



Communication

Pb(II) Determination in a Single Drop Using a Modified Screen-Printed Electrode

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Abstract: This work presents the development and validation of an electroanalytical method for Pb(II) determination in a single drop. The electrochemical sensors used were an unmodified screen-printed electrode (SPE) and a Bi-film SPE (BiFSPE). Anodic square wave stripping voltammetry (SWASV) was performed at an accumulation potential of -1.5 V vs. Ag/AgCl and an accumulation time of 60 s. Electroanalysis with an unmodified SPE did not yield satisfactory results, whereas the BiFSPE was a much better analysis method. The linear concentration using the BiFSPE was in the range of 138.8–162.5 $\mu\text{g/L}$. The accuracy and precision were evaluated for different spiked concentrations, but the method using the unmodified SPE was neither accurate nor precise. Using the BiFSPE, the method was found to be both accurate and precise for Pb(II) determination at a concentration of 140.0 $\mu\text{g/L}$, with recovery and relative standard deviation (RSD) of 106.6% and 12.1%, respectively. In addition, using the BiFSPE, LOD and LOQ values of 1.2 $\mu\text{g/L}$ and 3.3 $\mu\text{g/L}$ were obtained, respectively. The possible interference effect on Pb(II) stripping signal was checked in the presence of Cd(II), Zn(II), Cu(II), Sn(IV), Sb(III), Hg(II), Fe(III), As(V), K(I), I^- , Ca(II), and NO_3^- . Electrochemical impedance spectroscopy measurements were also performed for the unmodified SPE and BiFSPE. The application of single drop Pb(II) analysis was tested by real water sample analysis.

Keywords: single drop analysis; lead; SPE; bismuth; screen printed



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1. Introduction

If only a small sample volume is available (for example, one drop, i.e., a volume of 60 μL), electrochemical analysis becomes essential. The latter became possible with the development of screen-printing technology. These screen-printed electrodes (SPEs) are promising for on-site analysis [1]. However, the analysis of a single drop has not yet been widely reported.

Few reports have been published on the determination of analytes in a sample drop on SPEs. For example, Knežević et al. [2] constructed a novel sensor based on gold nanoparticles decorated on manganese dioxide, which was used for histamine determination in a single sample drop. The method proved to be accurate, precise, selective, fast, and reproducible. In another study, norepinephrine was detected in a sample drop using a smartphone-based square-wave voltammetry (SWV) system with a graphene SPE [3]. Chiu et al. [4] reported the simultaneous determination of chloride, bromide, and iodide in aqueous solutions in a 50- μL solution. It was also shown that heavy metals can be determined in one drop of solution using a SPE [5,6].

Conventionally, for the determination of heavy metals atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) are employed [7–13]. On the other hand, electrochemical determination of heavy metals using anodic or adsorptive stripping voltammetry have also been frequently employed [7,8,14,15]. Electroanalysis has certain

advantages over conventional analytical methods, including low detection limits (LOD), high sensitivity and selectivity, relatively simple and inexpensive equipment, a short analysis time, and the possibility of on-site analysis [16,17]. Anodic stripping voltammetry is one of the most effective methods due to a preconcentration step that allows the trace analysis of heavy metals with high accuracy, precision, and sensitivity [1,18]. In 2000, Wang et al. [19] introduced bismuth electrodes. Bismuth has very low toxicity, a wide operational potential window, and favourable electroanalytical properties, and is insensitive to dissolved oxygen in the sample solution [20–24]. Bismuth-film electrodes can be prepared in situ and ex situ, and both give good electroanalytical performances in heavy metal determination [7,8,20,24–28]. The modification of the working electrode can be essential to ensure the desired analytical performance [8,29–44].

The main routes of lead exposure are via the inhalation of contaminated aerosols or dust particles and via the ingestion of contaminated food and water. In the human body, most lead accumulates in the kidneys, followed by the liver and other soft tissues such as the heart and brain. Most of it is also accumulated in the skeleton. Lead also has a major effect on the nervous system; early symptoms thereof include headaches, a short attention span, irritability, and memory loss. Lead induces cell damage through the formation of reactive oxygen species. Lead compounds can inhibit DNA synthesis and repair, cause oxidative damage, and interact with various proteins. Studies indicate that lead is carcinogenic and induces renal tumours in rats and mice and is therefore considered a probable human carcinogen [45,46]. On that basis, lead determination was and will be a fundamental analytical problem [18,30,47–51]. To ensure drinking-water quality, besides the already-established procedures, new methods are being developed to remove lead ions and other metal ions from water, such as adsorption by functionalized membranes or polymeric nanocomposites [52].

The aim of this work is the development and validation of an electroanalytical method for Pb(II) determination in a sample drop using an SPE. The latter was performed with a graphite-based working electrode printed on an SPE holder and modified in situ with Bi-film. On this basis, the analytical performance of an unmodified SPE and a Bi-film-modified SPE (BiFSPE) was validated as a unique analysis of a very small amount (one drop) of sample solution using only a handheld potentiostat/galvanostat, a connecting cable, and a small SPE sensor.

2. Experimental

All electrochemical experiments were performed with a PalmSens 3 potentiostat/galvanostat controlled by PStace 5.6 software (PalmSens, Houten, Netherlands). An SPE (type AC1.W4.R2, BVT Technologies, Brno, Czech Republic) was used as a three-electrode system. The working electrode was made of graphite and had a diameter of 1 mm. The area of the counter electrode was 4 mm². The reference electrode was made of silver and coated with AgCl. All potentials reported in this work referred to this Ag/AgCl reference electrode. After each measurement set, the SPE was thoroughly washed with ultrapure water (obtained by means of the Milli-Q system, Millipore Corporation, MA, USA; resistance 18.2 MΩ cm). All measurements were performed at 23 °C.

The solutions of standards were diluted with ultrapure water. For cyclic voltammetry (CV) analyses, a 1.0-mM solution of potassium hexacyanoferrate, K₃[Fe(CN)₆] (99%, Sigma-Aldrich, St. Louis, MO, USA) in 0.1 M KCl (Carlo Erba Reagents, Milan, Italy) was used.

For the square-wave anodic stripping voltammetry (SWASV) analyses, a 0.1 M acetate buffer was used as the supporting electrolyte with or without 0.5 mg/L Bi(III). The buffer was prepared using sodium acetate trihydrate (≥ 99%, Fisher Scientific, Loughborough, UK) and acetic acid (≥ 99.8%, Sigma-Aldrich, St. Louis, MO, USA). The Bi(III), Zn(II), Cd(II), As(V), Sn(IV), Sb(III), Cu(II), and Hg(II) standard solutions (1000.0 mg/L) were supplied by Merck KGaA (Darmstadt, Germany). The Pb(II) standard solution (1000.0 mg/L) was supplied by Merck KGaA and used for the analysis of metal traces. For the electroanalysis,

this solution was diluted as required in the supporting electrolyte (0.1 M acetate buffer) with or without 0.5 mg/L Bi(III). The Fe(III) and KNO₃ were supplied by Sigma-Aldrich (St. Louis, MO, USA). The KI was supplied by Carlo Erba Reagents (Val de Reuil, France). The CaCl₂ was supplied by Acros Organics (Morris, NJ, USA).

2.1. Real Sample Analysis

The real sample analysis was performed using BiFSPE by preparing 0.1 M acetate buffer with tap water from our laboratory. The tap water was first allowed to run for 5 min before being used for solution preparation. All experimental conditions were the same as those used for the method validation.

2.2. CV of [Fe(CN)₆]^{3−}/[Fe(CN)₆]^{4−} Redox Couple

Before the SWASV measurement, each SPE was checked by CV measurement to determine if the sensor was functioning properly. A 60-μL drop of 1.0-mM potassium hexacyanoferrate solution in 0.1 M KCl was applied to the SPE, so that all three electrodes were covered by the drop. The CV scan started at 0.8 V and scanned in the cathodic direction to −0.3 V (switching potential) and then switched to 0.8 V, at a linear scan rate. Different scan rates were used, i.e., 10.00, 20.00, 50.00, 75.00, 125.00, 150.00, and 200.00 mV/s. The results obtained had to follow the characteristics of the reversible and diffusion-controlled process [53] in order to accept the SPE electrode as suitable. In cases of large deviations from the expected results (linearity of the current peak height vs. scan rate, the change in peak potential vs. scan rate, the ratio of the anodic and cathodic peak heights, and the expected potential difference for anodic and cathodic peaks, i.e., 59 mV [53]), the sensor was discarded and another one was used. After this test, the sensor was thoroughly rinsed with ultrapure water and dried under the stream of Ar gas.

2.3. SWASV Measurements

The analyses were performed with unmodified and modified SPEs. In this work, the term unmodified SPE refers to an SPE that has a graphite working electrode. A BiFSPE, on the other hand, refers to a Bi-film SPE where the working electrode was modified in situ during the preconcentration step with a thin bismuth film. The supporting electrolyte in this case was a 0.1-M acetate buffer containing 0.5 mg/L Bi(III).

First, a 60-μL drop of supporting electrolyte (without Pb(II)) was pipetted onto the SPE and the voltammogram of the solution without analytes (blank solution) was measured. The multiple standard addition method was used for quantification; each addition was 10 μL of a solution with Pb(II). After adding the 10-μL solution to the drop, the liquid on the sensor was drawn up into the pipette and dispensed again to form a drop on the sensor (several times) to ensure homogeneity of the solution. SWASV analyses were performed using a conditioning potential of 0.3 V, a conditioning time of 30 s, an accumulation potential (E_{acc}) of −1.5 V, an accumulation time (t_{acc}) of 60 s, a start potential of −1.5 V, an end potential of 0.3 V, a potential step of 0.004 V, an amplitude of 0.05 V, a frequency of 25.0 Hz, a standby potential of 0.3 V, and a standby time of 60 s.

It should be noted that there are some limitations to the method using a single drop of solution. First, a maximum of nine standard additions of 10-μL drops could be added to the initial drop (the final volume of the drop was 150 μL), as larger volumes would result in the drop being spilled over the SPE holder. Secondly, there was a limit with the potential window: the oxidation of water takes place at more positive potentials (from about 0.8 V) and gaseous oxygen is produced. Furthermore, hydrogen starts to develop at more negative potentials (starting from approximately −1.4 V).

2.4. Electrochemical Impedance Spectroscopy Measurements

Electrochemical impedance spectroscopy (EIS) measurements were performed at −0.7 V using the sinusoidal perturbation signal with an amplitude of 10 mV (peak-to-peak). These measurements were performed in the frequency range from 100 kHz to 50 mHz

with 5 measurement points per decade. Prior to the EIS measurements, the same preconcentration step as for SWASV was performed. These measurements were performed with a 60- μ L drop.

3. Results and Discussion

3.1. LOD and LOQ Determination

The LOD and limit of quantification (LOQ) were determined by the determination of the signal-to-noise ratio (S/N). For the LOD, this ratio had to be equal to or greater than 3 (but less than 10), and for the LOQ, this ratio had to be equal to or greater than 10 (but close to 10). The signal S is the peak height, i.e., $\Delta i_{\text{Pb(II)}}$, and N is the background noise (determined as the difference between the highest and the lowest current measured for the background contribution). First, a volume consisting of a 60- μ L drop of supporting electrolyte (blank) was dropped on the SPE and the SWASV measurement was performed, followed by 10- μ L additions of the solution with a known Pb(II) mass concentration ($\gamma_{\text{Pb(II)}}$) added to this drop, and voltammograms were measured until S/N ratios ≥ 3 (but < 10 , for LOD) and $S/N \geq 10$ (for LOQ) were obtained. This procedure was repeated at least three times.

Using the unmodified SPE, the baseline of an initial drop (blank solution) at the potentials for the Pb(II) stripping peak was intense and had a peak-like shape. We checked the ultrapure water by ICP-MS for possible contamination, but no Pb(II) was detected in the blank solution. Due to this intensive background contribution, the determination of the LOD and LOQ was less reliable, since the resulting S/N ratios were much more significant than 10 when clear peaks were developed with the addition of a solution containing Pb(II) to the initial drop (despite the subtraction of the background contribution). On the other hand, using the BiFSPE, the LOD and LOQ were determined to be 1.2 $\mu\text{g/L}$ and 3.3 $\mu\text{g/L}$, respectively.

3.2. Linear Concentration Range Determination

The linear concentration range was determined by successively adding 10 μL of solution of the diluted Pb(II) standard (as needed) to the drop previously placed on the SPE sensor. Several repeated linearity determinations were performed with the same SPE and using additional new SPEs (several repeat measurements were also performed with these new SPEs). The same procedure was performed for the BiFSPE. Linearity was accepted when the square of the correlation coefficient, R^2 , was greater than 0.980.

The voltammograms measured using the unmodified SPE are shown in Figure 1a, the linear calibration curve is shown in Figure 1b, and the change in the peak potentials ($E_{\text{Pb(II)}}$) vs. $\gamma_{\text{Pb(II)}}$ is shown in Figure 1c. The same is also shown for the BiFSPE in Figure 2. Figures 1a and 2a show a slight shift in the Pb(II) stripping peak potential. The reason is most likely that Pb can form an intermetallic compound (i.e., PbBi) or Pb is adsorbed on the Bi surface. On the one hand, the energy requirements to desorb/strip Pb from a carbon surface compared to the Bi surface/compound must be very different.

The linear concentration ranges obtained for the SPE and BiFSPE were 171.4–225.0 $\mu\text{g/L}$ and 138.8–162.5 $\mu\text{g/L}$, respectively. It should be noted that repeat measurements with different SPEs had different linear concentration ranges (depending on each SPE used). However, the linear concentration ranges given here for the SPE and BiFSPE are the most representative ranges among the repeat measurements (all measurements covered the given linear concentration range). The lower limit of the linear concentration range of Pb(II) was at lower $\gamma_{\text{Pb(II)}}$ when the BiFSPE was used compared to the unmodified SPE, while the upper limit of the linear concentration range was at higher $\gamma_{\text{Pb(II)}}$ for the unmodified SPE. The sensitivity of the BiFSPE was higher compared to the unmodified SPE (the sensitivity was evaluated based on the slope of the calibration curve, Figure 1b vs. Figure 2b). In both cases, using the unmodified SPE and the BiFSPE, the Pb(II) stripping peak potential shifted to more positive potentials as $\gamma_{\text{Pb(II)}}$ increased (Figures 1c and 2c).

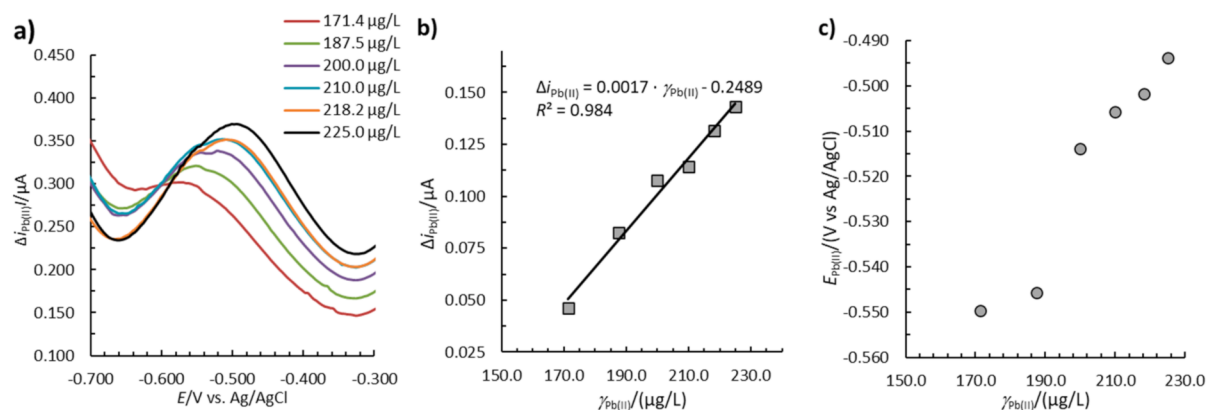


Figure 1. (a) Voltammograms measured for the unmodified SPE at different $\gamma_{\text{Pb(II)}}$ for a single drop placed on the SPE holder, (b) the linear concentration range, and (c) the change in $E_{\text{Pb(II)}}$ vs. $\gamma_{\text{Pb(II)}}$. The measurements were performed at $E_{\text{acc}} = -1.5$ V and $t_{\text{acc}} = 60$ s in 0.1 M acetate buffer (without Bi(III)).

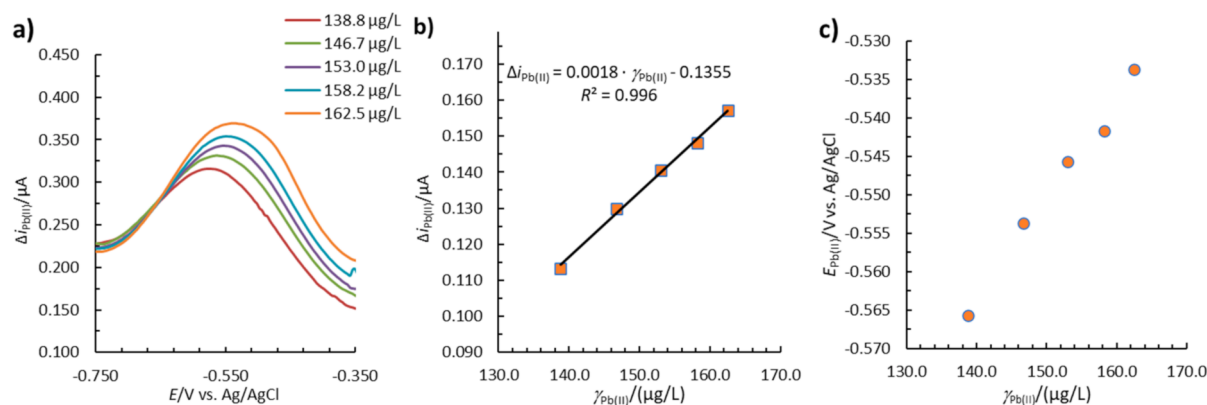


Figure 2. (a) Voltammograms measured for the BiFSPE at different $\gamma_{\text{Pb(II)}}$ for a single drop placed on the SPE holder, (b) the linear concentration range, and (c) the change in $E_{\text{Pb(II)}}$ vs. $\gamma_{\text{Pb(II)}}$. The measurements were performed at $E_{\text{acc}} = -1.5$ V and $t_{\text{acc}} = 60$ s in 0.1 M acetate buffer containing 0.5 mg/L Bi(III).

3.3. Determination of Accuracy and Precision

For a single drop of the test solution, the accuracy of both methods using the unmodified SPE and BiFSPE was evaluated by determining the Pb(II) concentration of the spiked 0.1 M acetate buffer solution. Quantification was performed using the multiple standard addition method (the results for the BiFSPE are shown in Figure 3). Based on the $\gamma_{\text{Pb(II)}}$ obtained, the average recovery (Re) and relative standard deviation (RSD) were calculated. The method was considered accurate when the average Re was between 80.0% and 120.0%. Furthermore, the method was considered precise if the RSD was equal to or lower than 20.0% [54].

The accuracy and precision of $\gamma_{\text{Pb(II)}}$ determination were tested at a Pb(II) concentration of 140.0 $\mu\text{g/L}$. The method with the unmodified SPE did not yield satisfactory accuracy and precision, i.e., the Re was not within the 80.0–120.0% interval and the RSD was higher than 20.0%. On the other hand, the method with the BiFSPE reported an average Re of 106.6% and thus accurate results. In addition, the RSD value when using the BiFSPE was 12.1%. On this basis, the method with the BiFSPE for the determination of Pb(II) can be considered accurate and precise. Therefore, the addition of Bi(III) to form Bi-film in situ on the SPE is necessary to obtain both accurate and precise results. Figure 3 shows that each individual measurement set produces differences in slopes and y-intercepts between successive measurements. Therefore, the multiple standard addition methodology is required to determine Pb(II), while the calibration curve method can be problematic [46].

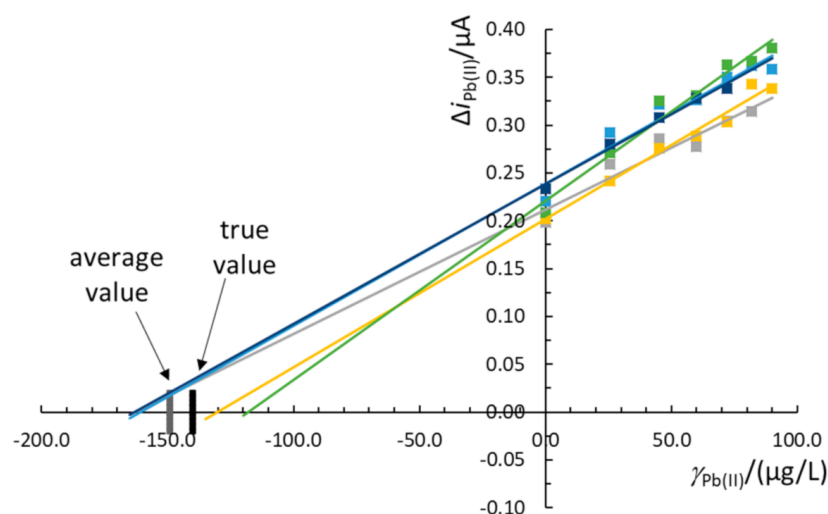


Figure 3. Method of multiple standard addition using the BiFSPE for the determination of $\gamma_{\text{Pb(II)}}$ in a single drop of solution consisting of the 0.1 M acetate buffer spiked with 140.0 $\mu\text{g/L}$ Pb(II).

3.4. EIS Measurements

EIS measurements were performed at $E = -0.7$ V vs. Ag/AgCl, which is the potential at which the Pb(II) stripping peak begins to develop while sweeping towards more positive potentials (Figures 1 and 2). The semicircles on the Nyquist plots (Figure 4) show that the real part of the impedance (Z_{real}) is at lower values for the BiFSPE compared to the unmodified SPE, indicating a lower charge transfer resistance (and also polarisation resistance) for the BiFSPE. Polarisation resistance is a measure of the resistance that the electrode presents to the transfer of electrons to or from the electrode to the electroactive species in solution. The lower it is, the more favourable the electron transfer and the better electroanalytical properties of the sensor are obtained. Therefore, the lower resistance behaviour of BiFSPE compared to unmodified SPE indicates a better analytical sensor [55].

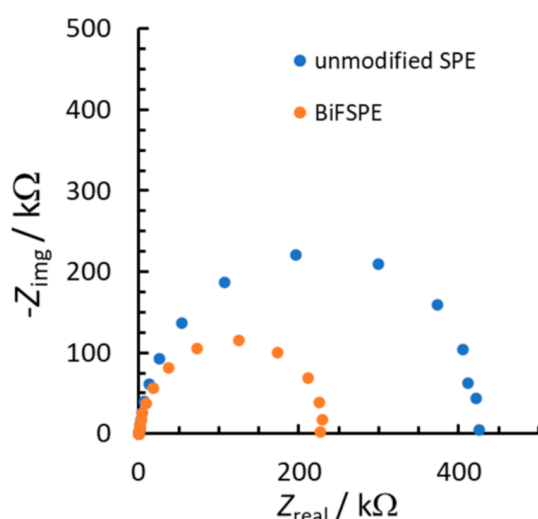


Figure 4. EIS Nyquist spectra measured for unmodified SPE and BiFSPE in 0.1 M acetate buffer containing 140.0 $\mu\text{g/L}$ Pb(II).

3.5. Interference Study and Real Sample Analysis

The interference effect of the following possible interfering substances Cd(II), Zn(II), Cu(II), Sn(II), Sb(III), Hg(II), Fe(III), As(V), K(I), I^- , Ca(II), and NO_3^- on the Pb(II) stripping signal was checked by SWASV in 0.1 M acetate buffer by preparing solutions with a

Pb(II):interferent mass concentration ratio of 1:1. The tested interferents could be present in real water samples. The influence of possible interferents on the Pb(II) stripping signal was calculated as the change in % = $\left(\frac{\Delta i_p^{\text{interferent}} - \Delta i_p^{\text{Pb(II)}}}{\Delta i_p^{\text{Pb(II)}}} \right) \cdot 100$, where $\Delta i_p^{\text{interferent}}$ and $\Delta i_p^{\text{Pb(II)}}$ are the Pb(II) stripping signals with and without the presence of a particular interferent, respectively. The calculated changes are shown in Table 1. The interference effect may occur due to the competition between Pb(II) and interferents in preconcentration step, thus the increase (positive change) or decrease (negative change) of the Pb(II) stripping signal is observed [56].

Table 1. The influence of different ions on the Pb(II) stripping signal at Pb(II):interferent mass concentration ratio of 1:1.

Interferent	Change in %
Cd(II)	20.3
Zn(II)	−17.8
Cu(II)	3.3
Sn(IV)	−57.9
Sb(III)	49.4
Hg(II)	22.8
Fe(III)	−16.2
As(V)	−15.8
K(I)	15.3
Ca(II)	−16.7
I [−]	−13.8
NO ₃ [−]	10.5

A significant effect on the Pb(II) stripping signal is observed with Sn(IV) (the stripping signal for Pb(II) decreased by 57.9%) and Sb(III) (the stripping signal for Pb(II) increased by 49.4%). This was expected since Sn(IV) and Sb(III) are used to form in-situ modified electrodes [18,57]. Ions such as Cd(II), Zn(II), Hg(II), Fe(III), As(V), K(I), Ca(II), I[−], and NO₃[−] had a minor effect on the Pb(II) stripping signal as the change in the Pb(II) signal was between $\pm 23.0\%$. Cu(II) did not have a significant influence on Pb(II) stripping signal (Pb(II) signal increased by only 3.3%).

For real sample analysis, the BiFSPE was employed, as it showed accurate and precise results in the method validation procedure (as given above). The accuracy and precision were tested in the same manner, as described in Section 3.3. The real sample analysis showed the accurate and precise result as the average Re was between 80.0 and 120.0% with RSD value of less than 20.0%.

4. Conclusions

The aim of this work was to develop and validate an electroanalytical method for the determination of Pb(II) in a single drop (60 μL). An unmodified SPE (a bare SPE with an Ag/AgCl reference electrode) and a Bi-film SPE (BiFSPE) were used for the trace determination of Pb(II) by means of square wave anodic stripping voltammetry (SWASV). The electrode modification of the BiFSPE was performed in situ with 0.5 mg/L Bi(III) in 0.1 M acetate buffer.

For Pb(II), the LOD and LOQ with the unmodified SPE could not be determined due to the intensive background contribution. For the BiFSPE, the obtained LOD was 1.2 $\mu\text{g/L}$ and the obtained LOQ was 3.3 $\mu\text{g/L}$. The linear concentration ranges of Pb(II) obtained for the SPE and BiFSPE were 171.4–225.0 $\mu\text{g/L}$ and 138.8–162.5 $\mu\text{g/L}$, respectively. It was also found that the sensitivity of the BiFSPE was higher compared to the unmodified SPE, which was assessed by the slope of the calibration curve. Using the unmodified SPE, the accuracy and precision were not satisfactory (the average recovery was not within 80.0–120.0% and the RSD was higher than 20.0%). However, when the BiFSPE was used, the average recovery and the RSD were 106.6% and 12.1%, respectively; thus, the BiFSPE

provides accurate and precise results for Pb(II) determination in a single drop solution. The interference effect was studied in the presence of Cd(II), Zn(II), Cu(II), Sn(IV), Sb(III), Hg(II), Fe(III), As(V), K(I), I^- , Ca(II), and NO_3^- . The most significant change in the Pb(II) stripping signal was found in the presence of Sn(IV) and Sb(III). The method showed accurate and precise results in the analysis of real water sample using BiFSPE. In addition, electrochemical impedance spectroscopy measurements showed that the BiFSPE has lower charge transfer resistance compared with bare SPE, which makes the BiFSPE more useful for electroanalytical studies.

This work demonstrates a very promising research area due to the potential for rapid on-site analysis of heavy metals and other pollutants. This work shows some shortcomings of SPEs and may therefore contribute to further research on this topic, where various surface modifications can lead to even better analytical performance.

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Conflicts of Interest: The authors declare no conflict of interest.

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