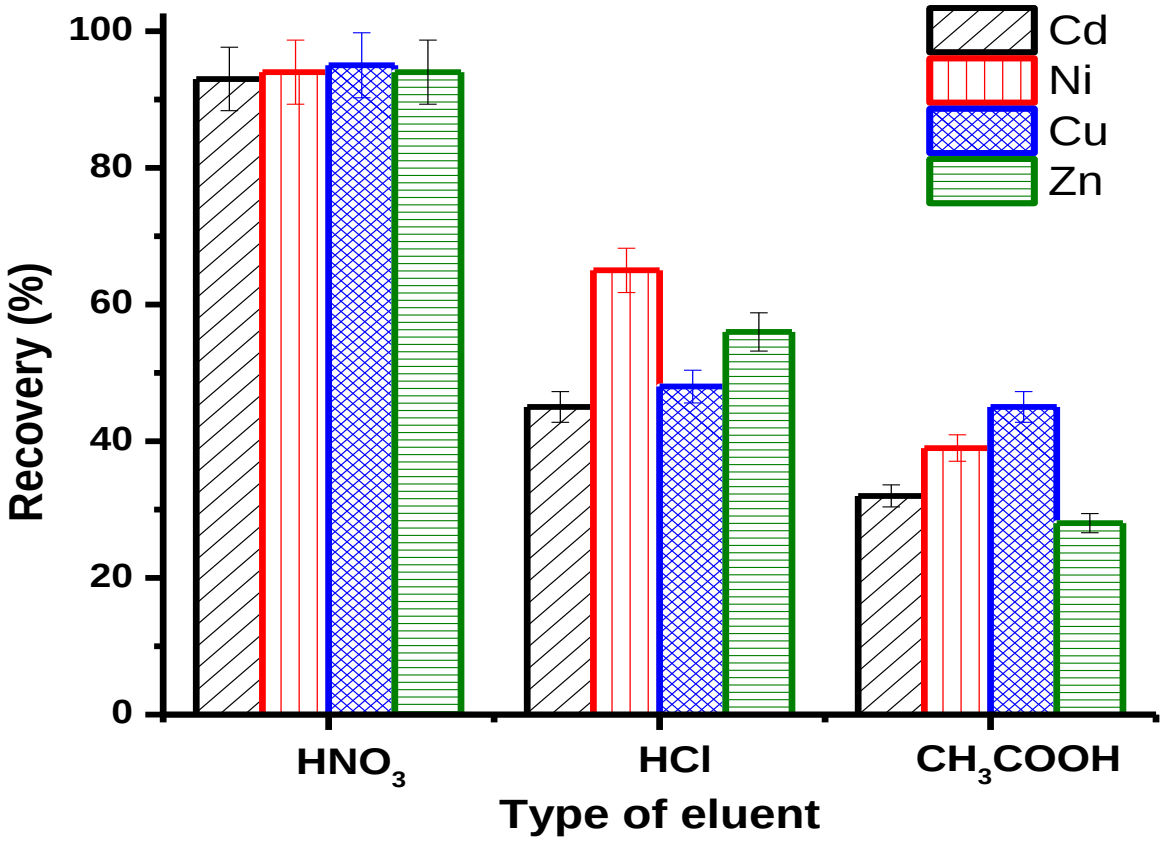
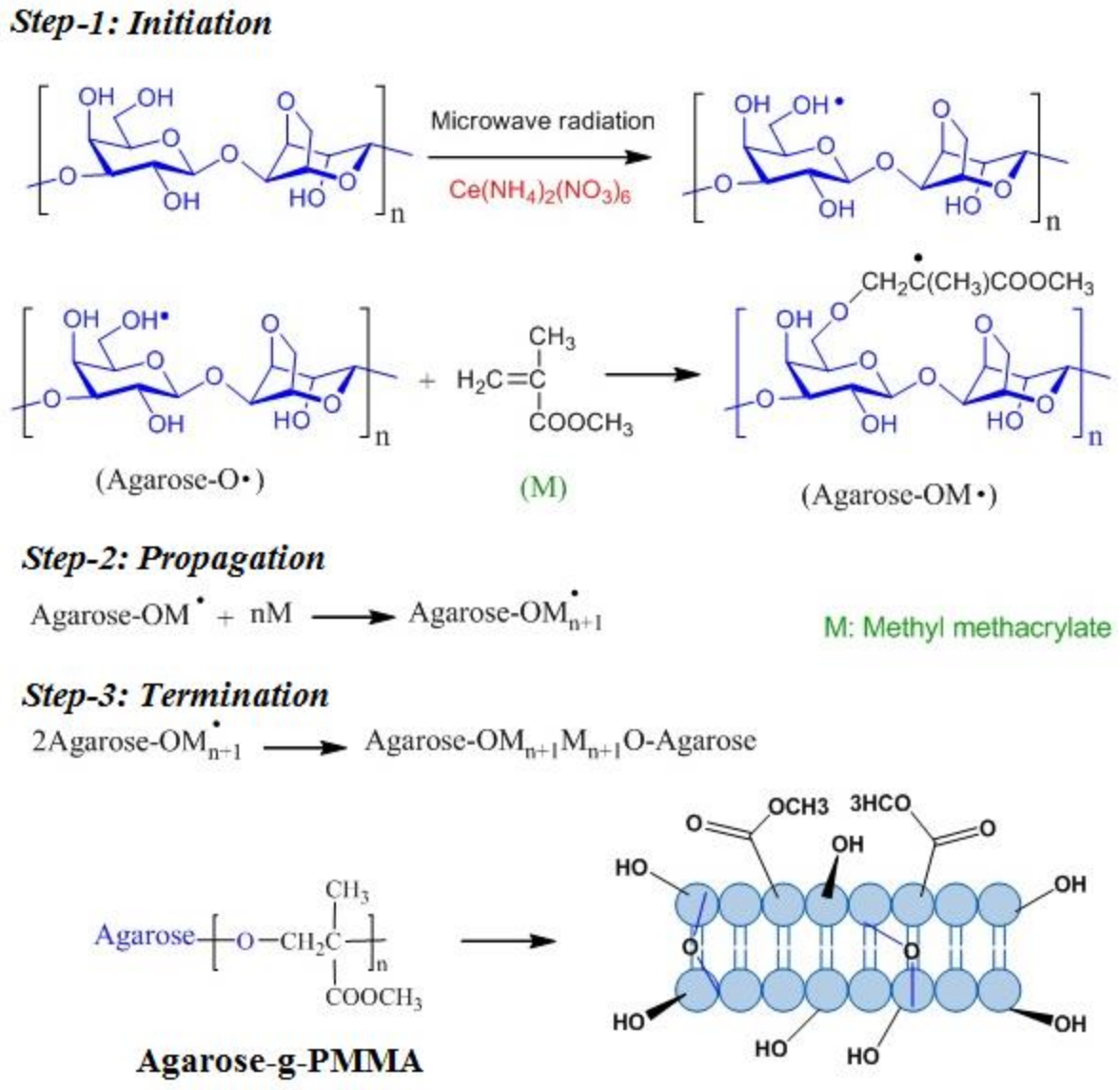
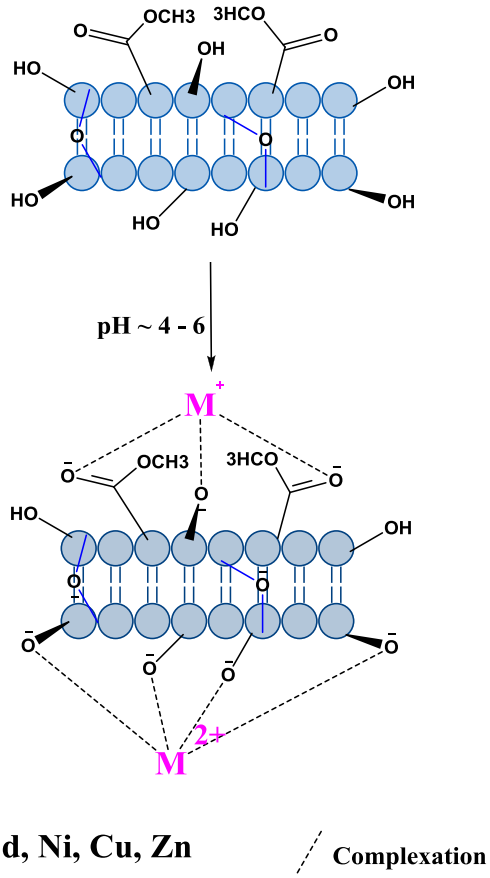


Fig. 8 Effects of type of eluent on the recovery of analytes (conditions: sample volume, 50.0 mL; pH 6; extraction time, 4 min; eluent volume, 1 mL; acid concentrations, 0.5 mol L^{-1}; n = 5).



Scheme 1 Schematic of proposed mechanism for microwave-assisted synthesis of Agarose-g-PMMA.



Scheme 2 Schematic depiction of the Agarose-g-PMMA extraction of Cd, Ni, Cu and Zn.

Table 1 Operating conditions for ICP-MS.

Parameter	Setting
Nebulizer gas flow rate (L min^{-1})	0.47
Lens voltage	7.25
Analog stage voltage	-2200
Pulse stage voltage	1275
ICP radiofrequency power (W)	1000
Discriminator threshold	70
AC rod offset	-13.50
Analytical masses	^{114}Cd, ^{58}Ni, ^{63}Cu, ^{64}Zn

Table 2 Results of effects of monomer concentration, initiator concentration, microwave irradiation time on the GE (%), G (%) and C (%) of samples polymerized at different conditions.

Weight of Agarose (g)	Weight of MMA (g)	Weight of CAN (g)	Microwave irradiation time (s)	[a]W (g)	[b] GE (%)	[c] G (%)	Conversion (%)
1.0	5.0	-	60	-	-	-	-
1.0	2.5	0.2	60	1.6	26	66	66
1.0	5.0	0.2	60	2.2	25	127	45
1.0	7.0	0.2	60	3.2	32	225	46
1.0	8.0	0.2	60	3.6	33	264	45
1.0	9.0	0.2	60	4.1	34	310	45
1.0	10.0	0.2	60	2.5	15	155	25
1.0	12.0	0.2	60	2.1	9.7	116	18
1.0	9.0	0.2	60	4.1	34	310	45
1.0	9.0	0.3	60	4.8	42	380	53
1.0	9.0	0.4	60	5.1	45	409	56
1.0	9.0	0.5	60	2.4	15	140	26
1.0	9.0	0.4	30	4.5	39	354	50
1.0	9.0	0.4	40	4.5	39	358	50
1.0	**9.0**	**0.4**	**60**	**5.1**	**45**	**409**	**56**
1.0	9.0	0.4	90	3.1	23	209	34

[a]W weight of copolymer after soxhlet extraction
[b]GE (%) grafting efficiency
[c]G (%) percentage grafting

Table 3 Effect of the concentration and time of eluent for desorption of target analytes from Agarose-g-PMMA. Conditions: concentration of sample 4 µg L^{-1}; solution volume, 50 mL; amount of adsorbent, 80 mg and pH = 6; eluent, HNO_3.

Concentration (mol L^{-1})	Elution time (min)	Recovery (%)			
		Cd^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
0.5	5	93	94	95	94
1	5	96	97	97	98
1.5	5	94	95	95	96
2	5	90	91	94	92
1	1	75	79	84	81
1	2	97	98	101	98
1	3	96	97	98	97
1	4	96	97	97	98

Table 4 Comparisons between the analytical performances of the Agarose-g-PMMA with other reported adsorbents.

Adsorbent	Detection	Element	q_{max}[a] (mg g^{-1})	PF[b]	LOD (µg L^{-1})	Ref.
Alumina hollow fiber	ICP-OES	Cu, Mn and Ni	6.6, 8.7 and 13.3	10	0.61 - 0.38	32
MWCNTs impregnated with D2EHPA-TOPO	AAS	Co, Ni and Zn	4.90, 4.78, 4.82	25	50	17
Chitosan biopolymer/8-hydroxyquinoline	FAAS	Zn	-	EF[c](17.6)	0.8	33
Fe_3O_4@SiO_2@TiO_2	ICP-MS	Cu	33.2	100	0.0023	34
Fe_3O_4@SiO_2@polyaniline-graphene oxidecomposite	ICP-MS	Trace elements	7.2 – 9.9	50	0.00004 – 0.00149	35
Agarose grafted with PMMA	ICP-MS	Cd, Ni, Cu, Zn	31.8, 42.5, 48.3 and 34.3	100	0.0006 - 0.0018	This work

a q_{max} Adsorption capacity
b Preconcentration factor (ratio of sample volume to eluent volume)
c Enrichment factor

Table 5 Analysis of Cd(II), Ni(II), Cu(II) and Zn(II) in environmental water samples. (Mean ± SD, $n = 5$).

Sample	Analytes	Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)	RR (%)
Tap water	Cd	0	–	
		0.5	0.492 ± 0.004	98.4
		2.0	2.031 ± 0.015	101
	Ni	0	–	
		0.5	0.510 ± 0.031	102
		2.0	2.062 ± 0.014	103
	Cu	0	1.536 ± 0.078	
		0.5	2.031 ± 0.019	99.0
		2.0	3.472 ± 0.013	96.8
	Zn	0	0.216 ± 0.005	
		0.5	0.713 ± 0.034	99.4
		2.0	2.161 ± 0.014	97.2
River water[a]	Cd	0	0.040 ± 0.025	
		0.5	0.552 ± 0.012	102
		2.0	2.004 ± 0.031	98.1
	Ni	0	0.283 ± 0.028	
		0.5	0.801 ± 0.009	103
		2.0	2.232 ± 0.024	97.4
	Cu	0	5.104 ± 0.007	
		0.5	5.590 ± 0.016	97.2
		2.0	7.021 ± 0.034	95.8
	Zn	0	3.103 ± 0.014	
		0.5	3.612 ± 0.019	102
		2.0	5.093 ± 0.025	99.5
Lake water[b]	Cd	0	0.524 ± 0.009	
		0.5	1.023 ± 0.006	99.8
		2.0	2.510 ± 0.018	99.3
	Ni	0	0.426 ± 0.009	
		0.5	0.912 ± 0.011	97.2
		2.0	2.436 ± 0.032	100
	Cu	0	3.471 ± 0.039	
		0.5	3.983 ± 0.021	102
		2.0	5.463 ± 0.036	99.6
	Zn	0	1.713 ± 0.041	
		0.5	2.193 ± 0.029	96.0
		2.0	3.692 ± 0.036	98.9

a Johor bahru, Malaysia
b Universiti Teknologi Malaysia (UTM), Skudai, Johor bahru, Malaysia

Table 6 Analysis of Cd(II), Ni(II), Cu(II) and Zn(II) in vegetable samples. (Concentration ± SD, $n = 5$).

Sample	Analytes	Added ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)	RR(%)
Spinach	Cd	0	0.003 ± 0.002	
		0.05	0.053 ± 0.031	101
		0.10	0.105 ± 0.004	102
	Ni	0	0.006 ± 0.016	
		0.05	0.057 ± 0.026	102
		0.10	0.104 ± 0.005	98.0
	Cu	0	0.645 ± 0.032	
		0.20	0.831 ± 0.016	96.0
		0.50	1.140 ± 0.011	99.0
	Zn	0	0.248 ± 0.018	
		0.05	0.297 ± 0.003	98.0
		0.10	0.341 ± 0.009	93.0
Cabbage	Cd	0	–	
		0.05	0.049 ± 0.034	98.0
		0.10	0.097 ± 0.017	97.9
	Ni	0	0.017 ± 0.004	
		0.05	0.068 ± 0.019	102
		0.10	0.115 ± 0.006	98.0
	Cu	0	0.409 ± 0.002	
		0.20	0.610 ± 0.027	100
		0.50	0.912 ± 0.021	100
	Zn	0	0.067 ± 0.035	
		0.05	0.114 ± 0.007	94.0
		0.10	0.159 ± 0.005	92.0
Potato	Cd	0	0.007± 0.018	
		0.05	0.055 ± 0.005	96.0
		0.10	0.101 ± 0.008	94.0
	Ni	0	0.049 ± 0.026	
		0.05	0.096 ± 0.023	94.2
		0.10	0.150 ± 0.009	101
	Cu	0	0.589 ± 0.007	
		0.20	0.791 ± 0.015	101.0
		0.50	1.052 ± 0.012	92.6
	Zn	0	0.045 ± 0.023	
		0.05	0.093 ± 0.007	96.0
		0.10	0.147 ± 0.037	102