Molecular recognition and detection of Pb(II) ions in water by aminobenzo-18-crown-6 immobilised onto a nanostructured SERS substrate

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Abstract

In this work, we propose a new sensitive, selective and portable surface enhanced Raman spectroscopy (SERS) methodology for the rapid on site detection of Pb(II) pollution in water. The new method utilises aminobenzo-18-crown-6 (AB18C6) as a selective recognition molecule to form a spontaneous complex with Pb(II) ions. The formed AB18C6-Pb(II) complex was rapidly immobilised onto a nanostructured gold substrate via Au-N bond formation and reproducibly screened by SERS using a handheld Raman device. For the SERS measurements, a substrate was fabricated by electrochemical deposition of gold nanostructures onto a flat gold disc, creating multiple hotspots for ultrasensitive SERS measurements. The limit of quantification (LOQ) for Pb(II) ions by the SERS method was 2.20 pM. The limit of detection (LOD) was 0.69 pM which is five orders of magnitude lower than the maximum Pb(II) level of 72 nM allowed by the US Environmental Protection Agency. The high sensitivity of the SERS substrate is attributed to the coupling between the Surface Plasmon Polariton (SPP) of its gold surface, the localised Surface Plasmon Resonance (SPR) of the gold nanostructures and the Raman radiation from the immobilised AB18C6-Pb(II) complex. The new SERS detection method was successfully applied for the selective and rapid screening of Pb(II) ion contamination in water proving its practical application for environmental analysis.

Keywords: SERS Nanosensor, Nanostructured gold substrate, Field deployable Selective Pb(II) detector

1. INTRODUCTION

There is a continuous search for rapid, selective, and sensitive techniques for the in-field screening of Pb(II) ions in the environment [1]. The toxicity of Pb(II) ranges from chronic inflammation of the kidney and heart to inhibited brain development and poor nerve conduction [2]. Though traditional methods such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Atomic Emission spectroscopy (ICP-AES) are useful for the detection of these toxins, they are usually not suitable for on-site applications since they require sophisticated instruments with significant footprints [3]. Other detection methods such as fluorescence spectroscopy [4], UV visible spectroscopy [5] and dynamic light scattering [6] have been proposed in the literature for the detection of Pb(II) ions. However, these methods suffer from disadvantages such as poor selectivity, interferences from spectral, chemical and background, low sensitivity at trace concentrations and lack of optimisation for the in-field screening of Pb(II) ions [7, 8].

Surface enhanced Raman spectroscopy (SERS) is a very sensitive mode of Raman spectroscopy where an enhanced electromagnetic field near the surface of a metal nanostructure causes significant amplification of the inherently weak Raman signal of analytes by several orders of magnitude [9]. This significant enhancement of the Raman signal allows for the detection of analytes at ultra-low concentrations down to the single molecule level. In addition, SERS can be used for the multiplexed analysis of multiple analytes in a sample [10]. Furthermore, with the emergence of commercial handheld Raman spectrometers and sensitive SERS substrates, in-field screening of environmental pollutants has been made easier [11]. Hence, SERS based nanosensors have attracted much attention over the past decade.

Metal ions are generally known to have a small Raman scattering cross-section and, in most cases, lack vibrational modes. Therefore, the direct detection of heavy metal ions by SERS is challenging and the use of Raman active recognition molecules that can bind these metal ions to the surfaces of SERS substrates is required [12, 13]. For this reason, SERS detection of Pb(II) ions by recognition molecules such as nucleic acid sequences [14], 4-(2-pyridylazo)resorcinol [15] and citrates [16] have been demonstrated in the literature where observable changes in the Raman fingerprint of these recognition molecules were used to indicate the presence of Pb(II) ions in samples. However, some of these recognition molecules may have poor selectivity towards the metal ion and their immobilisation onto the surface of SERS substrates is challenging.

Crown ethers and their benzo derivatives are Raman active ionophores that spontaneously form stable complexes with metal ions and, in many instances, do so selectively due to their unique coordination chemistry with the ion of interest [17]. For example, we recently demonstrated the use of aminodibenzo-18-crown-6 (ADB18C6) for the detection of Hg(II) ions in water [18]. The availability of electron donor atoms in their molecular structure facilitates their immobilisation on noble metal substrates such as gold via Au-O, Au-N, and Au-S bond formation [18].

The in-field SERS detection of metal ions requires substrates that can produce high SERS enhancement factors and be easily manufactured at low cost with a potential to reuse the substrate for multiple sample tests. We previously demonstrated a nanostructured gold disc electrode, that was fabricated by a cost-effective and relatively simple electrochemical method, providing very high SERS enhancement factors due to the presence of multiple hotspots on its surface [18, 19]. The substrate was also characterised by high SERS signal reproducibility due to the very low relative standard deviation in SERS measurements that were obtained. Therefore, in the current work, this substrate was utilised in combination with aminobenzo-18-crown-6 (AB18C6) for the SERS detection of Pb(II) ions. The aim of the work is to develop a simple and ultra-sensitive method for the rapid detection of Pb(II) ions by SERS for the in-field environmental monitoring of Pb(II) contamination. Additionally, the new SERS detection method is designed to offer high selectivity towards Pb(II) ions over other metal ions. Besides the ease of transporting this SERS substrate, it can be easily recycled by a simple and rapid electrochemical cleaning procedure and used for repeated SERS measurements. This new method can therefore be used for on-site detection of Pb(II) contamination with a handheld Raman device. To demonstrate the potential of the new method for real time analysis, it was utilised for the determination of Pb(II) in drinking water, spiked with lead, at ultra-trace concentration levels down to 0.97 pM which is significantly

lower than the maximum allowable concentration (72 nM) for Pb(II) ions in drinking water [20].

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All reagents and solvents were of analytical grade and used without further purification. Sulfuric acid (98%), potassium hydroxide, hydrogen peroxide solution (30%), 4-amino-benzo -18-crown-6 (AB18C6), gold (III) chloride ($HAuCl_4$), dimethyl sulphoxide (DMSO), $Pb(NO_3)_2$, $Cd(NO_3)_2$, $NaNO_3$, $Hg(NO_3)_2$, $Ca(NO_3)_2.4H_2O$, $CuSO_4$, BaO, KCl, LiCl, $NiCl_2.6H_2O$ and $CoCl_2.6 H_2O$, were all purchased from Sigma-Aldrich (USA). All solutions were prepared using ultra-pure deionised water (18.2MΩ.cm@25°C, Milli-Q).

2.2. Instrumentation

The nanostructured gold substrate was characterised by Scanning Electron Microscopy (SEM) to determine the geometry, size and distribution of the developed gold nanostructures. UV absorption spectra were recorded using a Cary 60 UV–visible spectrophotometer (Agilent Technologies, USA). Fluorescence measurements were carried out using a Fluorescence Cary eclipse spectrophotometer. All electrochemical studies were conducted using a µAutolab potentiostat (Metrohm Autolab) with a custom made three-electrode cell setup. SERS measurements were carried out using a handheld ID Raman Mini2 (ocean optics, USA).

2.3. Study of AB18C6-Pb(II) complex formation by fluorescence spectroscopy

The complex formation between AB18C6 and Pb(II) was investigated by fluorescence spectroscopy. For the fluorescence measurements, 1 mL of Pb(II) aqueous solutions in the concentration range of 1 µM to 800 µM was added to 1 mL of 1 mM AB18C6 (in DMSO: H_2O, 1:1 v/v) solution. The pH of the formed AB18C6-Pb(II) was 4.5. Aliquots of the formed complex, at different Pb concentrations, were transferred into fluorescence-free quartz cuvettes and their fluorescence emission was measured between 296 and 800 nm using an excitation wavelength of 295 nm. The K_f value for the complexation reaction was also calculated using the expression given by Shah et al [21] and 1pM Pb(II) ions in 10 µM AB18C6 solution.

2.4. Study of AB18C6 selectivity towards Pb(II) by UV-Vis spectroscopy

To determine the selectivity of AB18C6 towards Pb (II) over other environmentally relevant metal ions, 1 mL aliquots of 1 mM Pb(II), Cd(II), Cu(II), Ca(II), K(I), Ba(II), Li(I), Ni(II), Co(II), Na(I) and Hg(II) were added to 1 mL of 1 mM AB18C6 solution (in DMSO: H_2O, 1:1 v/v) and the UV absorption spectra of the prepared mixtures were acquired within the wavelength range 200 to 600 nm.

2.5. Electrochemical deposition of gold nanostructures on gold substrate

A gold disc electrode cut into dimensions with geometric area of 8 mm was used as a substrate to electrochemically deposit gold nanostructures on its surface. The surface of the gold substrate was polished with 0.5, 0.3 and 0.05 µm grain sized alumina slurry,
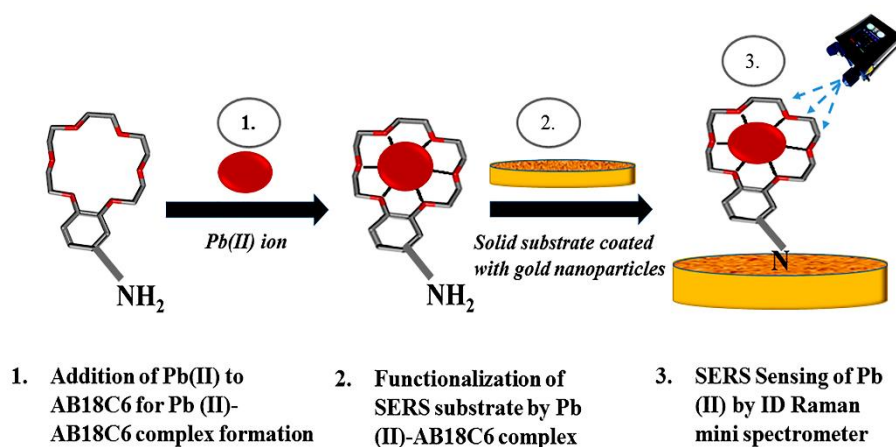
respectively, and rinsed with water and sonicated in deionised water for 15 minutes to remove all the physically adsorbed alumina slurry. The polished substrate was then soaked in piranha solution [(H_2SO_4 (98%): H_2O_2 (30%), 3:1 v/v] for 10 minutes and rinsed with deionised water. $HAuCl_4$ solution (4 mM in 0.1 M $HClO_4$) was used for the electrochemical deposition of gold nanostructures using the method described in our previous studies [18] to achieve a high number of deposited nanostructures per surface area. After the electrochemical deposition, the substrate was treated with oxygen plasma for 15 minutes to remove adsorbed organic contaminants. The nanostructured gold substrate was characterised and used for the SERS detection of Pb(II) ions.

2.6. *SERS detection of Pb (II) using AB18C6 crown ether and nanostructured gold substrate*

2 mL of 5 µM AB18C6 (in DMSO: deionised water 1:1 v/v) were mixed with 2 mL of 5 µM Pb(II) aqueous solution, to spontaneously form a complex between the lead ion and the crown ether. 1 mL of the formed complex was loaded onto the gold nanostructured substrates and incubated overnight to form a self-assembled monolayer of the crown ether-Pb(II) complex on the nanostructured substrate. The substrate was then rinsed with deionised water and screened by SERS to acquire the spectrum of the AB18C6-Pb(II) complex. The AB18C6-Pb(II) complex formation and its immobilisation onto the gold nanostructured substrate for SERS screening by handheld Raman spectrometer is depicted in scheme 1.

For comparison purposes, 1 mL of the crown ether only was loaded onto another gold nanostructured substrate, incubated overnight, rinsed with deionised water and screened to acquire the Raman spectrum of the crown ether alone.

The SERS measurements were carried out by a handheld Raman spectrometer at an excitation wavelength of 785 nm, and a laser power of 5 mW. The sample interrogation time was 2 seconds at 7 accumulations. The spectra were collected in the raster orbital scanning mode over a wavelength range of 400 cm^{-1} to 2000 cm^{-1}. In this mode, spectra from a large area of the sample is acquired at low average power while the integrity of the sample is preserved. In addition, the Raman spectra were collected with the "reference spectrum" and the "clean peaks" modes enabled. These scanning modes remove ambient light, and fluorescence background using an automated built-in correction algorithm.

1. Addition of Pb(II) to AB18C6 for Pb (II)-AB18C6 complex formation
2. Functionalization of SERS substrate by Pb (II)-AB18C6 complex
3. SERS Sensing of Pb (II) by ID Raman mini spectrometer

Scheme 1. Schematic illustration of the formation of AB18C6-Pb(II) complex and its immobilisation onto the nanostructured gold substrate for the detection of Pb(II) ions by SERS.

2.7. SERS quantification of Pb(II) in water

For the quantitative analysis of Pb(II) by SERS, crown ether-Pb(II) complexes at various Pb(II) concentrations were prepared by mixing 2 mL of Pb(II) standard solution (from 1 µM to 1 pM) with 2 mL of the crown ether solution. Aliquots of the formed complex were loaded onto the nanostructured substrates to form a Au-N bond between the AB18C6-Pb(II) complex and the gold nanostructured substrate. The substrates were then rinsed with deionised water and screened by SERS. A calibration curve was plotted from the SERS signal intensity at 820 cm^{-1} versus concentrations of Pb(II) ions.

The detection limit and limit of quantification for Pb(II) ions by SERS were estimated by loading the gold nanostructured substrate with Pb(II)-AB18C6 (1 pM) complex and screening with the handheld Raman spectrometer (n = 7). After determining the concentration, the LOD and LOQ were calculated as the standard deviation (δ) x t$_{99\%}$ and δ x10 respectively (where t is the threshold value of student t-distribution with a degree of freedom (n - 1) at 99% confidence interval) [22, 23].

To detect Pb (II) contamination in the environment, 1 mL of drinking water containing 0.12 µM Pb(II) was mixed with 1 mL of AB18C6 solution to form a Pb(II)-AB18C6 complex. The resulting complex was then loaded onto the gold nanostructured substrate and allowed to stand for an hour. SERS measurements were carried out using the handheld Raman device (n=3). The concentration of Pb (II) in water was calculated from the calibration curve and the percent recovery determined using the following equation:

$$\% \text{ Recovery} = \frac{CPb(x) - CPb(b)}{CPb(s)} \; X \; 100$$

Where C*Pb(x)* is concentration of spiked Pb(II) in the water (calculated from the SERS calibration curve); C*Pb(b)* represents the concentration of Pb(II) in blank water (before spiking) and C*Pb(s)* is the known concentration of the spiked Pb (II) standard solution.

The drinking water containing 0.12µM Pb(II) was also analysed using ICP-MS. SERS spectra for AB18C6 in mixed metal ion aqueous solution (containing 1µM each of Cd(II), Cu(II), Ca(II), K(I), Ba(II), Li(I), Ni(II), Co(II), Na(I) and Hg(II)] was acquired before and after adding 1µM Pb(II) ions (ESI 1).

2.8. Regeneration of the nanostructured gold substrate

1 nM AB18C6-Pb(II) complex were prepared, loaded onto a nanostructured gold substrate and screened by SERS. To regenerate the substrate surface and re-use it for SERS screening of Pb(II) ions, the AB18C6-Pb(II) complex was electrochemically desorbed from the substrate. For this purpose, the substrate bearing the AB18C6-Pb(II) complex was connected as the working electrode in an electrochemical cell. The electrochemical desorption process was carried out in 0.1M KOH using Ag/AgCl (KCl sat.) as a reference electrode and a platinum wire as an auxiliary electrode. Repetitive scans (20 cycles) were carried out between 0.10 and -1.40 V at a scan rate of 0.1 V/s. Afterwards, the substrates were screened with the handheld Raman device to ascertain the presence or absence of the AB18C6-Pb(II) complex on the substrate's surface. After regeneration, a new aliquot of the AB18C6-Pb(II) complex was loaded onto the substrate and re-screened by the handheld Raman device.

3. RESULTS AND DISCUSSION

3.1. SERS Substrate characterisation and Properties

The morphology and size of the gold nanostructure on the gold substrate were studied by SEM at different magnifications (Fig. 1a). The SEM measurements showed a high density of spherical gold nanostructures (average size 10 and 100 nm) that are distributed all over the gold disc [19]. Therefore, the nanostructured gold disc has a high coverage of hot spots where the Raman spectrum of the analyte experiences significant electromagnetic field enhancement. This enhancement is due to the coupling between the Surface Plasmon Resonance (SPR) of the nanostructures, the Surface Plasmon Polariton (SPP) of the underlying gold and the Raman scattering of adsorbed molecules and leads to the ultra-sensitive SERS detection of analytes [19, 24, 25] (Fig 1b).
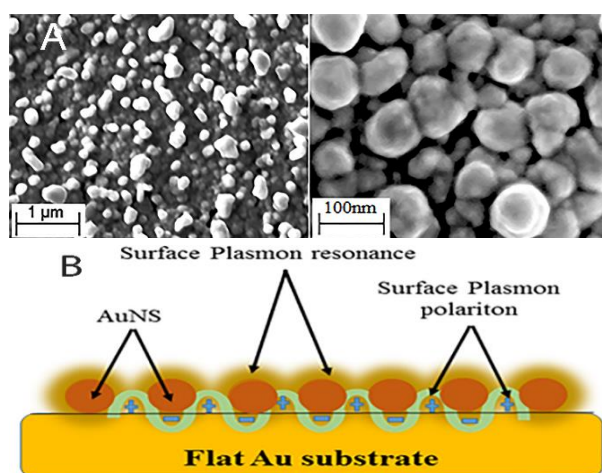

Fig. 1. (A) SEM images of gold nanostructures electrochemically deposited onto a flat solid Au material at different magnifications (B) Coupling of the SPP (from Au solid base material) and SPR (from gold nanostructures) for high SERS signals

3.2. AB18C6 and Pb (II) ion binding studies

Crown ethers and their derivatives have been reported to form complexes with heavy and transition metal cations through ion-dipole interaction with metal ions [26-28]. The benzo-18-crown-6 and its derivatives have been reported to selectively form stable complexes with Pb(II) ions [17, 29, 30]. This is attributed, in part, to the similarity of the host cavity size of the 18-crown-6 crown ether (2.2–3.2 A°) and the diameter of the Pb(II) ions (2.4 A°), which allows a stable complex to be formed [31]. In this work, the complex formation between AB18C6 and Pb(II) ions was studied by spectrofluorimetry. Fig. 2 shows the fluorescence spectra of AB18C6 in the presence and absence of Pb(II). In the absence of Pb(II), a strong band at 360 nm and a relatively weaker band at 582 nm were observed. The band at 360 nm was quenched while the band at 582 nm increased concomitantly with increasing Pb(II) concentration from 1 µM to 0.8 mM. This change in the crown ether spectra with the change of the Pb(II) ion concentration indicates complex formation between the crown ether and the metal ion. The calculated K_f value for this reaction was 1×10^5. This is similar to that reported by Rounaghi et al [32] using 18-Crown-6 to form a complex with pb(II).
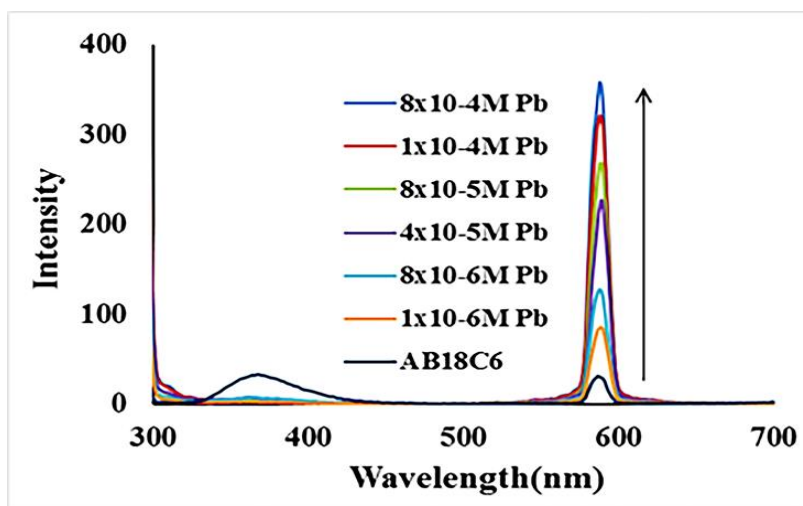
Fig. 2. Fluorescence spectra of AB18C6-Pb(II) complex at different concentrations of Pb (II) ions.

3.3. *Selectivity and interference studies*

The selectivity of AB18C6 towards Pb(II) ions was investigated by reacting the crown ether with Pb(II) ions and other environmentally relevant ions: K(I), Li(I), Na(I), Mg(II), Ba(II), Ca(II), Cd(II), Mn(II), Co(II), Cu(II), Hg(II), and Ni(II). The reaction mixtures were then screened by UV-Vis spectroscopy to monitor any changes in the UV-visible spectrum of AB18C6. The crown ether showed a characteristic absorption band at 295 nm (Fig. 3), which is attributed to the n→π* transitions between the electron lone pairs of the oxygen atoms and the amino benzene moiety of the crown ether [33, 34]. As shown by Fig. 3, the crown ether absorption band at 295 nm was quenched with the emergence of another band at 282 nm only in the presence of the Pb(II) ions. This change in the crown ether UV-visible spectrum is attributed to the formation of an ABC186-Pb(II) complex where the metal ion acts as a Lewis acid to withdraw the electron density from the oxygen atoms of the crown ether and cause the n→π* transitions within the formed complex to occur at higher energy [35]. The other metal ions had no significant effect on the crown ether absorption due to their inability to form a stable complex with AB18C6.
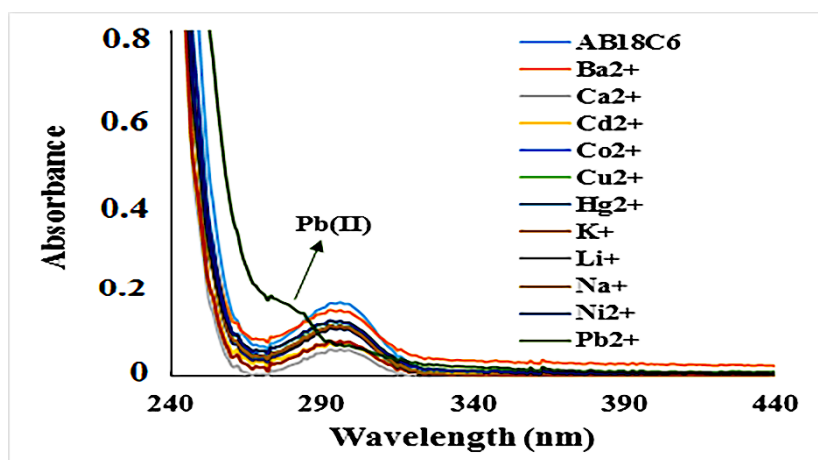

Fig. 3. UV-visible absorption spectra of mono and divalent ions after interaction with AB18C6

3.4. Detection of Pb (II) by SERS

To test the feasibility of the nanostructured gold substrate as a SERS sensor for Pb(II) ions, equal volumes of equimolar solutions of the crown ether and Pb(II) were mixed to form a complex in solution. The Pb(II)-AB18C6 complex was loaded on the gold nanostructured substrate to form a self-assembled monolayer where the amino group of the crown ether binds to the gold nanostructures of the substrate. The SERS spectrum of the AB18C6-Pb(II) complex was acquired and compared to that of the bare substrate (Fig. 4). The Raman spectrum of the AB18C6 crown ether showed vibration modes at 589 cm^{-1}, 628 cm^{-1}, 670 cm^{-1}, 720 cm^{-1}, 740 cm^{-1}, 785 cm^{-1}, 910 cm^{-1}, 985 cm^{-1}, 1011 cm^{-1}, 1047 cm^{-1}, 1124 cm^{-1}, 1185 cm^{-1}, 1271 cm^{-1}, 1349 cm^{-1} 1427 cm^{-1}, 1575 cm^{-1}, 1584 cm^{-1} and 1602 cm^{-1}. The band assignments for these vibration modes are given in Table 1. As indicated by Fig. 3 and Table 1, when AB18C6 is reacted with the Pb(II) solution, many of the crown ether vibration modes shift to higher wavenumbers. In addition new bands at 820 cm^{-1}, 958 cm^{-1}, 1149 cm^{-1}, 1284 cm^{-1}, 1440 cm^{-1}, 1458 cm^{-1}, 1483 cm^{-1} appear. The band at 820 cm^{-1} can be attributed to the complex formation between the crown ether and the divalent lead ion [36]. The bands at 958 cm^{-1}, 1149 cm^{-1}, 1284 cm^{-1}, 1440 cm^{-1}, 1458 cm^{-1}, 1483 cm^{-1} may be attributed to conformational changes of the crown ether moiety upon binding with the lead ion [37].
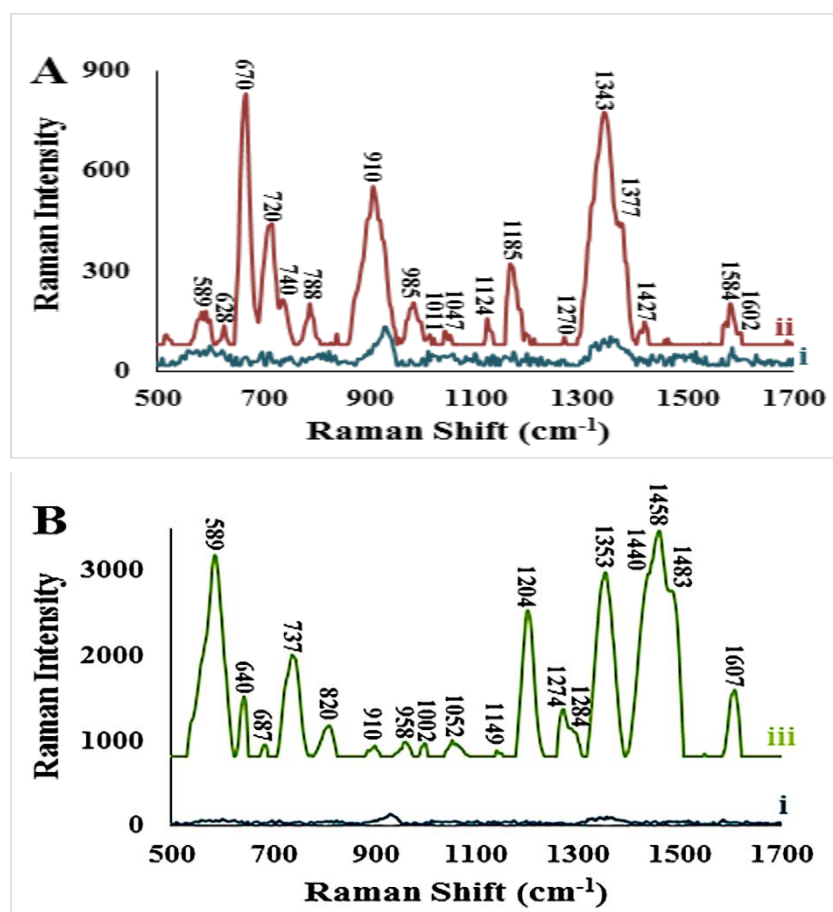

Fig. 4A: SERS spectra of (i) nanostructured gold substrate, (ii) AB18C6 crown ether and **B:** (iii) AB18C6-Pb(II) complex

Table 1

SERS wavenumbers/cm of Pb(II)- AB18C6 complex and AB18C6 and their band assignments

Raman shift/cm		Band assignment		References
AB18C6	Pb(II)-AB18C6	AB18C6	Pb(II)-AB18C6	
589	589	C-C-O deformation C-O-C bending Ring deformation	C-C-O deformation C-O-C bending	[38]
628	640	Ring deformation	Ring deformation	[39, 40]
670	687	ring deformation	ring deformation	[39, 41]
720	-	ring deformation , CH wag		[39, 40]
740	737	CH bend	CH bend	[40, 41]
788	-	CH, ring breathing		[39, 40]
-	820		CH_2 rock, crown ether-lead complex	[36]
910	910	CH bend, C-C- stretch, C-O stretch	CH bend, C-C- stretch, C-O stretch	[36, 42]
-	958		CH_2 rock, C-C, C-O vibrations in D_{3d} conformer of the crown ring	[43]
985	-	CH_2 rock, C-O vibrations in C_i conformer of the crown ring		[36, 43, 44]
1011	1002	Ring breathing	Ring breathing	[41]
1047	1052	C-C, C-O vibrations in C_i conformer of the crown ring	C-C, C-O vibrations in C_i conformer of the crown ring	[43, 44]
1124	-	C-C, C-O vibrations of the crown ring		[36, 43, 44]
-	1149		CH_2 rock vibration in D_{3d} conformer of the crown ring	[43]
1185	1204	CH_2 wagging, CH bend	CH_2 wagging, CH bend	[41, 45]
1271	1274	CH_2 twist , C_i, D_{3d} conformers of the crown ring	CH_2 twist , C_i, D_{3d} conformers of the crown ring	[43]
	1284		CH_2 twist , CH_2 deformation in the $D3d$ conformers of the crown ring	[36, 43, 44]
1343, 1377	1353	CH bend and ring deformation	CH bend and ring deformation	[39-41]
1427	-	CH_2 wagging and CH_2 scissoring vibrations in C_i conformer of the crown ring		[43]
-	1440, 1458, 1483		CH_2 deformation in D_{3d} conformer of the crown ring	[36, 44]
1584		C-C		[42]
1602	1607	C-C, ring stretch	C-C,ring deformation and ring stretch	[39, 41, 42]

3.5. Reproducibility of the nanostructured gold substrate in the SERS detection of Pb(II)

To confirm the reproducibility of the nanostructured gold substrate for the Raman detection of Pb(II) ions, 100 µL aliquots of 1 pM AB18C6-Pb (II) complex were loaded on 7 independent nanostructured gold discs and screened by SERS. As indicated by Figure ESI 2,

matching Raman spectra with similar intensities were acquired by the handheld Raman spectrometer, thus confirming the reproducible SERS enhancement of the electrochemically prepared nanostructured gold substrates.

3.6. Recycling the nanostructured SERS substrate

In order to regenerate the nanostructured gold substrate for repeated SERS detection of Pb(II) ions, the deposited AB18C6-P(II) complex was desorbed from the substrate's surface by using cyclic voltammetry. Fig. 5 shows a clear cathodic desorption peak of the AB18C6-P(II) complex from the substrate surface at − 0.27 V. This peak at -0.27 V disappears after 20 cycles of cathodic desorption which indicates the complete removal of the adsorbed AB18C6-Pb(II) complex.
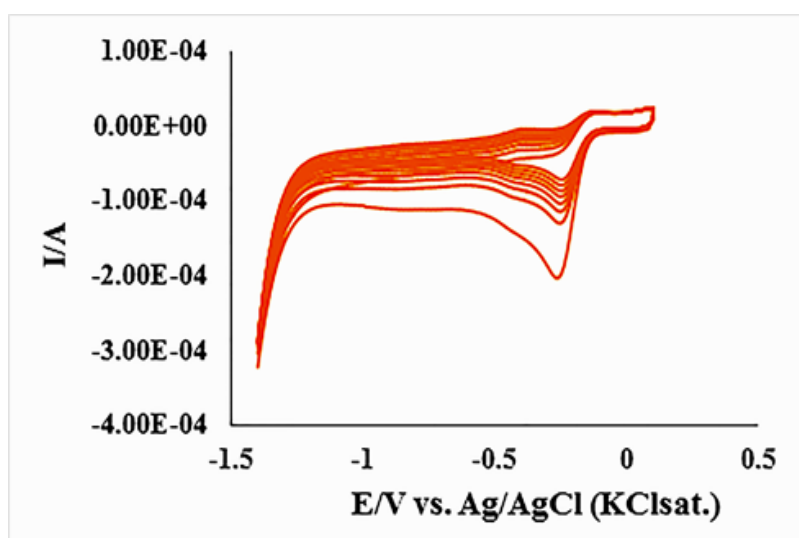

Fig. 5. Cyclic voltammograms between 0.10 and -1.40 V at a scan rate of 0.1 V/s for 10 µM AB18C6-Pb(II) functionalised substrate.

In order to confirm the removal of the AB18C6-Pb(II) complex, the substrate was screened by SERS before and after the electrochemical desorption process. The SERS spectra before and after desorption are shown in Fig. 6. It clearly indicates the disappearance of the AB18C6-Pb(II) complex Raman fingerprint. When a new aliquot of the complex was deposited on the substrate after its regeneration, the Raman fingerprint of the AB18C6-Pb(II) complex re-emerged (Fig. 6c) confirming that the electrochemical treatment did not compromise the SERS activity of the nanostructured substrate.
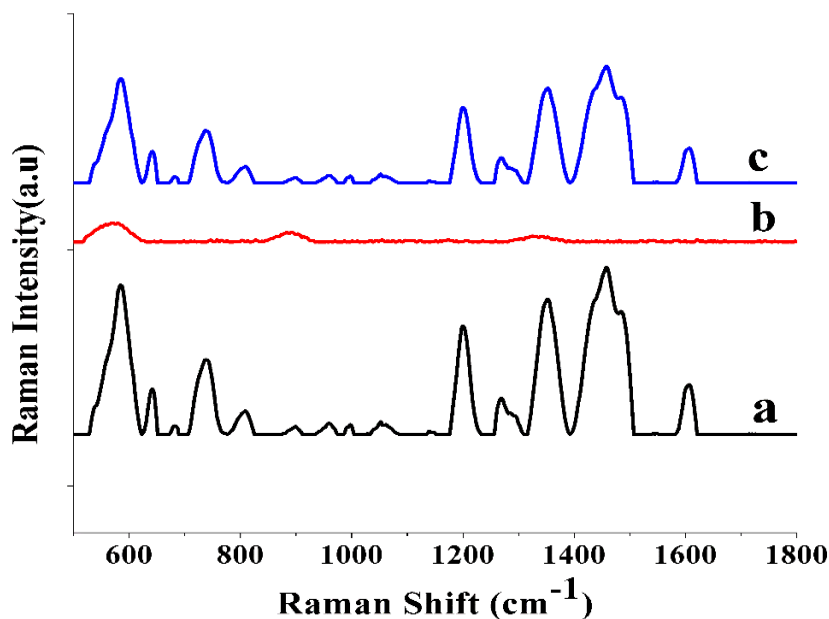
Fig. 6. (a) Raman spectrum of the AB18C6-Pb(II) complex on the nanostructured gold substrate before electrochemical desorption, (b) Raman spectrum of the substrate after desorption, (c) Re-emergence of the AB18C6-Pb(II) complex Raman fingerprint after depositing a new aliquot of the complex.

3.7. Quantitative SERS detection of Pb (II)

For quantitative detection of Pb(II) ions by SERS, the intensity of the AB18C6-Pb(II) complex Raman band at 820 cm^{-1} was recorded and found to be directly proportional to the concentration of Pb(II) ions (Fig. 7). A linear relationship was obtained between the SERS signal intensity and the corresponding log concentration of Pb(II) ions over the concentration range from 1×10^{-12} M to 1×10^{-6} M with a correlation coefficient (R^2) of 0.9742. The LOQ and LOD of Pb(II) ions by SERS were found to be 2.20×10^{-12} and 6.90×10^{-13} respectively (n=7), which are lower than the maximum allowable concentration (72 nM) for Pb(II) in drinking water as defined by the United States Environmental Protection Agency [20]. In addition, the new SERS detection platform for Pb(II) ions by AB18C6 and the gold nanostructured substrate showed significantly improved sensitivity when compared to other Pb (II) detection methods that utilise 18 crown 6 derivatives (Table 2).
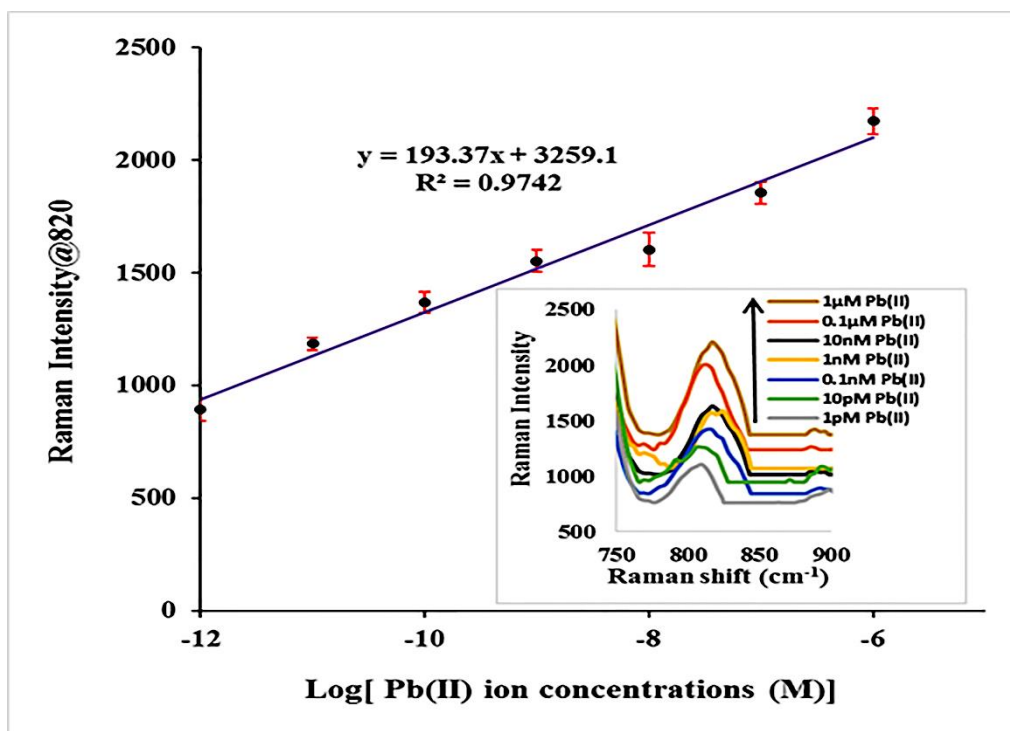
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Fig. 7. A plot of the Raman intensity (at 820 cm^{-1}) versus the logarithm of Pb(II) concentration (1x10^{-12} M to 1x10^{-6} M) and the corresponding SERS spectra from 750 to 900 cm^{-1}(Inset).

Table 2
Comparison of the proposed Pb(II) sensing method with some reported methods

Sensing material	Analytical technique	Medium	LOD	Reference
4-aminobenzo-18-crown-6	UV - visible spectroscopy	water	5.0x10^{-8} M	[29]
Dicyclohexano-18-crown 6-ether	Electrothermal atomic absorption spectrometry	water	1.0x10^{-11} M	[46]
Benzo-18-crown-6	Electrochemistry	water	1.0x10^{-9} M	[17]
4-aminobenzo-18-crown-6	SERS	water	6.9x10^{-13} M	This work

3.8. Environmental application

To demonstrate the potential application of the new SERS detection method for the environmental analysis of Pb(II) ions, it was used to screen a drinking water sample that was spiked with 1.2x10^{-7} M Pb(II). Another aliquot of the same spiked water sample was re-screened by ICP-MS for cross validation. The amount detected and the % recovery of each method are shown in Table 3. Although the % recoveries obtained by both methods are within the usually acceptable limit of 85-115%, the % recovery by the ICP-MS analysis was higher than that of the SERS analysis, the SERS detection is comparatively rapid and easily applied to in-field screening of lead contamination.

Table 3
Recovery studies with spiked drinking water (n = 3)

Amount added (M)	Amount detected (M)		% Recoveries	
1.2x10^{-7}	SERS	ICPMS	SERS	ICP-MS
	1.06 x 10^{-7}	1.37 x 10^{-7}	88.83 ± 9.19	114.17 ± 1.97

4. CONCLUSIONS

In summary, we demonstrated a new SERS sensor for the selective detection of Pb(II) ions. The new method utilises aminobenzo-18-crown-6 for the molecular recognition of Pb(II) via the formation of a coordination complex with the metal ion. The formed complex is immobilised onto a highly sensitive nanostructured gold substrate, through Au-N bond formation, for the indirect SERS detection of Pb(II) ions by a handheld Raman spectrometer. The new sensor showed excellent selectivity towards Pb(II) ions as well as low LOD and LOQ values of 0.69 pM and 2.20 pM respectively and was used successfully to determine the lead ion content of drinking water. Therefore, this SERS nanosensor has excellent potential for the rapid on-site detection of Pb(II) ions in the environment.

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Supporting Information

Molecular recognition and detection of Pb(II) ions in water by aminobenzo-18-crown-6 immobilised onto a nanostructured SERS substrate

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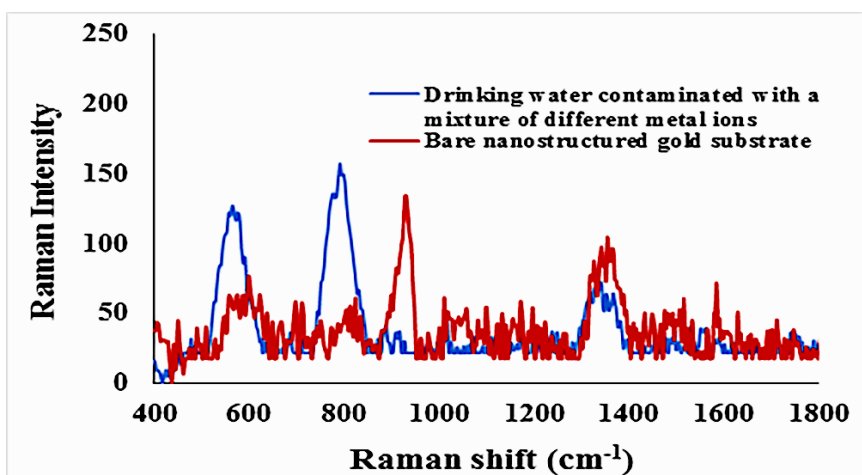
*Corresponding Author: g.ayoko@qut.edu.au
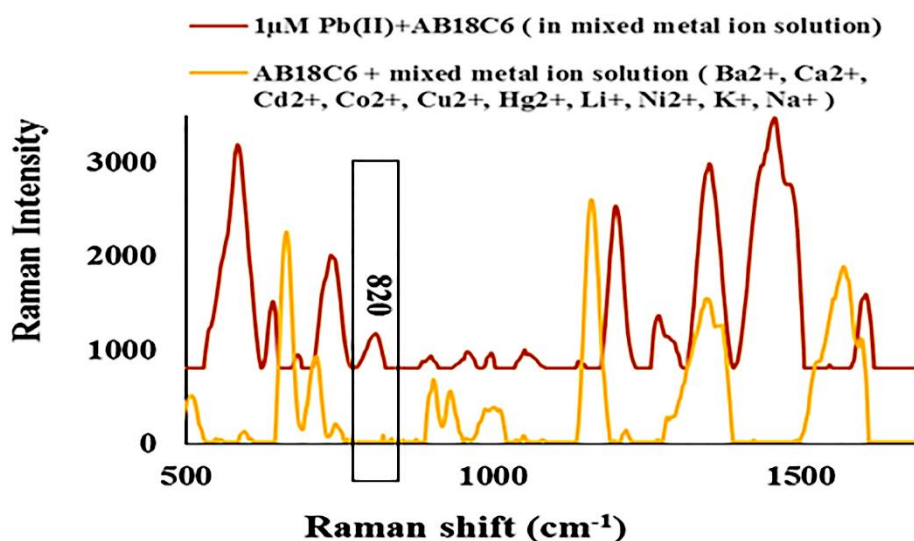
ESI 1, SERS spectra of AB18C6 in mixed metal ion solution (yellow line) and in mixed metal ion solution containing Pb(II) ions (red line)
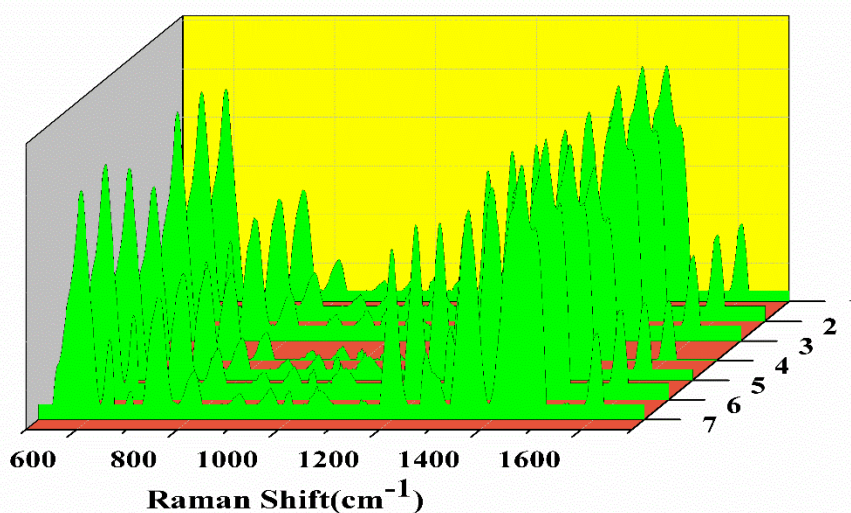
ESI 2, SERS spectra of 1pM Pb (II)-AB18C6 complex on solid gold SERS substrate (n=7)

ESI 3, SERS spectra of the bare nanostructured gold substrate (red line) and drinking water spiked with K(I), Li(I), Na(I), Mg(II), Ba(II), Ca(II), Cd(II), Mn(II), Co(II), Cu(II), Hg(II), Pb(II) and Ni(II) ions (blue line)
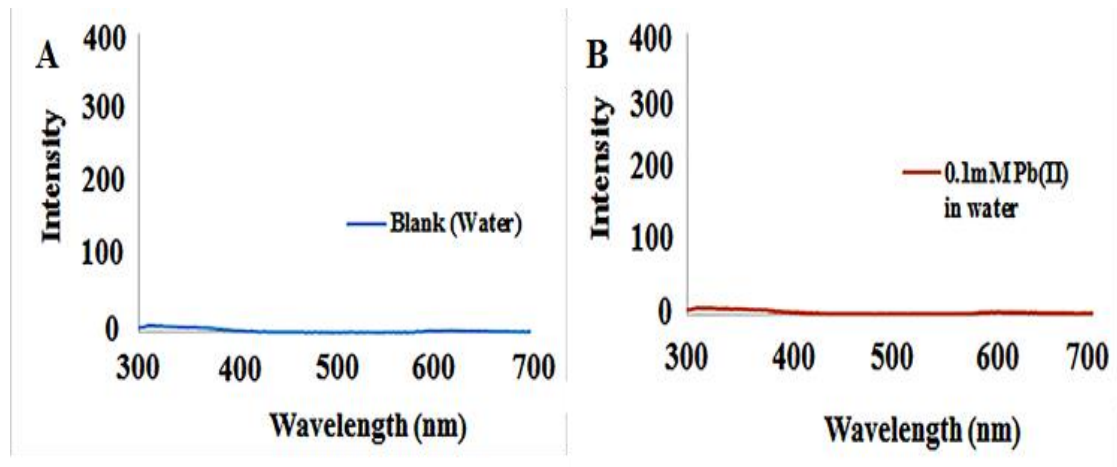
ESI 4, Fluorescence spectrum of (A) water and (B) 0.1mM Pb(II) in water