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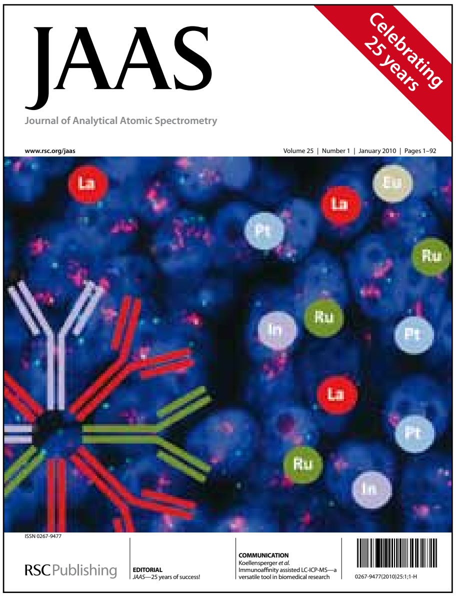

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A novel Cu-BSA nanocomposite based vapour generation approach for the rapid determination of mercury in aqueous media by cold vapour atomic absorption spectrometry (CVAAS) and on-line flow injection inductively coupled plasma mass spectrometry (FI-ICPMS)

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Abstract

The present study reports, for the first time, a novel Copper-Bovine Serum Albumin nanocomposites (Cu-BSA NCs) based vapor generation as a simple, rapid and reliable approach for the sensitive determination of mercury in waters. Copper nanoparticles in the form of Cu-BSA NCs acted as reductant to convert mercury ions to elemental mercury (Hg^0) which was subsequently quantified using cold vapour atomic absorption spectrometry (CVAAS) and on-line flow injection inductively coupled plasma mass spectrometry (FI-ICPMS). The basic experimental parameters such as reaction time, amount of copper nanoparticles, pH and temperature of sample solution related to chemical vapour generation (CVG), have been optimized for both inorganic mercury (iHg) and methyl mercury (MeHg) species using CVAAS in batch mode. These studies indicated that the reduction process was very rapid (<20 s) when the pH and temperature of the sample solution was maintained at ≥4.0 and ~90°C respectively. After optimizing the conditions by CVAAS, further studies were performed with on-line

FI-ICPMS. The recoveries of mercury species were found to be in the range of 97-104%. The absolute limits of detection of the developed method in conjunction with FI-ICPMS were 2.8 pg and 4.1 pg for iHg and MeHg respectively. Interference of concomitant ions and possible mechanism of the Cu-BSA NCs induced vapor generation of mercury have been discussed in detail. The proposed CVG method was applied to the analysis of total mercury in various natural water samples.

Key words

Mercury, Chemical Vapor Generation (CVG), Copper Nanoparticles, Bovine Serum Albumin (BSA), CVAAS and FI-ICPMS

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Introduction

Mercury is well recognized as a highly toxic element among heavy metals because of its accumulative and persistent character in the environment which has resulted in its wide spread occurrence at varying concentrations in the entire food chain.[1-6] The predominant pathway for human exposure to mercury is drinking water and dietary intake. As a safeguard for human health, the U. S. Environmental Protection Agency (USEPA) has set a limit of 2 ng mL^{-1} of mercury in accordance with the primary drinking water standard, while the European Community has indicated a maximum Hg concentration of 1 ng mL^{-1} in drinking water. Based on these facts, the development of highly efficient "green" analytical methodologies for the determination of mercury at ultra-trace levels is very important for environmental protection and food safety.

Consequently, a number of studies on the development of sensitive and accurate methods for the determination of mercury in various matrices have been widely reported as well as reviewed.[7-13]

Chemical vapor generation (CVG) in combination with atomic spectrometry techniques remains the most successful and widely adopted sample introduction approach for the sensitive determination of mercury. CVG offers several advantages which include efficient matrix separation, high analyte transport efficiency, high selectivity, simple instrumentation and ease of automation, compared to that of traditional pneumatic nebulization of solution samples.[14-16] Various reductants such as tin chloride ($SnCl_2$), tetrahydroborate (THB) (sodium or potassium salt) and non-tetrahydroborate based reagents have been widely used for the vapour generation of mercury and highlighted in the literature.[13-18,23,24] Despite many advantages, THB-acid based reductive system suffers from chemical interferences, unstable reductant solution, vigorous chemical reactions and significant waste generation.[19] Consequently, alternative approaches such as photo – induced CVG [20-27] and ultrasound assisted CVG [28] have been developed for the generation of volatile form of mercury. However, development of new vapour generation systems within the context of “green chemistry” is increasingly demanded.

Because of the “cold vapour” property of mercury, CVAAS[12,18,29] and atomic fluorescence spectrometry (AFS)[30] have become widely used analytical techniques employed for the determination of mercury in a wide variety of matrices. Among the ICP-based techniques, inductively coupled plasma mass spectrometry (ICP-MS) is the most powerful technique for the determination of mercury because of its outstanding capabilities including simplicity of interface, excellent sensitivity, wide dynamic range and ability to provide isotope ratio measurements.[31-37]

Applications of nano-materials are being highly researched in diverse fields of science and technology due to their peculiar physical and chemical properties.[38-40] Their major areas of application include catalysis, heat transfer, chemical and biological sensors, optoelectronics, photonic and electronic devices.[41-44] Authors from this laboratory have reported a simple method for the synthesis of Cu-BSA NCs without need of inert environment.[45] The synthesized composite exhibited very good antibacterial activity thus promising potential applications in the field of antimicrobial agents and wound dressing.

The aim of the work is to explore a completely new application of Cu-BSA NCs as a reductant for vapor generation of mercury (Hg^0) from sample solutions which is analysed by atomic absorption or mass spectrometry systems for a sensitive determination of mercury. The copper nanoparticles in the most stable form of Cu-BSA NCs were prepared in this laboratory and utilized for the efficient reduction of both inorganic mercury (iHg) and methylmercury (MeHg) species into volatile forms without further addition of chemical reducing agent or mineral acid and subsequent determination by CVAAS in batch mode and on-line FICPMS. The purpose of this work is also to describe the potential advantages of copper nanoparticles for the CVG of mercury when coupled with on-line FI-ICPMS. To the best of our knowledge, this is the first report on the use of copper nanoparticles in the form of Cu-BSA NCs as a reductant for the vapour generation of mercury.

Experimental

Instrumentation

Mercury was determined using a Cold Vapor Atomic Absorption Spectrometer (Model MA 580E, Electronic Corporation of India Limited (ECIL), Hyderabad, India) and it was used for preliminary optimization studies in batch mode.

A VG Plasma Quad 3 ICP-MS (VG Elemental, Winsford, Cheshire, U.K) system located in a class 100 area of Ultra-trace analysis laboratory of our Centre, was used for the determination of mercury after vapour generation. The mercury signal was monitored through the high abundant isotopes of mercury m/z 199, 200 and 202 using on-line flow injection chemical vapour generation (FI-CVG) sample introduction mode. The on-line FI-CVG system was assembled as shown in Fig 1 which comprised a six-port injection valve (Cole-Parmer, UK) with a 100 µL sample loop with a manual injector, a peristaltic pump (Gilson, USA), reaction coil comprises a 0.75 m PTFE tubing of dimensions of 0.8 mm i.d. x 1.2 mm o.d. and a U-type gas-liquid separator. In all FI-ICPMS related experiments, water was used as carrier solution.

Elemental mercury generated from the vapour generating system was directly transported to the ICP torch. The least possible distance was maintained between vapour generating system and ICP to avoid sample dispersion. The sensitivity of the instrument was checked daily before starting the actual experiments. The optimized ICP-MS operating conditions that yielded the best sensitivity for the mercury are; RF power-1400 W, cool gas flow-13.5 L min^{-1}, auxiliary gas glow-0.95 L min^{-1}, nebulizer gas flow-0.82 L min^{-1} and flow rate of carrier solution-0.5 mL min^{-1}.

The MS instrumentation and data acquisition were controlled using the Plasma Lab data software. A time resolved mode of data acquisition (TRA) was used for obtaining FI-ICPMS plots. After data acquisition in TRA mode, the data was exported into graphical software for further processing which includes generation of plots and calculation of peak areas. In the case of quantitative determinations of mercury, the total area under peak after subtracting the base line was used.

Reagents and materials

Ultra-pure water with >18 MΩ-cm resistivity, generated using a Milli-Q high purity water system (Millipore, Bedford, MA, USA), located in class 100 area was used for the preparation of all solutions and all the reagents were of the highest available purity. All containers were cleaned by soaking in a 20% nitric acid and were rinsed several times with water before use. Inorganic mercury standard solution (1000 mg L^{-1}) in 5% HNO_3 traceable to NIST 3133 (SD Fine-Chem Ltd, Mumbai, India) was used as a stock standard. A methyl mercury stock standard solution (100 mg L^{-1}) was prepared from methyl mercury chloride (Aldrich) by dissolving an appropriate amount of the solid in minimum quantity of acetone and making up to required volume with high purity water. The calibration solutions for both iHg and MeHg were prepared just before use by sequential dilution of a 1 mg L^{-1} stock standard with high purity water.

All the water samples were collected in polypropylene bottles (previously cleaned by soaking for 24 h in 10% HNO_3 solution and finally rinsed thoroughly with high purity water before use). Immediately after collection, all water samples were filtered by using 0.45 µm cellulose filters from Millipore.

Synthesis of Cu-BSA NCs

Cu-BSA Ncs containing embedded copper nanoparticles was synthesized as described in our earlier reported method.[45] Briefly**,** an ammonical solution of $CuSO_4$ (10 mM, pH-10) and a fresh 0.02% BSA solution were mixed and stirred for 1 hr followed by the addition of 100 mM hydrazine in a tightly closed bottle. The reaction bottle was then kept in a water bath preset at 50°C for 3 hrs. Appearance of dark red color after a period of 3 hrs indicated the formation of metallic copper nanoparticles. Phase analysis, purity and morphology of the product have been studied earlier by various physical techniques. The important characteristic of the synthesized Cu-BSA NCs is that the product is prepared without any inert atmosphere and could be stored at 4ºC for long term use. The final concentration of copper nanoparticles in the form of Cu-BSA NCs was 10 mM.

Typical procedure for Cu-BSA NCs based vapour generation of mercury

In order to maximize vapour generating conditions for the formation of Hg^0 by the addition of Cu-BSA NCs, a set of optimization experiments in batch mode was carried out by CVAAS. In general, iHg and MeHg species show different reduction behavior and different efficiencies in the conventional vapour generation processes. CVG efficiency of mercury species (iHg and MeHg) with Cu-BSA NCs was studied by spiking individually to obtain the best compromised operating conditions for the vapor generation system. Known amount of iHg or MeHg standard was spiked separately into a reaction vessel (of CVAAS system) containing 5 mL of pre-heated water (~90ºC) followed by the addition of 100 µg of Cu-BSA NCs. The reaction mixture was stirred well for 20 s in closed environment before passing the Hg^0 vapors to the AAS for quantification.

After the initial optimization studies with CVAAS, further studies were continued on the present vapour generation approach using an on-line FI-ICPMS (Fig 1). Parameters such as after optimizing length of the PTFE reaction coil, flow rate of Ar and carrier solution were optimized. A series of standard solutions spiked with a known amount of iHg or MeHg and Cu-BSA NCs were loaded into the injection loop with a syringe and injected into the carrier solution (water, in this case). Vapour generation takes place at the hot zone of the 1 m length PTFE reaction coil immersed in hot water maintained at ~90^0C (Fig 1). The generated mercury vapor (Hg^0) was separated from sample solution by a gas liquid separator (GLS) and directed to the ICP-MS system mercury determination.

Calibration curves were obtained by injecting a series of individual standard solutions of iHg or MeHg in the concentration range 0 (analytical blank)-5 ng mL^{-1} (0.5, 1.0, 2.5 and 5 ng mL^{-1}) along with a fixed amount of Cu-BSA NCs (20 µg mL^{-1}) and were used for quantification purposes. Standard addition method was also applied, in order to determine possible matrix interferences, if any. To quantify mercury concentration, the area of the peak generated in TRA mode after subtracting the blank signal was used. The analysis of each sample was repeated at least thrice. A blank was injected periodically after the analysis of sample to assess any possible memory effect, which was found to be insignificant. A control solution with a known mercury concentration was injected at regular intervals during the working day to assess the overall stability and sensitivity of the ICP-MS system.

Results and discussion

Optimization of vapour generating conditions for the determination of mercury

In order to evaluate the suitability of the proposed CVG method to both off-line (batch) and on-line systems, detailed studies were carried out by using two instrumental techniques CVAAS (for batch mode) and FI-CVG-ICPMS (for on-line mode).

Batch studies with CVAAS

Based on the initial set of experiments in batch mode, various basic parameters such as concentration of Cu-BSA NCs, temperature of reaction mixture, pH of sample solution and reaction time were found to be critical for promoting the CVG process of mercury species (iHg and MeHg). For the optimization of these variables, a reaction vessel containing 5 mL of pre-heated water and a standard solution of 50 ng (absolute) of iHg or MeHg was used. A one-at-a-time method (i.e., changing one parameter each time while keeping others constant), was used in order to obtain optimal conditions for the vapour generation of mercury and subsequent determination by CVAAS.

Temperature of sample solution: Initially, a set of experiments was carried out to examine the vapour generating efficiency of Cu-BSA NCs at room temperature. These studies indicated that CVG efficiency for both the mercury species was relatively low (<10%) when sample solution was kept at room temperature. The pre-heating of sample solution may serve to increase the efficiency of vapour generation. Therefore, the effect of the temperature (varied from 40 to 100^{0}C) was tested in order to evaluate its influence on the CVG efficiency of mercury by keeping amount of Cu-BSA NCs (100 μg), amount of mercury (50 ng), pH of sample solution (~7) and reaction time (~20 s) constant and general procedure was followed.

Figure 2 shows the response of absorbance signal of iHg and MeHg arising from a 50 ng standard solution as a function of temperature of sample solution. As can be observed in Fig 2, absorption values were increased for both the mercury species (i.e., increase of CVG efficiency of mercury) when the temperature of the sample solution increased from 40^{o}C to 90^{o}C and then remained almost constant. The optimum efficiency conditions for both the mercury species were obtained when the temperature of the sample solution was kept at ~90ºC which is considered as reaction temperature.

Concentration of Cu-BSA NCs: Depending on the type of aqueous medium, it may be possible that the amount/concentration of Cu-BSA NCs may influence the reduction of mercury. Hence, the influence of concentration of Cu-BSA NCs upon the CVG efficiency of mercury was assessed.

Figure 3 illustrates that the CVG efficiency for iHg and MeHg increased with increase in concentration of Cu-BSA NCs. As shown in Fig 3, it is clear that the efficiency of Cu-BSA NCs for the vapour generation of iHg increased almost linearly with the amount of Cu-BSA NCs in the range of 10-50 µg (absolute in 5 mL of water) and then became constant in the range of 50 to 100 µg which suggested that the minimum amount of Cu-BSA NCs for obtaining quantitative CVG efficiency of iHg was required to be 50 µg. Reduction of mercury in sample solution was incomplete when an amount of Cu-BSA NCs was below 50 µg.

In the case of studies related to MeHg, similar observations as in the case of iHg were noticed. However, optimal vapour generation efficiency of MeHg was obtained with 100 µg of Cu-BSA NCs which was double the amount required as in case of iHg. In consideration of both efficiency and reagent consumption, 100 µg of Cu-BSA NCs (in 5 mL of water) was used for vapour generation of both iHg and MeHg species.

pH of sample solution: Since the solution pH affects the extent of stability of *nanoparticle* nature of copper which in turn determines CVG efficiency of mercury from solutions, effect of sample pH was studied. Sample solutions were adjusted to different pHs in the range of 3-12 by the addition of HNO_3 or NaOH. Fig 4 shows the response of vapour generation of mercury with respect to change in the sample solution pH in the range of 3-12 at constant Cu-BSA NCs (100 μg), iHg/MeHg (50 ng), reaction time (20 s) and temperature of sample solution (~90^0C). As can be seen in Fig 4, the absorbance signal of two mercury species increased with pH, most intensively in the pH range 3 to 5. CVG efficiency was relatively poor when pH of the sample solution was maintained at pH <3 while optimum absorption signal (i.e., maximum CVG efficiency) for both the mercury species were obtained in the pH range of 6–12. The low recoveries at pH<3 could be due to the instability of Cu- BSA NCs under these conditions.

Reaction time: One of the prime requirements of sample preparation method is that it must be simple to use and reasonably fast for obtaining high sample throughput. The reaction time (i.e., time require for the quantitative conversion of mercury to elemental mercury) must be as short as possible. The effect of reaction time (stirring time) upon the CVG efficiency was evaluated at different time intervals ranged from 5-40 s by keeping other parameters constant. This parameter primarily determines the stirring time of the solution and efficiency of the vapour generation. The optimum reaction time required to obtain maximum CVG efficiency for iHg and MeHg was obtained by varying stirring time and the results are presented in Fig 5. As seen here, reaction (i.e., reduction process) between Cu-BSA NCs and mercury species is very rapid. It was found that a reaction time of about 20 s was sufficient for quantitative CVG of both the species of mercury.

Mechanism of Cu-BSA NCs induced vapor generation

After addition of Cu-BSA NCs to a mercury solution, it was observed that the red color of solution fades and finally turns to colorless when the temperature of the sample solution was kept at ~90^{0}C. According to the proposed mechanism, it is expected that after addition of Hg^{2+} to the Cu NPs embedded in the composite material, Cu^0 acts as reducing agent because of its standard reduction potential being much less than that of Hg^{2+} thus producing Hg^0.[15] Copper in turn gets oxidized which is the main reason for the color change from red to colorless. The mechanism is based on the following reduction process.

$$Hg^{2+} + Cu^0 \rightarrow Hg^0 + Cu^{2+}$$

For the above reaction, the Gibbs energy can be calculated using the standard potentials from the electrochemical series ($Cu^{2+} + 2e \rightarrow Cu^0$, $E^0 = +0.340$ V; $Hg^{2+} + 2e \rightarrow Hg^0$, $E^0 = +0.851$ V; $\Delta E = 0.5108$ V):

$$\Delta G = -zF\Delta E = -2 \text{ X } 96487 \text{ C/mol X } 0.5108 \text{ V} = -98.57 \text{ kJ/mol}$$

Where in ΔG= Gibbs energy, z = valence, F= Faraday constant, and ΔE = standard potential difference. The resulting negative Gibbs energy shows that the mercury reduction process as shown in the above equation is a highly favoured reaction.

On-line experiments with FI-ICPMS

After initial optimization experiments with CVAAS in batch mode, further studies were continued with on-line FI-ICPMS system. Advantages of on-line FI-ICPMS include automation, low sample consumption, improved detection limits and increased sample throughput. Three main parameters related to FI-ICPMS, length of the reaction coil, Ar and carrier solution flow rate were found to be critical in obtaining optimum vapour generating efficiency and transport efficiency to the ICP-MS.

From the initial set of optimization studies with CVAAS, it was found that mercury reduction process with Cu-BSA NCs was very rapid (<20 s) when temperature of the sample solution kept at ~90^{0}C. However, in the case of on-line FI-ICPMS system, length of the PTFE reaction coil determines the reaction time. Length of the reaction coil was optimized by taking reaction coil of different lengths (0.5 m, 0.75 m and 1 m) and followed the general vapour generation procedure as described in earlier section. For carrying out on-line FI-ICPMS studies, an aqueous solution made with a mixture of 1 ng mL^{-1} of iHg and 20 μg mL^{-1} of Cu-BSA NCs was used. From these studies, it was observed that a reaction coil of length 0.75 m was found to be optimum for quantitative conversion of mercury in sample solution to elemental mercury.

The Ar flow rate through GLS which also served as the sample gas of ICP-MS is one of the important parameter affecting the efficiency of gas-liquid separation and transport of mercury vapour. The effect of Ar flow-rate was investigated in the range of 0.6 to 1.0 L min^{-1} using constant sample flow rate of 0.45 mL min^{-1}. A significant improvement in the signal intensity was observed with the increase of gas flow-rate up to 0.82 L min^{-1}, while decreased with further increase of the flow rate. The optimal signal (narrower peaks with minimum tailing together with higher S/N ratios) was obtained at 0.82 L min^{-1}. The observed variation in the signal intensity of mercury with the change in gas flow rate is mainly due to displacement of analytical region in the plasma. For further experiments, Ar flow-rate of 0.82 L min^{-1} was thus employed.

The effect of sample introduction flow rate (carrier solution flow rate) ranging from 0.4 to 0.6 mL min^{-1} was investigated for the determination of Hg at 1 ng mL^{-1}. This parameter primarily determines the residence time of the sample solution in the reaction coil which in turn influences the CVG efficiency. From the results, it was observed that the sensitivity increases almost linearly with an increase in sample introduction flow rate of up to 0.5 mL min^{-1} and then decreased marginally at higher flow rates (>0.5 mL min^{-1}). These observations also validate the fast kinetics of mercury vapour generation by Cu-BSA NCs. A carrier solution flow rate of 0.5 mL min^{-1} was selected for all subsequent measurements.

Throughout these studies, the relative sensitivities of the two mercury species differ due to their difference in physical and chemical properties. The sensitivity difference between the species is more significant in CVAAS compared to FI-ICPMS. In the case of iHg, CVG process (i.e., formation of Hg^0) occurring directly (as described in earlier section) while in the case of MeHg, part of the Cu-BSA NCs are utilized for cleavage of the stable C-Hg bond and rest are used for the generation of elemental mercury. During the reduction process of MeHg, the formation of other species (which are not detected by CVAAS) along with Hg^0 may also be the cause of different sensitivities of iHg and MeHg species. Similar observations were noticed in our earlier studies with $NaBH_4$-HCl reduction system.[12,36] Since the proposed CVG approach has been attempted first time by authors, further studies are needed to understand the CVG process better before it could become fully established.

Effect of concomitant ions

As mentioned in earlier sections, one serious problem with the conventional vapour generation techniques for the determination of mercury is chemical interferences caused by the transition metals mainly Co, Cu, Zn and Ni. In photo-induced CVG these interferences are alleviated due to low reducing ability of small organic molecules used and higher reduction potential of mercury over transition metal ions [16]. In the current investigation also, the effect of various concomitants (Na, K, Ca, Mg, Fe, Mn, Co, Cu, Zn and Ni) on the vapour generation efficiency of Cu-BSA NCs was studied by FI-ICPMS. The results indicated that no significant interferences were observed with the standard solutions containing 100 μg mL^{-1} of Na, K, Ca, Mg and 50 μg mL^{-1} of Fe, Mn, Co, Cu, Zn and Ni and 1 ng mL^{-1} mercury (50000-fold) with satisfactory recoveries of higher than 95%. In the present Cu-BSA NCs based vapor generation approach, the reduction of mercury is mainly driven by difference in electrochemical reduction potential of Cu^{2+}/Cu and Hg^{2+}/Hg, the interference from other transition elements does not occur because their standard reduction potential is less than that of Cu.

Analytical figures of Merit

The analytical characteristics for both the test mercury species were established under optimal conditions for the proposed Cu-BSA NCs based approach. As mentioned previously, precision between the measurements and memory effects are one of the largest challenges presented by the analysis of mercury in various environmental matrices. To evaluate the presence of such effects in the developed on-line FI-ICPMS system, six 100 μL replicate injections of a 1 ng mL^{-1} inorganic mercury standard solution were acquired in TRA mode. The percent relative standard deviation (%RSD) of the integrated peak areas was calculated to be 3.8% using this manual flow injection approach, illustrating the overall stability characteristics of the present CVG system. Variation in the base line signal (essentially due to noise induced by gas flow through GLS and ICPMS) was found to be about 6.5% RSD indicating no appreciable accumulation of mercury occurs in the FI-ICPMS system.

The respective response functions and correlation coefficients obtained for two mercury species using CVAAS and on-line FI-ICPMS systems are presented in Table 1. The data in Table 1 illustrate, the calibration curves for both the species of mercury in the studied range showed good linearity with satisfactory correlation coefficients (R^2>0.995). The limits of detection (LOD) calculated as the concentration of mercury yields a signal equivalent to the three times the standard deviation of the blank value (3σ, n=6) obtained for the proposed CVG approach in conjunction with FI-ICPMS was calculated to be 0.028 ng mL^{-1} and 0.041 ng mL^{-1} for iHg and MeHg respectively. The repeatability expressed as the relative standard deviation (RSD, n=6) was <5% for mercury determination at the concentration levels of 1 ng mL^{-1}. With the use of 0.75 m length reaction coil, 90^0C reaction temperature and Ar flow rate of 0.82 L min^{-1}, the sampling frequency was 12-14 h^{-1} with three replicate injections using FI-ICPMS system.

Comparison of the developed vapor generation method with the recently reported methods

Table 2 presents a comparison of detection limits for a range of atomic spectrometry methods for the test species evaluated here. As can be seen, the LOD values obtained for the present CVG approach in conjunction with FI-ICPMS are very competitive with recently reported conventional vapour generation methods, but are an order of magnitude higher than preconcentration methods. The achievements of lower LODs would require a preconcentration step.

However, the LOD values obtained with our FI-ICPMS system is enough to meet the requirements of regulations where permissible levels in drinking water are 1-2 ng mL^{-1} Hg. This suggests that the proposed method can be a better choice for the sensitive determination of iHg and MeHg compared to $NaBH_4$ method because there was negligible interference from various concomitant ions. As mentioned in earlier sections, the $NaBH_4$-acid reduction system has been widely used for mercury determination in wide range of samples. However, one of the main problems associated with $NaBH_4$-acid system is that the rapid reaction between $NaBH_4$ and HCl generates large amount of hydrogen gas which is troublesome in maintaining stable plasma conditions. Depending on type of matrix, $NaBH_4$ concentration of 0.1 to 2% along with 2-5% acid is generally used as a carrier solution for the generation of elemental mercury while in the case of developed CVG method, water can be used as carrier.

Analytical application

In order to further check the practical applicability of the proposed Cu-BSA Ncs based CVG method for the determination of mercury, experiments were carried out with different water samples-ground water (collected in the premises of our laboratory), lake water (Hussain Sagar, Hyderabad, India) and water samples collected from bore-wells (~150 m depth) located near-around a battery manufacturing factory. The water samples were collected in pre-cleaned polyethylene bottles, filtered through 0.45 μm filter and analysed immediately after collection. Total dissolved solids (TDS) of the collected samples was ~650 μg mL^{-1} and pH of various water samples as received were in the range of 6.8-7.1 and samples were analysed immediately after collection without any pH adjustment.

Optimization studies have shown that Cu-BSA NCs are able to reduce both iHg and MeHg but albeit with different sensitivities, similar to $NaBH_4$ based CVG studies. Prior separation of the mercury species is therefore necessary for carrying out speciation studies. But, the main objective of the present work is on the utilization of Cu-BSA NCs as a reducing agent for the generation of elemental mercury and subsequent determination. All the water samples were analysed for total mercury after subjecting to UV-irradiation.

Ground water sample: Analysis of ground water sample by FI-ICPMS indicated that the Hg level in the collected water sample was well below the detection limit of the instrument. Therefore, spiking experiments were performed at different concentration levels (1-5 ng mL^{-1}) of iHg or MeHg species which are the most prevalent forms of mercury in the environment and then general procedure, as described previously, was followed. The results showed good recoveries in the range of 98-104% demonstrating the capability of the proposed method for the analysis of mercury in ground water samples on routine basis.

Hussain Sagar lake water: The Hussain Sagar Lake in Hyderabad, India is an enchanting and the largest man-made Lake in Asia which is presently in an advanced state of eutrophication due to discharges released from various industrial activities.[50] The determination of mercury is very important from the point of environmental protection. Two water samples were collected from Hussain Sagar lake to determine the levels of total Hg using the proposed CVG approach. Fig 6 shows the plot of Hussain

Sagar lake water sample showing mercury peak whose concentration was determined to be about 0.75 ng mL^{-1} which is slightly less than the permissible level set by regulating authorities.

Ground water samples collected near a battery manufacturing factory: Alkaline batteries used to be manufactured with mercury (up to 20 mg) as one of their components to control side reactions at cathode. Due to high content of mercury and the resulting environmental concerns, the sale of mercury batteries is banned in many countries.[51]

To determine the mercury levels in ground waters near a battery making factory located in Hyderabad, India (presently not operational), three water samples were collected from different locations outside the factory. After collection of the samples, water samples were filtered through 0.45 μm filter and general procedure was carried out. Typical profile of a water sample collected near the battery making factory obtained by FI-ICPMS detection showed significant quantity of mercury (Fig 6). As seen from Table 3, the concentration of total Hg was determined to be ~15 ng mL^{-1}, ~13 ng mL^{-1} and ~11 ng mL^{-1} for samples 1, 2 and 3 respectively. In all the analysed water samples, mercury concentration was found to be same before and after UV-irradiation which indicates that there were no detectable levels of MeHg in the collected samples. The concentration levels of mercury species in the tested water samples except for tap water sample were much above the permissible levels of 1-2 ng mL^{-1} set by USEPA and EC where as water samples collected from Hussain Sagar lake contain mercury at slightly elevated levels.

Conclusions

A novel Cu-BSA NCs based vapour generation method within the context of green chemistry was demonstrated for the determination of mercury in wide variety of aqueous media in both batch (CVAAS) and on-line FI-ICPMS systems. The Cu-BSA NCs used in this method could be synthesized in the laboratory and found to be stable for period of two months thus avoiding the need of freshly preparing the reagents. Furthermore, the developed method is a relatively green analytical method since no additional chemical reagents (such as $NaBH_4$ or $SnCl_2$) are needed in the vapour generation process. The results obtained in this study clearly demonstrate that Cu-BSA NCs based vapor generation method is simple in operation and can be used as an alternative to conventional vapour generation techniques for the determination of mercury at ultra-trace levels and its application to various natural waters to safeguard human health. As the present CVG method is able to determine total mercury in waters, further studies are currently being carrying out for speciation of mercury.

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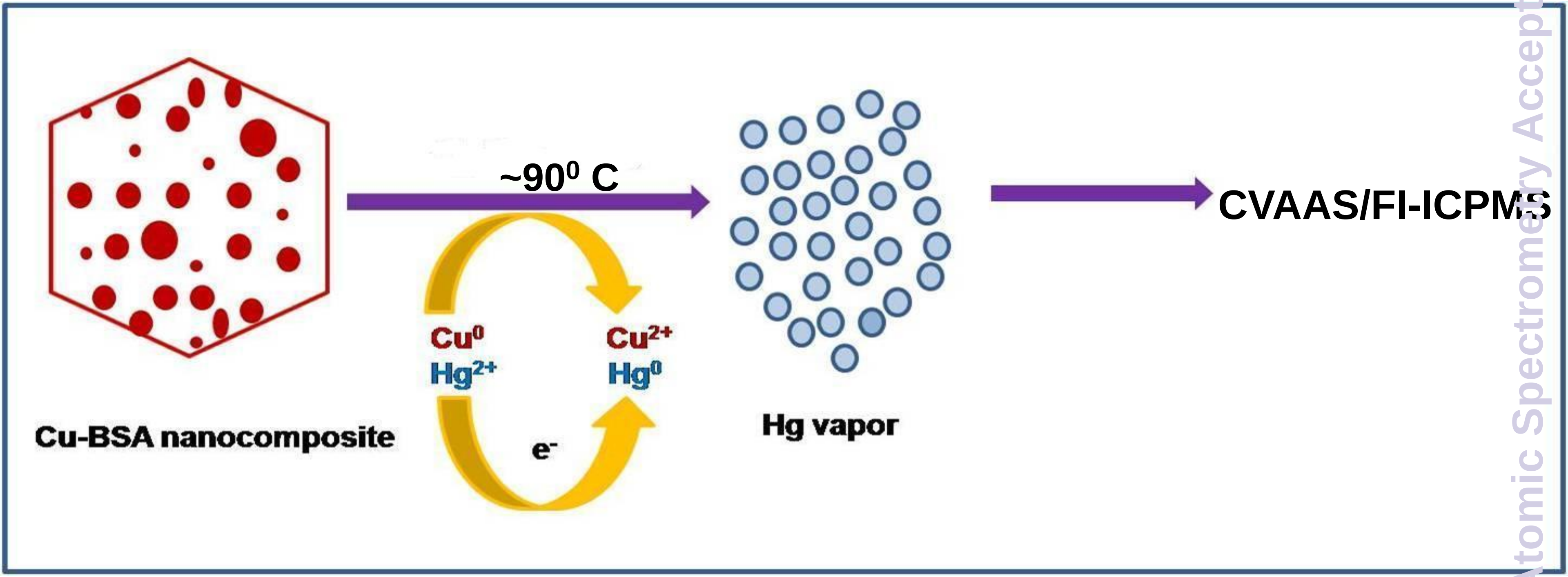
Figure Captions

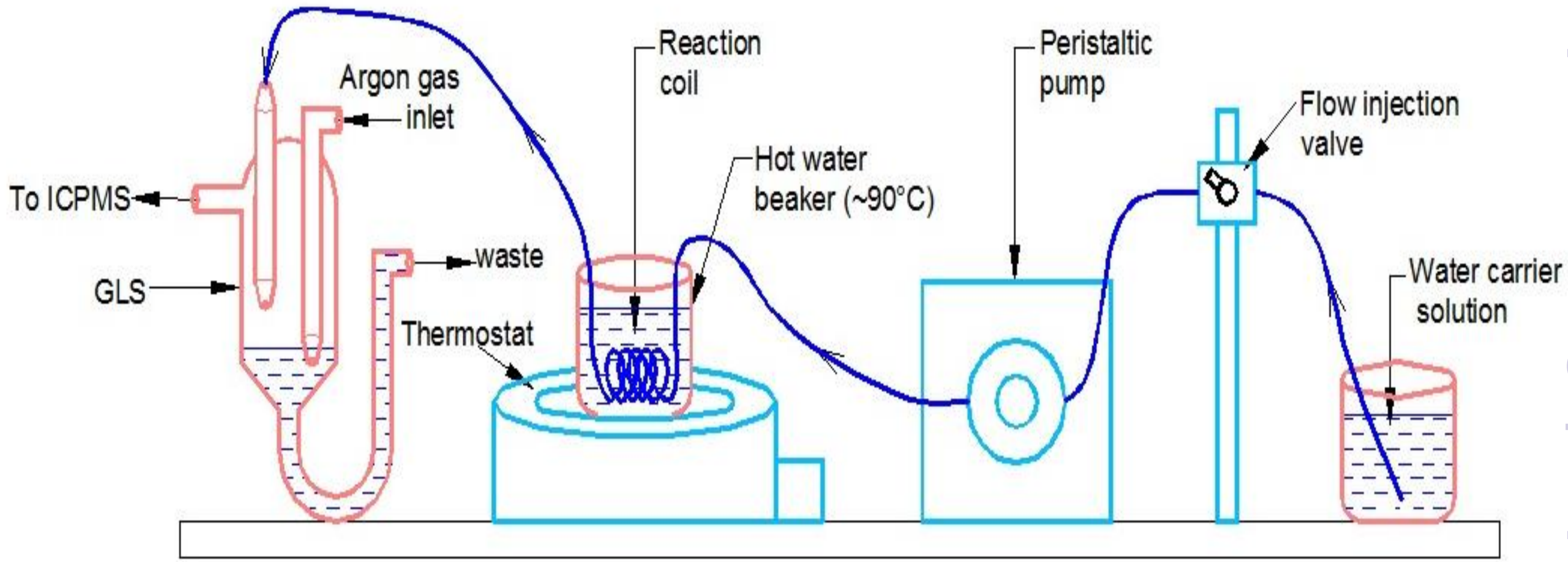
1. Schematic diagram for the Cu-BSA NCs based vapour generation set-up for the determination of mercury by FI-ICPMS
2. Effect of temperature of sample solution on the vapour generation efficiency of Cu-BSA NCs and CVAAS detection: Sample volume = 5 mL, amount of Cu-BSA NCs added = 100 µg, amount of mercury taken = 50 ng, pH of sample solution = ~7 and stirring time = ~20 s
3. Effect of concentration of Cu-BSA NCs (in the form of Cu-BSA NCs) on the vapour generation efficiency of iHg and MeHg species and CVAAS detection: sample volume = 5 mL, temperature of sample solution = ~90^{0}C, amount of mercury taken = 50 ng, pH of sample solution = ~7 and stirring time = ~20 s
4. Influence of pH of sample solution on the vapour generation efficiency of iHg and MeHg species with Cu-BSA NCs and CVAAS detection: Sample volume = 5 mL, temperature of sample solution = ~90^{0}C, amount of mercury taken = 50 ng, amount of Cu-BSA NCs added = 100 µg and stirring time = ~20 s
5. Effect of reaction time on the vapour generation efficiency of iHg and MeHg species with Cu-BSA NCs and CVAAS detection: Sample volume = 5 mL, temperature of sample solution = ~90^{0}C, amount of Cu-BSA NCs added = 100 µg, amount of mercury taken = 50 ng, pH of sample solution = ~7 and reaction time = ~20 s

6. Typical profile of a water samples collected (a) at Hussain Sagar lake and (b) near battery manufacturing factory showing total mercury obtained using developed Cu-BSA nanocomposite based vapour generation approach in conjunction with FI-ICPMS detection (three peaks represent replicate injections of same sample); pH of sample solution = ~7, sample injection volume = 100 µL, concentration of Cu-BSA NCs = 20 µg mL^{-1} and flow rate of carrier solution = 0.5 mL min^{-1}.

Table of Contents



For the first time, copper nanoparticles in the form of Cu-BSA NCs used as reductant to convert mercury ions to elemental mercury (Hg^0) which was subsequently quantified by CVAAS and on-line FI-ICPMS. The proposed vapour generation method is a simple, rapid and viable approach for the determination of mercury in natural waters on routine basis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pump
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Flow injection
valve
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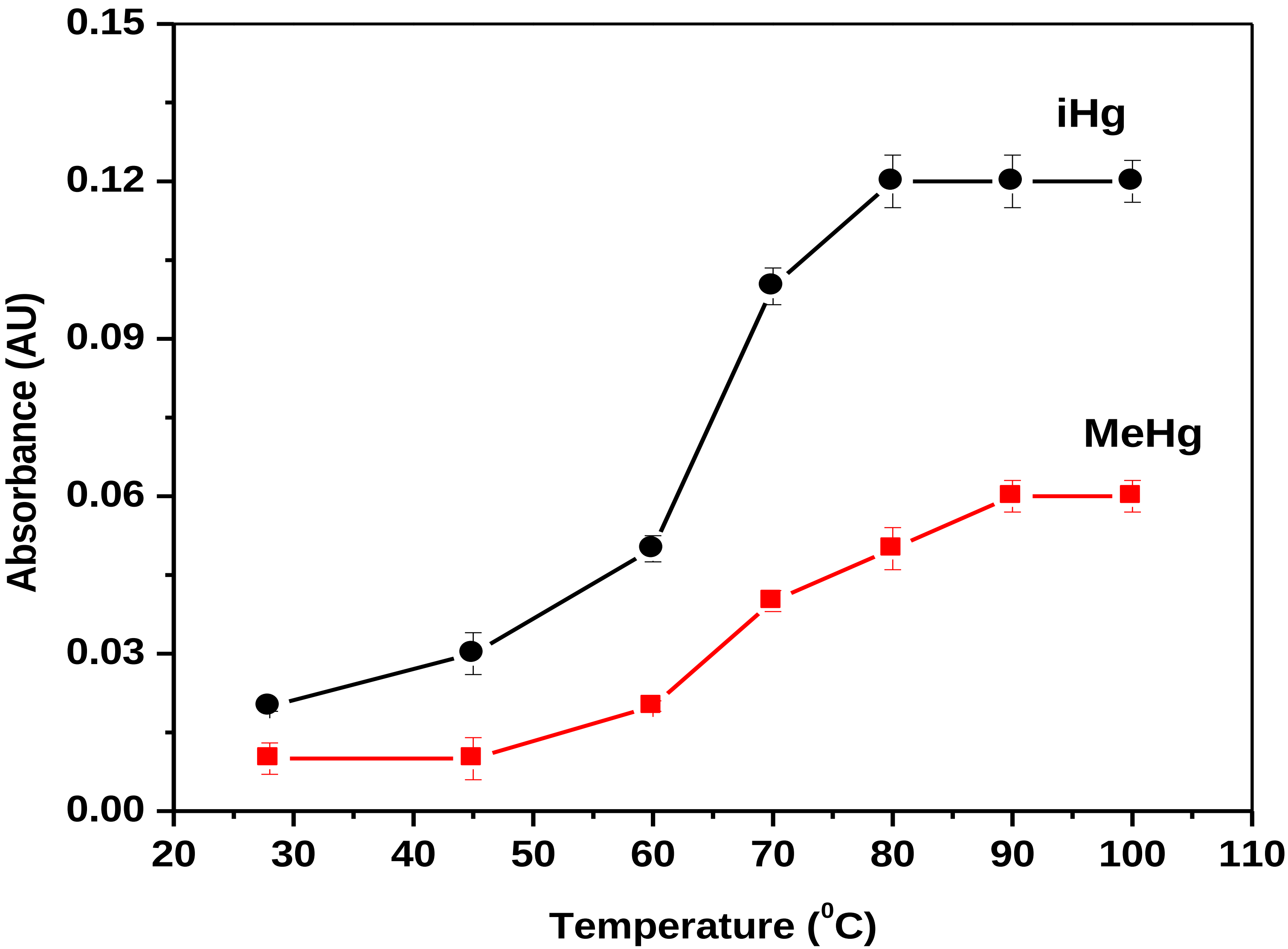
beaker (~90°C)
To ICPMS
waste
Water carrier
solution
GLS
Thermostat

Fig 1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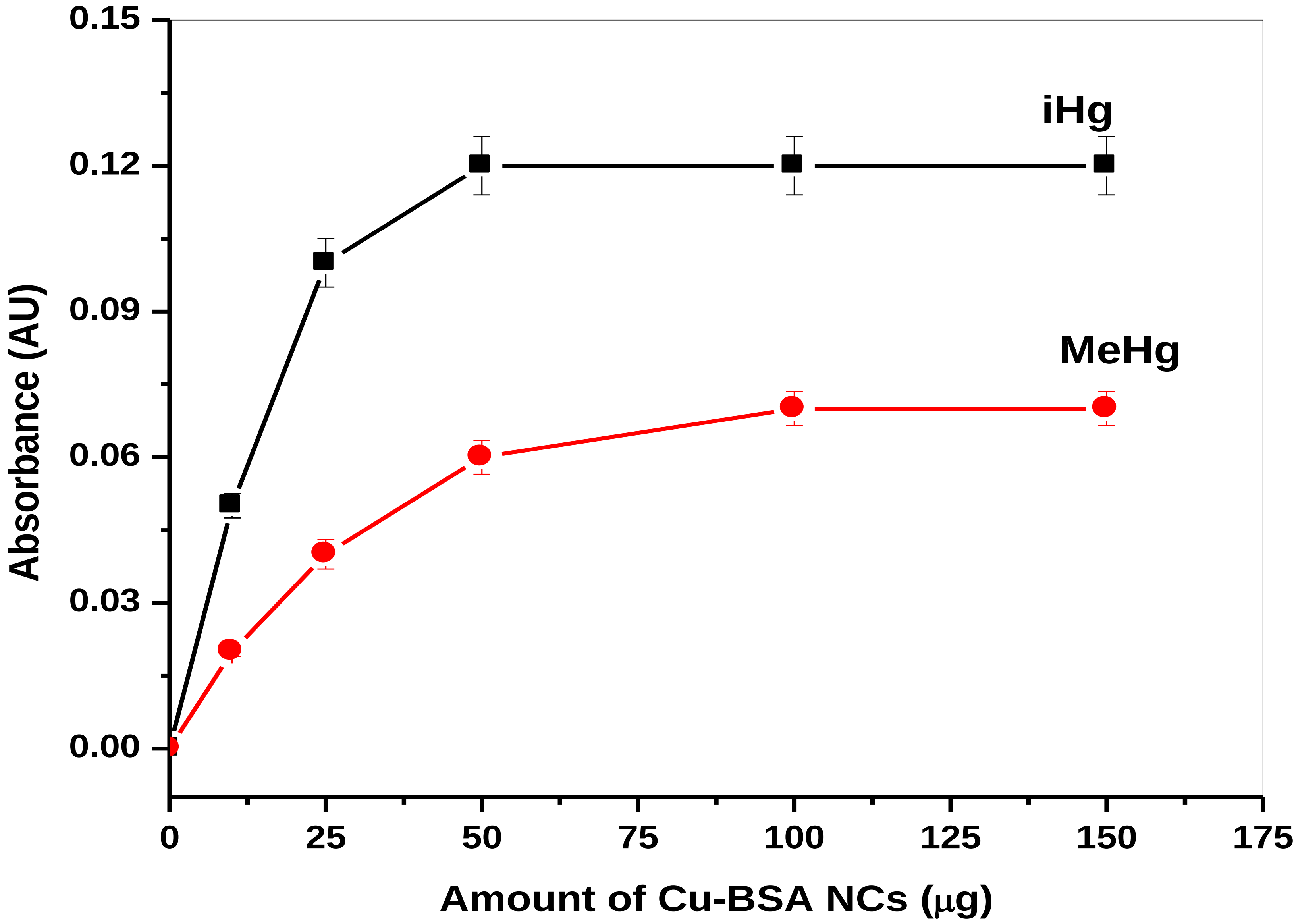
100
110
Temperature (^{0}C)
iHg
MeHg

Fig 2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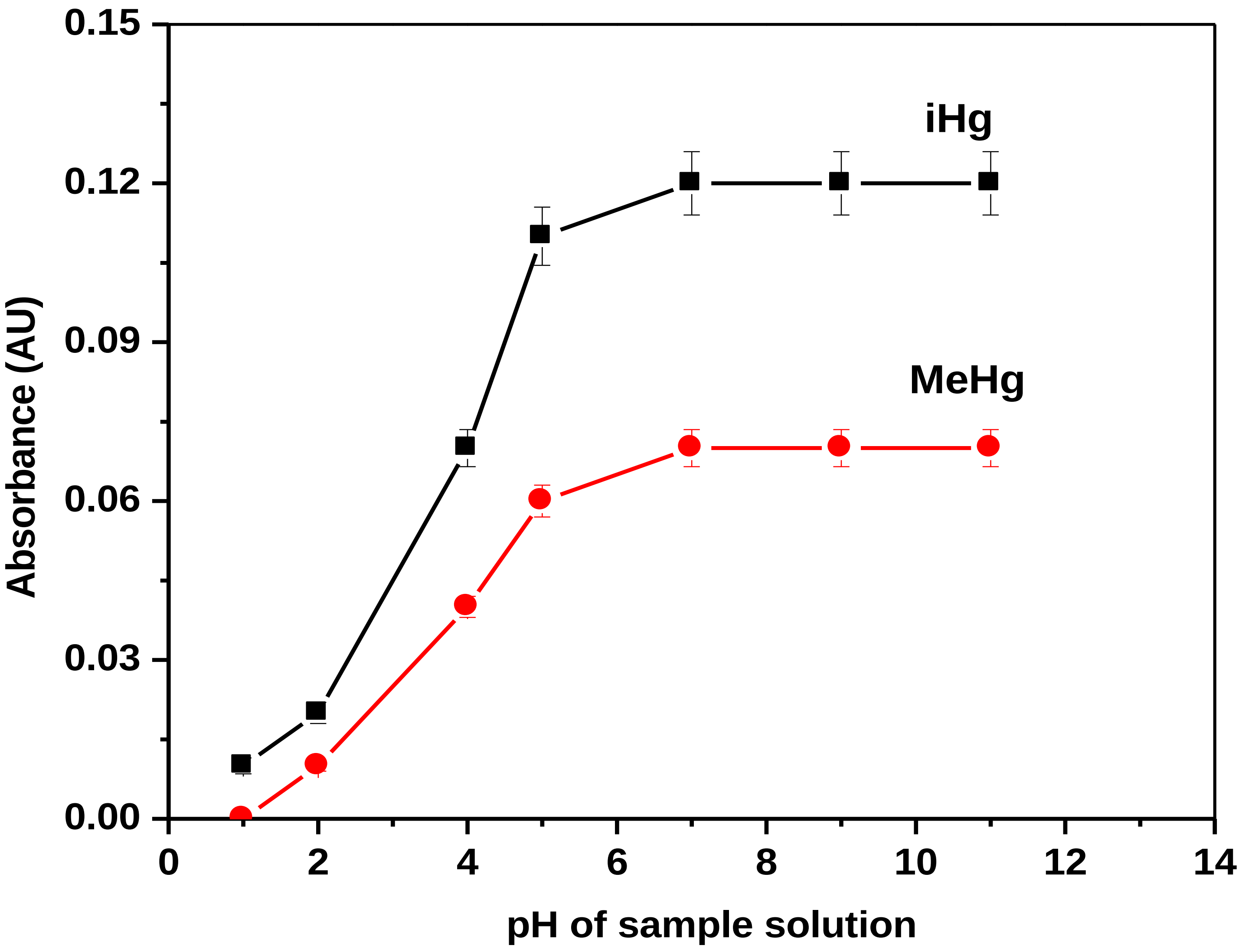
100
125
150
175
Amount of Cu-BSA NCs (µg)

Fig 3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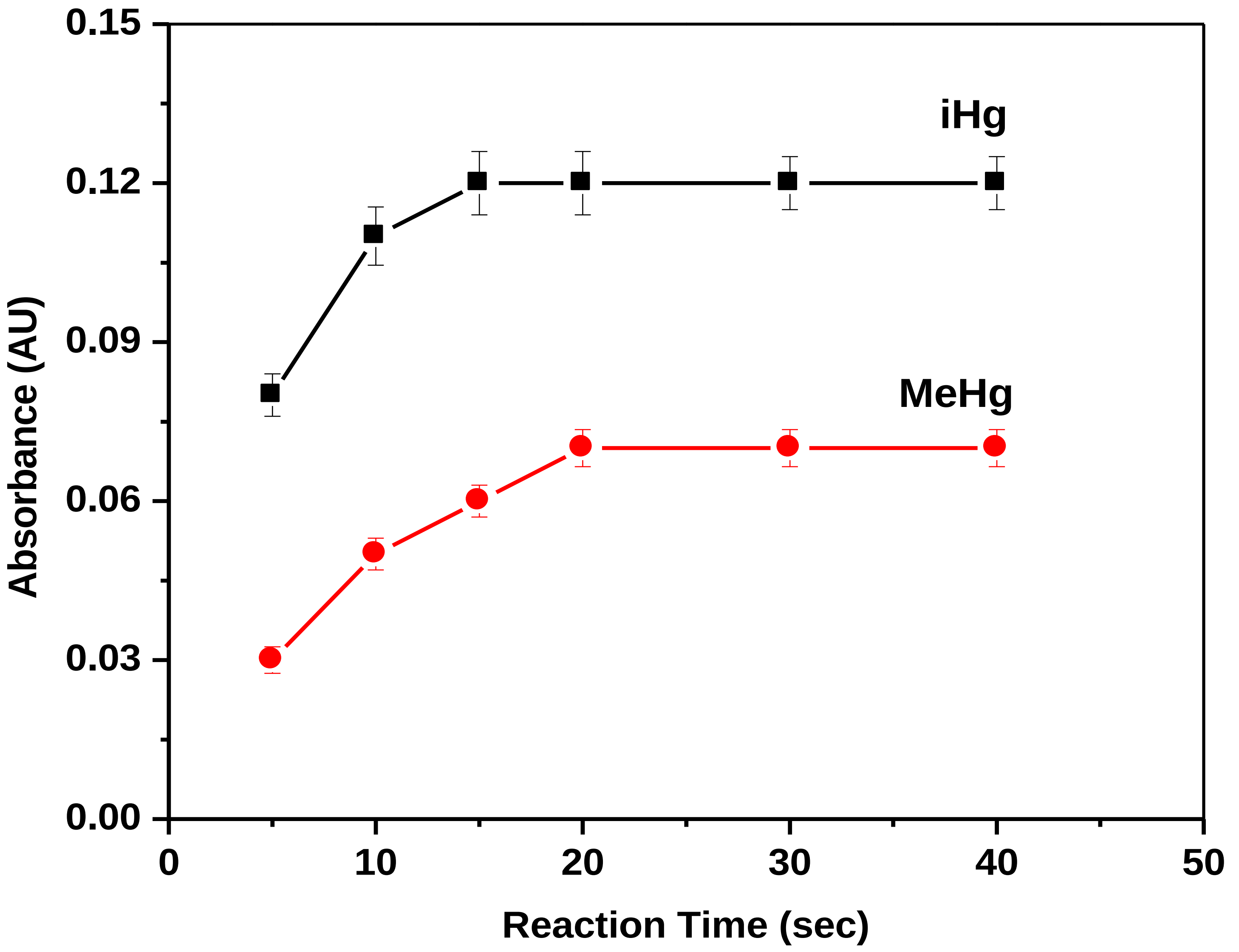
8
10
12
14
pH of sample solution

Fig 4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MeHg
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10
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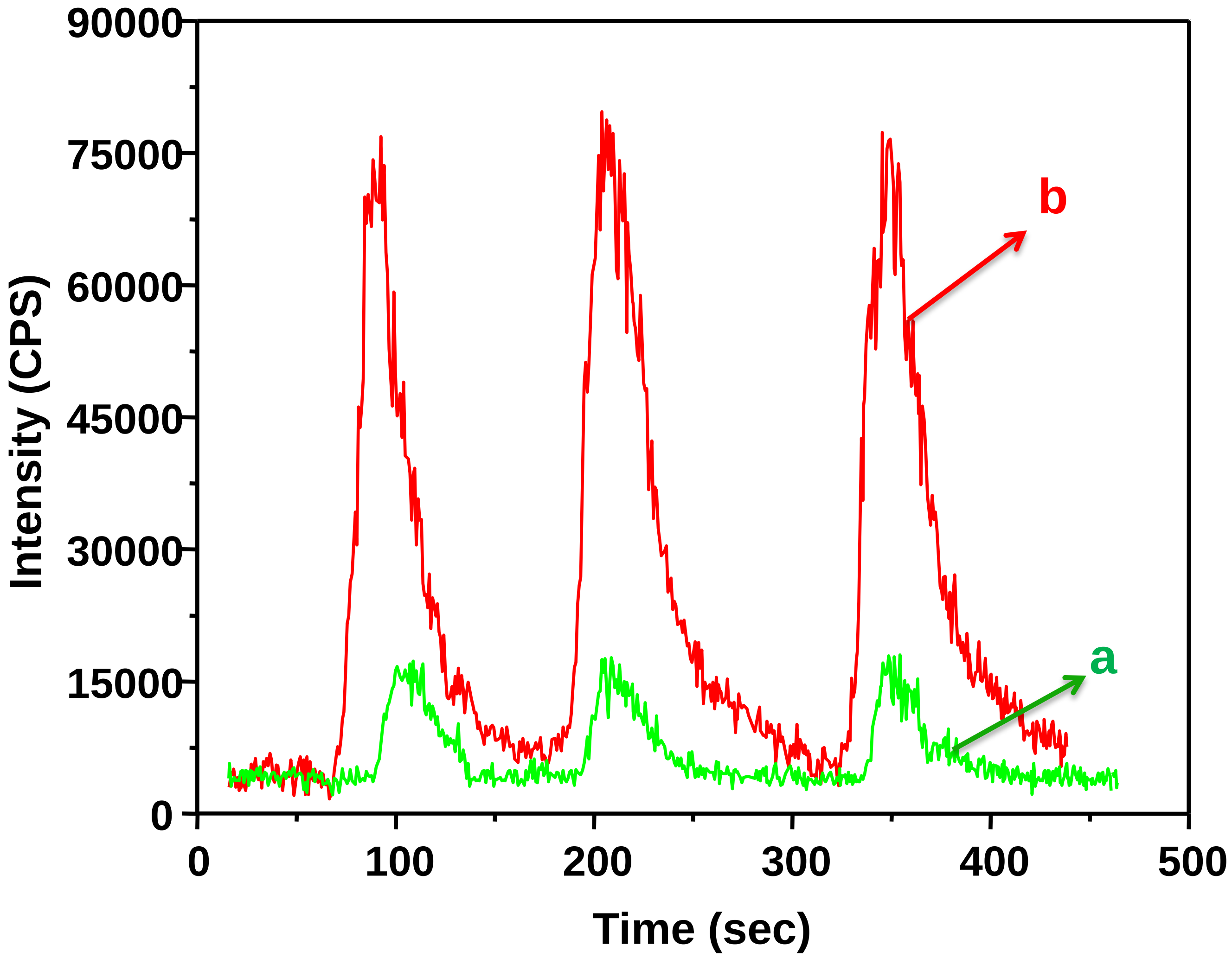
30
40
50
Reaction Time (sec)

Fig 5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100
200
300
400
500
Time (sec)
b
a

Fig 6

Table 1. Analytical response characteristics of mercury species with the proposed Cu-BSA NCs based vapour generation method

Medium	Type of mercury species	Response function		R^2	
		CVAAS Calibration range: 0-100 ng mL^{-1}	FI-ICPMS Calibration range: 0-5 ng mL^{-1}	CVAAS	FI-ICPMS
Milli-Q water	Inorganic Mercury	y = 0.067x- 0.021	y = 42608 x –125	0.998	0.999
	Methyl Mercury	y = 0.024x - 0.002	y = 31284 x + 7198	0.997	0.999
Ground Water	Inorganic Mercury	y = 0.063x - 0.005	y = 41649 x + 6043	0.998	0.999
	Methyl Mercury	y = 0.023x - 0.005	y = 30472 x -970	0.998	0.999

Table 2: Comparison of detection limits obtained by the present Cu-BSA NCs based vapour generation method with the recently reported methods

Species and sample matrix	Method and mode of detection	Detection limit	Reference
iHg and MeHg; Natural waters	Speciation using polyaniline column coupled to FI-CVG-ICPMS	2.52 pg and 3.24 pg (absolute)	36
Pb, Cd and Hg; natural and sea-waters	Chelating resin column for preconcentration and FI-CVG-ICPMS	0.012 ng mL^{-1} for Hg	46
Total Hg; Waters	Electrochemical cold vapour generation coupled to microchip microwave induced helium plasma OES	1.1 ng mL^{-1}	47
Total Hg; Natural Waters	UV-Vapour generation AFS by on-line solid phase extraction	0.03 ng L^{-1}	25
Total Hg; Alcoholic drinks	ICPMS after matrix-assisted photochemical vapour generation	0.003 ng mL^{-1}	48
Hg; Natural waters	On-line UV photoreduction by FI-CVAAS	0.3 ng mL^{-1}	29
Hg; Marine sediment and Fish tissues	Photo- and themo-chemical VG of mercury after solubilization in formic acid	0.68 ng absolute	22
Hg; Liquid samples	Ultrasound-promoted CVG in the presence of formic acid and AAS	0.1 ng mL^{-1}	28
Hg; Waters	Cu-BSA NCs based VG approach and FI-ICPMS	0.004 ng mL^{-1}	Present work

Table.3. Analysis of various water samples for total mercury using Cu-BSA NCs based vapour generation approach

Type of water sample	Found (ng mL^{-1})		
	Present Method		Direct Nebulization by ICP-MS
	CVAAS	FI-ICPMS	
Hussain Sagar lake water-1	0.93±0.04	0.95±0.02	0.91±0.05
Hussain Sagar lake water-2	0.86±0.04	0.85±0.03	0.85±0.04
Hussain Sagar lake water-3	0.79±0.03	0.76±0.04	0.79±0.05
Water sample collected 100 m away from battery factory	14.96±0.54	14.88±0.51	15.11±0.55
Water sample collected 200 m away from battery factory	12.87±0.44	12.94±0.48	12.6±0.52
Water sample collected 400 m away from battery factory	10.96±0.46	11.37±0.44	11.25±0.51