Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Data

For

Simultaneous determination of heavy metals in biological samples by multiple-template imprinting technique: An electrochemical study

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Table S1: Interaction energies (ΔE, kJ mol^{-1}) of various monomer-template complexes in gas phase.

Monomer	Copper (II)	Cadmium (II)	Lead (II)
Lysine	−19.29	−17.31	−14.23
Alanine	−60.41	−57.13	−56.78
Glutamic Acid	−48.67	−43.87	−41.93
Histidine	−44.47	−39.65	−38.91
Cysteine	−48.48	−41.82	−48.48
Arginine	−127.71	−118.92	−112.09
Tyrosine	−33.05	−30.74	−28.93
2xArginine	−234.91	−230.01	−225.68
3xArginine	−189.45	−182.65	−176.92

Table S2: Comparative study between HF and ONIOM method for interaction energy of monomer-template complex along with MWCNTs in kcal mol^{-1}.

Compound name	HF method		ONIOM method	
	Interaction energy	Time (h)	Interaction energy	Time (h)
Cu^{+2}-$(Arginine)_2$-MWCNTs	−572.91	172	−586.48	23
Cd^{+2}-$(Arginine)_2$-MWCNTs	−561.43	168	−572.64	22
Pb^{+2}-$(Arginine)_2$-MWCNTs	−556.04	168	−562.98	23

Table S3: Validation of proposed method by Student's t-test and F-test

S.N.	Metal ions	T-test value (Reported)*	T-test value (Calculated)	F-test value (Reported)*	F-test value (Calculated)
1.	Copper	4.30	0.00075	9.27	0.0055
2.	Cadmium	4.30	0.0015	9.27	0.01
3.	Lead	4.30	0.018	9.27	0.004

*At 95% confidence limit.

Table S4: Comparison of proposed method with standard technique (AAS) for determination of metal ions in various samples.

S. N.	Sample*	Copper (mg)			Cadmium (mg)			Lead (mg)		
		Satndard Method	Our method	% Error	Standard Method	Our method	% Error	Satndard Method	Our method	% Error
1.	Soil	1.30	1.32	1.54	2.79	2.80	0.36	0.160	0.160	0.00
2.	Water	1.44	1.45	0.69	2.80	2.79	0.36	0.129	0.130	0.77
3.	Tomato Leaf	1.28	1.28	0.00	2.20	2.21	0.45	0.139	0.140	0.72
4.	Tomato Fruit	1.29	1.28	0.77	2.29	2.28	0.44	0.189	0.190	0.53

* Samples taken were from coal mine area was used.

S.1. UV-visible spectroscopic evaluation of monomer template interaction

UV-visible spectroscopic analysis provides a means for determining the extent of monomer-template binding interaction as well as it also prove the extent of complex formation between arginine and three different metal ions. The spectra for the reaction mixture are shown in Figure S1 (A to C). The results showed that addition of metal ions like Cd^{2+}, Cu^{2+} and Pb^{2+} caused apparent spectral changes due to the strong interaction between arginine and metal cation. Among the investigated cations, the arginine has stronger interaction with Cu^{2+} and weakest with Pb^{2+} ions because binding interactions depends upon radius of the metal ions [S1]. According to the literature, the radius of Cu^{2+}, Cd^{2+} and Pb^{2+} are 73, 95 and 119 pm respectively [S1]. Smaller the radius of metal ions greater will be binding with MIP. The above mentioned fact is further prooved by computational data given in Table S1, where inetraction energy is highest for Cu (II) complex and lowest for Pb (II) complex with arginine.

Spectroscopic evaluation of the interaction between different template and monomers was conducted in water medium. So, UV-visible spectroscopic evaluationconfirms the presence of the metal in the complex and strong binding between arginine and metal ions. The expected binding mechanism between metal ions, MWCNTs and MIP cavities are shown in Scheme 2 in main manuscript.

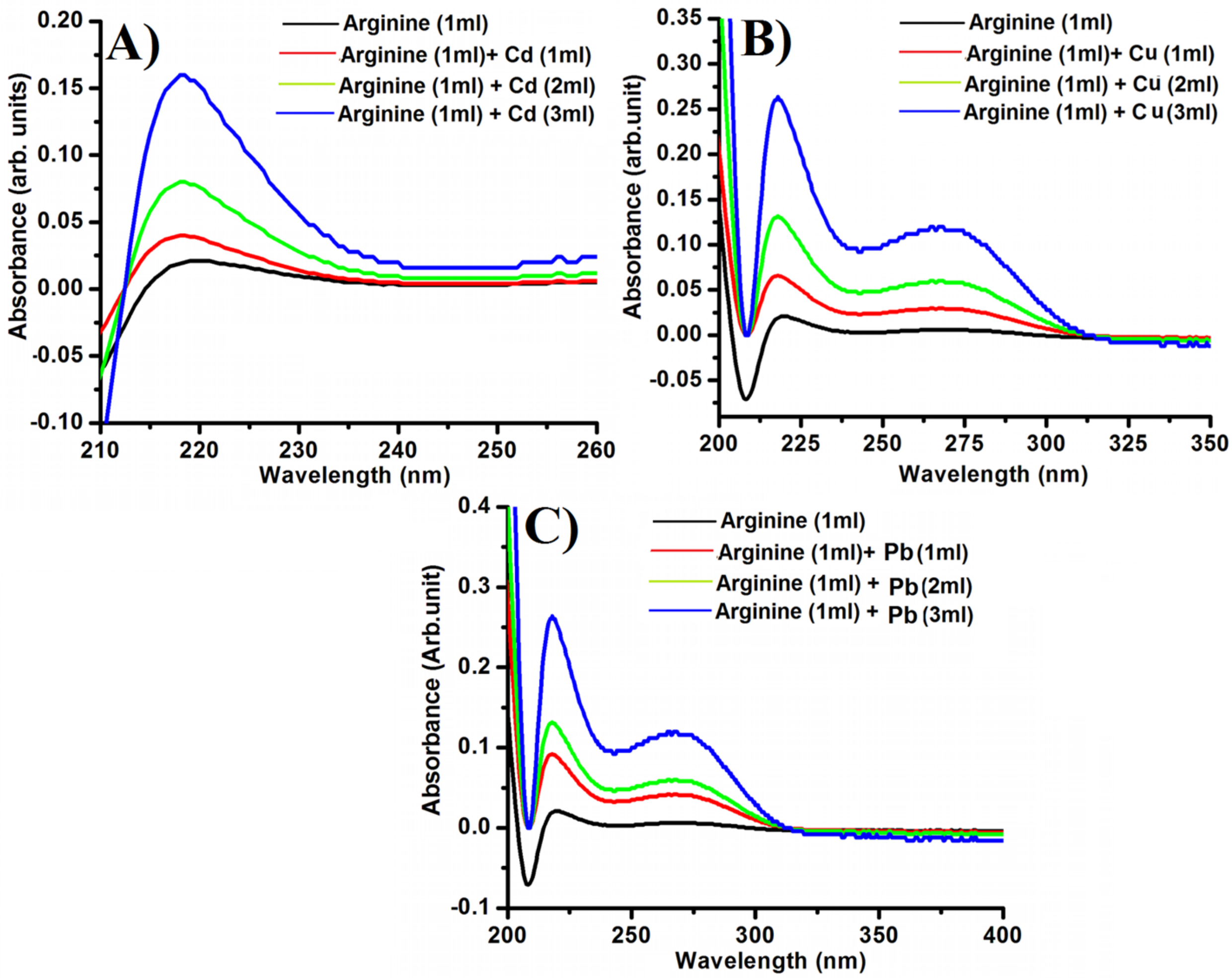

Figure S1: UV-visible spectra for mixture of monomer (arginine) and template (Cd^{+2}, A; Cu^{+2}, B and Pb^{+2}, C) in the mole ratio 1:1, 1:2 and 1:3.

S.2. Selection of appropriate conditions for preparation of the MWCNTs modified PGE electrode

In order to obtain an optimal recognition film, the influences of electropolymerization conditions on the response of the sensor to metal ions were investigated. The thickness of the composite layer has an important impact on the electrochemical responses of the modified electrode. Since the electron transfer takes place at the interface of a solid phase (i.e. electrode) and a liquid phase (i.e. electrolyte), the film thickness should be optimized to obtain the best performance for the modified PGE. In order to study the effect of film thickness, the different number of CV cycles (number 5 to 12) was casted on PGE surface to form the 'sensing' layer with a series of experiments. With increasing the number of cycles till ten, the accessible specific surface area of PGE was increased. By further increasing the number of CV cycles, the peak current decreases due to the formation of more thicker sensing film which provides a less accessible imprinted site. So, the maximum current response of MIP electrode for metals was obtained by applying ten cycles for electropolymerization. Therefore, the optimum polymerization cycle was found to be ten (Fig. S2, A).

For optimization of the extraction cycle of template, different numbers of cycles (3-12 cycles) were applied to extract the template molecule from the polymeric film with 10ml phosphate buffer solution in the potential range from +2.5 to -2.0V. The best response of current for the electrode was obtained after the 5th cycles, implies the optimized extraction cycle for the template removal (Fig. S2, B).

The concentration of arginine has an obvious effect for the response of PGE. There is a sharp increase in the peak current with the increase of arginine concentration from 0.05 to 0.1mM and then decreases, getting a highest peak current at the concentration 0.1mM. Thus, we were proceeding with 0.1 mM arginine for further study (Fig. S2, C).

The influence of pH value on the current response of 0.5 mM Cd (II), Cu (II) and Pb (II) respectively at the PGE was examined by DPSV with 0.03 M buffer solution in the pH range from 2-10 which depicts that current increases with the increase in pH value from 2.0 to 7.0 and then decreases with an increasing pH from 7.0 to 10.0 as at higher pH, the metal ions started to hydrolyse due to the kinetic reasons [S2]. Therefore, the optimum pH of the solution was set at 7.0 (Figure S2, D).

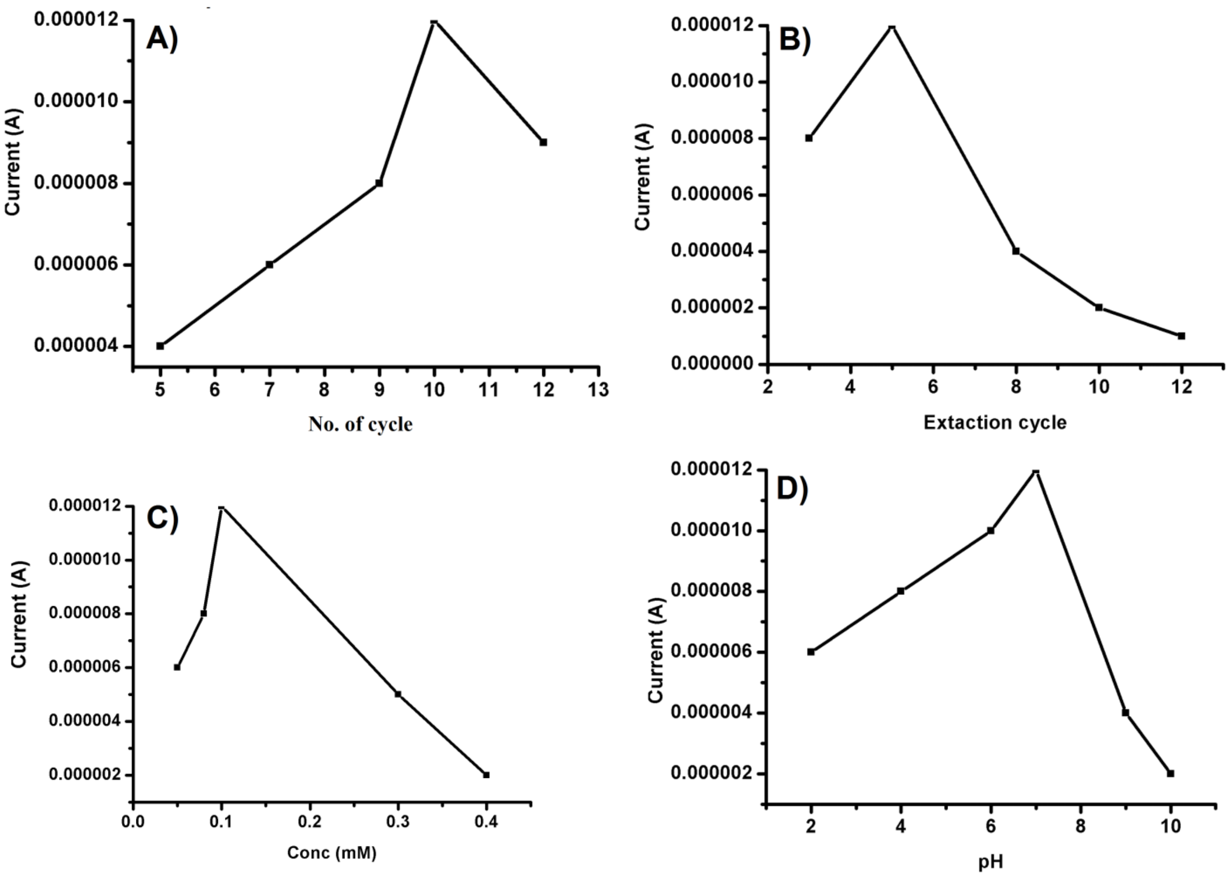

Figure S2: Optimization of different parameters affecting sensor development: A) number of cycle for electropolymerization of arginine, B) number of cycle for extraction of metal ions, C) concentration of monomer and D) pH versus current.

S.3. Reproducibility, repeatability and storage stability of the imprinted sensor

After studying all the practical aspect of the proposed sensor, one important parameter in the sensor fabrication is their reproducibility and durability. To investigate the reproducibility of the prepared sensor, a known concentration of metal ions (20µM each) was measured by three modified electrodes, which were prepared independently under identical experimental conditions. The response shows a RSD of 1.8%, which indicates an excellent reproducibility during electrode fabrication. To measure the repeatability of the proposed sensor six consecutive runs were taken for the same concentration of the metal ions (20µM each) and the calculated RSD was found to be about 0.9%, which confirms the reproducibility in results. Furthermore, when the proposed sensor was stored in air at room temperature, the sensor can retain 95.5% of its initial response after 45 days. These results indicate that the proposed sensor has acceptable reproducibility and good stability for the detection of the metal ions.

References:

S1. J. A. Dean, N. A. Lange, Lange's handbook of chemistry, Fifteenth edition, McGraw-Hill, 1999.

S2. A. A. Afkhami, F. Soltani-Felehgari, T. Madrakian, H. Ghaedi, M. Rezaeivala, Fabrication and application of a new modified electrochemical sensor using nano-silica and a newly synthesized Schiff base for simultaneous determination of Cd^{2+}, Cu^{2+} and Hg^{2+} ions in water and some foodstuff samples, Anal. Chim. Acta 771 (2013) 21– 30.