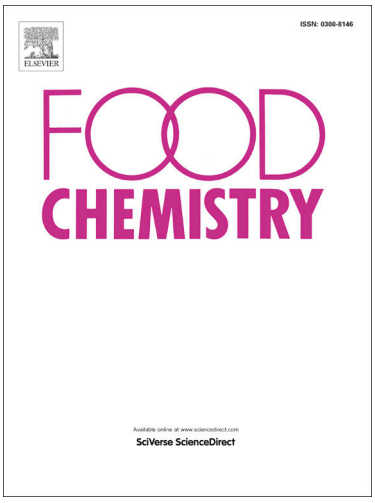

Analytical Methods

Synthesis, characterization and application of cross-linked functional terpolymer through epoxy group as sorbent for extraction of cadmium from waters and foods: Multivariate optimization

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Synthesis, characterization and application of cross-linked functional terpolymer through epoxy group as sorbent for extraction of cadmium from waters and foods: Multivariate optimization

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Abstract

The purpose of this study was to develop a selective sorbent for cadmium ions (Cd(II)) enrichment in orbital shaker assisted solid phase microextraction (OS-SPME) from different aqueous and food samples. A maleic anhydride-styrene-glycidyl methacrylate (MA-St-GMA) terpolymer was synthesized and characterized in detail. Experimental variables of sample preparation step were optimized using a central composite design (CCD). The final determination step was performed using flame atomic absorption spectroscopy (FAAS). The MA-St-GMA sorbent exhibited a high adsorption capacity (195.9 mg g^{-1}) for the Cd(II) ion. The developed method under optimal conditions provides satisfactory performance and a significant improvement compared to other protocols available in the literature. The linear range and detection limit of the method is 0.1-130 ng mL^{-1} and 0.03 ng mL^{-1}, respectively. The robustness, intraday/interday precision, selectivity, and accuracy of the method were investigated. To further validate the method, a dedicated series of analysis was performed using certified reference materials (CRMs). This part of the study confirmed the applicability of the method for routine analysis. The OS-SPME-FAAS method was validated using water and food samples. Relative standard deviations and recovery for real-world samples were in ranges 1.7–2.2 % and 95.5-98.5 %, respectively. As a result, the MA-St-GMA sorbent showed that it could quantitatively extract Cd(II) ions from aqueous solution.

Keyword: Functional materials; Adsorbents; Food analysis; Water environmental monitoring; Heavy metals; Environmental pollution

1. Introduction

Water reservoirs in the environment and foodstuff are the most important factors that reside on exposure of living baings on cadmium (Nordberg et al., 2015, Järup, 2003). Since cadmium (Cd) has high toxicity, in 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) determined a temporary tolerable monthly intake of 25 μg kg^{-1} body weight for Cd, corresponding to a weekly value of 5.8 μg kg^{-1} body weight (Joint, 2010). In addition, in 2010 the consumer product safety commission recommended the daily acceptable Cd intake value as 0.1 μg kg^{-1} body weight (Mead, 2010). The negative effects of cadmium on health and the restrictions imposed by authorized institutions require the control of the amount of Cd in water and food.

For the accurate and reliable determination of cadmium at trace levels, a selective sample preparation step is required. Since food samples have a complex matrix, the sample preparation step is very important. In recent years, , studies involving various sample preparation techniques such as dispersive liquid-liquid microextraction (DLLME) (Meira & de Souza Dias, 2017), switchable solvent-based liquid phase microextraction (Chaikhan et al., 2022), ionic liquids (ILs) microextraction (Hafez et al., 2020), solid phase microextraction (SPME) (Er et al., 2018), solidified floating organic drop microextraction (Fındıkoğlu et al., 2018), spray assisted droplet formation-liquid phase microextraction (Topal et al., 2021), cloud point extraction (CPE) (Kasa et al., 2019), solid phase extraction (SPE) (Habila et al., 2018), hollow fiber liquid-liquid microextraction (HFLLME) (Hsu et al., 2016) have been carried out by researchers. As prepared extracts are analysed by flame atomic absorption spectroscopy (FAAS) or Inductively Coupled Plasma (ICP) with atomic emission spectroscopy (ICP-OES) or Mass spectrometry (ICP-MS). Among mentioned sample prep. techniques, the most important one is SPME. In this technique, sampling, separation of the analyte, and enrichment take place in one step. In this context, it is quick and easy. Also, SPME can be combined with

gas chromatography and high-performance liquid chromatography. In the method, the consumption of organic solvents is minimized or even eliminated (Xu et al., 2016; Jalili et al., 2020). All these advantages make SPME stand out among other techniques.

The primary attributes anticipated in polymers synthesized through copolymerization encompass durability against high temperatures, adaptability, see-through quality, chemical robustness, preference for specific target substances, capability to adsorb, resilience to solvents, and so forth. (Kismet & Wagner, 2018; Uspenskii &Zelenetskii, 2019). The significance of producing novel polymeric materials through chemical modification techniques and suitable building blocks has increased notably in the field of SPME applications, particularly over the past ten years. (Zhang et al., 2020; Mejía-Carmona, & Lanças, 2020; Jalili et al., 2020).

Optimization of experimental conditions is very important for the reliability and quality of the results. Multivariate statistical techniques such as composite design (CCD) (Altunay et al., 2020), Box-Behnken (Elik et al., 2022), Doehlert matrix (Meira & de Souza Dias, 2017) are more advantageous than univariate optimization as they are faster, less costly and reduce the number of experiments as well as provide information about the interaction between variables (Elik & Altunay, 2022).

The aim of this study was to synthesize a new maleic anhydride-based sorbent for the selective and rapid extraction of Cd(II) ions and to demonstrate its feasibility. In this context, MA-St-GMA terpolymer (as sorbent) was synthesized in a cross-linked form due to the presence of GMA-derived epoxy group in its main chain. The as-obtained material was characterized in detail. Then, using this MA-St-GMA sorbent, a simple and economic orbital shaker SPME procedure was developed for the highly selective determination of Cd from water and food samples. To the best of our knowledge, this MA-St-GMA sorbent was used for the first time in the literature for this purpose.

2. Materials and methods

2.1. Apparatus

The determination step of Cd was performed using an AAS-6300 model flame atomic absorption spectroscopy instrument (Shimadzu, Kyoto, Japan). The operating parameters of the FAAS for the analysis of Cd are given in Supplementary Material Table S1. Multi Bio RS-24 model orbital shaker (BioSan, Berlin, Germany) was used to disperse the sorbent in the sample solution. A pH adjustment of the solutions during the synthesis, extraction and analysis steps was performed under the control of digital pH meter (Sartorius PB20, Göttingen, Germany). The digestion step was performed using a microwave system (Milestone Ethos D model, Sorisole-Bg, Italy). A vortex (VG3 model, IKA GmbH, Staufen, Germany) was used to accelerate the desorption step. Ultrapure water obtained from a Milli-Direct Q3 purification (Merck-Millipore, Darmstadt, Germany) was used in all studies. A Perkin Elmer 400 model Fourier transformed infrared spectrometer (Perkin Elmer, Massachusetts, USA) was used for the structural analysis studies. Thermal analysis, differential scanning calorimetry (DSC) curves were acquired under nitrogen atmosphere at a flow rate of 25 $mL \cdot min^{-1}$ using a DSC7020 model thermal analyser (Hitachi-Hightech, Chiyoda, Japan).

2.2. Chemicals and materials

All chemicals used in the synthesis and extraction experiments were of at least analytical purity and no further purification steps were applied. The stock solution (1000 mg L^{-1}) of Cd(II) ion was prepared by dissolving the appropriate amount of $Cd(NO_3)_2$ (Merck, Darmstadt, Germany) in the DI water. Calibration solutions of Cd(II) ion in extraction studies were prepared by sequential dilution of this stock solution in the water. Phosphate buffer solution (0.2 $mol \cdot L^{-1}$, pH 7.5) was prepared by mixing 42 mL of dibasic sodium phosphate (Sigma Aldrich, St. Louis, MO, USA) with 8 mL monobasic sodium phosphate (Merck) in 50 mL of water. Ethanol (EtOH, Merck), methanol (MeOH, Merck), acetonitrile (ACN, Sigma) and

tetrahydrofuran (THF, Sigma) were tested as elution solvents. Maleic anhydride (MA, Merck), Styrene (St, Merck) and Glycidyl methacrylate (GMA, Merck) were used as monomers in the synthesis of the terpolymer. Benzoyl peroxide (BPO, Merck) was used to form the first free radicals in the synthesis step. A butan-2-one (Methyl ethyl ketone /MEK, Merck) was used to dissolve MA and to create a reaction medium in the synthesis stage. A n-hexane (Sigma) was used as a washing solvent to remove monomer residues, remaining in the polymer after synthesis. Accuracy of the method was verified using the standard reference materials (SRMs) including 1643e-Trace elements in water, 1570a-Trace elements in spinach leaves and 1573a-Trace elements in tomato leaves.

2.3. Synthesis of MA-St-GMA terpolymer

The MA-St-GMA terpolymer was synthesized through the formation of charge transfer complexes. The mole ratios of MA:St:GMA during synthesis were 2:1:1. A 19.6 g of MA was placed in a reaction flask and dissolved in approximately 50 mL of butan-2-one. Then, 11.6 mL of St with a density of 0.9 $g \cdot mL^{-1}$ and 13,2 mL GMA with a density of 1.08 $g \cdot mL^{-1}$ was added. Afterwards, additional 50 mL of MEK was added to this mixture and a homogeneous solution was obtained. A 0.50 g of BPO initiator was weighed and added to this solution and dissolved at room temperature. This solution containing monomers, solvent and initiator was placed in a shaking water bath set at 80 ^{0}C and left for 1 hour. After this time, a viscous solution was obtained. The solution was allowed to cool at room temperature. An excess amount (10 mL) of ethyl alcohol was poured into the cooled solution to precipitate the terpolymer. The precipitate was then washed with n-hexane. The MA-St-GMA terpolymer was obtained as a white precipitate. It was allowed to dry at room temperature for over 24h. To observe whether the synthesized MA-St-GMA terpolymer dissolves in common solvents; water, tetrahydrofuran, acetone, benzene and toluene were used. However, it was not dissolved in any of these solvents.

2.4. Sampling and microwave digestion

The spring water was collected from the hot spring area in Sivas/Turkey. Wastewater was collected in the industrial area in Sivas/Turkey. Tap water was used from our laboratory. Well water was collected from the agricultural field in Sivas/Turkey. Food samples such as green tea, rice flour, corn, tomato, spinach, mushroom, black tea, parsley, lettuce, tuna fish were collected from local markets in Sivas/Turkey. Collected water samples were first filtered through a 0.45 um hydrophilic PTFE syringe filter (Merck-Millipore), then the OS-SPME-FAAS method was directly applied.

Digestion of food samples was carried out by the microwave digestion procedure outlined below. First, especially the vegetables were washed with water and their edible parts were separated by a knife. They were then homogenized with a laboratory blender. Then, homogenized samples (3 g) and standard reference materials (SRMs, 0.5 g) were weighed using an analytical balance and transferred to Teflon tubes including 9.0 mL of conc. HNO_3 (65%) and 3.0 mL of conc·H_2O_2 (30%). Following the placement of the Teflon tubes in the microwave system. The microwave digestion program was as follows: step-1 (2 min, 80 °C, 250 W), step-2 (5 min, 130 °C, 500 W), step-3 (3 min at 180 °C, 600 W) and step-4 (10 min at 220 °C, 600 W). The final volume of the resulting mixture was diluted to 10 mL with DI water.

2.5. Experimental design methodology

The central composite design (CCD) was used for the optimization of the OS-SPME procedure. The formula for the total number of experiments in the CCD is presented below.

$$Number\ of\ experiments = 2^K + 2K + X \quad (1)$$

Where K is the number of investigated parameters (sorbent amount, pH, eluent volume, adsorption time), and X is the number of repetitions in the center point. In this study, K and X were 4 and 6, respectively. Replicates of samples for CCD and calibration samples were made in triplicate (technical replicates), i.e. One sample was analyzed 3 times by the device and the

average of the results was used in the CCD. Also, in CCD optimization, it is desirable that the relationship between analytical results and parameters fit the following quadratic model.

$$A = \beta_0 + \beta_1\alpha_1 + \beta_2\alpha_2 + \beta_3\alpha_3 + \beta_{12}\alpha_1\alpha_2 + \beta_{13}\alpha_1\alpha_3 + \beta_{23}\alpha_2\alpha_3 + \beta_{11}\alpha_1^2 + \beta_{22}\alpha_2^2 + \beta_{33}\alpha_3^2 \quad (2)$$

Where (A) is the analytical results, (α1, α2, and α3) is the variables, (β_{11}, β_{22}, *and* β_{33}) is the quadratic coefficients, (β_1, β_2, *and* β_3) is the linear coefficients, (β_{12}, β_{13}, *and* β_{23}) is the binary interaction coefficients and (β_0) is the constant. Selected parameters (see Table S2) were sorbent amount (A), pH (B), eluent volume (C), adsorption time (D). Levels for sorbent amount (mg) were 20, 35, and 50 while star points (α=±1.2) were 5 and 65. Levels for sorbent pH were 4.5, 5.5, and 7.0 while star points were 2.5 and 8.5. Levels for eluent volume (mL) were 0.79, 1.25, and 1.7 while star points were 0.35 and 2.15. Levels for Adsorption time (min) were 8.0, 12.5, and 17.0 while star points were 3.5 and 21.5.

2.6. The OS-SPME-FAAS method

The experimental steps of the developed method for the selective and sensitive determination of Cd(II) ion are as follows. First, the pH of the conical tubes containing 10 mL of digested sample was adjusted to pH 7.5 by using 0.2 mol·L^{-1} of phosphate buffer solution. Then, 40 mg of newly prepared MA-St-GMA sorbent was added to the conical tubes. In order to ensure the adsorption of Cd(II) ion from the sample solution onto the MA-St-GMA sorbent, the conical tubes were placed in an orbital shaker and shaken at 200 rpm for 19 min. After orbital shaking, the conical tubes were placed in a centrifuge and centrifuged at 300 rpm for 2 min. At this stage, the MA-St-GMA phases containing Cd(II) ions were collected at the bottom of the conical tubes. Then, the aqueous solution was drained by decantation. Acidic EtOH (0.9 mL) was added to the MA-St-GMA sorbent remaining in the conical tubes and then vortexed for 1 min. In this step, which is expressed as the desorption step, Cd(II) ions adsorbed on the MA-St-GMA sorbent were desorbed. Finally, analyzes were carried out by injecting the eluent

liquid into the atomization section of the FAAS. All described steps were performed in triplicate with the sample blank.

2.7. Calculation of percent recovery

In the OS-SPME experiments, the percent recovery (%) of Cd(II) ion was used as a reference to select the optimum values of the investigated parameters. The formula for calculating the recovery is presented below.

$$\text{Recovery (\%)} = \frac{Amount\ of\ the\ Cd(II)\ measured\ after\ the\ OS-SPME}{Amount\ of\ the\ Cd(II) spiked\ to\ the\ model\ solution}\ x100 \quad (3)$$

3. Results and discussion

3.1. Characterization of the MA-St-GMA terpolymer

Selection of proper polymer-based material for sample preparation demands some knowledge to plan the synthesis stage, taking into account the desired functionality. In this case, the epoxy group of glycidyl methacrylate monomer is characterized by a high reactivity and multi-reactivity ability. Since epoxides have the geometry of a three-membered ring with angles of 60°, the orbitals forming the ring bonds cannot overlap maximally. For this reason, epoxy rings are tensioned. Opening of a three-membered strained ring yields a lower energy and more stable product. Thus, due to the epoxy functional groups in the glycidyl methacrylate monomer in the main chain of the synthesized MA-St-GMA terpolymer, it acts as an auto crosslinker in the co/terpolymers, providing effective and reproducible polymer cross-linking.

The chemical formula of the MA-St-GMA terpolymer is given in Figure S1. Its FTIR spectrum is also given in Figure 1. The characteristic FTIR bands of the terpolymer in Figure 1 relate to anhydride rings and three-ring epoxy groups. Accordingly, the bands at the wave number of 1860 and 1224 cm^{-1} observed on the spectrum belong to the anhydride ring in maleic anhydride (Imren Koç & Koç, 2016). The 1153 cm^{-1} band belongs to the asymmetric aliphatic C-O vibrations. The 1022 and 939 cm^{-1} bands are the vibration bands of the C-O-O-C groups that form when the epoxy ring opens during the reaction and the cross-link is formed. These

bands confirm that the epoxy ring has opened. If the ring had not opened, bands would been observed at 970 and 914 cm^{-1} (Maity et al., 2008). The absence of these bands on the spectrum indicates that the three-ring strained epoxy groups open during synthesis and form crosslinks. The bands at 1780,1715 and 1640 cm^{-1} wave numbers belong to the ester groups present in the chemical structure. The schematic representation showing the crosslink formation during the synthesis of the cross-linked MA-St-GMA terpolymer is given in Figure S2.

3.2. Differential scanning calorimetry analysis of MA-St-GMA terpolymer

The easiest way to determine the glass transition temperature (Tg) of polymers with linear or branched chain structure is the Differential Scanning Calorimetry (DSC) technique. A second-order phase transition in the DSC plot obtained with this technique gives the Tg value of the polymer. There is no such phase transition in the DSC plot of cross-linked polymers. The DSC curve of the MA-St-GMA cross-linked terpolymer was acquired in the temperature range from room temperature to 300 ^{0}C in nitrogen atmosphere and is given in Figure S3. As it can be observed on the DSC curve, there is no second-order phase transition. This is further proof that the synthesized MA-St-GMA terpolymer is cross-linked as well as insoluble in any solvent used. The dissolution information of the MA-St-GMA terpolymer and the findings obtained from the FT-IR spectrum showed that during the synthesis of the terpolymer, the epoxy ring in the GMA monomer opened and acted as an auto crosslinker (see Figure S2). Due to this cross-linked structure, no endothermic or exothermic phase transition was observed in the polymer heated up to 300 ^{0}C. The interaction of cross-linked MA-St-GMA terpolymer with Cd(II) during adsorption is schematically given in Figure 2.

3.3. Preliminary studies before CCD

Our study includes the adsorption and desorption steps. For efficient, selective and easy separation and preconcentration of Cd(II) ions from the sample solution, the amount of sorbent,

pH and adsorption time should be optimized in the adsorption step, while the eluent type, volume and desorption time should be optimized in the desorption step. All variables in the adsorption step were optimized by CCD. However, preliminary experiments were made for the selection of the eluent type in the desorption step. In this context, ACN, EtOH, MeOH, THF, pH.7.5 buffer solution, water and acidic EtOH were investigated as eluent solvent at equal volume. According to the results in Supplementary Material Figure S4, the best recovery of Cd(II) was obtained using acidic EtOH. In particular, low recovery in the pH 7.5 buffer solution used in the adsorption step has a key role for the reliability of the results. Therefore, acidic EtOH was selected as the eluent solvent in the CCD optimization step.

The reusability of the MA-St-GMA sorbent is an important factor in terms of method economic feasibility. As the number of sorbent usage increases, the efficiency of the adsorption decreases. The main reason for this behaviour, can be attributed to partial dissolution of the sorbent depending on the chemicals used in the sample solution as well as adsorption of compounds present in the real matrix. Recovery of Cd(II) ion was over 90% for 40 mg sorbent up to 22 repeated experiments. At higher uses, recovery of Cd(II) ion dropped below 70%. These results showed that the MA-St-GMA sorbent exhibited high reusability.

3.4. Optimization of OS-SPME-FAAS method by CCD

The CCD approach, defined in Supplementary Material Table S3 was applied for the optimization, while ANOVA analysis and surface response plots of the parameters were used for evaluation of the influence of each optimized facotr.

3.4.1. ANOVA analysis

Based on CCD experimental design, a 30 experiments for optimization were performed. The optimized value was the recovery of target analyte (Table S3). These values were used in ANOVA analysis. The results of this part of the studies are presented in Table 1. The CCD design is named as significant (for linear, binary and quadratic interactions) for p-value less

than 0.05 at the 95% probability level. The CCD model was significant as its p value was < 0.0001. Examining the results in Table 1, it is clear that all interactions were significant because their p-value was less than 0.05. The other evaluation is to identify the interactions that contribute most to the recovery of Cd(II) ions. In this context, it means that as the numerical value of the F-value increases, the contribution to the model also increases. Based on this explanation, the linear, binary and quadratic interactions that contribute the most to the recovery of Cd(II) ion are D (F-value:1944.54), AB (F-value:571.07) and D^2 (F-value:1918.10), respectively. The effect of uncertain errors on results is evaluated by the Lack of Fit p-value. Here, the p-value should be greater than 0.05 for uncertain errors to have a significant effect on the results. The results supported the explanation. In this context, it can be said that the uncertain error does not have a significant effect on the recovery of Cd(II) ions. Finally, the equation for the full quadratic model is given below.

$$Recovery\ (\%) = +76.48 +0.9523A +0.6939B -3.83C +4.26D +2.03AB -0.8187AC +0.8562AD +2.51BC +1.61BD -1.02CD -0.8181A^2 +3.90B^2 -5.75C^2 +8.07D^2 \quad (4)$$

The agreement between the experimental results and the estimated values of the CCD is explained by the adjusted-R^2 and the predicted-R^2. The adjusted-R^2 (0.9966) adjusts the number of variables contributing to the CCD and decreases as the number of variables in the CCD increases. This indicates a good correlation between experimental results and CCD. For CCD to be the appropriate model for the extraction of Cd(II) ions, the difference between adjusted-R^2 and predicted-R^2 (0.9912) must be less than 0.2. The CCD was suitable for the optimization of the Cd(II) ion extraction process since the difference between the two values was considerably smaller than the critical value.

3.4.2. Surface response graphs

The effect of the binary interactions of the parameters on the recovery of Cd(II) ions was evaluated by drawing surface response graphs. Figure 3a explains the effect of pH and sorbent

amount on the recovery of Cd(II). When the sorbent amount is above 40 mg, quantitative recoveries were achieved in the pH range of 7.3-8.5. In particular, in the experiments performed under pH 6, phase separation could not be achieved due to the excessive dispersion of the MA-St-GMA sorbent in the sample solution. For this reason, it was more suitable to study the extraction at basic pH values, where phase separation was easy to achieve.

The effect of sorbent amount and eluent volume on recovery of Cd(II) ions is presented in Figure 3b. The eluent ensures that the Cd(II) ions absorbed on the MA-St-GMA sorbent are quantitatively desorbed into the measurement phase. The results showed that the recovery of Cd(II) ions was quantitative while the MA-St-GMA sorbent amount and eluent volume were in the range of 40-60 mg and 0.7-1.4 mL, respectively. Recovery of Cd(II) ions was low at almost all MA-St-GMA sorbent amounts above 1.7 ml of eluent volume. This is because the concentration of Cd(II) decreases as the eluent volume in the measurement phase increases. The effect of the interaction between the adsorption time and the sorbent amount on the recovery of Cd(II) is presented in Figure 3c. To obtain reliable results, the MA-St-GMA sorbent must exhibit high interaction with Cd(II) ions in the sample solution. This is achieved by dispersing the MA-St-GMA sorbent into the sample solution. Here, an orbital shaker was used to disperse the sorbent into the sample solution. This part of the studies revealed that quantitative recoveries of almost all MA-St-GMA sorbent amounts are achieved when the adsorption time exceeds 15 min.

3.4.3. Optimisation step

The last step of the CCD is to determine the optimum values of the parameters. The aim of this study is to obtain the maximum recovery of Cd(II) ion. In this context, the recovery of Cd(II) ion was estimated as 91.1% by the CCD when the sorbent amount, pH, eluent volume, and adsorption time were used as 40 mg, 7.5, 0.9 mL and 19 min, respectively (see Supplementary Material Figure S5). Using these optimum conditions, the recovery of Cd(II) ion was calculated

as 92.4%. The results showed that there was no significant difference between the predicted recovery and the experimental recovery. Therefore, these values were used as the optimum value for the parameters in the analysis and validation experiments of real samples.

3.5. Adsorption capacity

The adsorbent capacity (Qe) is expressed as the maximum amount of analyte adsorbed by a certain amount of sorbent. To test the Qe of the MA-St-GMA sorbent, 40 mg of the MA-St-GMA was added to 120 mL of the sample solution containing 80 ng mL^{-1} of Cd(II) ions. Then the mixture was shaken for 20 min to ensure the adsorption of Cd(II) ions onto the MA-St-GMA sorbent. The MA-St-GMA sorbent was removed from the sample solution. Finally, the aqueous solution was analyzed in the FAAS. The formula for calculating the adsorbent capacity of the MA-St-GMA sorbent is presented below.

$$Q_e = (C_i - C_e) \cdot V \cdot W^{-1} \quad (5)$$

Where Qe (mg g^{-1}), Ci (mg L^{-1}), Ce (mg L^{-1}), V (mL) and W (g), were the adsorbent capacity, the initial concentration of Cd(II), and the measured concentration of Cd(II), the volume of the model solution and amount of the MA-St-GMA sorbent, respectively. The Q_e was found to be 195.9 mg g^{-1}. This result showed that the MA-St-GMA sorbent exhibited high adsorption ability for Cd(II) ion.

3.6. Performance of the OS-SPME-FAAS method

The analytical properties including calibration equation, linear range, limit of quantification (LOQ), limit of detection (LOD), and enhancement factor (EF) of the OS-SPME-FAAS method were determined under optimized conditions. The linear range of the OS-SPME-FAAS method was achieved by the analysis of sample solutions containing known amounts of Cd(II) and plotting the corresponding concentration of Cd(II) by the analytical signal. The linear range of the method for Cd(II) ion was in the range of 0.1-130 ng mL^{-1} with a coefficient of determination (R^2) of 0.997. LOD (0.03 ng mL^{-1}) and LOQ (0.1 ng mL^{-1}) were calculated using

3Sb/m and 10Sb/m, respectively. Where, Sb and m are the standard deviation of the twelve replicate analysis of the sample blank and the slope of the calibration plot, respectively. The EF (186) is calculated from the ratio of the slopes of the calibration graphs created before and after the OS-SPME-FAAS method. All data are presented in Supplementary Material Table S4.

3.6.1. Selectivity

The selectivity of the OS-SPME-FAAS method for Cd(II) ions primarily depends on the selectivity of MA-St-GMA sorbent and the optimized sample preparation conditions. In order to evaluate these effects, the matrix ions in Supplementary Material Table S5 were added to the model solution and then the OS-SPME-FAAS method was applied. Based on the analytical results obtained, the recovery and RSD of Cd(II) ion were calculated in the presence of matrix ions. Recovery of Cd(II) was in the range of 92±6 to 99±1% and the highest RSD was 2.2%. Furthermore, the highest and lowest tolerable limits were calculated as 5000 (for Na^{+}, Ca^{2+}, Mg^{2+}, SO_4^{2-} ions) and 20 (for Sn^{2+} and Cu^{2+} ions). Acceptable results showed that the OS-SPME-FAAS method exhibits high selectivity for Cd(II) ions. The reason for the high selectivity can be attributed to the high selectivity of the MA-St-GMA sorbent in respect to Cd(II) ions.

3.6.2. Robustness

The robustness was evaluated by the effect of small changes in optimized conditions on the results. In this context, the robustness of the OS-SPME-FAAS method was investigated with ±20% change in the optimized parameters such as sorbent amount, pH, eluent volume and adsorption time. The results in Supplementary Material Table S6 showed that the small changes of operation conditions did not make a significant difference in RSD. In addition, quantitative recoveries were achieved in all studies. These results confirmed that the developed OS-SPME-FAAS method under optimized conditions provide high robustness, which is very important for application in routine laboratory practice.

3.6.3 Intra/inter-day precision studies

The precision of the OS-SPME-FAAS method was investigated by intraday and interday studies on the quality control (QC) sample. In this study, mushroom was chosen as the QC since it was thought that the possible matrix effect would be the strongest. Firstly, low (1 ng mL^{-1}), medium (25 ng mL^{-1}) and high (100 ng mL^{-1}) amounts of the Cd(II) were added to the QC sample. Then, these samples for the intraday study were analyzed in five replicates in one day, while the same samples for the interday study were analyzed in five replicates on three consecutive days. The results in Supplementary Material Table S7 show that the RSDs are in the range of 1.7-2.6% for the intraday study, while the RSDs are in the range of 2.4-3.2% for the interday study. The lack of significant difference between the RSDs obtained in both studies showed that the OS-SPME-FAAS method had high precision.

3.6.4. Analysis of SRMs for accuracy assessment

The accuracy of the method was studied by analyzing three standard reference materials (SRMs) using the OS-SPME-FAAS method. Analysis results of the SRMs are presented in Supplementary Material Table S8. Experimental quantities from the analysis of SRM-1643e-Trace elements in Water (6.568±0.073 ng mL^{-1}), SRM-1570a-Trace elements in spinach leaves (2.876±0.058 mg kg^{-1}) and SRM-1573a - Trace elements in tomato leaves (1.517±0.027 mg kg^{-1}) were 6.469±0.326 ng mL^{-1}, 2.750±0.297 mg kg^{-1} and 1.462±0.076 mg kg^{-1}, respectively. It is seen that there is a high agreement between experimental values and reference values. The recovery of Cd(II) was obtained in the range of 95.6-98.5% for the SRMs. The t-exp obtained (0.68-1.62) for SRMs at a confidence level of 95% and four degrees of freedom was smaller than the t-critical (2.78). This showed that the obtained differences between measured and certified values didn't differ in the statistical manner.

3.7. Sample analysis

Following extensive validation studies, the applicability of the OS-SPME-FAAS method was investigated in respect to real water and food samples. While the OS-SPME-FAAS method was applied to water samples including spring water, wastewater, well water and tap water, a 20 ng mL^{-1} of Cd(II) solution was spiked to them. Three of each real sample (for instance, 3 mushrooms) were taken; each was prepared separately for analysis; Separate analysis (3 replicates on the analysis of each sample) was performed for each sample prepared; Results from 3 independent samples were averaged. As a result of the study (see Table 2a), the Cd(II) could not be detected in well water and tap water. In case of spring water and wastewater a 0.7 ng mL^{-1} and 3.3 ng mL^{-1}, respectively, contamination with cadmium was determined. Recovery for spiked samples was in the range of 95.5-98.5%. The OS-SPME-FAAS method was applied also to several food samples - green tea, rice flour, corn, tomato, spinach, mushrooms, black tea, parsley, lettuce and tuna fish. From the results (see Table 2b), the Cd was not detected in green tea and lettuce. The highest and lowest Cd levels were found in mushrooms (1.35±0.17 µg g^{-1}) and corn (0.18±0.01 µg g^{-1}), respectively. All results were below the WHO allowed limits for water and food samples.

3.8. Comparison with related methods

The analytical parameters of the OS-SPME-FAAS method were compared with other methods available in the literature. This analysis was performed for most important parameters, i.e. LOD (0.03 ng mL^{-1}), RSD, EF (186), linear range (0.1-130 ng mL^{-1}) and adsorption capacity (195.9 mg g^{-1}) . Comprehensive data are presented in Supplementary Material Table S9. The adsorption capacity of the method was higher than reported for other sorbents used in already published studies. Also, its RSD was lower than that reported for other protocols. The detection limit of our study was lower comparing to other protocols based on expensive techniques including differential pulse anodic stripping voltammetry (DPASV) and thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS). The liner range and PF values of our

method were comparable to all other studies. In overall, it can be concluded, that developed method provide a significant improvement to existing protocols.

4. Conclusion

This study aimed to synthesize a new sorbent for the OS-SPME procedure and to demonstrate its applicability for the extraction of Cd(II) in different water and food samples before FAAS determination. In this regard, maleic anhydride-styrene-glycidyl methacrylate (MA-St-GMA) terpolymer was synthesized and characterised in details. Important experimental variables were optimized by CCD to enable selective extraction of Cd(II) ions from the samples with high recovery. Using optimized conditions, the linear range, LOD and EF of the method were 0.1-130 ng mL^{-1}, 0.03 ng mL^{-1} and 186, respectively. In the presence of different ions, the MA-St-GMA sorbent exhibited high selectivity for Cd(II). Compared with different sorbents, the MA-St-GMA has high adsorption capacity. The OS-SPME-FAAS method is economical as the sorbent exhibits high reusability and is also environmentally friendly due to minimum reagent consumption. The method does not include a heating step. According to our knowledge, this is the first study that show that the MA-St-GMA sorbent can be applied to extract Cd(II) ions. Fully validated method revealed usefulness for analysis of real water and food samples. High robustness ensures possibility of its implementation in routine laboratory practice. Finally, we believe, that future studies should focus on MA-St-GMA sorbent applicability in the extraction of other toxic organic and inorganic analytes.

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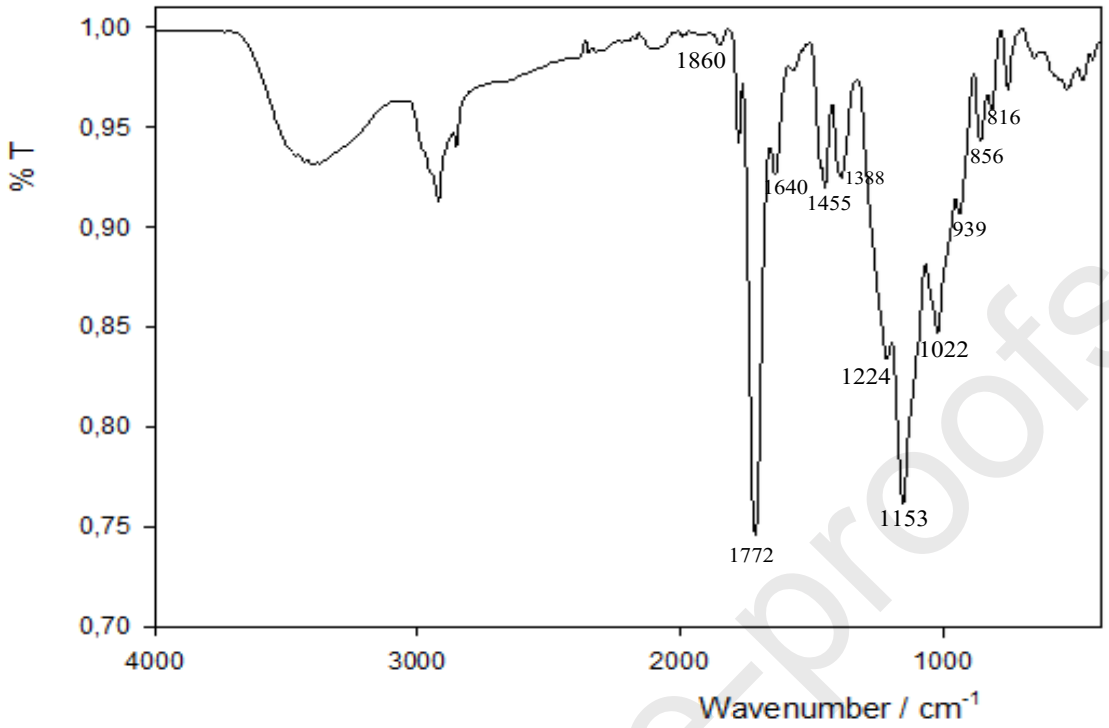


Figure 1. FTIR Spectrum of MAStGMA Terpolymer

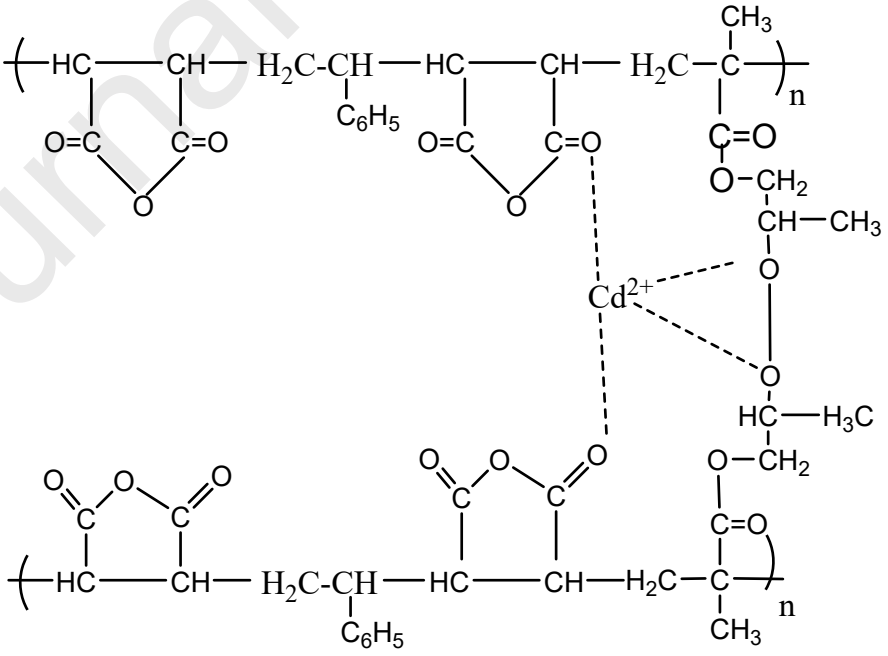
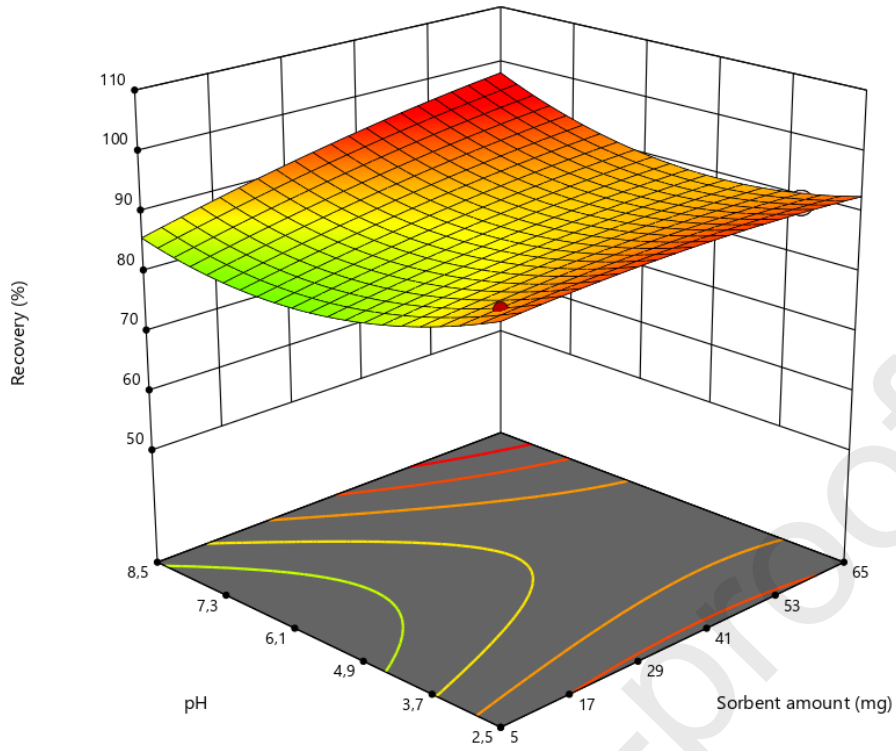
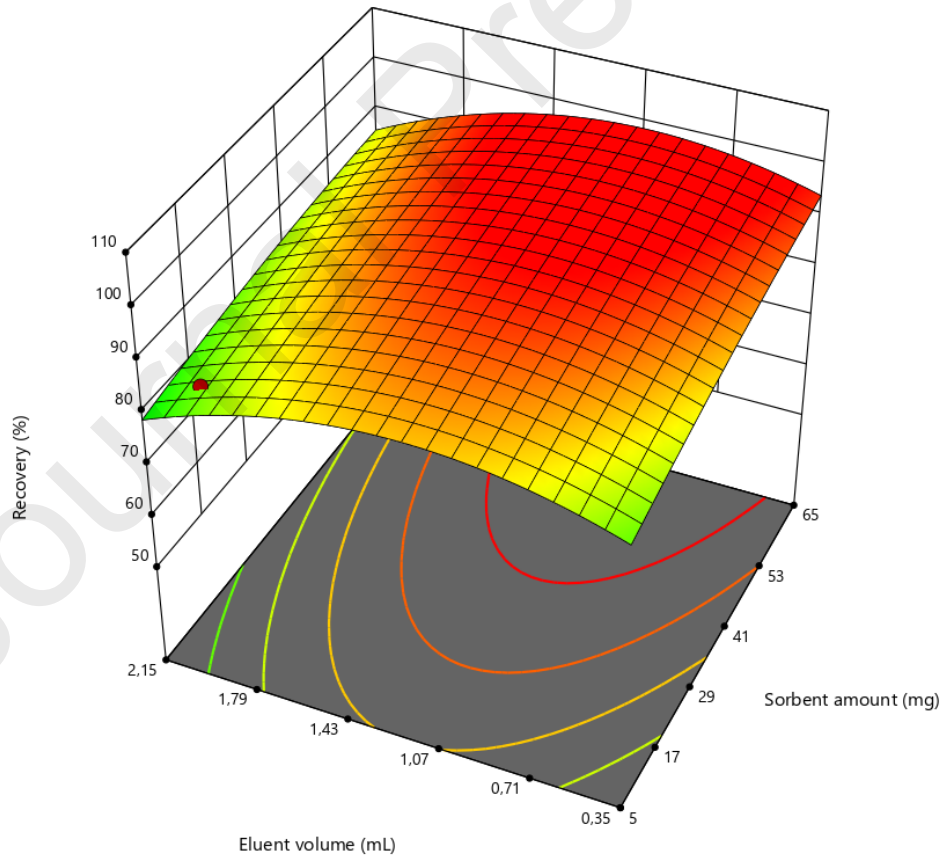


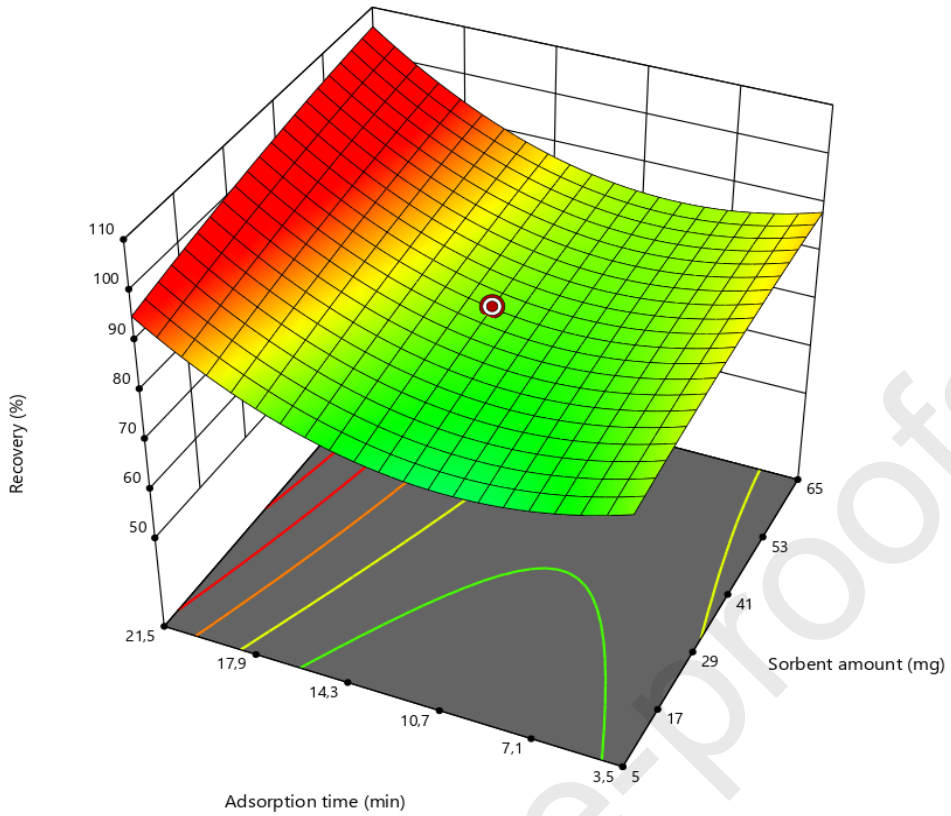
Figure 2. Interactions between cross-linked MAStGMA and Cd(II) ions



(a)



(b)



(c)

Figure 3(a-c). 3D response surface plots for the assessment of the desirability function during OS-SPME procedure when the following pair of the main variables have been optimized: (a) pH vs. sorbent amount; (b) sorbent amount vs eluent volume; (c) adsorption time vs. sorbent amount

Table 1. ANOVA analysis and quality parameters of the full quadratic model

Source	F-value	p-value	
Model	603.17	< 0.0001	significant
A-Sorbent amount	97.30	< 0.0001	
B-pH	51.65	< 0.0001	
C-Eluent volume	1574.99	< 0.0001	
D-Adsorption time	1944.54	< 0.0001	
AB	571.07	< 0.0001	
AC	60.95	< 0.0001	
AD	66.66	< 0.0001	
BC	375.12	< 0.0001	
BD	234.57	< 0.0001	
CD	94.36	< 0.0001	
A^2	19.71	0.0005	
B^2	448.84	< 0.0001	
C^2	973.11	< 0.0001	
D^2	1918.10	< 0.0001	
Lack of Fit	2.58	0.1534	not significant
Quality parameters			
Adjusted-R^2	0.9966	Predicted-R^2	0.9912

Table 2a. Test of addition/recovery for the application of OS-SPME-FAAS method (N = 3).

Water	Spiked (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	RSD (%)
Spring water	-	0.7	-	2.2
	20	20.4	98.5	1.9
Waste water	-	3.3	-	2.4
	20	22.7	97.0	2.1
Well water	-	n.d*	-	-
	20	19.1	95.5	1.7
Tap water	-	n.d	-	-
	20	19.5	97.5	2.0

n.d: not detected

Table 2b. Results for the determination of Cd in food samples by using OS-SPME-FAAS method (N=3)

Foods	Found (µg g^{-1})
Green Tea	n.d*
Rice flour	0.26±0.02
Corn	0.18±0.01
Tomato	0.23±0.02
Spinach	0.71±0.05
Mushroom	1.35±0.17
Black tea	0.58±0.04
Parsley	0.47±0.05
Lettuce	n.d
Tuna fish	0.91±0.12

n.d: not detected

Highlights

- Innovative sorbent design for targeted sample preparation
- High adsorption capacity (195.9 mg g^{-1}) for Cd(II) ion of MA-St-GMA sorbent
- Enhanced selective Cd(II) extraction efficiency from different water and food samples
- 4 orders of magnitude linear range, sub-ppb sensitivity for environmental analysis
- High recovery, very good reproducibility in complex matrix samples routine analysis

Declaration of interests

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

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Roberto Castro-Muñoz: Writing - review & editing

Nail Altunay: Investigation, Writing -original draft, Experimental design