

Research Article

Development of Tannin Modified Membrane for Spectrophotometric Determination of Lead

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ABSTRACT

Tannin modified membrane (TM) was developed for preconcentration and detection of trace level of lead (Pb) using a complex formation between Pb^{2+} and (4-2-pyridylazo)-resorcinol (PAR). The extraction membrane was prepared by immobilizing tannin on a rectangular cellulose filter paper sized 7 x 9 cm² and then cut into a small circular shape of 13-mm diameter to fit with a commercially available syringe filter holder. The 4-layered of the TMs was employed for preconcentration of Pb^{2+} . An aliquot of 50 mL of standard or sample containing Pb^{2+} was loaded by using a 50-mL syringe that connected with a filter holder. To accelerate speed of analysis, peristaltic pump was used by connecting to a bottom side of the filter holder. For loading step, a flow rate of 4.4 mL/min was used. Elution of Pb^{2+} was accomplished by manually passing 5 mL of 0.1 M HCl through the membrane. An aliquot of 3 mL of the eluent was then mixed with the PAR reagent under the controlled pH of 9. Absorbance of 522 nm was monitored. Various optimization parameters affecting the immobilization of tannin on the cellulose filter paper were investigated. Under optimized conditions, linear calibration was obtained from 0.1 mg/L to 1.25 mg/L of Pb^{2+} solution. Acceptable precision of 2.0% (n = 5) was obtained. Good recoveries of 90.40 and 91.99 were achieved for drinking water samples.

Keywords: Lead, Tannin, Gelatin, Paper-based extraction unit, Preconcentration

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Introduction

Environmental pollution is an important issue due to its potentially toxic effects on living beings and the ecosystem [1]. Contamination of many heavy metals to natural water results in several public health problems [2]. Lead (Pb) is one of the heavy metals frequently determined in water samples due to high toxicity and it has an accumulative effect, causing anemia, brain, and kidney malfunction problems [3]. Therefore, the quantitative analysis of trace level of Pb is an important for health care and environmental monitoring.

Determination of Pb^{2+} in natural samples needs to be performed at low concentrations as mg/L or less. Therefore, analytical methods need to be high selectivity and sensitivity, such as atomic absorption spectrometry (AAS) [4], inductively coupled plasma atomic emission spectrometry (ICP-AES) [5] and inductively coupled plasma mass spectrometry (ICP-MS) [6]. However, there are some disadvantages such as expensive, time consuming, and requiring skilled operators arising from the above-mentioned techniques. On the contrary, UV-visible spectrometry has a several advantages including simplicity, flexibility, low cost, and convenience. In order to achieve determination of low concentrations of Pb^{2+} by using the UV-VIS spectrometric method, various sample pretreatment procedures have been designed as a preconcentration step including liquid-liquid extraction [7] and solid phase extraction (SPE) [8].

Over the years, tannins have been used as adsorbents for the removal of Pb^{2+} from aqueous solutions due to the structure of tannins contains multiple adjacent hydroxyl groups, which are able to chelate with a variety of transition-metal ions. Immobilize tannins onto various water-insoluble matrices, such as tannin-immobilized cellulose fiber from coconut husk [9], collagen fiber immobilized bayberry tannin [10] and tannin-immobilized gelatin/PVA nanofiber [11] have been developed for adsorption of Pb^{2+} in aqueous solutions.

Application of the mentioned tannins is a study mainly to be used for the treatment of lead-contaminated water. However, in the best of our knowledge, there have been no report on using of tannins in preconcentration of Pb^{2+} . In this work, the development of a tannin modified membrane (TM) for the preconcentration and determination of Pb^{2+} using complex formation between Pb^{2+} and 4-(2-pyridylazo)-resorcinol (PAR) is presented. Application of the proposed method was carried out for drinking water samples.

Materials and Methods

Reagents and chemicals

All the chemicals used were analytical reagent grade, and the solutions were prepared in deionized water. Gelatin from bovine skin (Type B, Sigma-Aldrich) and Tannic acid powder were obtained from HIMEDIA (India). The stock standard solution of lead (Pb^{2+}) a concentration of 1000 mg/L was obtained from Merck (Darmstadt, Germany) and was diluted with deionized water. Sodium hydroxide and hydrochloric acid were purchased from Merck (Darmstadt, Germany).

A solution of 1×10^{-3} M 4-(2-Pyridylazo) resorcinol (PAR) (monosodium salt, Sigma-Aldrich) was obtained by dissolving 0.022 g in 100 mL of deionized water. A 0.1 M borate buffer solution was prepared by dissolving 4.226 g of sodium tetraborate and 1.793 g of boric acid (Ajax Finchem, New Zealand) in 500 mL of deionized water, and the pH was adjusted to 9 with NaOH.

Instrumentation

A peristaltic pump (Ismatec, Germany) and reusable polycarbonate syringe filter holder, 13 mm (Sartorius Stedin, Biotech, Germany) were used for a preconcentration step. A pH meter (F20 pH meter, Mettler Toledo) was used for adjusting pH of tannin, gelatin and Pb^{2+} solutions. Jasco spectrophotometer, model V-750 (Japan) was used for recording the absorption spectrum in the wavelength ranges 400 – 700 nm. A visible spectrophotometer (V-1200, Mapada, Shanghai, China) was employed for measuring absorption at 522 nm of Pb^{2+} - PAR_2 complex.

Preparation method of TM

Tannic acid was immobilized with a gelatin cross-linking agent [12] by mixing 30 mL of 1%w/v gelatin with 30 mL of 3%w/v tannic acid followed by adjusting the pH of 9. The mixed solution was heated at 60 °C for 1 hour. Next, a cellulose filter paper (7 x 9 cm) was immersed in the solution. After immersing for about 10 second, the tannin-modified paper was dried on a rack overnight at room temperature. The dried tannin-modified paper was then cut into a small piece of 13-mm diameter circular shape TM, as shown in Figure 1.



Figure 1 Photo of a dried TM with 13-mm diameter.

Analytical procedure

For preconcentration of Pb^{2+} , four pieces of TMs were placed into a 13 mm-syringe filter holder. An aliquot of 50.0 mL of standard Pb^{2+} or sample solutions (pH 5, adjusted with 0.1 M HCl or 0.1 M NaOH) was loaded to a 50-mL syringe barrel which was connected to the filter holder as shown in Figure 2. Peristaltic pump was employed for controlling the flow rate of the Pb^{2+} solution passing through the TMs. Washing TM from unbounded anion was carried out with 1.0 mL of DI water, and then Pb^{2+} ion was eluted with 5 mL of 0.1 M HCl solution.

Determination of Pb^{2+} was performed by pipetting 3 mL of eluted solution into 10.0 mL-volumetric flask. Then, 2 mL of 1×10^{-3} M PAR and 3 mL 0.1 M borate buffer solutions pH 9 were added, and then the solution was diluted to 10 mL with DI water in a 10 mL-volumetric flask.

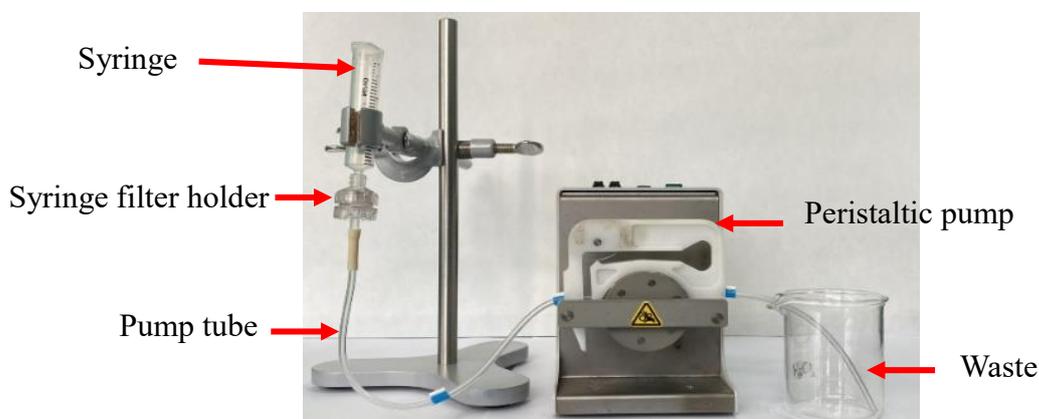


Figure 2 Experimental set-up for preconcentration of Pb^{2+} by using developed TM.

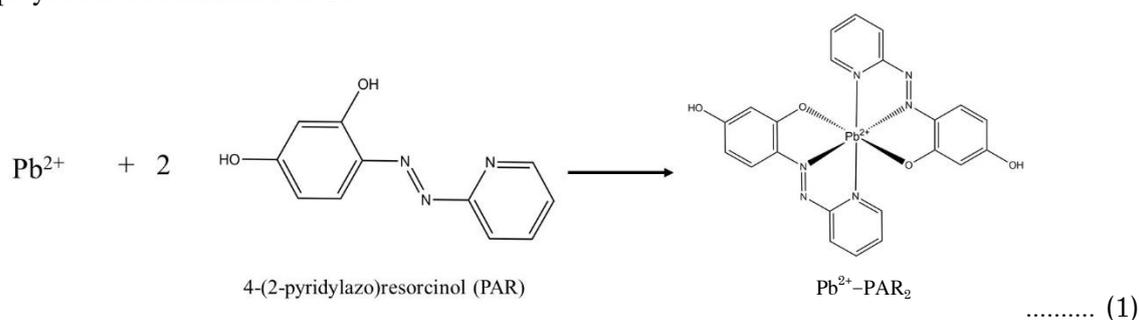
Sample preparation

Water samples were purchased from local shop in Bangkok, Thailand. Samples were filtered through a Whatman[®] no.1 filter paper before analysis.

Results and Discussion

Preliminary study on absorption Spectra of Pb^{2+} - PAR_2 complex

In this work, complexometric reaction between Pb^{2+} and PAR as shown in equation 1 was employed for determination of Pb^{2+} .



Optimum wavelength was determined by mixing the eluted Pb^{2+} solution with PAR to form Pb^{2+} - PAR_2 complex. Figure 3 presents absorption spectra of the blank reagent (PAR in borate buffer pH 9) and the Pb^{2+} - PAR_2 complex solution (prepared as described in “Analytical Procedure” section) when DI water was used for baseline measurement. Maximum wavelength was found to be 414 nm and 522 nm for the PAR reagent and the Pb^{2+} - PAR_2 complex, respectively. In addition, absorbance of the complex at 522 nm can be measured with no effect of residual PAR reagent, therefore, wavelength of 522 was selected for further detection of Pb^{2+} - PAR_2 complex.

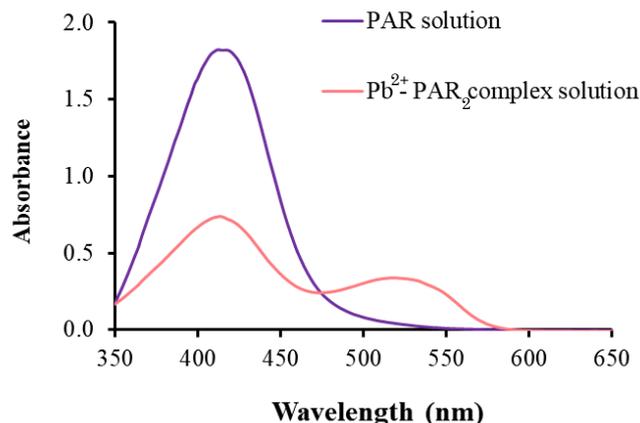


Figure 3 Absorption spectra of the blank reagent and the Pb²⁺-PAR₂ complex solution: C_{PAR} = 1.0 × 10⁻³ mol/L, C_{Pb(II)} = 2.0 mg/L.

Optimization of TM preparation

A. Tannic acid concentration

Tannic acid has strongest ability to form complex with various metal ions including Pb²⁺ [13]. Amount of Tannin loaded onto a cellulose filter membrane plays an important role of lead adsorption. Effect of tannic acid concentration in the modification of the paper-based extraction membrane step was investigated in the range of 0 to 7 %w/v when gelatin used as a cross-linking agent was fixed at 1%w/v. The TMs obtained from various tannic acid concentrations were further employed in the “Analytical procedure” for evaluation of Pb²⁺ adsorption efficiency. The more Pb²⁺ adsorption, the more Pb²⁺ concentration was found in the eluent. Results in Figure 4 show that 3 % w/v of tannic acid provides the highest absorbance of the Pb²⁺-PAR₂ complex. This indicates that using of 3% of tannic acid can provide the TM which has the highest efficiency for Pb²⁺ adsorption. It should be noted that adsorption of Pb²⁺ was also observed for the condition with 0% tannic acid. This may be attributed to the functional groups present in gelatins, such as -OH, -COOH, and -NH₂, which can chelate towards Pb²⁺ [11]. However, the concentration of 3% tannic acid was selected for subsequent studies.

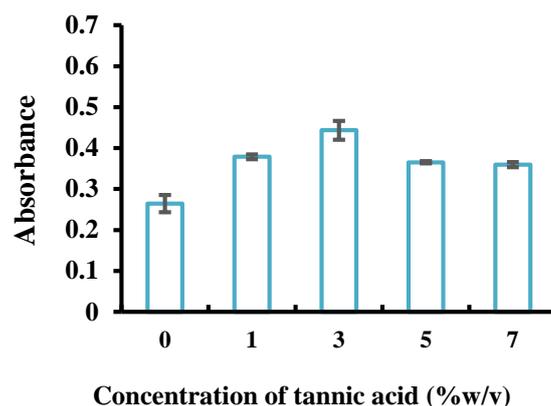


Figure 4 Effect of concentrations of tannic acid on Pb^{2+} adsorption efficiency on the TM. The experiments were carried out using standard 1 mg/L of Pb^{2+} . All experiments were performed in triplicate.

B. Crosslinking reaction time

In this work, gelatin was used as a cross-linking agent for tannin immobilization on cellulose paper filter. Gelatin has functional groups such as $-NH$, $-NH_2$, and $-C=O$ in their molecular structure. The amino group itself can interact with tannins through hydrogen bonds [13]. In addition, according to the reaction characteristics of tannins, the hydroxyl groups in tannins can also undergo an esterification reaction with carboxyl groups of gelatins [14]. For preparation of TM, cross-linking reaction between tannic acid and gelatin was occurred before dip coating on the cellulose filter paper. To investigate the effect of cross-linking reaction time, the TM was prepared by using different cross-linking reaction time in the range of 0 to 210 min. The appropriate reaction time was accessed by the time that provided good adsorption efficiency of Pb^{2+} by TM. Results in Figure 5 show that absorbance of $Pb^{2+}-PAR_2$ complex increased when increasing reaction time from 0 to 30 min. After that, the absorbance of the $Pb^{2+}-PAR_2$ complex is remained constant. Therefore, 60 min was chosen as reasonable time for preparation of TM.

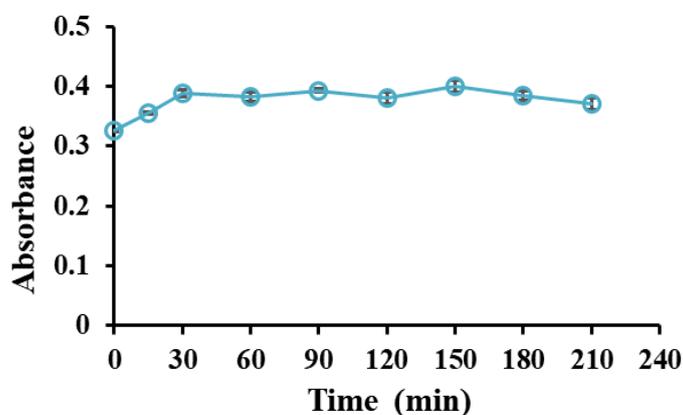


Figure 5 Effect of crosslinking time on Pb^{2+} adsorption efficiency on the TM. The experiments were carried out using standard 1 mg/L of Pb^{2+} . All experiments were performed in triplicate.

C. pH of the cross-linking solution

pH is an important parameter for the crosslinking reaction between tannic acid and gelatin. Hence, the effect of pH in a range of 7 to 11 of the mixed reaction solution was studied (Figure 6). It was found that the pH over pH 8 did not affect the signal or the precision of the measurements. However, the phenol hydroxyl group of the tannin was found to be easily oxidized at the pH greater than 9 [15]. Thus, the crosslinking solution of pH 9 was chosen as appropriate.

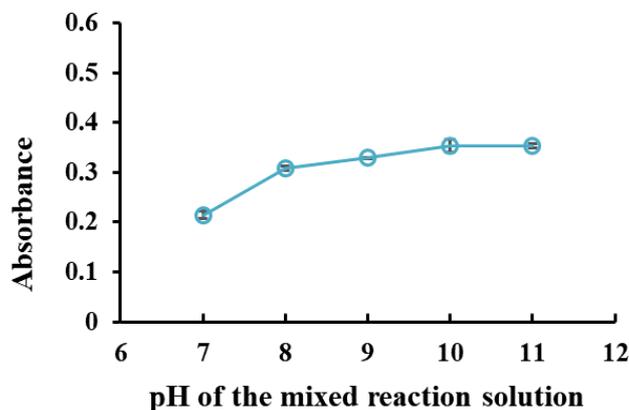


Figure 6 Effect of pH of the cross-linking solution on Pb^{2+} adsorption efficiency on the TM. The experiments were carried out using standard 1 mg/L of Pb^{2+} . All experiments were performed in triplicate.

Optimization for the preconcentration of Pb^{2+}

A. pH of Pb^{2+} solution

The pH of metal solution is one of the parameters plays an important role in metal–chelate formation and preconcentration technique [16-17]. The effect of pH on the Pb^{2+} standard solution was studied in the pH range of 4.0 to 10.0. The results in Figure 7 demonstrate that the absorbance of the Pb^{2+} – PAR_2 complex increased as the pH increased from 4.0 to 5.0, possibly due to the acidic dissociation of the phenolic hydroxyl group of tannin, resulting in a stronger complexing ability with the metal ion (the pKa of tannic acid is approximately 6).

However, at a higher pH of 6.0, there was a decrease in absorbance. This could be due to the precipitation of Pb^{2+} as $Pb(OH)_2$, caused by the high concentration of OH^- ions in the solution, resulting in poor biosorption experiment results. Additionally, at higher pH levels, the phenolic hydroxyl groups of the tannic acid are more readily oxidized, making it impractical to apply this approach above pH 8. Therefore, in further experiments, the standard or sample solution containing Pb^{2+} was adjusted to pH 5 before loading through the TM, as this provided the highest absorption signal.

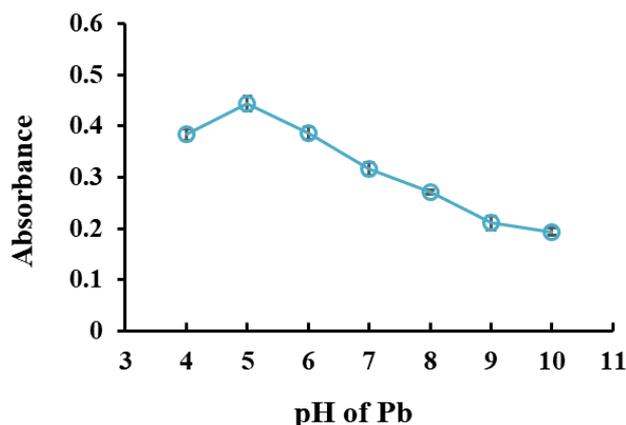


Figure 7 Effect of pH of Pb^{2+} solution before loading through the TM. The experiments were carried out using standard 1 mg/L of Pb^{2+} . All experiments were performed in triplicate.

B. Volume of Pb^{2+} solution

Volumes of the standard 1.0 mg/L Pb^{2+} solution loaded on the TM were investigated from 10 to 80 mL. As expected, absorbance of the Pb^{2+} - PAR_2 complex increased with increasing volume of the standard Pb^{2+} solution for the entire range studied (Figure 8). This indicated that the TM was capable for adsorption of Pb^{2+} under this condition. In addition, sensitivity of the analysis might be increased by increasing volume of the loaded solution. In this work, 50 mL of 1 mg/L Pb^{2+} was chosen as a model for further study.

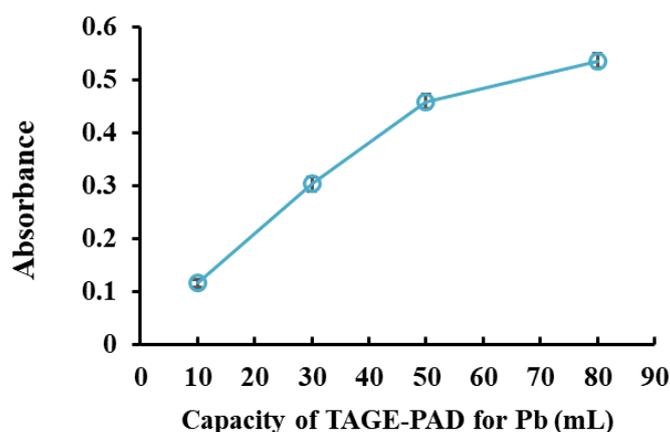


Figure 8 Effect of loading volume of standard 1 mg/L Pb^{2+} . The experiments were carried out using standard 1 mg/L of Pb^{2+} . All experiments were performed in triplicate.

Analytical performance

The calibration curve for the determination of Pb^{2+} was obtained under the optimum conditions as described in the previous sections. In Figure 9, a linear calibration curve was achieved for the loading Pb^{2+} concentration ranging from 0.1 to 1.25 mg/L with a coefficient of determination (r^2) of 0.9953. The limit of detection (3σ blank/slope) was 0.07 mg/L.

Five replicate injections of 1.0 mg/L Pb^{2+} were carried out giving a precision of 2.0 % relative standard deviation (RSD). The enrichment factor (EF) was calculated as the ratio between the analyte concentration of the eluent and the loading concentration. Table 1 presents enrichment factors (EF) obtained from standard Pb^{2+} concentrations ranging from 0.25 to 1.25 mg/L. An average enrichment factor of 7.1 was achieved, when 50 mL of Pb^{2+} solution was loaded and eluted with 5 mL of 0.1 M HCl.

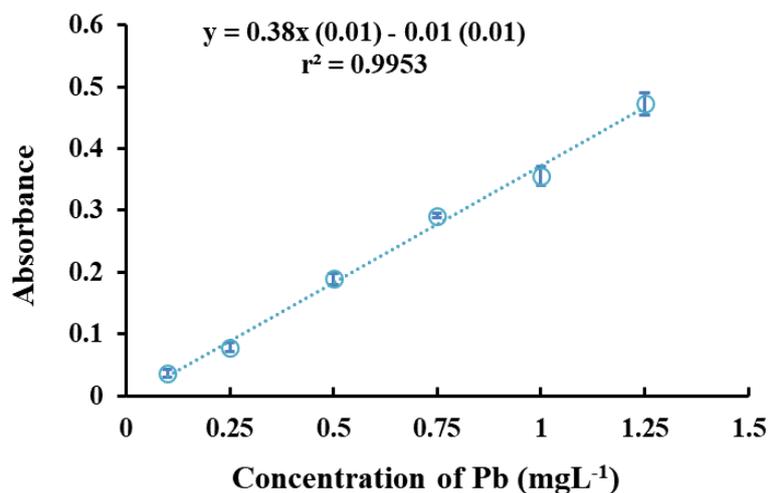


Figure 9 Calibration curve for the determination of Pb^{2+} ranging from 0.1 – 1.25 mg/L.

Table 1 Enrichment factors (EF) obtained from standard Pb^{2+} concentration ranging from 0.25 to 1.25 mg/L.

Concentration of Pb^{2+} (mg/L)	Measured concentration of Pb^{2+} (mg/L)	EF
0.25	1.69	6.8
0.50	3.71	7.4
0.75	5.57	7.4
1.00	6.66	6.7
1.25	8.88	7.1

Sensitivity of the developed method using TM was demonstrated by comparing the slope of the calibration equations obtained from the standard Pb^{2+} solution with and without preconcentration with the developed TM. As shown in Figure 10, slope of the linear equation was enhanced about 2 times with the preconcentration step. The result confirmed good potential of the developed TM for preconcentration of Pb^{2+} .

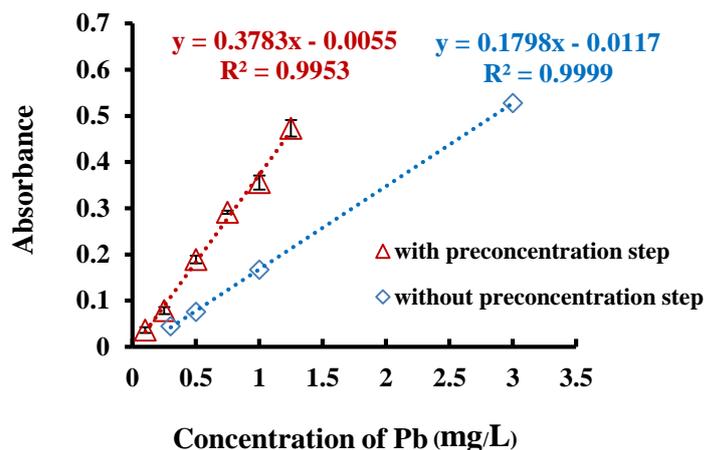


Figure 10 Calibration curves and equations obtained from Pb²⁺ solution using Pb²⁺-PAR₂ method with (Red dash line) and without preconcentration step (blue dash line).

Application to water samples

In order to validate the proposed methodology, the developed TM with Pb²⁺-PAR₂ method was evaluated by analyzing samples of drinking water spiked at 0.5 mg/L. The results are shown in Table 2. The recoveries were obtained from Pb²⁺ added to the samples. The results indicated that the developed TM for preconcentration of Pb²⁺ had high accuracy as observing good recoveries in the range of 90 to 92%.

Table 2 Percent recovery of Pb²⁺ in drinking water samples.

Sample	Based value (mg/L)	Quantity of Pb ²⁺ added (mg/L)	Quantity of Pb ²⁺ found (mg/L)	Recovery (%)
A	N.D.	0.00	N.D.	-
		0.50	0.45 ± 0.01	90.40
B	N.D.	0.00	N.D.	-
		0.50	0.46 ± 0.01	91.99

N.D.: Not detected.

Mean ± SD (n = 2).

As part of the validation process for the developed method, the extracted Pb²⁺ solution obtained from spiked samples (with a concentration of 0.5 mg/L Pb²⁺) was measured using AAS. After pre-concentrating Pb²⁺ using the developed TM, the Pb²⁺ contents in the extracts obtained through the spectrophotometric method were compared with the values obtained through the AAS method (as shown in Table 3). The results indicated that Pb²⁺ content as determined by the two methods were agree well with each other, as confirmed by a statistics t-test at 95% confidence level. The results of the recovery

study result and the AAS data further confirmed the accuracy of this method for quantitative analysis of Pb^{2+} at the ppm level.

Table 3 Pb^{2+} content in spiked drinking water samples determined using the developed TM with Pb^{2+} - PAR_2 method and from the AAS method.

Sample	Measured concentration of Pb^{2+} (mg/L) in the extracts		t_{cal} ($t_{\text{table}} = 6.314$)
	TM with Pb^{2+} - PAR_2 method	AAS method	
A	0.45 ± 0.01	0.57 ± 0.05	2.97
B	0.46 ± 0.01	0.54 ± 0.03	2.91

Conclusions

In this work, Tannin modified membrane (TM) was developed for preconcentration of Pb^{2+} solution. The analytical method employed spectrophotometric detection of the Pb^{2+} - PAR_2 complex. The method for immobilizing tannin on a rectangular cellulose filter paper was simple and low cost. Moreover, the developed TM was ready to use with a 13-mm syringe filter holder. By using the preconcentration with TM together with the spectrophotometric detection of Pb^{2+} - PAR_2 complex, a linear range of 0.1 – 1.25 mg/L Pb^{2+} , with detection limit of 0.07 mg/L Pb^{2+} was achieved. The method was applied to the determination of Pb^{2+} in spiked drinking water samples with acceptable recoveries (90 to 92%). For comparison of Pb^{2+} content, the results obtained from the developed method were agree well with the AAS method. The proposed method is robust, cost-effective and can be used for quantitative analysis of Pb^{2+} in ppm level.

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References

1. Jalbani N, Soylak M. Preconcentration/separation of lead at trace level from water samples by mixed micelle cloud point extraction. *J Ind Eng Chem.* 2015;29:48-51.
2. Naeemullah, Kazi TG, Shah F, Afridi HI, Khan S, Arian SS, et al. A green preconcentration method for determination of cobalt and lead in fresh surface and waste water samples prior to flame atomic absorption spectrometry. *J Anal Methods Chem,* 2012;2012:713862:1-8.
3. Maratta A, Vázquez S, López A, Augusto M, Pacheco PH. Lead preconcentration by solid phase extraction using oxidized carbon xerogel and spectrophotometric determination with dithizone. *Microchem J.* 2016;128:166-71.

4. Elci L, Kartal AA, Soylak M. Solid phase extraction method for the determination of iron, lead and chromium by atomic absorption spectrometry using Amberlite XAD-2000 column in various water samples. *J Hazard Mater.* 2008;153(1-2):454-61.
5. Zougagh M, García de Torres A, Vereda Alonso E, Cano Pavón, JM. Automatic on line preconcentration and determination of lead in water by ICP-AES using a TS-microcolumn. *Talanta.* 2004;62(3):503-10.
6. Townsend AT, Miller KA, McLean S, Aldous S. The determination of copper, zinc, cadmium and lead in urine by high resolution ICP-MS. *J Anal At Spectrom.* 1998;13(11):1213-9.
7. Alkan M, Kara D. Preconcentration of Heavy Metal Ions from Wastewater by Solvent Extraction Using N,N'-bis(2-Hydroxy-5-bromo-benzyl)-1,2 diaminopropane Prior to Their Determination by Flame Atomic Absorption Spectrometry. *Instrum Sci Technol.* 2004;32(3):291-301.
8. Pytlakowska K. Dispersive micro solid-phase extraction of heavy metals as their complexes with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol using graphene oxide nanoparticles. *Microchimica Acta.* 2015;183(1):91-9.
9. Taksitta K, Sujarit P, Ratanawimarnwong N, Donpuksa S, Songsrirote K. Development of tannin-immobilized cellulose fiber extracted from coconut husk and the application as a biosorbent to remove heavy metal ions. *Environ Nanotechnol Monit Manag.* 2020;14:100389.
10. Wang R, Liao X, Shi B. Adsorption behaviors of Pt (II) and Pd (II) on collagen fiber immobilized bayberry tannin. *Ind Eng Chem Res.* 2005;44(12):4221-6.
11. Meng J, Lin X, Zhou J, Zhang R, Chen Y, Long X, et al. Preparation of tannin-immobilized gelatin/PVA nanofiber band for extraction of uranium(VI) from simulated seawater. *Ecotoxicol Environ Saf.* 2019;170:9-17.
12. Zhang X, Do MD, Casey P, Sulistio A, Qiao GG, Lundin L, et al. Chemical modification of gelatin by a natural phenolic cross-linker, tannic acid. *J Agric Food Chem.* 2010;58(11):6809-15.
13. Collins RA, Ng TB, Fong WP, Wan CC, Yeung HW. Removal of polyphenolic compounds from aqueous plant extracts using polyamide minicolumns. *Biochem Mol Boil Int.* 1998;45:791-6.
14. Quideau S, Deffieux D, Douat-Casassus C, Pouységu L. Plant polyphenols: chemical properties, biological activities, and synthesis. *Angew Chem Int Ed.* 2011;50:586-621.
15. Sengil IA, Özacar M. Biosorption of Cu (II) from aqueous solutions by mimosa tannin gel. *J Hazard Mater.* 2008;157(2-3):277-85.
16. Elçi A, KabakçI E, Elci L. Solid-phase extractive preconcentration of trace copper as its calmagite anionic chelate using a polyaniline column for flame atomic absorption spectrometric determination. *Anal Lett.* 2015;48:632-46.
17. Narin I, Soylak M. Enrichment and determinations of nickel(II), cadmium(II), copper(II), cobalt(II) and lead(II) ions in natural waters, table salts, tea and urine samples as pyrrolydine

dithiocarbamate chelates by membrane filtration– flame atomic absorption spectrometry combination. *Anal Chim Acta.* 2003;493(2):205-12.