pH-controlled charge transfer sensitive 2-aminobenzimidazole modified poly(styrene-co-maleic anhydride) copolymer for selective extraction, pre-concentration and determination of trace Hg^{2+} and CH_3Hg^+ in vinegar by combination of ultrasound assisted-cloud point extraction with UV–VIS spectrophotometry

H.B. Zengin, R. Gürkan [*]

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

A R T I C L E   I N F O

A B S T R A C T

In this study, a separation/pre-concentration procedure, ultrasound assisted-cloud point extraction with modified 2-aminobenzimidazole (2-ABzI), was developed for the extraction of trace Hg^{2+} and CH_3Hg^+ and from vinegar samples- modified copolymer coupled with Hg-binding chelator, 2-ABzI. The imidic copolymer was characterized, and used for detection of the enriched Hg species by spectrophotometer at 332 nm. The method is based on their pH-induced charge transfer sensitive complexation with copolymer matrix via 2-AmBzI moiety at pH 6.0 and 4.5, and their extraction into the mixed micellar phase of the mixed non-ionic/ionic surfactants as extractant/sensitivity enhancer. The variables influencing the extraction efficiency were optimized. From pre-concentration of 25-mL sample, with a changing calibration sensitivity and pre-concentration factor of 62.5, a good rectilinear relationship between absorbance and concentrations of Hg species in ranges of 1–15, 15–150 μg L^{-1} was obtained. The detection limits are 0.30 and 0.31 μg L^{-1}, respectively. Its precision/accuracy were in range of 3.1–7.6 % and 90.5–96 % after spiking (n: 5, for measurements of 5, 25 and 100 μg L^{-1} in same day and three consecutive days). The matrix effect was also studied (n: 3, 100 μg L^{-1}). The method is simple, cost-effective, fast, accurate, safe, highly stable and selective, which has successfully been applied to the speciation of mercury in vinegar.

1. Introduction

Vinegar is a condiment used worldwide in food preparation, which has been also employed as an antibacterial agent (Costa et al., 2009; Jafari et al., 2012; Kuda et al., 2013; Parnell and Harris, 2003; Pereira et al., 2013; Sengun and Karapinar, 2005). Toxic heavy elements such as mercury, lead, and cadmium can occur in vinegar by contamination during production or storage. Mercury (Hg) is toxic and non-degradable element, and its concentration can build up in food chains to the toxic level to human being. Vinegar may contribute to mercury intake due to its everyday life's nature. Vinegar is a product obtained from the alcoholic fermentation followed by acetic fermentation of raw materials that have a high carbohydrate content like wine, apple, and molasses (Akpınar-Bayızıt et al., 2010). Hg as a toxic heavy metal that can be harmful for human health, can occur in foodstuffs due to environmental pollution (Akpınar-Bayızıt et al., 2010). Since Hg is usually present at trace/ultra-trace levels in vinegar, its determination requires sensitive analytical techniques. Given the importance of this ingredient in human food, the development of sensitive and selective new analytical methods for quality control of vinegar is always of great importance (Camin et al., 2013; Tsai and Kao, 2012; Ubeda et al., 2012).

So far, sensitive and selective but expensive, time-consuming and complex analytical detection techniques generally needing expert user in his or her area, including stripping chronopotentiometry (Ndung'u et al., 2004; Da Silva et al., 2007; Dessuy et al., 2011; Saei-Dehkordi et al., 2012), have been used successfully for the analysis of essential (Se, Zn, Cu, Cr, Mn, Co Mo) and non-essential metals (Cd, Pb, Al, Sn, As, Sb) as well as major ions in vinegar samples (Fu et al., 2013). However, in addition to ultrasonic and microwave digestion with acid or acid mixture in the sample preparation step, these techniques require to use

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internal standard, a suitable chemical modifier and dynamic reaction cell in order to minimize signal fluctuation, improve signal stability and control the memory effect at low concentration levels in detection step. All these processes increase the cost of the method considerably. The limited number of published studies for direct determination of Hg in alcoholic drinks such as vinegar, wine and/or liquor samples without pre-concentration were developed by authors (Liu, 2010; Li et al., 2006; Gao et al., 2012; Junior et al., 2007; Dressler et al., 2012), who used the matrix-assisted photo-chemical vapor generation in acid media for sample introduction with atomic fluorescence spectrometry (AFS), inductive coupled plasma-mass spectrometry (ICP-MS), cold vapor-atomic absorption spectrometry (CV-AAS), flow injection-cold vapor generation- inductive coupled plasma-mass spectrometry (FI-CVG-ICP-MS) and gas chromatography- inductive coupled plasma-mass spectrometry (GC-ICP-MS) with derivatization step as the detection tool. Although these methods are very fast, sensitive and has low detection and quantification limits, they have generally poor precision, low recovery rates, and are not available in almost every analytical research laboratory due to be expensive and complex.

Also, the detection techniques like CV-AAS, AFS, ICP-MS after pretreatment with suitable reductants and photoreduction were successfully used for selective reduction, extraction and determination of mercury in various sample matrices such as certified dogfish liver, water, certified lake sediment and fish, fish otoliths, seawater, river water, sediments, sewage sludge, coal/coals and coal fly ash (Bendl et al., 2006; Zheng et al., 2005; Yin et al., 2007; Kenduzler et al., 2012; Abadi et al., 2012; Ribeiro et al., 2004, 2009).

In comparison with the sensitive methods mentioned above, spectrophotometric methods are among the relatively low-cost, simple, easy to use, highly sensitive and selective methods in especially presence of chromogenic reagents having pH-dependent charge transfer (CT) transition as well as good accuracy and precision, and therefore are very popular. They are preferably used in especially underdeveloped analytical research labs for the determination of low levels of Hg in different matrices. Hg is a global pollutant identified as a highly toxic element due to its accumulative and persistent character in the environment and living organisms (Mane et al., 2015). Hg can induce damage to the central nervous system, lead to renal function impairment, and result in chest pain and dyspnea (Motahar et al., 2018). One of the main routes of incorporation of Hg into the human body is vinegar. Therefore, accurate and reliable determination of trace amounts of Hg is of great importance, especially in the food and beverage matrices.

The sensitivity of spectrophotometric methods is greatly improved by use of new modified polymeric/co-polymeric chelators and cross-linked chitosan enriched with amine groups for the determination of trace/ultra-trace levels Hg species (Zengin and Gürkan, 2019; Abolhasani et al., 2015; Donia et al., 2011); for most applications, a separation and pre-concentration step is often required before detection due to its extremely low concentration in sample matrices. For this purpose, several procedures such as cloud point extraction (CPE) (Gürkan and Kir, 2013; Afkhami et al., 2006; Samaddar and Sen, 2016), ultrasound assisted-cloud point extraction (UA-CPE) (Zengin and Gürkan, 2019), ionic liquid based dispersive liquid–liquid microextraction (IL-DLLM) (Gharehbaghi et al., 2009), natural deep eutectic solvent-based ultrasound-assisted-microextraction (NADES-UA-ME) (Altunay et al., 2019), switchable solvent based liquid phase microextraction (SS-LPME) (Khan and Soylak, 2016) in combination with spectrophotometry, including ionic liquid-based single-drop microextraction (IL-SDME) combined with high-performance liquid chromatography-photodiode array detection (HPLC-DAD) (Pena-Pereira et al., 2009), have been reported for pre-concentration of trace mercury. The UA-CPE investigated in this study is a simple, fast and eco-friendly microextraction technique. The UA-CPE procedure presented by our research group (Zengin and Gürkan, 2019) in combination with spectrophotometry, is a simple, easy to use, highly selective, efficient, and fast technique for the separation, pre-concentration and speciation of traces of Hg^{2+} and CH_3Hg^+ ions in

seafood.

In the present study, newly synthesized 2-aminobenzimidazole or 2-iminobenzimidazoline (2-ABzI) modified poly(styrene-co-maleic anhydride) copolymer matrix, poly(SMAnh), as the metal chelating ligand, (see Scheme S1 for open molecular structure) was used and its application for selective extraction and pre-concentration of Hg^{2+} and CH_3Hg^+ ions from sample matrix was investigated. pH dependent heterocyclic benzimidazole compounds, characterized by amide-imidic acid or amine–iminol tautomerisation (–C(=O)-NH– and –C(OH)=N-) after modification, have been considered and studied in this study, owing to their heterocyclic structural and metal binding properties via amine N-, and benzimidazole ring N-, N- donor atoms for efficient metal chelation. Recently, it has also been reported that 2-ABzI ligand as a good chromophore and fluorophore in UV–VIS region is interestingly applicable for the corrosion inhibition and its mechanism of copper ions (Cu^+ and Cu^{2+}) in presence of Cl^- ions by cyclic voltammetry (CV), chronopotentiometry, electrochemical impedance spectroscopy (EIS), and potentiodynamic curve (PD) techniques (Xhanari and Finšgar, 2017; Finšgar et al., 2018), pH independent inclusion complexation of 2-ABzI with β-cyclodextrin (Rajamohana et al., 2019), and chelation and simultaneous determination of heavy metals, Pb^{2+}, Cd^{2+} and Hg^{2+} on the carbon paste electrode (CPE) modified with 2-benzimidazole thiol by square wave voltammetry (SWV), CV and EIS (Charaf et al., 2018).

In this study, UA-CPE, based 2-ABzI modified poly(SMAnh), was used, as a newly synthesized and modified imidic chelating agent, poly (SMIm) via ring closure after thermal pretreatment at 150 °C, for separation and pre-concentration of Hg^{2+} and CH_3Hg^+ ions in vinegar samples. Various analytical parameters, including pH, buffer volume, chelating copolymer matrix amount, ionic and nonionic surfactant amounts as extractant, temperature and time of sonication, centrifuge rate and time were evaluated and optimized in detail. The method has been extended to include the selective extraction of Hg^{2+} and CH_3Hg^+ ions from vinegar samples in the presence of matrix components.

2. Experimental

2.1. Standards, reagents and apparatus

In the present study, all the chemicals used were at least of analytical purity; they were purchased from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany). At initial, all the containers were kept in 10 % (v/v) HNO_3 for a day and subsequently washed five times with ultrapure water. Its stock solution, 0.1 % (w/v), were prepared by dissolving 0.1 g of the2-ABzI modified imidic copolymer in acetone–$CHCl_3$ mixture (1:3, v/v), due to precipitation in alcohol. The 25 mg L^{-1} working solutions of Hg^{2+} and CH_3Hg^+ were prepared by serial dilution of the stock solutions of 1000 mg L^{-1} with water. A solution of 5.0 % (v/v) non-ionic surfactant, Triton X-114, in water was used as the extractant. The diluting agents (methanol, ethanol, acetonitrile, acetone, acetone-$CHCl_3$ (1:3, v/v), methanol and ethanol acidified with 1.0 mol L^{-1} HNO_3) were used as either pure or a mixture in extraction process. The 3.0 mmol L^{-1} of SDS was prepared in water and used together with Triton X-114 as sensitivity enhancer in the extraction process. The KCl solution of 2.0 mmol L^{-1} for salting-out effect was prepared by dissolving a suitable mass of solid KCl salt in water. The Britton-Robinson buffer solutions (in range of pH 2.5–12.0), providing an improvement in selectivity, were prepared by mixing 1500 μL acid mixture, 110 μL 1.0 mol L^{-1} NaOH and 390 μL ultrapure water for pH 4.5, and 1500 μL acid mixture, 190 μL 1.0 mol L^{-1} NaOH and 310 μL ultrapure water for pH 6.0, so as to obtain a buffer of 1250 and 1500 μL for Hg^{2+} and CH_3Hg^+ in optimization step. The concentration of each buffering component (citric acid, phosphate, barbital, and boric acid, including glycine) was 20 mmol L^{-1}. In this study, the glycine was adopted as a weak acid additive due to selectively bind Hg^{2+} and CH_3Hg^+ ions in medium. The certified samples: SRM 1515 Apple leaves and SRM 1641e Mercury in water, both supplied from NIST (Gaithersburg, Canada), were used for validation of the method.

Also, the 0.02 % (w/v) $K_2Cr_2O_7$ in 5.0 % (v/v) HNO_3, acid mixture of conc. HNO_3-H_2SO_4-$HClO_4$ (2:1:1, v/v), dilute acid mixtures of $HClO_4$-HCl-CH_3COOH (each one, 2.0 mol L^{-1}; 1:2:2, v/v), 4.0 mol L^{-1} HCl and 0.75 % (w/v) thiourea mixture (2:1, v/v) for analysis of total Hg, free Hg^{2+} and CH_3Hg^+ were used in the extraction of the SRMs and vinegar samples under sonication conditions at 35 °C.

2.2. Instrumentation

A UV–visible spectrophotometer PC (UV-1800 Shimadzu model, Kyoto, Japan) was used for measurements of Hg in the samples against analyte blank at 332 nm by placing a 0.4 mL of the diluted micellar phase with acetone-$CHCl_3$ (1:3, v/v) into a quartz cell of micro-capacity (1-cm light path). FT-IR spectra were taken using a Bruker (Tensor II model, Billerica, MA, USA) spectrometer (with direct sampling at ATR mode without KBr pellet). ^{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. An ultrasonic bath (UCS-10 model, Seoul, Korea) (40 kHz and 300 W) was used in the selection extraction of Hg from the sample matrix by UA-CPE. A vortex (VM96-B model, Seoul, Korea; 50 Hz, 12 W) was used in the homogenization of the samples. A centrifuge (Universal 320 Hettich model, London, England) was used for phase separation. The pH measurements were made using a pH meter (Selecta 2001 Sartorius docu-model, North America). A Labconco ultrapure water system (Kansas City, USA) was used to obtain ultrapure water with a resistivity of 18.2 MΩ cm^{-1}.

2.3. Synthesis of 2-ABzI-modified copolymer

In this study, bulk copolymer of styrene and maleic anhydride monomers (at molar ratio of 1:1) with azobisisobutyronitrile (AIBN) initiator in benzene was synthesized at 70–80 °C. The resulting copolymer was dried in the open air. After dissolving the dried copolymer in THF, it was modified with 2-ABzI at 55 °C with continuous stirring. The copolymer formed by the modification was precipitated with ethyl alcohol, and after a certain period of time in n-hexane, it was filtered and dried. After dissolving a certain amount (50 %) of the obtained 2-ABzI modified poly(SMAm) in DMF, imidation process was performed at 150 °C for five hours with continuous stirring. After precipitation with ethanol, the obtained 2-ABzI modified styrene-maleimide copolymer ((2-ABzI/poly(SMIm)) was left to dry after waiting in n-hexane. Thus, the modified copolymers obtained were submitted to ATR-FTIR, ^{1}H NMR, TGA/DTG and DTA for characterization of their structural and thermal properties.

2.4. Sampling, sample preparation

As Hg contents of the samples, including SRMs, is very low, even minor contamination at any stage of the sampling, sample storage, handling and analysis has the potential to affect the accuracy of the results. The method accuracy was controlled by analysis of the two SRMs, SRM 1641e Mercury in water and SRM 1515 Apple leaves, supplied from NIST, Gaithersburg, Canada. The certified values are available for total Hg for the assessment of the method's accuracy. The solid SRM was used as stored, without further grinding and sieving. The vinegar samples, including lemon juice with garlic, were randomly supplied from the local markets in Sivas, Turkey. The vinegars were mostly in glass bottles, but some were in plastic bottles. According to the procedures of some author groups with slight modification (Liu, 2010; Junior et al., 2015; Silva et al., 2012), all the samples were ultrasonically extracted as follows:

Aliquots of (10 mL and 1.0 g) of certified liquid/ solid samples were independently transferred to the centrifuge tubes of 50-mL, to which 5 mL water was added to facilitate homogenization of sample by vortexing (2 min at 1200 rpm). After that, for total Hg analysis, 10-mL of liquid

sample was pretreated and oxidized with 5.0 mL of 0.02 % (w/v) $K_2Cr_2O_7$ in 5.0 % (v/v) HNO_3 while 1.0 g of solid sample was pretreated with 10.0 mL of acid mixture of conc. HNO_3-H_2SO_4-$HClO_4$ (2:1:1, v/v) by heating it over 150 °C in a fume hood over a hot-plate in a controlled manner until white sulfuric acid fumes emerge, and followed by addition of dilute $K_2Cr_2O_7$ until each solution turned yellow. For analysis of free Hg^{2+} and CH_3Hg^+, 10.0-mL and 1.0-g aliquots of original liquid and solid samples were independently pretreated with dilute acid mixture of HNO_3 (or $HClO_4$, for certified solid plant sample)-HCl-CH_3COOH (each one, 2.0 mol L^{-1}; 1:2:2, v/v) for 20 min at 35 °C in ultrasonic bath (40 kHz, 300 W) for destruction of the sample matrix and extraction of the Hg.

Before analysis by the matrix-matched calibration curve with five calibration point in range of 1–15 µg L^{-1} (each ion), in order to facilitate the mass transfer between two phase and to ensure the repeatability/ stability of the results against possible matrix effect, and to minimize analyte loss via volatilization and interconversion of mercurial species by methylation or demethylation, vinegar samples were submitted to two different ultrasonic soft sample preparation procedures for CH_3Hg^+ and Hg^{2+} ions at pH 4.5 and 6.0, respectively, as follows:

(i) 5.0 mL of vinegar sample was pretreated and extracted with 3.0 mL of 4.0 mol L^{-1} HCl and 0.75 % (w/v) thiourea mixture (2:1, v/v) for 15 min at 60 °C in ultrasonic bath (40 kHz, 300 W). Inhere, function of each reagent is to form stable complexes in acidic medium as a stabilizer against interconversion with great stability difference, allowing leaching from the sample matrix to the aqueous solution.

(ii) 5.0 mL of vinegar sample was pretreated and extracted using 10 mL of dilute acid mixture of $HClO_4$-HCl-CH_3COOH (each one, 2.0 mol L^{-1}; 1:2:2, v/v) for 20 min at 35 °C in ultrasonic bath (40 kHz, 300 W). Inhere, function of each reagent is to destruct the organic matrix, form stable complexes in acidic medium against interconversion as a stabilizer, respectively, allowing leaching from the sample matrix to the aqueous solution.

Thus, the pretreated- and extracted vinegar samples were diluted at ratios of 1:5 with 2.0 % (v/v) HNO_3 and HCl solutions at equal volume to overcome the possible matrix effect. In a similar way, for total Hg analysis, 5.0 mL of sample extracts were mixed and subjected to oxidation with 5.0 mL of mixture of 0.02 % (w/v) $K_2Cr_2O_7$ in 5.0 % (v/v) HNO_3.

To check the matrix effect in terms of reliability of the method, the matrix-matched calibration curves with five calibration point (1.0, 2.5, 5.0, 10.0 and 15.0 µg L^{-1}) according to AOAC recommendations for validation of the method performance criteria (AOAC Official, 2016) was adopted in the range of 1–15 µg L^{-1} by spiking with known amounts of Hg^{2+} and CH_3Hg^+ around the quantification limit of the method. For all the pre-treatment processes, a blank control was carried out in the same way. Then, an aliquot (5.0 mL) of the final clear extract or pre-treated sample solutions with and without spiking after dilution at 10- and 5-folds, respectively, was submitted to pre-concentration by UA-CPE prior to analysis by spectrophotometry at 332 nm. The Hg^{2+}, CH_3Hg^+ and total Hg contents of the samples were determined using the matrix-matched calibration curves.

2.5. UA-CPE procedure

Aliquots of 5.0 mL of the pre-treated sample solutions or a standard solution containing Hg^{2+}/ CH_3Hg^+ (50 µg L^{-1} in optimization step) in the range of 5–15 and 15–150 µg L^{-1}, 1.0/1.2 mmol L^{-1} of the improved B-R buffer solution (pH 4.5 and 6.0), $3.5 \times 10^{-4}/3.0 \times 10^{-4}$ % (w/v) of 2-ABzI-modified imidic copolymer solution, 45/30 µmol L^{-1} of SDS, 6.0 µmol L^{-1} KCl, and 0.075/0.025 % (v/v) of Triton X-114 were mixed in a centrifuge tube of 50-mL and diluted to mark with water, and sonicated for phase separation in an ultrasonic bath (40 kHz, 300 W) for 5/7 min at
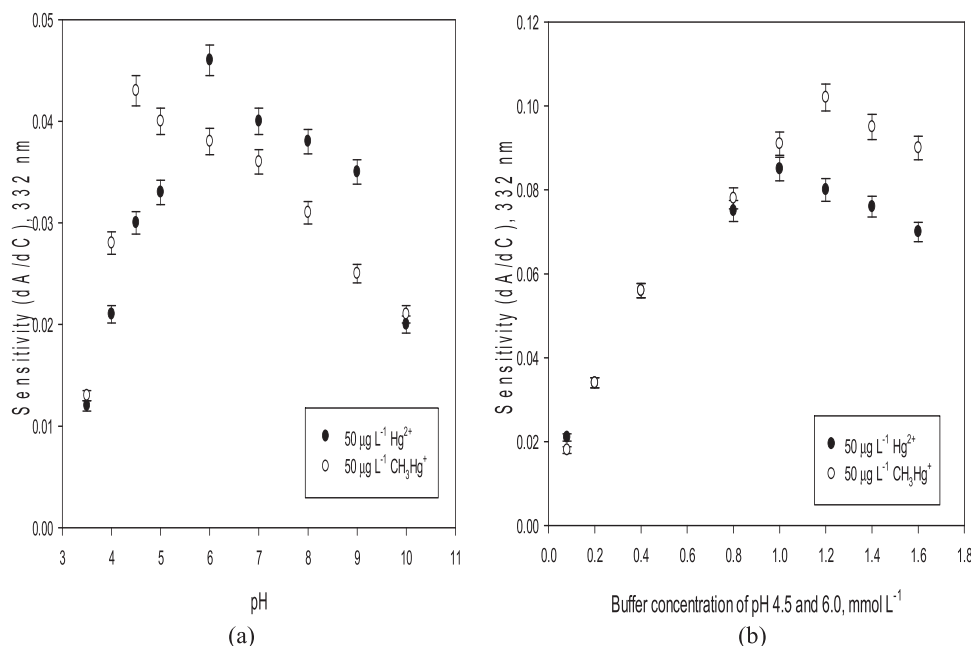
Fig. 1. (a, b) Effect of (a) pH and (b) B-R buffer concentration on the sensitivity of Hg^{2+} and CH_3Hg^+ ions (each, 50 μg L^{-1}) at pH 4.5 and 6.0.

40 °C. The phase separation was accelerated by centrifuging at 3000 rpm for 5 min. Without cooling, the surfactant-rich phases became viscous. Then, the aqueous phase was separated by using a syringe. Subsequently, the surfactant-rich phase was diluted to a volume of 0.4 mL with acetone-CHCl$_3$ (1:3, v/v) in order to decrease its viscosity and make the final volume feasible to transfer into the optical cell of 1.0 cm for the absorbance measurement of the Hg-complexes spectrophotometrically at 332 nm against an analyte blank prepared under similar conditions. The Hg levels of the samples were determined by using the matrix-matched calibration curves to suppress the matrix effect. To determine the signal contributions resulting from the used reagents, a blank control solution subjected to the same procedure was measured in parallel with the samples or standard calibration solutions.

2.6. Statistical analysis

For optimization studies, the standard deviations of the absorbance measurements were calculated (n: 3) and represented as error bars. According to AOAC Official, (2016), the repeatability were expressed as the RSDs for five replicate measurements at three different concentration levels. The average plus standard deviation of the analyte concentrations (n: 3) were calculated for each sample. The results were statistically evaluated by employing the Student's t test, and the calculated t-values were compared with the tabulated value for two degrees of freedom at the 95 % confidence level.

3. Results and discussion

The preliminary experiments showed that the extractable stable chelate complex formed from the pH-dependent complex formation reaction among reagents and CH_3Hg^+ or Hg^{2+} is extracted into ionic and non-ionic surfactant mixture, SDS-Triton X-114. As a result of this observation, an UA-CPE was developed for the simultaneous determination of each ion. The absorption spectra of the surfactant-rich phase diluted with acetone-CHCl$_3$ (1:3, v/v) showed a maximum band at 332 nm without a blue or red shift in absorption wavelength except for pH difference in terms of selectivity of extraction, which was selected for absorbance measurements. The UV spectra for increasing CH_3Hg^+ and Hg^{2+} concentration in range of 1–15 μg L^{-1} at pH 4.5 and 6.0 respectively as a function of measurement wavelength under optimized

reagent conditions were given in Fig. S1(a, b). The effect of the different variables was independently optimized in order to achieve the maximum sensitivity for their fixed concentrations of 50 μg L^{-1}.

3.1. Characterization of the amide and/or imide copolymers by instrumental tools

From the IR spectra in Fig. S2(a), it has been observed that SMAnh copolymer has the expected anhydride units at 1855 and 1804 cm^{-1}, which belong to symmetric and asymmetric C=O stretching vibrations of maleic anhydride (MAnh), respectively. The C–O–C stretching vibrations, resulting from the MAnh ring, were observed at 1025 and 935 cm^{-1}. Instead of the MAnh units, new peaks appeared in the range of 1778–1727 cm^{-1}, which probably corresponds to the maleamidic acid and maleimide groups. The results indicate that the MAnh rings in the SMAnh backbone is greatly opened by the reaction with 2-ABzI selected as modifier, and a new copolymeric matrix is formed.

Also, ^{1}H NMR spectra (in DMSO-d6, single pulse at 400 MHz) in Fig. S2(b) confirmed and supported the FT-IR results. In the SMAnh copolymer, chemical shifts, resulted from two methane –CH– protons on the furan unit at 6.3 ppm and aromatic hydrogen of the benzene ring of styrene at 6.7–7.4 ppm, were observed. The chemical shifts between 7.2 and 7.4 ppm belong to the aromatic ring hydrogen of styrene, indicating a proton signal of the styrene residue. These aromatic hydrogen peaks indicate that SMAnh copolymer is partially modified with 2-ABzI. The characteristic peaks observed in the ^{1}H NMR spectra of the SMAnh are as follows: (1) a chemical shift of two protons on the MA groups at 3–4 ppm (overlapped with the water signal) and (2) distinct spectral features of the SMAnh copolymer with two methane –CH– protons on the furan unit at 3.3 ppm, methylene –CH$_2$– protons at 2 ppm close to that of DMSO, D6 solvent, and a –CH peak at 1 ppm. After modification with 2-ABzI, the characteristic peaks in range of 2–4 ppm and a sharp peak at 6.2–6.3 ppm were either disappeared or shifted to down fields and up fields. By amidation and imidation processes, a gradual decrease in peak intensities in up field (at 7.5–7.6 ppm) has been observed while a sharp increase in peak intensities in down field (in range of 1.2–2.2 ppm) is observed, confirming that 2-ABzI is bound to the SMAnh by amidation and imidation reactions. Considering weak and wide bands observed around 7.0 and 6.2 ppm unlike that of poly(SMAnh), it is also a proof of modification with 2-ABzI.
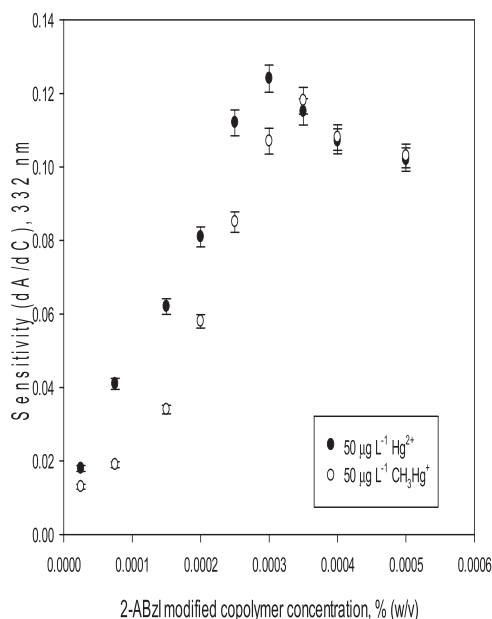
Fig. 2. Effect of 2-ABzI modified imidic copolymer concentration in acetone-CHCl$_3$ (1:3, v/v) on the sensitivity of Hg^{2+} and CH$_3$Hg$^+$ ions (each, 50 μg L^{-1}) at pH 4.5 and 6.0.

Thermal properties of poly(SMAnh), and their amidic and imidic copolymers modified with 2-ABzI were also established by thermal analysis techniques such as TGA, DTG and DTA (by heating from room temperature to 600 °C at heating rate of 10 °C/min and N$_2$ flow of 25 cm^3/min). From TGA and DSC results in Fig. S2(c, d), it has been observed that the thermal stability of the poly(SMAnh) is greatly enhanced through the incorporation of 2-AmBzI in the copolymers. The glass transition temperatures (T$_g$) of each copolymer with and without modification were determined separately in the first transformation intervals of the DTA thermograms using the tangential slope method. From the results, it was observed that the thermal stability of the imide

structure is higher than those of others, with increasing glass transition temperatures of 115, 155 and 195 °C in order of poly(SMAnh)< 2-ABzI/poly(SMAm)< 2-ABzI/poly(SMIm).

3.2. Optimization of UA-CPE parameters

Several factors which affect the UA-CPE procedure such as pH, buffer concentration, amount of chelating 2-ABzI modified copolymer, type and concentration of ionic/nonionic surfactants, salt amount, diluent type and amount, operational parameters like sonication temperature and time, centrifugation rate and time were optimized to obtain the maximum extraction efficiency or the best sensitivity of the proposed method. In this study, the factors were optimized by changing only one factor at a time while the other remaining factors were keep constant. The optimization was carried out on the aqueous solution (in a centrifugation tube of 50-mL) containing 50 μg L^{-1} of each analyte. All the experiments were performed in triplicate and the mean plus standard deviation of the results were used for optimization.

3.2.1. Effect of pH

The pH of the aqueous phase is one of the most important factors in extraction of Hg ions from various media for the formation of a stable metal complex. In order to obtain the maximum extraction efficiency for each ion at fixed concentration of 50 μg L^{-1}, the effect of the pH on the sensitivity was studied in Fig. 1(a). The effect of pH on the complex formation of Hg^{2+} and CH$_3$Hg$^+$ was studied in a range of 3.5–10.0. For 2-ABzI modified imidic copolymer matrix, in range of pH 4.5–6.0, the sensitivity at 332 nm increases linearly, reaching values of 4.5 and 6.0 respectively for CH$_3$Hg$^+$ and Hg^{2+}, that gradually decrease for pH values in the range of 6.0–10.0. At lower pHs (< 4.5 or 6.0), CH$_3$Hg$^+$ and Hg^{2+} were in the free ionic forms, and the H$^+$ ions may have competed with both CH$_3$Hg$^+$ and the Hg^{2+} ions for binding sites on the carbonyl (C=O) and benzimidazole donor N-atom groups on the surface of the copolymer matrix. Once these groups were protonated, the strong electrical repulsion prevented each ion from contacting the surface of the copolymer matrix, resulting in lower metal binding capacities at lower pH, which can be attributed to the hydrolysis of Hg^{2+} (pK$_{a1,2,3}$: 3.40, 5.98, 21.1; K$_{sp}$: 3.2 ×10^{-26}) and CH$_3$Hg$^+$ (pK$_{a1}$: 4.65, including pK$_{a,2, 3}$: 1.73
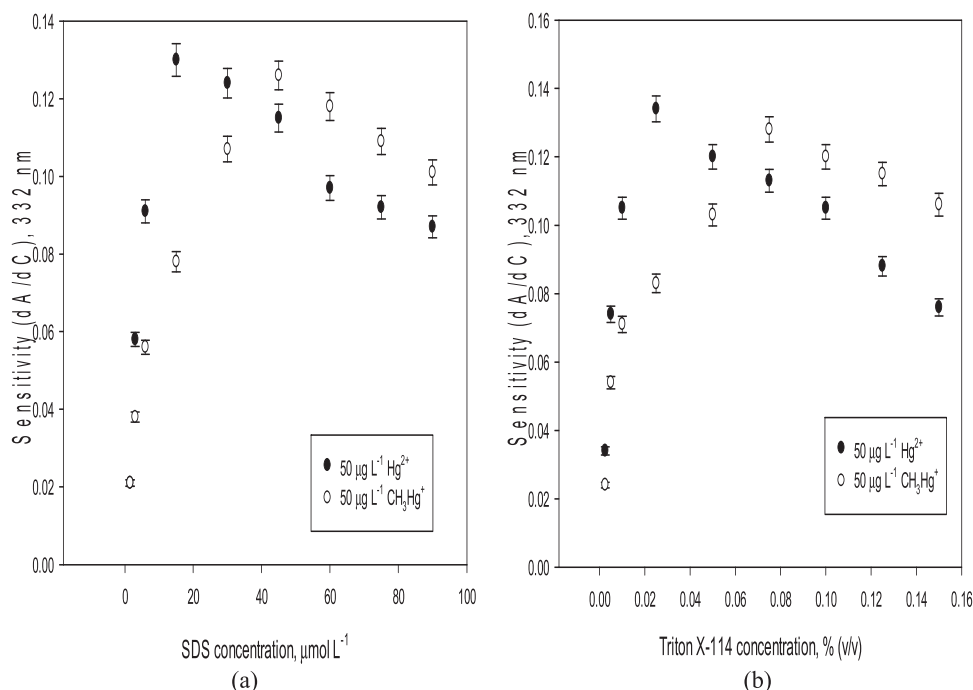

(a)

(b)

Fig. 3. (a, b) Effect of (a) SDS concentration and (b) Triton X-114 concentration on the sensitivity of Hg^{2+} and CH$_3$Hg$^+$ ions (each, 50 μg L^{-1}) at pH 4.5 and 6.0.
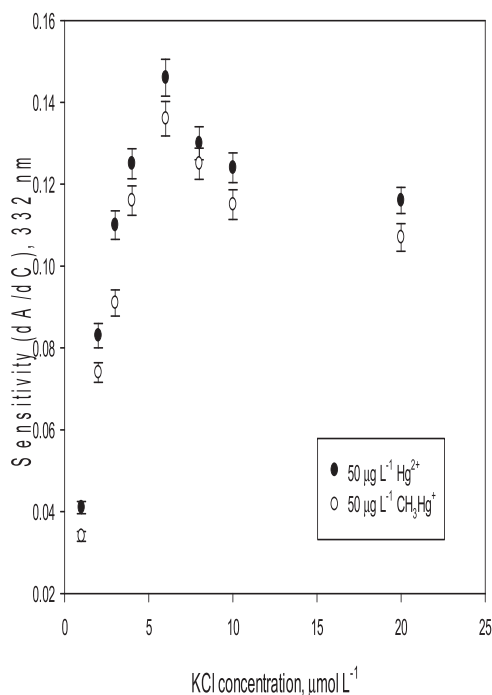
Fig. 4. Effect of KCl concentration on the sensitivity of Hg^{2+} and CH_3Hg^+ ions (each, 50 μg L^{-1}) at pH 4.5 and 6.0.

and 5.64 for further self-aggregation) at high pHs (Jawaid et al., 1978; Powell et al., 2005; De Robertis et al., 1998; Strumm and Morgan, 1996). At higher pHs (>6.0), the surface of copolymer matrix was negatively charged due to deprotonation of benzimidazole moiety (pK$_a$: 5.3). Consequently, the sensitivity sharply decreased at pH values higher than 4.5 and 6.0, respectively. Therefore, a pH of 4.5 and 6.0 for each ion was selected as optimal for the further studies. In these selected pHs, it is thought that CH_3Hg^+ and Hg^{2+} ions have participated in complexation by coordination via carbonyl (C=O) and benzimidazole donor sp^2 N-atom groups in form of free CH_3Hg^+ (or CH_3HgOH) and neutral Hg

(OH)$_2$ in presence of SDS, so as to form a complex in a linear and tetrahedral geometry as a function of pH. In order to control the pH during the analytical procedure, it was adjusted to 4.5 and 6.0 for CH_3Hg^+ and Hg^{2+} ions with an improved B-R buffer solution that consist of citric acid, boric acid, phosphate, barbital and glycine mixture especially for stabilization of Hg species in medium (each, 20 mmol L^{-1}).

Also, the effect of buffer concentration on the sensitivity was studied in range of 0.08–1.6 mmol L^{-1}. From the results in Fig. 1(b), it can be seen that the best sensitivity was observed in a buffer volume of 1.0 and 1.2 mmol L^{-1} for Hg^{2+} and CH_3Hg^+ at pH 6.0 and 4.5, respectively. At lower and higher buffer volumes, the sensitivity was significantly decreased. Therefore, these values were considered as optimal for further studies.

3.2.2. Effect of 2-ABzI modified imidic copolymer

The effect of the quantity of 2-ABzI modified imidic copolymer on the sensitivity was studied in Fig. 2. To study this effect, the copolymer matrix concentration as effective chelator was tested in range of 2.0×10^{-5}-5.0×10^{-4} % (w/v). In range of $(3.0–3.5) \times 10^{-4}$ %, the sensitivity was reached to a maximum for 50 μg L^{-1} Hg^{2+} and CH_3Hg^+ at pH 6.0 and 4.5, respectively. At lower and higher concentrations, the sensitivity was significantly decreased. Thus, in all studies, a concentration of 3.0×10^{-4} % and 3.5×10^{-4} % of co-polymeric chelator was adopted as optimal.

3.2.3. Effect of ionic and nonionic surfactants

In UA-CPE, choosing an appropriate surfactant is important, since the temperature corresponding to cloud point is correlated with the hydrophilic property of a surfactant. A successful UA-CPE should maximize the extraction efficiency by minimizing the phase volume, thus increasing its concentrating capability. In the separation/pre-concentration process, the effect of SDS concentration on the sensitivity was studied in range of 1.5–90 μmol L^{-1} in Fig. 3(a). The sensitivity for Hg^{2+} increased with increasing volume from 1.5 to 45 μmol L^{-1}, and reached a maximum at a volume of 45 μmol L^{-1} while it increased with increasing volume from 1.5 to 30 μmol L^{-1} for CH_3Hg^+, and reached a maximum at a volume of 30 μmol L^{-1}. However, at higher concentrations than 30 or 45 μmol L^{-1}, the sensitivity was gradually decreased. This indicated that the quantity of SDS is quantitatively enough to
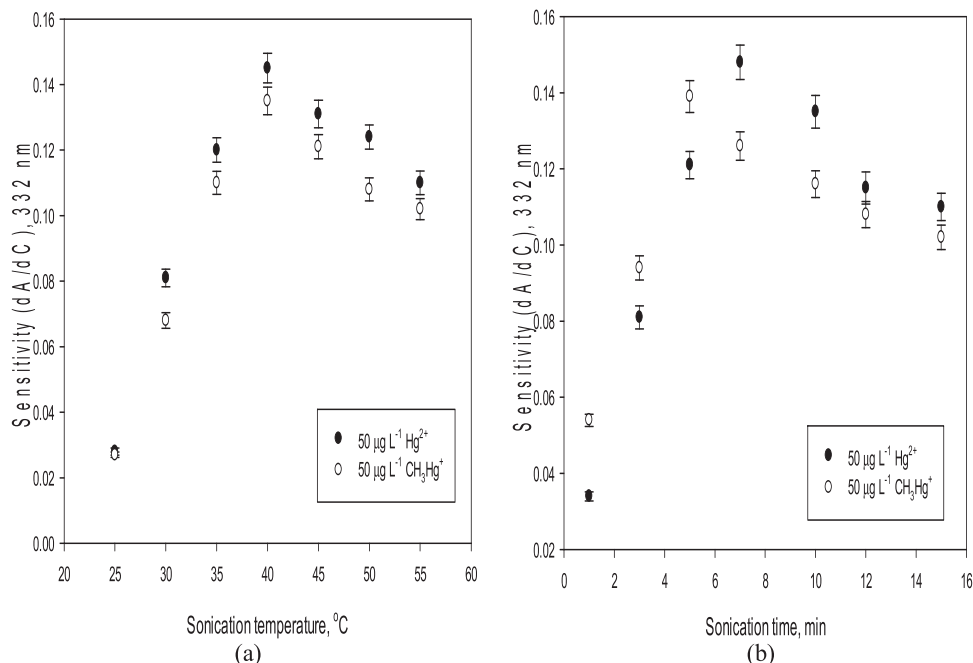

Fig. 5. a, b) Effect of (a) sonication temperature, °C and (b) sonication time, min on the sensitivity of Hg^{2+} and CH_3Hg^+ ions (each, 50 μg L^{-1}) at pH 4.5 and 6.0.

Table 1

Analytical figures of merit of the methods developed for detection of CH_3Hg^+ and Hg^{2+} by 2-ABzI-functionalized poly(SMIm) copolymer in presence of anionic surfactant, SDS at pH 4.5, 6.0 respectively.

Analytes	Calibration type	From regression analysis at 332 nm in acetone-CHCl₃ (1:3, v/v)				[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_r % for same day, 5, 25 and 100 µg L⁻¹, n: 5)	Intermediate precision (as RSD_R % for three consecutive days, 5, 25 and 100 µg L⁻¹, n: 5)	Recovery %
For CH_3Hg^+ ions at pH 4.5	By the rectilinear calibration curve in solvent	1–15, 15–150	$(1.38 \pm 0.12) \times 10^{-2}$, $(2.40 \pm 0.1) \times 10^{-3}$	$(1.41 \pm 0.14) \times 10^{-2}$, 0.205 ± 0.01	0.9930/0.9971,	0.30	1.01	3.5–5.5	4.3–6.5	92.0–95.5
	*By the matrix-matched calibration curve prepared from blank sample extracts	1–15	$(1.51 \pm 0.13) \times 10^{-2}$	$(1.65 \pm 0.13) \times 10^{-2}$	0.9941	0.26	0.86	3.4–5.8	4.1–7.6	90.5–94.0
For Hg^{2+} ions at pH 6.0	By the rectilinear calibration curve in solvent	1–15, 15–150	$(1.24 \pm 0.10) \times 10^{-2}$, $(2.80 \pm 0.1) \times 10^{-3}$	$(1.81 \pm 0.13) \times 10^{-2}$, 0.183 ± 0.01	0.9934/0.9963,	0.31	1.05	3.1–5.2	4.3–7.0	92.1–96.0
	*By the matrix-matched calibration curve prepared from blank sample extracts	1–15	$(1.35 \pm 0.12) \times 10^{-2}$	$(1.90 \pm 0.14) \times 10^{-2}$	0.9946	0.31	1.04	3.5–6.0	5.0–8.3	90.2–93.5
[b]Matrix effect, %	+ 9.42 %, + 8.87 %									
[c]Sensitivity enhancement factor (EF)	43.0, 48.5									
[d]Pre-concentration factor (PF)	62.5									

* Based on externally spiking into the blank sample extracts at concentration levels of 1, 2.5, 5, 10 and 15 µg L⁻¹ of each species before analysis

[a] Limits of detection and quantification ((LODs, LOQs) for CH_3Hg^+ and Hg^{2+} ions were determined according to the expressions at $3.0 \times \sigma_{blank}/m$ and $10 \times \sigma_{blank}/m$, respectively (where σ_{blank} is the standard deviation of ten consecutive blanks (n: 10), and m is the slope of the calibration curves prepared in solvent and from sample extracts).

[b] Based on comparison of slopes of two calibration curves established in solvent and sample extracts by using formula, ME %= $(1 - m_{solvent} / m_{matrix-matched}) \times 100$

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

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

obtain the maximum sensitivity. Thus, a SDS concentration of 30 and 45 µmol L⁻¹ was adopted as optimal for further studies.

Triton X-114 is one of the non-ionic surfactant extensively used in UA-CPE. Because that is its benefits such as commercial availability with high purity, low toxicity and cost, high density of the surfactant-rich phase can promote the phase separation by centrifugation, and relatively low cloud point temperature. As can be in Fig. 3(b), UA-CPE of Hg^{2+} and CH_3Hg^+ at pH 6.0 and 4.5 was carried out in range of 0.0025–0.15 % (v/v) Triton X-114. The sensitivity for Hg^{2+} increased with increasing concentration from 0.0025 % (v/v) to 0.075 % (v/v), and reached a maximum at a concentration of 0.075 % (v/v) while it increased with increasing volume from 0.0025 % (v/v) to 0.025 % (v/v) for CH_3Hg^+, and reached a maximum at a concentration of 0.025 % (v/v). However, at higher concentrations for both ions, the sensitivity was gradually decreased. This indicated that the quantity of TritonX-114 is enough for the chelate extracted absolutely to ensure completeness of the extraction and improve the sensitivity. Thus, a Triton X-114 concentration of 0.0025/0.075 % (v/v) was adopted as optimal for further studies.

3.2.4. Effect of salting-out

Studies on the effects of some additives, such as ionic and non-ionic surfactants and electrolytes, as NaCl, NaNO₃ and Na₂SO₄ on the cloud point behavior of non-ionic surfactants have been reported (Hinze and Pramauro, 1993). It was observed that the presence of electrolytes decreases the cloud point (salting-out effect), resulting in a more efficient extraction. The lower cloud point is attributed to electrolytes promoting dehydration of the poly(oxyethylene) chains (Armstrong et al., 1998). According to Komaromy-Hiller et al. (1996), the salting-out phenomenon is directly related to desorption of ions to the hydrophilic parts of the micelles, increasing inter-attraction between micelles and consequently leading to the precipitation of surfactant molecules. Based on this discussion, the salting-out effect was studied in the presence of different concentrations of KCl (1–20 µmol L⁻¹, for each ion) at 40 °C in Fig. 4. It was found that KCl resulted in the maximum sensitivity, and the extraction efficiency increased with increasing KCl concentration until reach a maximum at 6.0 µmol L⁻¹. This effect may be due to the enhanced hydrophobic interactions among the surfactant aggregates and chelate complexes of Hg^{2+}/CH_3Hg^+ ions as well as the decrease in the cloud point temperature of Triton X-114 in the presence of KCl. At higher concentrations than 6.0 µmol L⁻¹, the sensitivity decreased gradually. High salt concentration can increase the density of water drops accompanied by the surfactant-rich phase, and hence disturb the phase separation. Hence, a concentration of 6.0 µmol L⁻¹ was accepted to be enough for extraction of both ions.

3.2.5. Effect of sonication temperature and time

The temperature in UA-CPE procedure is an important factor affecting the formation of a stable chelate complex. In this study, the temperature was evaluated from 25° to 55 °C in Fig. 5(a). The results illustrated that the sensitivity, in fact it is linearly related to extraction efficiency, increased by increasing the temperature to 40 °C.

Table 2
The possible matrix effect on the selective extraction of 100 μg L^{-1} CH$_3$Hg$^+$ and Hg^{2+} at pH 4.5, 6.0 respectively by UA-CPE prior to analysis by UV spectrophotometry (n: 3).

Co-existing ions	Tolerance ratio, [Interferent]/[analyte ion]	Recoveries %	RSDs %
NH$_4^+$, K$^+$, Na$^+$	1500:1	98.0–101.5	2.0–4.0
Ca^{2+}, Mg^{2+}, Sr^{2+}, NO$_3^-$, F$^-$	1250:1	97.0–99.1	2.5–3.6
Ba^{2+}, Cl$^-$, SO$_4^{2-}$, HPO$_4^{2-}$, C$_2$O$_4^{2-}$	1000:1	96.5–101.0	2.5–4.1
Al^{3+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Ni^{2+}	750:1	98.0–103.0	2.0–3.6
HCO$_3^-$, Br$^-$, Tartaric acid, Citric acid	500:1	96.0–98.7	2.0–3.8
As^{5+}, Sb^{5+}, Zn^{2+}, Ag$^+$, EDTA	400:1	93.5–96.5	3.2–4.3
Cd^{2+}, MoO$_2^{2+}$, VO^{2+}, VO$_2^+$, Urea	300:1	95.0–97.2	2.1–4.0
Pb^{2+}, Bi^{3+}, NO$_2^-$, I$^-$	250:1	95.5–97.6	3.0–3.7
Fe^{3+}[a]	200:1 (1500)*	103.5	4.5
(As^{3+}, Sb^{3+})[b]	150:1 (1000)*	94.3–97.0	2.5–4.3
(Formaldehyde)[c]	100:1 (750)*	95.2	3.7
(Sn^{2+}, Mn^{2+})[d]	50:1 (300, 500)*	92.5–95.5	3.1–4.0
(Co^{2+}, Cu^{2+})[e]	35:1 (350)*	102.3–105.5	3.8–4.7

* The values in parentheses refer tolerance limits in presence of masking agents
[a] By using 1.0 mL of 0.02 mol L^{-1} NH$_4$F solution in medium buffered to pH 4.0–4.5
[b] After pre-oxidation of As^{3+}/Sb^{3+} to As^{5+}/Sb^{5+} with 0.01 mol L^{-1} H$_2$O$_2$ solution in alkaline medium
[c] After pre-treatment of 0.25 mL of 0.02 mol L^{-1} Na$^-$metabisulfite, Na$_2$S$_2$O$_5$ around pH 5.0
[d] By using 0.2 mL of 20 mmol L^{-1} TEA/2 mmol L^{-1} oxalic acid mixture near to pH 5.0–5.5 or 0.2 mL of 0.025 mol L^{-1} Na$_2$H$_2$P$_2$O$_7$ solution in a medium buffered to pH 7.0
[e] By using selectively 2.0 mL of 10 mmol L^{-1} ethylendiamine/ 1.0 mmoL L^{-1} oxalic acid mixture (3:1, v/v) in a medium buffered to pH 5.0

Accordingly, a temperature of 40 °C for both CH$_3$Hg$^+$ and Hg^{2+} ions at pH 4.5 and 6.0 was chosen as optimal for further studies.

Sonication time is a reflection of extraction efficiency, which determines the time required for quantitative extraction. Different sonication times in the range of 1–15 min in the ultrasonic bath (40 kHz, 300 W) were studied at 40 °C according to the method. The sensitivity versus sonication time is indicated in Fig. 5(b). As can be seen, the maximum sensitivity was accomplished during 5 and 7 min. Thus, a duration of 5 and 7 min was used in further studies.

3.2.6. Effect of centrifugation rate and time

At initial, the effects of centrifugation rate (1000–4000 rpm) and time (1–15 min) on the sensitivity of each ion were independently studied. It was also found that when the solution was centrifuged at 3000 rpm for 5 min, the sensitivity (or extraction efficiency) linearly increased and reached rapidly to a maximum. Hence, a duration of 5 min was adopted as optimal.

3.2.7. Effect of suitable solvent for dilution of surfactant-rich phase

As the surfactant-rich phase was very viscous, diluent (such as methanol, ethanol, acetonitrile, acetone, acetone-CHCl$_3$ (1:3, v/v), acidic methanol and ethanol (1.0 mol L^{-1} HNO$_3$) was in triplicate added to the surfactant-rich phase after UA-CPE to facilitate its transfer into spectrophotometric cell. From the results obtained, it was found that acetone gave the maximum sensitivity and good signal reproducibility/stability for Hg^{2+} while giving poor signal reproducibility/stability at pH 4.5 for CH$_3$Hg$^+$. However, the sensitivity was smaller in presence of other solvents. Perhaps, this case is due to the fact that Hg^{2+} or CH$_3$Hg$^+$ ions kinetically promoted pH-dependent hydrolysis of 2-ABzI modified imidic copolymer with a pK$_a$ value of 5.5 (Goyal and Srivastava, 1992). When the acetone-CHCl$_3$ (1:3, v/v) is used in dilute Cl$^-$ medium instead of acetone, this fluctuation in signal was greatly eliminated. Also, this case can be explained by the fact that acetone (as a polar aprotic solvent, H-bond acceptor and miscible with water) and CHCl$_3$ (as a nonpolar solvent, H-bond donor and immiscible with water) behave like a synergistic solvent mixture compatible with hydrophilicity/hydrophobicity of both free ligand and CT-sensitive Hg-ligand complexes for absorbance to stabilize between blank and sample under optimized reagent conditions. In this study, the diluent mixture is thought to act as a stabilizer to minimize possible analyte loss by pH-dependent disproportionation via charge transfer (Hg$_2^{2+}$ = Hg^{2+} and Hg or 2CH$_3$Hg$^+$ = (CH$_3$)$_2$Hg + Hg^{2+}). Also, the addition of acetone was found to be useful in ASV determination of Pb^{2+} ions in terms of reproducibility without any suppression effect on the peak current (Goyal and Srivastava, 1992; Ertaş et al., 2000). Therefore, the surfactant-rich phase was diluted to a volume of 0.4 mL with acetone-CHCl$_3$ (1:3, v/v) to have an appropriate amount of sample for transferring and measuring the sample absorbance.

3.3. Analytical figures of merit

The validation study for assessing standard method performance requirements was performed according to AOAC Official (2016), and the achieved analytical parameters were evaluated as follows: Under the selected optimal conditions, the calibration curves in solvent were observed to be rectilinear in the concentration range of 1–15, 15–150 μg L^{-1} CH$_3$Hg$^+$ and Hg^{2+} at pH 4.5 and 6.0, respectively while the five pointed matrix-matched calibration curves (1.0, 2.5, 5.0, 10.0, 15.0 μg L^{-1}) in sample extract were linear in concentration range of 1–15 μg L^{-1} with an enhancement in signal of 9.42 % and 8.87 % where the Student's t-test is applied to compare slope of the calibration curves

Table 3a
The speciation analysis results of binary mixtures containing Hg species, CH$_3$Hg$^+$, Hg^{2+} and total Hg at known concentration ratios before and after pre-oxidation with acidic dichromate (n: 5).

Added, μg L^{-1}		*,** Found, μg L^{-1}			Accuracy/precision of speciation analysis					
					RSD %			Recovery %		
CH$_3$Hg$^+$	Hg^{2+}	CH$_3$Hg$^+$	Hg^{2+}	*** Total Hg	CH$_3$Hg$^+$	Hg^{2+}	Total Hg	CH$_3$Hg$^+$	Hg^{2+}	Total Hg
0	30	–	26.8 ± 1.2	27.1 ± 1.2	–	4.5	4.4	–	89.3	90.3
5	25	4.5 ± 0.2	23.4 ± 1.0	27.5 ± 1.2	4.4	4.3	4.4	90.0	93.6	91.7
10	20	9.4 ± 0.4	18.4 ± 0.7	28.3 ± 1.2	4.2	3.8	4.2	94.0	92.0	94.3
15	15	13.6 ± 0.6	13.4 ± 0.6	28.4 ± 1.2	4.4	4.5	4.2	90.7	89.3	94.7
20	10	18.3 ± 0.7	9.5 ± 0.4	28.1 ± 1.2	3.9	4.2	4.3	91.5	95.0	93.7
25	5	23.5 ± 1.0	4.6 ± 0.2	28.1 ± 1.2	4.2	4.3	4.3	94.0	92.0	93.7
30	0	28.1 ± 1.3	–	28.3 ± 1.3	4.6	–	4.6	93.7	–	94.3

* The mean and standard deviations of five replicate measurements under optimal reagents' conditions
** Obtained at analysis conditions conducted using 2.0 mL of 5.0 mol L^{-1} HCl and 0.2 % (w/v) KCl (3:1, v/v) in methanol as stabilizer under optimal reagents' conditions to ensure the repeatability and stability of the results, and especially to prevent possible mercury losses via interconversion of mercurial species by complexation, methylation and demethylation above pH 4.5
*** After pre-oxidation with 0.02 % (w/v) K$_2$Cr$_2$O$_7$ in 5.0 % (v/v) HNO$_3$

Table 3b

The validation of the method developed for CH_3Hg^+ and Hg^{2+} ions at pH 4.5 and 6.0, respectively for speciation analysis of CH_3Hg^+, Hg^{2+} and total Hg levels in two certified samples (n: 5).

CRMs	Spiked, µg kg^{-1}	[a]Certified, µg kg^{-1}		[b]Observed, µg kg^{-1}		RSD %	Recovery %	[c]Total Hg, µg kg^{-1}	The one paired Student's t-test[d], t_{exp}
		CH_3Hg^+	Hg^{2+}	CH_3Hg^+	Hg^{2+}				
[e]SRM 1641e Mercury in water from NIST, Gaithersburg, Canada	–	–	–	<LOD	9.8 ± 0.6	-, 6.1	-, 96.4	9.70 ± 0.60 (10.16 ± 0.17)	1.71
	10, 10	–	–	9.3 ± 0.6	19.4 ± 1.0	6.4, 5.2	94.6, 96.0	–	–
SRM 1515 Apple leaves from NIST, Gaithersburg, Canada	–	–	–	<LOD	8.10 ± 0.5	-, 6.2	-, 93.8	8.20 ± 0.50 (8.64 ± 0.46)	1.97
	10, 10	–	–	9.2 ± 0.5	17.6 ± 0.8	5.4, 4.5	92.0, 95.0	–	–

[a] The certified and expected values for CH_3Hg^+ and Hg^{2+} respectively after dilution at 1:10, 1:5, respectively

[b] The mean plus SD of five replicate measurements obtained by application of the proposed method after ultrasonic extraction of 10.0-mL and 1.0-g aliquots of original liquid and solid samples with dilute acid mixture of HNO_3 (or $HClO_4$, for solid plant sample)-HCl-CH_3COOH (each one, 2.0 mol L^{-1}; 1:2:2, v/v) for 20 min at 35 °C in ultrasonic bath (40 kHz, 300 W), 5.0 mL of the sample extracts pretreated and diluted at ratios of 10- and 5-fold dilution for CH_3Hg^+ and Hg^{2+} ions, respectively

[c] Total Hg values measured after pre-oxidation of 5.0 mL sample extracts after pre-oxidation of 10-mL liquid sample with 5.0 mL of 0.02 % (w/v) $K_2Cr_2O_7$ in 5.0 % (v/v) HNO_3, and pre-treatment of 1.0 g solid sample with 10.0 mL of acid mixture of conc. HNO_3-H_2SO_4-$HClO_4$ (2:1:1, v/v) until white sulfuric acid fumes emerge, and followed by addition of dilute $K_2Cr_2O_7$ until each solution turned yellow where the values in parenthesis are certified for total Hg after dilution at 1:10, 1:5, respectively

[d] The experimental t-values calculated by using the one paired Student's t-test, t = $N^{1/2}$ (μ - $x_{average}$) /s for five replicate measurements of total Hg (as CH_3Hg^+ plus Hg^{2+}) as there is no reported certified value for free Hg^{2+} and CH_3Hg^+ in terms of speciation analysis, where the critical t-value is 2.78 for 4 degrees of freedom at confidence interval of 95 %

[e] Neither it is certificated, nor it is reported as a mean information value in 95 % confidence limit

Table 4a

The accuracy and precision studies of CH_3Hg^+, Hg^{2+} and total Hg levels measured in the selected two quality control samples via the matrix-matched calibration curves.

Sample	[a]Total Hg, µg L^{-1}	Added, µg L^{-1}	Accuracy/precision for speciation analysis of CH_3Hg^+ and Hg^{2+} with and without spiking					
			On the same day (n: 5)			On three consecutive days (n: 3 × 5)		
			[b]Found, µg L^{-1}	Recovery %	RSD %	[b]Found, µg L^{-1}	Recovery %	RSD %
Apple vinegar	2.30 ± 0.12	–	<LOD, 2.15 ± 0.10	–	-, 4.6	<LOD, 2.10 ± 0.12	–	-, 5.7
		10, 10	9.2 ± 0.5, 11.2 ± 0.5	92, 90.5	5.4, 4.5	9.1 ± 0.5, 11.2 ± 0.5	91, 91	5.4, 4.5
[c]Lemon juice with garlic	0.85 ± 0.05	–	<LOD, 0.90 ± 0.05	–	-, 5.6	<LOD, 0.86 ± 0.05	–	-, 5.0
		10, 10	9.3 ± 0.5, 10.3 ± 0.5	93, 94	5.4, 4.8	9.2 ± 0.5, 9.8 ± 0.5	92, 90	5.4, 5.1

[a] Total Hg values measured after pre-oxidation of 5.0 mL sample extract with 5.0 mL of mixture of 0.02 % (w/v) $K_2Cr_2O_7$ in 5.0 % (v/v) HNO_3

[b] The mean plus its standard deviation of five replicate measurements obtained by using the matrix-matched calibration approach where 5.0 mL of the pretreated- and extracted-sample solutions were independently analyzed with and without spiking with 5, 10 and 15 µg L^{-1} before pre-oxidation

[c] Garlic plus lemon juice mixture, may improve cholesterol level, lower blood pressure, and often used instead of vinegar in salad dressings

when using 25-mL of the sample solution. Without a serious matrix effect, it can be concluded that the calibration curves in solvent will be reliably able to use in analysis of Hg contents of vinegar samples instead of standard addition method for possible matrix effect. The regression equations were Abs = $(1.38 ± 0.12) × 10^{-2}$ C + $(1.41 ± 0.104) × 10^{-2}$, Abs = $(2.40 ± 0.1) × 10^{-3}$ C + 0.205 ± 0.01 for CH_3Hg^+ at pH 4.5, and Abs = $1.24 ± 0.10) × 10^{-2}$ + $(1.81 ± 0.13) × 10^{-2}$, Abs = $(2.80 ± 0.1) × 10^{-3}$ C + 0.183 ± 0.01 for Hg^{2+} at pH 6.0 (where A is the absorbance and C is mercury concentration in µgL^{-1}), with a determination coefficient (r^2) of 0.9930/0.9971 and 0.9934/0.9963, which indicates good linearity in the mentioned concentration ranges. From the calibration curves in solvent, the limits of detection and quantification (LODs, LOQs), based on a signal-to-noise ratio (S/N) of 3 and 10, were 0.30/1.01 and 0.31/1.05 µg L^{-1}. The precision (as RSDs) of the method, determined by analyzing the standard solutions at 5, 25 and 100 µg L^{-1} of CH_3Hg^+ and Hg^{2+} five times in same day and three consecutive days, have been in range of 3.5–6.5 % and 3.1–7.0 % while the recovery rates are in range of 90.2–96.0 %. When compared with the threshold values (with a RSD$_r$ / RSD$_R$ of 22–45, 15–30 % and a recovery rate of 60–115, 80–110 %) recommended by the AOAC Official (2016) in the used concentration range, it can be concluded that the results are quantitatively acceptable. It has been found that the sensitivity enhancement factors, defined as the slope ratio of two calibration curves for each ion with and without pre-concentration, are 43.0 and 48.5 for CH_3Hg^+ and

Hg^{2+} respectively. From pre-concentration of 25-mL sample solution, a pre-concentration factor of 62.5 was obtained for each ion in optimal conditions. The other analytical figures of merit achieved were given in detail in Table 1.

3.4. Interferences

The effects of potential interfering species in Table 2 were tested in tolerance ratio ranging from 1:35–1:1500 by the high selectivity for Hg-ligand complex formation at pH 4.5 and 6.0 by using solutions containing 100 µg L^{-1} CH_3Hg^+ and Hg^{2+} and adding various concentrations of potentially interfering ions. The tolerance limits of the co-existing ions, defined as the largest amount decreasing the recovery of each ion to less than 5.0 %. Table 2 shows the tolerance limits of the interfering neutral and ionic species' concentrations. Most of the studied neutral and ionic interfering species did not interfere with the determination of CH_3Hg^+ and Hg^{2+} in tolerance range of 250–1500-fold, Fe^{3+}, As^{3+}/Sb^{3+} and formaldehyde did not interfere at more than a 200-, 150- and 100-fold excess, and the Sn^{2+}/Mn^{2+} and Cu^{2+}/Co^{2+} ion pairs did not disturb determination at more than a 50- and 35-fold excess where the recovery rate is in range of 92.5–105.5 % with a precision ranging from 2.0 % to 4.7 % from triplicate measurements. According to the obtained data, most foreign cations and major cations in the samples have no obvious influence on determination of each ion under the

Table 4b

. The analysis results of CH_3Hg^+, Hg^{2+} and total Hg levels in the selected vinegar samples by the developed UV spectrophotometric method with and without spiking (n: 5).

Sample	[a]Total Hg, µg L^{-1}	Added, µg L^{-1}	By three-point standard addition method							[b]The two sample t-test[c], t_{exp}
			[d]After extraction of 5.0 mL vinegar sample with 3.0 mL of 4.0 mol L^{-1} HCl and 0.75 % (w/v) thiourea mixture (2:1, v/v) for 15 min at 60°C in ultrasonic bath (40 kHz, 300 W)				[d]After extraction of 5.0 mL vinegar sample using 10 mL of dilute acid mixture of $HClO_4$-HCl-CH_3COOH (each one, 2.0 mol L^{-1}; 1:2:2, v/v) for 20 min at 35°C in ultrasonic bath (40 kHz, 300 W)				
			[e]Found, µg L^{-1}	Recovery %	RSD %		[e]Found, µg L^{-1}	Recovery %	RSD %	
Apple cider vinegar	3.50 ± 0.2	–	<LOD, 3.20 ± 0.2	–	-, 6.2		<LOD, 3.25 ± 0.2	–	-, 6.1	0.40
		10, 10	9.2 ± 0.5, 12.5 ± 0.6	92, 93	5.4, 4.8		9.10 ± 0.5, 12.7 ± 0.6	91, 94	5.5, 4.7	–
White grape vinegar	2.80 ± 0.2	–	<LOD, 2.55 ± 0.2	–	-, 7.8		<LOD, 2.60 ± 0.2	–	-, 7.7	0.40
		10, 10	9.3 ± 0.5, 11.8 ± 0.6	93, 92	5.4, 5.1		9.2 ± 0.5, 12.1 ± 0.7	92, 95	5.4, 5.8	–
Red grape vinegar	4.10 ± 0.2	–	<LOD, 3.70 ± 0.2	–	-, 5.4		<LOD, 3.80 ± 0.2	–	-, 5.3	0.79
		10, 10	9.3 ± 0.5, 13.1 ± 0.6	93, 94	5.4, 4.6		9.4 ± 0.5, 13.3 ± 0.6	94, 95	5.3, 4.5	–
Red grape vinegar	4.50 ± 0.2	–	<LOD, 4.20 ± 0.2	–	-, 4.8		<LOD, 4.30 ± 0.2	–	-, 4.7	0.79
		10, 10	9.3 ± 0.4, 13.5 ± 0.5	93, 93	4.3, 3.7		9.2 ± 0.4, 13.7 ± 0.5	92, 94	4.1, 3.6	–
Pomegranate vinegar	5.50 ± 0.3	–	0.50 ± 0.03, 4.70 ± 0.2	–	6.0, 4.3		0.55 ± 0.03, 4.80 ± 0.2	–	5.5, 4.2	2.64 (0.79)
		10, 10	9.2 ± 0.5, 14.2 ± 0.6	92, 95	5.4, 4.2		9.3 ± 0.4, 14.3 ± 0.6	93, 95	4.3, 4.2	–
Balsamic vinegar	6.00 ± 0.3	–	0.60 ± 0.03, 4.90 ± 0.2	–	5.0, 4.1		0.55 ± 0.03, 5.20 ± 0.2	–	5.4, 3.8	2.64 (0.24)
		10, 10	9.8 ± 0.5, 14.3 ± 0.5	92, 94	5.1, 3.5		9.7 ± 0.5, 14.7 ± 0.5	92, 95	5.2, 3.4	–
Natural organic vinegar	2.80 ± 0.2	–	<LOD, 2.60 ± 0.1	–	-, 3.8		<LOD, 2.55 ± 0.1	–	-, 3.9	0.79
		10, 10	9.6 ± 0.4, 12.1 ± 0.5	96, 95	4.2, 4.1		9.4 ± 0.4, 12.0 ± 0.5	94, 95	4.2, 4.2	–
Hawthorn vinegar	2.50 ± 0.2	–	<LOD, 2.20 ± 0.1	–	-, 4.5		<LOD, 2.30 ± 0.1	–	-, 4.3	1.58
		10, 10	9.5 ± 0.4, 11.7. ± 0.5	95, 95	4.2, 4.3		9.5 ± 0.4, 11.8 ± 0.5	95, 95	4.2, 4.2	–

[a] Total Hg values measured after pre-oxidation of 5.0 mL sample extract with 5.0 mL of mixture of 0.02 % (w/v) $K_2Cr_2O_7$ in 5.0 % (v/v) HNO_3

[b] The accuracy and precision results based on statistical comparison of the results obtained by two different ultrasonic soft sample preparation procedures for CH_3Hg^+ and Hg^{2+} ions at pH 4.5 and 6.0, respectively

[c] The experimental t -values were calculated by using the two sample t-test, 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 of CH_3Hg^+ and free Hg^{2+} ions according to two sample preparation procedures where the critical t-value is 2.78 for 8 degrees of freedom at confidence interval of 95 %.

[d] The mean plus its standard deviation of five replicate measurements obtained by using the three level standard addition method chosen for determination of CH_3Hg_+ and Hg^{2+} levels around the method quantification limits after (i) extraction of 5.0 mL vinegar sample with 3.0 mL of 4.0 mol L^{-1} HCl and 0.75 % (w/v) thiourea mixture (2:1, v/v) for 15 min at 60 °C, and (ii) extraction of 5.0 mL vinegar sample using 10 mL dilute acid mixture of $HClO_4$-HCl-CH_3COOH (each one, 2.0 mol L^{-1}; 1:2:2, v/v) for 20 min at 35 °C in ultrasonic bath (40 kHz, 300 W) to facilitate the mass transfer between two phase and to ensure the repeatability and stability of the results against possible matrix effect, and to minimize analyte loss via volatilization and interconversion of mercurial species by methylation or demethylation

[e] The mean plus its standard deviation of five replicate measurements obtained by using standard addition method in which aliquots (5.0 mL) of the pretreated, extracted and diluted sample solutions for two sample preparation procedures were independently analyzed by three level standard addition method after dilution at ratios of 1:5 with 2.0 % (v/v) HNO_3 and HCl solutions at equal volume to overcome the possible matrix effect.

selected conditions. Moreover, in the case of serious interference, the effect of these species can be controlled, and greatly suppressed with improvement of 300–1500-fold in tolerance ratio by the use of suitable masking agents at pH close to neutral before pre-concentration by UA-CPE. The values between parentheses in Table 2 refer to tolerance limits in presence of masking agents.

3.5. Speciation analysis

The performance and reliability of the method for the applicability to analysis of real samples were checked by determination of CH_3Hg^+, Hg^{2+}, and total Hg content in binary mixtures. In order to determine CH_3Hg^+ and Hg^{2+} before, and total Hg after pre-oxidation with acidic dichromate (n: 5), model solutions that contain different amounts of CH_3Hg^+ and Hg^{2+} with ratios of 1:1, 1:2 and 1:3 or 1:1, 2:1 and 3:1 as equivalent to total Hg level of 30 µg L^{-1} were prepared. Then, the CH_3Hg^+, Hg^{2+} and total Hg levels with and without oxidation in the test

solutions was performed by the procedure explained. The results show that the proposed method with a RSD of 3.8–4.6 % a recovery rate of 89.3–95 %could be successfully applied to the speciation analysis of mercury in Table 3(a).

Also, the method was validated by analysis of two certified samples in terms of their total Hg contents in Table 3(b). It was observed that there is not statistically a significant difference between the results found by the present method and their certified values, via use of the one-paired t-test. Also, after spiking with 10 µg L^{-1} for each ion, based on the threshold values of 21 % and 60–115 % recommended by the AOAC Official, (2016), the method in terms of accuracy and precision was reliable, and it was observed that the results obtained could quantitatively be accepted with RSDs of 4.5–6.4 % and recovery rates of 92.0–108.8 %. The recovery values without spiking refer to a comparison between observed and reported values given in parentheses, in terms of total Hg content of CRMs (in fact, it can be accepted as equivalent to the measured Hg^{2+} content due to be under the method LODs of CH_3Hg^+

Table 5

Comparisons of the method performance with those of the other detection methods reported in literature for selective reduction, extraction and determination of mercury species in vinegar at levels of μg L^{-1}.

Analyte	Sample matrix	Reductant type	Peak potential, E°, volt	[a]Pre-treatment step	[b]Detection step	Linear range, μg L^{-1}	DLs (or QLs), μg L^{-1}	PF (or EF)	RSDs %	Recovery %	Refs
Hg^{2+}	Wine	Ethanol	0.20	Matrix assisted-photoreduction/NanoTiO$_2$	CVG-AFS	2–10	0.02, 0.03, 0.07	–	2.2–12.0	97–126	Li et al. (2006)
Hg^{2+}	Wine, liquor	Ethanol	0.20	Matrix assisted-photoreduction	CVG-ICP-MS	0.05–2.0	0.003	–	2.3–3.1	94–110	Gao et al. (2012)
Hg^{2+}	Vinegar	Thiourea/HCl	0.75, 1.25–1.50	UAE	CV-AAS	0.0–3.0	0.014, 0.047	–	3.87–8.11	85–119	Junior et al. (2015)
Hg^{2+}	White vinegar	Acetic acid	1.25	Matrix assisted-photoreduction	CVG-AFS	Up to 1 mg L^{-1}	0.08	–	4.6–7.8	92–98	Liu, (2010)
Hg^{2+}, CH$_3$Hg$^+$, total Hg	Red wine	NaBH$_4$, NaBPh$_4$	1.24	Reduction/derivatization	GC-ICP-MS, FI-CVG-ICP-MS	1–25, 0.025–1	0.77, 0.88,0.01	–	<10	99–104	Dressler et al. (2012)
Total Hg, CH$_3$Hg$^+$	CRM Dogfish liver	Acetic acid	1.25	UV reduction	CV-AAS	Up to 500	2.1	–	2.9	92.3	Bendl et al. (2006)
Hg^{2+}, CH$_3$Hg$^+$	Water	Formic acid	0.20, 0.67	Irradiation with natural daylight or UV lamp	CVG-AFS	Up to 25 or 300	0.003 or 0.2	–	–	–	Zheng et al. (2005)
CH$_3$Hg$^+$, total Hg	Certified lake sediment, fish sample	2-Mercaptoethanol	0.33	UV irradiation	CV-AFS	0.2–3.0	0.06	–	2.0–6.4	92.7–96.7	Yin et al. (2007)
Hg^{2+}, CH$_3$Hg$^+$, total Hg	Fish otoliths	NaBH$_4$	1.24	Matrix assisted-photoreduction	CVG-ICP-MS	0.04–10	0.0042, 0.0064	–	<5.0	96.2–98.7	Kenduzler et al. (2012)
Hg^{2+}	Seawater	Pd-modifier	0.99	CPE	ETAAS	10–100	1.2	73	4.7	–	Abadi et al. (2012)
Hg^{2+}	River water	SnCl$_2$	0.15, 0.38	UV photoreduction	PVG-FAPES	0.25–1, 1–25	0.24, 0.25	–	2.2–2.5	91.6–100.4	Ribeiro et al. (2009)
Hg^{2+}, total Hg	Sediments, sewage sludge, coal and coals, coal fly ash	NaBH$_4$	1.24	Slurry sampling	CV-AAS	1–20	0.7	–	3.5–23.8	76.3–112.8	Ribeiro et al. (2004)
Hg^{2+}, CH$_3$Hg$^+$, total Hg	Vinegar	Thiourea/HCl	–	UAE/UA-CPE	CT-sensitive UV spectrophotometry	1–15, 15–150	0.30, 0.31	62.5 (43, 48.5)	3.1–7.0, 3.4–7.6	92.1–96, 92–95.5	This study

*Depending on ionic strength and pH of the medium

[a]UAE: Ultrasound assisted extraction; CPE: Cloud point extraction; UA-CPE: Ultrasound assisted-cloud point extraction

[b]CV-AAS: Cold vapor-atomic absorption spectrometry; CVG-AAS: Cold vapor generation-atomic absorption spectrometry; CVG-AFS: Cold vapor generation-atomic fluorescence spectrometry; CVG-ICP-MS: Cold vapor generation-inductive couple plasma mass spectrometry; ET-AAS: Electrothermal-atomic absorption spectrometry; PVG-FAPES: Photo vapor generation coupled with furnace atomization plasma emission spectrometry; GC-ICP-MS: Gas chromatography-inductive coupled plasma-mass spectrometry; FI-CVG-ICP-MS: Floe injection-cold vapor generation-inductive couple plasma mass spectrometry

contents).

3.6. Analysis of vinegar samples

The UA-CPE procedure, based on 2-ABzI modified imidic poly (SMIm) copolymer matrix, was applied for pre-concentration and simultaneous determination of CH_3Hg^+ and Hg^{2+} in the selected vinegar samples. At initial, via the matrix-matched calibration curves in two quality control samples, the accuracy/precision for speciation analysis of CH_3Hg^+ and Hg^{2+} with and without spiking (10 µg L-1, each) were tested on the same day and on three consecutive days. Different amounts of CH_3Hg^+ and Hg^{2+}, including total Hg were spiked to these samples in order to estimate the accuracy and applicability of the proposed method in Table 4(a). From the results in Table 4(a), it can be seen that the method gives the accurate and precise results with a higher recovery than 90 % and lower RSD than 5.7 % for CH_3Hg^+, Hg^{2+} and total Hg.

In similar way, the speciation analysis of vinegar samples was performed in Table 4(b). To control the matrix effect, the standard addition method with three point (5, 10 and 15 µg L^{-1}) after two soft sample preparation procedures based on ultrasonic effect was adopted and applied to the speciation analysis of samples. Also, the spiking at levels of 10 µg L^{-1} was made into each sample matrix, and their recoveries were established for five replicate measurements before and after pre-oxidation. In terms of CH_3Hg^+, Hg^{2+}, total Hg contents of samples, it was observed that there is statistically not a significant difference between the results found by the present method after two soft sample preparation procedures, according to the two sample t-test. The recoveries for the spiked samples were in the acceptable range of 91–95 % with lower RSD than 7.8 %. When the accepted maximum limits of 10 µg L^{-1} for total Hg in wine by OIV is considered (Płotka-Wasylka et al., 2018), it is clear that the results will not pose to a serious risk on human health where the total Hg contents of the selected vinegar samples are in range of 2.30–6.0 µg L^{-1}, including lemonade with value of 0.85 µg L^{-1}. All the results indicated that the newly developed analytical method could be satisfactorily used for the speciation analysis of Hg in vinegar samples.

3.7. Comparison of the method with other methods

It has been demonstrated that the 2-ABzI modified poly(SMIm) copolymer provide a new, efficient, eco-friendly and fast route for separation/pre-concentration and speciation analysis of CH_3Hg^+ and Hg^{2+}. This method is certainly simpler, faster and more convenient than other methods that have been proposed for simultaneous UA-CPE and speciation of mercuric ions. Micellar separation by means of UA-CPE, based on pH-controlled complexation with modified copolymer matrix, greatly shortened the analysis time of the method. This chelating matrix was successfully applied to efficient enrichment of trace levels of CH_3Hg^+ and Hg^{2+} ions from vinegar samples with low alcohol contents (lower than 0.5 or 1.0 %). Table 5 shows a comparison of the proposed method with other detection methods reported in literature. It could be concluded that some values obtained for the proposed method such as RSD %, recovery rate, enrichment or enhancement factor, linear range, and LODs and LOQs are either better or at least comparable with those of the previously reported-sensitive, selective but complex, costly, time-consuming and requiring expert user in his or her area detection methods such as CV-AAS, ETAAS, PVG-FAPES, GC-ICP-MS (especially in terms of detection limit) with and with FI, depending on the calculation equations used to determine LODs as a measure of calibration sensitivity, according to reductant type and pre-treatment step of sample matrix (Dressler et al., 2012; Bendl et al., 2006; Abadi et al., 2012; Ribeiro et al., 2004, 2009). Furthermore, it avoids the time-consuming sample pretreatment (suitable elution mode in LC or GC), derivatization and/or reduction of analyte to a volatile species, furnace and temperature programs in ETAAS and GC-MS, and no further sample processing and clean-up steps were required. The main benefits of this

methodology are simplicity, versatile and selectivity (due to formation of pH-dependent CT complex in pre-concentration step), pre-concentration factor, fast complexation/separation, low cost, and available/accessible in almost every analytical research laboratory.

4. Conclusions

In this study, the modified poly(SMAnh) copolymer with 2-ABzI as chelating ligand was prepared and applied to highly selective pH-controlled separation/pre-concentration of CH_3Hg^+ and Hg^{2+} ions from aqueous solution in UA-CPE procedure before analysis by UV spectrophotometry. Many effective factors in selective extraction of mercuric ions, such as pH, buffer volume, chelator amount, ionic and nonionic surfactant (SDS, Triton X-114), KCl amount, type and amounts of diluent, time and temperature of ultrasonic bath, rate and time of centrifugation were optimized. The optimal pHs for the extraction were found to be 4.5 and 6.0 for each ion with their fixed concentration level of 50 µg L^{-1}. Subsequently, the phase separation and complexation equilibrium in mixed micellar interface was achieved for Hg species with sonication times of 5 or 7 min at 40 °C and a centrifugation time of 5 min at 3000 rpm. In addition, the effects of matrix components potentially available in vinegar samples were tolerable by the present method.

The findings indicate that the modified poly(SMIm) copolymer with 2-ABzI can be used as an inexpensive, efficient, fast (due to complexation of CH_3Hg^+ and Hg^{2+} ions by pH-controlled CT) and environmentally safe metal binding chelator for separation/pre-concentration of each ion. With the proposed method, good LODs, precisions, accuracy and sensitivity and a RSD of 3.6–7.1 % at even low concentration levels were achieved. Moreover, due to be use of dilute solvents and oxidants in ultrasound assisted-extraction step, a unique green approach was introduced as an alternative to toxic organic solvents. Trace and ultra-trace mercury in vinegar samples could be reliably detected with good repeatability, reproducibility and recoveries after spiking.

CRediT authorship contribution statement

Hacı Bayram Zengin: Conceptualization, Investigation, Formal analysis, Conduction of experimental studies, Data collection, Interpretation of IR and ^{1}H NMR peaks, Writing – review & editing. **Ramazan Gürkan:** Supervision, Method development, Methodology, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2022.104729.

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