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Short Communication

Validation of the Polyacrylic acid/Glassy Carbon Differential Pulse Anodic Stripping Voltammetric Sensor for Simultaneous Analysis of Lead(II), Cadmium(II) and Cobalt(II) Ions

Moses O. Oyagi, John O. Onyatta, Geoffrey N. Kamau, Peterson M. Guto[*]

Department of Chemistry, University of Nairobi, P. O. Box 30197, Nairobi, Kenya
[*]E-mail: Peterson.guto@uonbi.ac.ke

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Electrochemical sensor validation ensures that the determination procedure employed for a specific test is suitable for its intended use. Accuracy and precision, linearity, range, limits of detection and quantification validation parameters were determined for the Polyacrylic acid/Glassy Carbon Differential Pulse Anodic Stripping Voltammetric Sensor used for the simultaneous analysis of Pb(II), Cd(II) and Co(II) in accordance with the guidelines of the ISO/IEC 17025. The recovery degree for accuracy were in the range of 101% to 110% for Pb(II), 100% to 105% for Cd(II) and 93% to 104% for Co(II). The precision was found to be less than 10% for ten determinations. Linear concentration range were also investigated and found to lie in the range of 125 – 7.8µM Pb(II), 16 – 2µM Cd(II) and 2 – 0.125mM Co(II). Limits of detection also were found to be 0.9nM Pb, 1.9mM Cd and 11.0µM Co and limits of quantitation were 3.0nM Pb, 6.3nM Cd and 36.7µM Co. The effects of foreign substances like Cu^{2+}, SO_4^{2-}, K^+, Na^+, Cl^-, NH_4^+ and O_2 were found to have no significant effect on the electrochemical responses of the three heavy metals. These results confirms that this sensor can provide accurate, reliable and consistent results for the determination of Pb(II), Cd(II) and Co(II) heavy metals in diverse samples.

Keywords: Validation, Polyacrylic acid/Glassy carbon electrode, Lead, Cadmium, Cobalt and differential pulse anodic stripping voltammetry.

1. INTRODUCTION

Validation of analytical methods requires assessment of their performance indicators which depend on the type of the method and its inherent characteristics [1, 2]. Equally important, all electroanalytical methods require same validation studies like any other analytical method regardless of their application(s) [3]. In the validation studies, method suitability has to be proven for its intended

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use. These studies are, but not limited to limit of detection, limit of quantification, linearity, linear concentration range, accuracy and precision [1 - 6].

Recently, we developed an electrochemical sensor based on polyacrylic acid/glassy carbon differential pulse anodic stripping voltammetry for simultaneous analysis of Lead(II), Cadmium(II) and Cobalt(II) in tap Water. With differential pulse anodic stripping voltammetry, the sensor was optimized and successfully applied in the simultaneous determination of Pb(II), Cd(II) and Co(II) in tap water sample [7]. This work aims to validate the optimized Polyacrylic acid/Glassy Carbon Differential Pulse Anodic Stripping Voltammetric Sensor in accordance with the guidelines of the International Organization for Standardization and the International Electrotechnical Commision 17025 (ISO/IEC 17025) with respect to accuracy, precision, linearity, range and limits of detection and quantification [8, 9]. Also, effect(s) of impurities on the performance of this Polyacrylic acid/Glassy Carbon Differential Pulse Anodic Stripping Voltammetric Sensor are also explored using well selected foreign substances.

2. EXPERIMENTAL

2.1. Chemicals and Solutions

Poly(acrylic acid), 25wt% solution in water, Cobalt(II) chloride (AR), cadmium nitrate (AR), lead nitrate (AR), acetic acid glacial (AR) and sodium acetate anhydrous (AR) were from fisher scientific and were used as received. For voltammetry, the electrolyte was acetate buffer. Water was de-ionized by Elgastat de-ionizer from fisher scientific. All other chemicals were reagent grade.

2.2. Apparatus

All the electrochemical experiments were performed with a CHI 1232B Electrochemical Station (CH Instrument Co., USA). A three-electrode system (CH Instrument Co., USA) consisted of a modified glassy carbon working electrode with diameter of 3 mm, a platinum wire auxiliary electrode and Ag/AgCl reference electrode. A pH meter Bench – Model CyberScan (Eutech Instruments) was used for all pH measurement. All experiments were carried out in a 10.0mL electrochemical cell at 23 ± 0.2 ^{o}C.

2.3. PAA Film Preparation and Optimization

Glassy carbon disk electrodes (3 mm diameter) were abraded on wet silicon carbide paper (400 grit, Buehler) followed by (600 grit, Buehler) [10]. Rinsed in water, then polished thoroughly with 0.05 micron micropolish (CH Instruments) slurry on a soft cloth before sonicated in ethanol and distilled water for 3 min each to remove particles and other possible contaminants [11]. The actual surface area was 0.071cm^2. Varying amounts of freshly prepared 4mM polyacrylic acid were deposited on the polished electrode and allowed to react at room temperature for varying times. Then the electrodes were rinsed with de-ionized water before use.

2.4. Procedure

All measurements were carried out in the differential pulse anodic stripping voltammetric (DPASV) mode. The differential pulse stripping voltammograms were recorded from 0V to -1.45V followed by a 10 s rest period. Prior to the next determination, the modified electrode was activated for 60 s in a pH 6.0 acetate buffer to remove the previous deposits completely.

2.4.1. Accuracy

The accuracy of an electrochemical sensor/method expresses the nearness between the expected value and the value found. It is expressed by calculating the percent recovery (%R) of the analyte recovered. In this case, to evaluate the accuracy of the developed electrochemical sensor, successive analysis of three different standard concentrations of the analyte (Pb(II), Cd(II) and Co(II)) solutions were added to the samples in different experiments. The standard solutions added were 50%, 100% and 150% of the expected working sample concentraion. The data of the experiment were statistically analyzed using equation 1 [6] to study the recovery and validity of the sensor.

$$\frac{\mathrm{Re}\,\mathrm{cov}\,ered}{Added}\,x100\% \qquad (1)$$

2.4.2. Precision

Precision of an analytical method is showing the closeness, matching or concordance degree of a measurement series obtained from several samples derived from the same homogeneous sample under specific conditions. Precision of the current method was considered at repeatability level only. Analysis was done on ten identical samples (reference material of Pb, Cd and Co standard solutions) and expressed as RSD% amongst responses using equation 2 [6].

$$RSD\% = \frac{SD}{Mean}\,x100\% \qquad (2)$$

2.4.3. Linearity, Linear Concentration range, Limit of Detection and Limit of Quantitation

Measurements of six replicates were made for the detection of Pb^{2+}, Cd^{2+} and Co^{2+} with the concentrations varying from 2mM to 0.1nM each in the acetate buffer, pH 6.0 for 300 seconds deposition time on PAA/GC electrode. The linearity of the sensor was evaluated by using calibration curve to calculate coefficient of correlation, slope and intercept values. Based on three times the standard deviation of the baseline (*equation 3*) [6], the limits of detection (LOD) were estimated for Pb(II), Cd(II) and Co(II).

$$C_{LOD} = \frac{3.s}{m} \tag{3}$$

While limit of quantitation (LOQ) being the lowest concentration of analyte that can be measured in the sample at an acceptable level of accuracy and precision was calculated using the standard deviation of the response and the slope method expressed as shown in equation 4 [6]:

$$C_{LOQ} = \frac{10.s}{m} \tag{4}$$

2.4.4. Effect of Impurities

The effect of interference on the performance of the electrochemical sensor was evaluated to ensure that there was no interference from other components present in the samples. This was studied by adding the expected possible interferants like Cu^{2+}, SO_4^{2-}, K^+, Na^+, Cl^-, NH_4^+ and O_2. These interferants were added first independently, then in combinations and their effect on the stripping currents of the analyte was monitored.

3. RESULTS AND DISCUSSION

3.1. Effect of the Amount of Polyacrylic acid

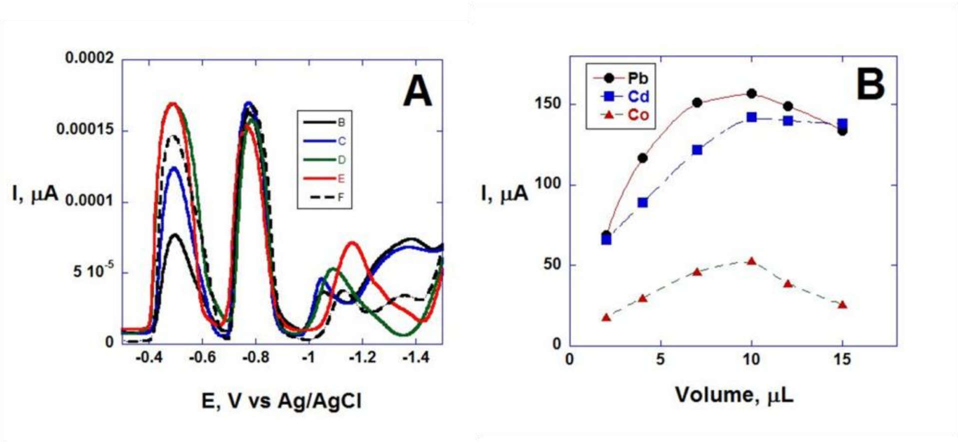

Figure 1. (A) Effects of the PAA concentration on the peak currents of 0.5 mM Pb(II), Cd(II) and Co(II) at the PAA/GC electrode. Deposition potential: -0.8V, Accumulation time: 300seconds, amplitude: 0.06V, pulse period: 0.02seconds, pulse width: 0.01seconds and sampling width: 0.0033seconds. The PAA amounts added were: B = 2 μL, C = 4 μL, D = 7 μL, E = 10 μL, F = 13 μL of 4 mM PAA on GC electrode. **(B)** Plots of currents (μA) versus volumes (μL) of 4 mM PAA.

To investigate the effect of the amount of PAA on the stripping responses, different amounts of the 4 mM PAA were deposited on the GC electrode. Increasing the amount of PAA on the GC electrode caused the sensitivity to increase linearly up to 10 μL, whereas beyond 10 μL caused the sensitivity of the electrode to decline due to the over-thick film, which hampered the electron transfer between metal ions and base electrode and also increased the background current. Similar observation was made by Tian et al [12] on MWCNTs-NADBS modified stannum film electrodes. Therefore, 10 μL of the 4 mM PAA was used on the GC electrode surface in all subsequent work.

3.2. Effect of the Drying Period of the Polyacrylic acid film

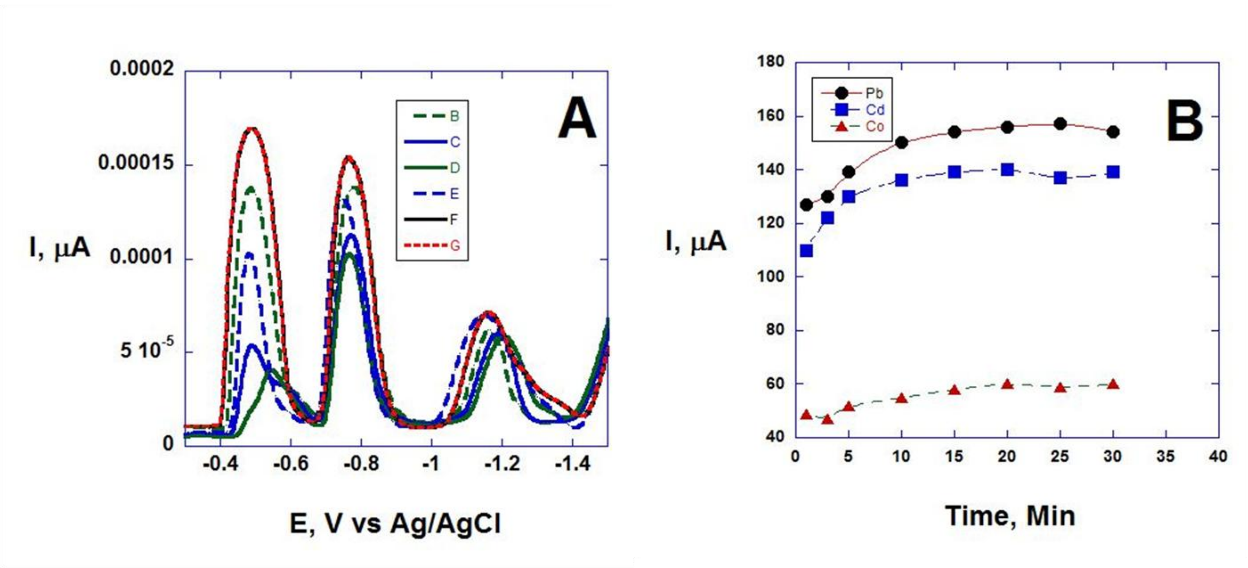

Figure 2. (A) Effects of drying time of 10 μL of 4 mM PAA on the peak currents of 0.5 mM Pb(II), Cd(II) and Co(II) at the PAA/GC electrode. Deposition potential: -0.8V, Accumulation time: 300seconds, amplitude: 0.06V, pulse period: 0.02seconds, pulse width: 0.01seconds and sampling width: 0.0033seconds. Drying time were: B = 1 min, C = 5 min, D = 10 min, E = 15 min, F = 20 min, G = 25 min of drying the PAA film on GC electrode. **(B)** Plots of currents (μA) versus drying time (min) of the PAA films.

The electrochemical responses of 0.5 mM Pb(II), Cd(II) and Co(II) at the PAA/GC electrode containing 10μL of 4mM PAA film drying times were studied by DPASV. The results are shown in figure 2. It can be seen from figure 2 that the maximum peak currents for Pb(II), Cd(II) and Co(II) were obtained when the films were dried for 20 minutes. There was no observable change in the current responses when the PAA films were dried beyond 20 minutes. Consequently, a 20 minute drying period was selected for further work.

3.3. Stability of the Polyacrylic acid Film on Glassy Carbon

The stability of the PAA/GCEs were tested for six weeks in a solution containing 1.0mM Pb(II), Cd(II) and Co(II) dissolved in acetate buffer, pH 6. After six weeks, the percentage decrease in the PAA film activity were 6.3% for Pb(II), 4.8% for Cd(II) and 17% for Co(II). Thus, the stability of the PAA/GCE is satisfactory. The small decrease can be attributed to the fouling of the electrode surfaces. The mechanical robustness of the PAA/GCEs is excellent compared to many of the reported polymer films in literature [13, 14].

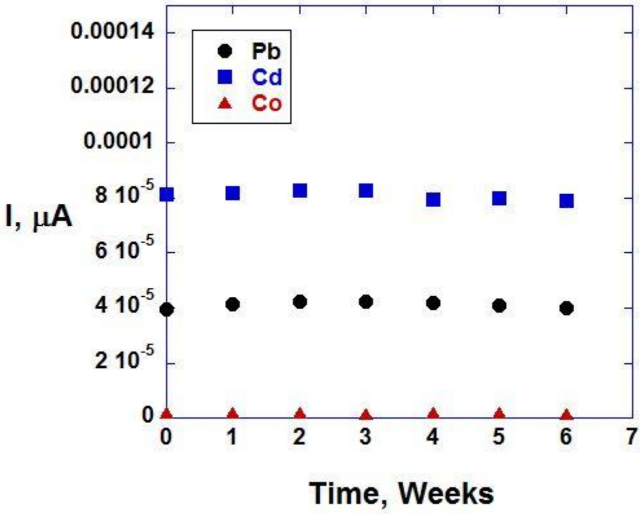

Figure 3. PAA/GC electrode stability in 2 mL of 1mM Pb(II), Cd(II) and Co(II) in acetate buffer, pH 6. Deposition potential: -0.8V, Accumulation time: 300seconds, amplitude: 0.06V, pulse period: 0.02seconds, pulse width: 0.01seconds and sampling width: 0.0033seconds. The films were dried for 20 minutes before investigating their stability.

Based on the results obtained on the electrochemical behavior of metals at the surface of PAA modified electrode, the following mechanism suggested by Nourifard et al [15] is represented for the electro-reduction/oxidation of metals at the surface of the PAA modified GC electrode;

$(M^{2+})_{sol.} + (PAA)_{surf.} \rightarrow (M^{2+}\text{-}PAA)_{adsorb.}$ ------------------- Pre-concentration step

$(M^{2+}\text{-}PAA)_{adsorb.} + 2e^- \rightarrow (M^o, PAA)_{adsorb.}$ ------------------- Pre-concentration step

$(M^o, PAA)_{adsorb.} - 2e^- \rightarrow (PAA)_{surf.} + (M^{2+})_{sol./surf.}$ ----------- Stripping step

Where M is the metal and PAA is the polyacrylic acid. Subscripts “sol” is solution, “surf” is surface, “adsorb.” is adsorbed and “sol/surf” is solution/surface. Comparing the differential pulse anodic stripping voltammetric currents of the modified and unmodified electrodes observed in both the current and our previous work [7] indicates that modification of the GC electrode with PAA caused easier and faster charge transfer at the electrode surface.

3.4. Accuracy

Accuracy of an analytical method is showing the closeness of the value accepted as conventional true value or as reference value and the measured value [1 - 5]. The accuracy was evaluated by adding a known amount of pure active consitituent to the known sample concentration, then analysing the mixture and comparision of the obtained and the expected results. The experimental results obtained and the calculated values are presented in table 1.

Table 1. The recovery values of the added analyte from spiked standard solutions of Pb(II), Cd(II) and Co(II) on PAA/GC electrode.

	Sample	Original (mM)	Current (A)	Added (mM)	Current (A)	Found (mM)	Recovery, %
1.	Sample (Pb)	3.0	$2.16x10^{-4}$	2.5	$1.82x10^{-4}$	2.53	101%
		3.0	$2.16x10^{-4}$	3.0	$2.38x10^{-4}$	3.30	110%
		3.0	$2.16x10^{-4}$	3.5	$2.70x10^{-4}$	3.75	107%
2.	Sample (Cd)	3.0	$2.05x10^{-4}$	2.5	$1.76x10^{-4}$	2.57	103%
		3.0	$2.05x10^{-4}$	3.0	$2.15x10^{-4}$	3.15	105%
		3.0	$2.05x10^{-4}$	3.5	$2.38x10^{-4}$	3.48	100%
3.	Sample (Co)	3.0	$2.79x10^{-5}$	2.5	$2.60x10^{-5}$	2.79	93%
		3.0	$2.79x10^{-5}$	3.0	$2.81x10^{-5}$	3.02	101%
		3.0	$2.79x10^{-5}$	3.5	$3.40x10^{-5}$	3.66	104%

Overly, the recovery degree obtained in this study were in the range of 101% to 110% for Pb(II), 100% to 105% for Cd(II) and 93% to 104% for Co(II). These lies between the imposed limits of 90 to 110% [1 - 5]. This indicates that the developed sensor is sensitive enough and accurate for the determination of Pb(II), Cd(II) and Co(II) ions in real samples.

3.5. Precision

Table 2. Experimental results and statistical calculated results obtained when proving repeatability on standard samples for Pb(II), Cd(II) and Co(II) on PAA/GC electrode.

	Calculated Statistical Parameter	Pb Standard Sample	Cd Standard Sample	Co Standard Sample
1.	Number of replicate Sample	10	10	10
2.	Average Value	$4.18x10^{-5}$	$8.12x10^{-5}$	$2.24x10^{-6}$
3.	Standard Deviation (SD)	$3.967x10^{-6}$	$5.802x10^{-6}$	$1.946x10^{-7}$
4.	RSD%	9.0%	7.0%	8.7%

Precision of an analytical method is showing the closeness, matching or concordance degree of a measurement series obtained from several samples derived from the same homogeneous sample under specific conditions. Precision of the current method was considered at repeatability level only. Repeatability is obtained when test/measurement is realized in one laboratory, by one operator, using only one type of measuring equipment and only one method in a short time period. Analysis was done on 10 identical reference material of Pb(II), Cd(II) and Co(II) standard solutions. Experimental and calculated results are shown in table 2. The precision was found to be less than 10%. These were within the acceptable range [1 - 5].

3.6. Linearity

Linearity of an electroanalytical method is a measure of how well calibration plot of response versus concentration approximates a straight line. Linearity was checked by plotting a response factor (RF) versus concentration. The RF is obtained as shown in equation 5 [6].

$$RF = \frac{Signal - y^{\text{int}ercept}}{Concentration} \tag{5}$$

The changes in the RF factor for Pb(II), Cd(II) and Co(II) were found to range from 1.5% to 2.5%. This was considered acceptable linearity [1 - 5]. The linear range was observed to vary from one metal to another.

3.7. Limit of Detection

Measurements were made for the detection of Pb^{2+}, Cd^{2+} and Co^{2+} with the concentrations varying from 2mM to 0.1nM each in the acetate buffer, pH 6.0 for 300 seconds deposition time on PAA/GC electrode. Based on three times the standard deviation of the baseline, the limits of detection were estimated for Pb(II), Cd(II) and Co(II). The limits of detection were recorded in table 3. They were found to vary from one metal to another.

Table 3. The values of the LCR, LOD, LOQ, R^2 and regression equation of Pb(II), Cd(II) and Co(II) on PAA/GC electrode.

	Analyte	**LCR**	**LOD**	**LOQ**	**RE**	**R^2 (*N = 6*)**
1.	Pb	125 – 7.8μM	0.9nM	3.0 nM	y=7.2625+421.32x	0.99964
2.	Cd	16 – 2μM	1.9nM	6.3 nM	y=2.4243+121.95x	0.99903
3.	Co	2 – 0.125mM	11.0μM	36.7 μM	y=28.346+75.089x	0.99794

LCR = Linear concentration range, LOD = Limit of detection, LOQ = Limit of quantitation, RE = Regression equation. **NOTE:** Regression equation y = ba + a, y = i(μA), x = Conc, b = Calibration curve slope, a = y-intercept.

Limit of quantitation (LOQ) being the lowest concentration of analyte that can be measured in the sample at an acceptable level of accuracy and precision was calculated using the standard deviation of the response and the slope method expressed as shown in equation 4 where the voltammetric current "S" was estimated from six replicate determinations of the blank signals. The linear calibration, concentration range, linear correlation coefficient, regression equation and detection limits were investigated and summarized in Table 3.

3.8. Effect of Impurities

Large numbers of easily oxidizable/reducible substances are normally present in most natural samples. Serious interference in metal ion determination occurs when there is competitive adsorption of the interferants into the polymer film(s) at uncontrolled concentrations. Possible interferences arise when the foreign substances reduce/oxidize at potentials close to the target ions and/or due to poor specificity of the adsorbing polymer film(s) on the electrode surface. The effects of Cu^{2+}, SO_4^{2-}, K^+, Na^+, Cl^-, NH_4^+ and O_2 foreign substances were tested in the determination of Pb(II), Cd(II) and Co(II) heavy metals at concentrations exceeding 50 fold. These foreign substances were found to have insignificant effect on the stripping current responses of the target heavy metals. However, in case of trace levels of these heavy metal ions in less polluted natural samples, their interference(s) will not be of paramount consequence [16].

4. CONCLUSION

Polyacrylic acid/Glassy Carbon Differential Pulse Anodic Stripping Voltammetric (PAA/GC-DPASV) sensor has been developed and validated according to ISO/IEC 17025 with respect to accuracy, precision, linearity, range and limits of detection and quantification. Also, foreign substances like Cu^{2+}, SO_4^{2-}, K^+, Na^+, Cl^-, NH_4^+ and O_2 were found to have no significant effect on the electrochemical responses of the three heavy metals. The present and previous [7] results confirms that this sensor can provide accurate, reliable and consistent results for the determination of Pb(II), Cd(II) and Co(II) heavy metals in diverse samples.

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