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# Optimization and Validation of Analytical Method for Determination of Thallium

## *Optymalizacja i walidacja metody ilościowego oznaczania talu*

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**ABSTRACT:** Thallium is a chemical element bearing the symbol Tl and the atomic number 81. It is a gray post-transition metal. Thallium, like mercury and lead, is a heavy metal and is highly toxic. It is also a neurotoxin for the central nervous system of mammals. Thallium also appears to bioaccumulate by some plants, including trees, and may also contaminate water. This is why it is essential to optimize and validate a method for the correct determination of thallium.

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## 1. Introduction

Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Validation of a method is essential for an experimental methods. It must validate non-standard methods, methods developed by the laboratory to confirm that the methods are fit for the intended use. The validation shall be as extensive as it is necessary to meet the needs of the given task. The laboratory must record the results obtained and the procedure used for validation [1]. Main validation parameters are: specificity, selectivity, precision, accuracy, linearity, range and detection limit. The precision is a term used to describe data from an experiment that has been repeated several times. If the experiment yields a tightly grouped set of data points, then it has high precision. To quantify precision, to describe it with a number, we can use such measures as Standard Deviation (SD) or Coefficient of Variation (CV). The accuracy of a measurement is how close a result comes to the true value. Determining the accuracy of a chemical analysis measurement may require verification of the analytical method with a known standards, standard solutions [3]. The linearity is the method's ability to obtain the results, which are directly proportional to the concentration of an analyte in the sample. The working range is the range where the method gives results with acceptable uncertainty. Working range can be wider than linear range [4]. It's expressed by stating the lower and upper range values. The limit of detection is concept and term used to describe the lowest concentration of an analyte that can be reliably measured by a particular measurement procedure [5].

The goal of this work is to optimize the measurement parameters and validate a method of voltammetric determination of the thallium. For this purpose, differential pulse voltammetry (DPV) with the control growth mercury drop electrode (CGMDE) is applied for the quantitative determination of thallium in the voltammetric cell. Thallium is a chemical element bearing the symbol Tl and the atomic number 81. It is a gray post-transition metal. Thallium, like mercury and lead, is a heavy metal and is highly toxic. It is also a neurotoxin for the central nervous system of mammals. Thallium also appears to bioaccumulate by some plants, including trees, and may also contaminate water.

## 2. Experimental

### 2.1. Instrumentation and Software

An Electrochemical Analyzer M161 with the electrode stand M161, both MTM-ANKO, made in Poland, were used for all voltammetric measurements. The classical three-electrode quartz cell, volume of 5 ml, consisting of the CGMDE type M164 (MTM-ANKO, made in Poland) with the surface area of 1.8 mm<sup>2</sup> as a working electrode, used in the hanging mercury drop electrode mode, a double junction reference electrode Ag/AgCl/3M KCl with replaceable outer junction (2M KNO<sub>3</sub>) and a platinum wire as an auxiliary electrode. All solution used for analyses were purged with argon. A magnetic Teflon-coated bar was used for stirring, approximately 600 rpm, during the accumulation period. Experiments were carried out at room temperature, approximately 24°C. The EAQt electrochemical analyzer software enabled electrochemical measurements, data acquisition, and advanced processing of the results (**Fig. 1**).



**Figure 1.** Experimental setup in the laboratory.

### 2.2. Reagents and Solutions

All solutions and the sample preparation were realized with double distilled water. An acetate buffer of pH 5.61 was used for the preparation of the supporting electrolyte. Also, Tl standard stock solution (10 mg·l<sup>-1</sup>, dilution of standard solution Certipur at 1000 mg·l<sup>-1</sup>, Merck) was applied (**Fig. 2**). Prior to use, all glassware and the electrode's body were cleaned by immersion in double distilled water to avoid contamination.



**Figure 2.** A bottle of standard solution Certipur at  $1000 \text{ mg}^{-1}$ , Merck brand.

### 2.3. DPV Procedure

The measurement was performed in the differential pulse (DP) mode. 4.5 ml of double distilled water and 0.5 ml of acetate buffer at 0.1 M (pH 5.61) was added in the cell and the solutions was purged with argon of 99.995% purity for 3 minutes. Then,  $10 \mu\text{l}$  of standard thallium solution was added to the cell while maintaining an argon over the solution before the record.

For the preconcentration time test, the measurement parameters were: current range= $1 \mu\text{A}$ ,  $E_p = -800 \text{ mV}$ ,  $E_k = -100 \text{ mV}$ ,  $E_{\text{step}} = 2 \text{ mV}$ ,  $dE = 50 \text{ mV}$ ,  $t_p = 20 \text{ ms}$ ,  $t_w = 20 \text{ ms}$ ,  $t_d = 500 \text{ ms}$ , accumulation potential:  $-800 \text{ mV}$ .

For the test of the potential range, the measurement parameters were: current range= $1 \mu\text{A}$ ,  $E_p = -800 \text{ mV}$ ,  $E_k = -100 \text{ mV}$ ,  $E_{\text{step}} = 2 \text{ mV}$ ,  $dE = 50 \text{ mV}$ ,  $t_p = 20 \text{ ms}$ ,  $t_w = 20 \text{ ms}$ ,  $t_d = 500 \text{ ms}$ , break: 60 sec, accumulation potential:  $-800 \text{ mV}$ .

For the test of the potential step, the measurement parameters were: current range= $1 \mu\text{A}$ ,  $E_p = -700 \text{ mV}$ ,  $E_k = -100 \text{ mV}$ ,  $dE = 50 \text{ mV}$ ,  $t_p = 20 \text{ ms}$ ,  $t_w = 20 \text{ ms}$ ,  $t_d = 500 \text{ ms}$ , break: 60 sec, accumulation potential:  $-700 \text{ mV}$ .

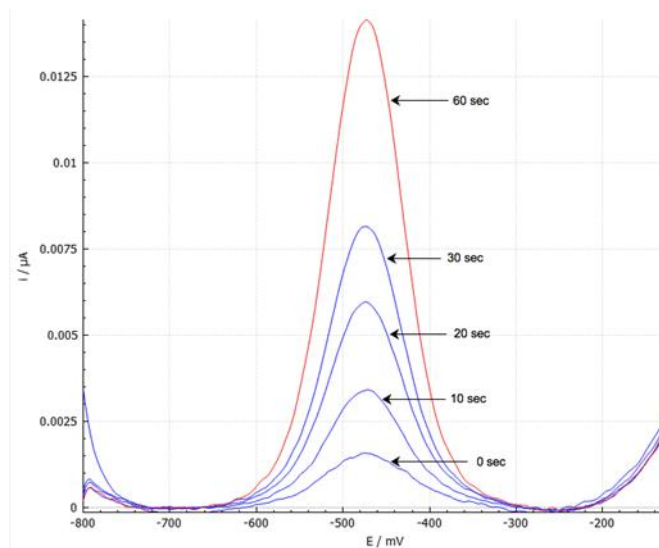
For the test of the potential pulse, the measurement parameters were: current range= $1 \mu\text{A}$ ,  $E_p = -700 \text{ mV}$ ,  $E_k = -100 \text{ mV}$ ,  $E_{\text{step}} = 3 \text{ mV}$ ,  $t_p = 20 \text{ ms}$ ,  $t_w = 20 \text{ ms}$ ,  $t_d = 500 \text{ ms}$ , break: 60 sec, accumulation potential:  $-700 \text{ mV}$ .

For the test of the sampling time and the waiting time, the measurement parameters were: current range= $1 \mu\text{A}$ ,  $E_p = -700 \text{ mV}$ ,  $E_k = -100 \text{ mV}$ ,  $dE = 50 \text{ mV}$ ,  $E_{\text{step}} = 3 \text{ mV}$ ,  $dE = 50 \text{ mV}$ ,  $t_p = 20 \text{ ms}$ ,  $t_w = 20 \text{ ms}$ ,  $t_d = 500 \text{ ms}$ , break: 60 sec, accumulation potential:  $-700 \text{ mV}$ . All measurements were performed in the same conditions.

## 3. Results and Discussion

### 3.1. Optimization of the measuring settings

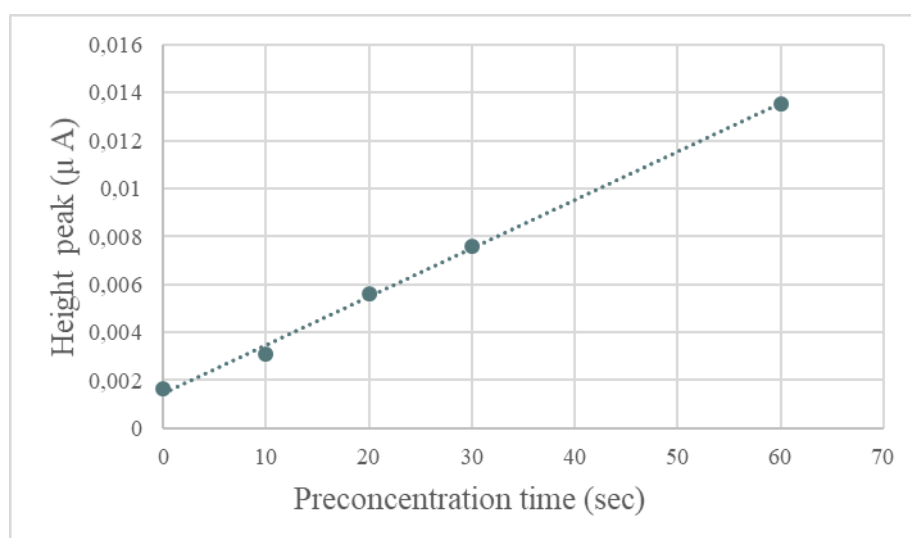
The first test concerns the preconcentration time, for this test, the measurement parameters were: current range= $1 \mu\text{A}$ ,  $E_p = -800 \text{ mV}$ ,  $E_k = -100 \text{ mV}$ ,  $E_{\text{step}} = 2 \text{ mV}$ ,  $dE = 50 \text{ mV}$ ,  $t_p = 20 \text{ ms}$ ,  $t_w = 20 \text{ ms}$ ,  $t_d = 500 \text{ ms}$ , accumulation potential:  $-800 \text{ mV}$ . We varied the preconcentration time five times, 0, 10, 20, 30 and 60 seconds (**Fig. 3**) (**Tab. 1**).



**Figure 3a.** Set of voltammogram according to different pre-concentration times with 10  $\mu\text{l}$  of Tl standard stock solution ( $0.1 \text{ mol}\cdot\text{l}^{-1}$ ).

**Table 1.** Table of the results of the different pre-concentration times.

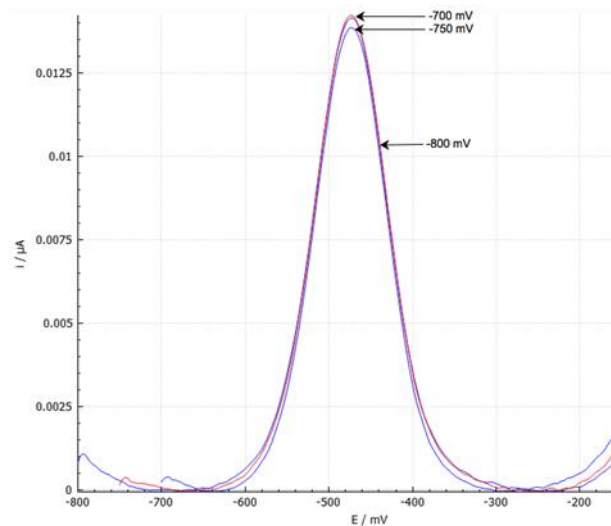
Preconcentration time (s)	Peak height ( $\mu\text{A}$ )	SD ( $\mu\text{A}$ )	% RSD
0	0.0017	0.00020	11.5000
10	0.0030	0.00028	7.88783
20	0.0056	0.00016	2.69923
30	0.0076	0.00021	2.54333
<b>60</b>	<b>0.0136</b>	<b>0.00031</b>	<b>2.17975</b>



**Figure 3b.** Graph showing the correlation between pre-concentration time (in sec) and peak height (in  $\mu\text{A}$ ).

We choose a preconcentration time of 60 seconds because it is with this time that we obtain the highest peak and the %RSD is very small.

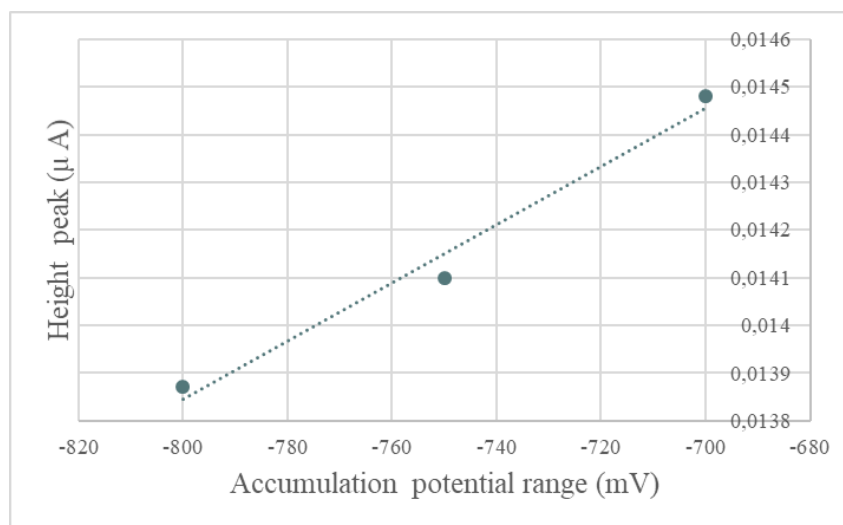
The second test concerns the accumulation potential, for this test, the measurement parameters are: current range=1 $\mu$ A,  $E_p$ =-800 mV,  $E_k$ =-100 mV,  $E_{step}$ =2 mV,  $dE$ =50 mV,  $t_p$ =20 ms,  $t_w$ =20ms,  $t_d$ =500ms, break: 60 sec, accumulation potential: -700 mV. We changed the accumulation potential range three times from -800 to -700 mV (**Fig. 4**) (**Tab. 2**).



**Figure 4a.** Set of voltammogram according to different accumulation potentials with 10  $\mu$ l of Tl standard stock solution ( $0.1 \text{ mol}\cdot\text{l}^{-1}$ ).

**Table 2.** Table of the results of the different accumulation potentials.

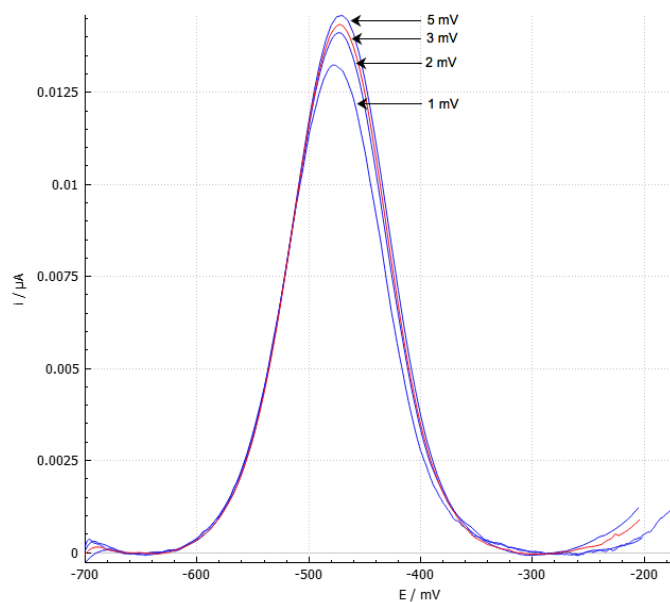
Accumulation Potential range (mV)	Height peak ( $\mu$ A)	SD ( $\mu$ A)	% RSD
-800	0.01387	0.000177	1.249266
-750	0.01410	0.000095	0.667755
<b>-700</b>	<b>0.01448</b>	<b>0.000142</b>	<b>1.003358</b>



**Figure 4b.** Graph showing the correlation between accumulation potential (in mV) and peak height (in  $\mu$ A).

We chose an accumulation potential of -700 mV because it was with this potential that we obtained the highest peak and the %RSD was higher than for -750 mV but it remained low.

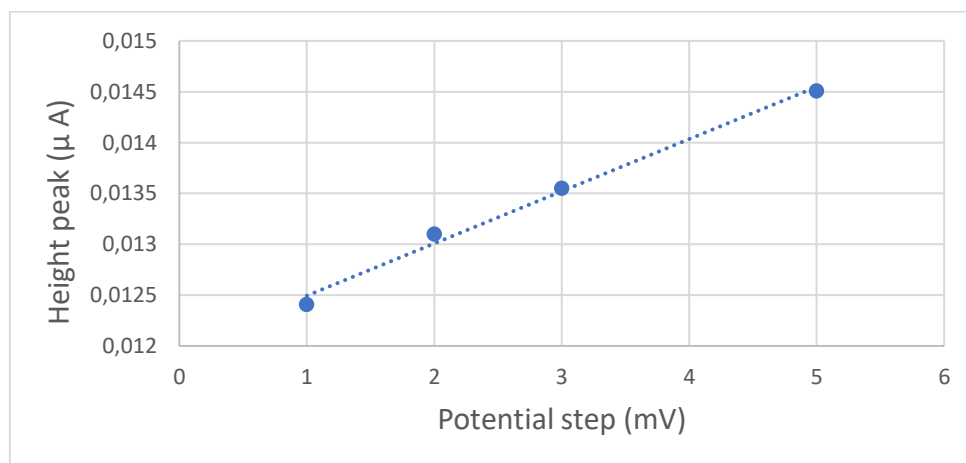
The third test concerns the potential step, for this test, the measurement parameters were: current range=1 $\mu$ A,  $E_p$ =-700 mV,  $E_k$ =-100 mV,  $dE$ =50 mV,  $t_p$ =20 ms,  $t_w$ =20ms,  $t_d$ =500ms, break: 60 sec, accumulation potential: -700 mV. We varied the potential step four times from 1 to 5 mV (**Fig. 5**) (**Tab. 3**)



**Figure 5a.** Set of voltammogram according to different potential steps with 10  $\mu$ l of Tl standard stock solution (0.1 mol.l<sup>-1</sup>).

**Table 3.** Table of the results of the different potential steps.

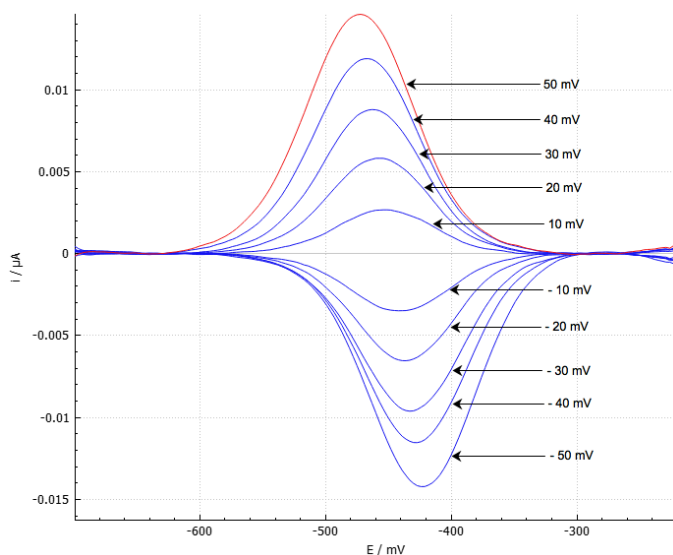
Potential step (mV)	Height peak ( $\mu$ A)	SD ( $\mu$ A)	% RSD
1	0.01241	0.000452	3.434867
2	0.0131	0.000191	1.378492
<b>3</b>	<b>0.01355</b>	<b>0.000049</b>	<b>0.341219</b>
5	0.01451	0.000303	2.059444



**Figure 5b.** Graph showing the correlation between potential step (in mV) and peak height (in  $\mu$ A).

We chose a potential step of 3 mV because it was with this threshold that we obtained the best precision while having a high peak and the %RSD was very small.

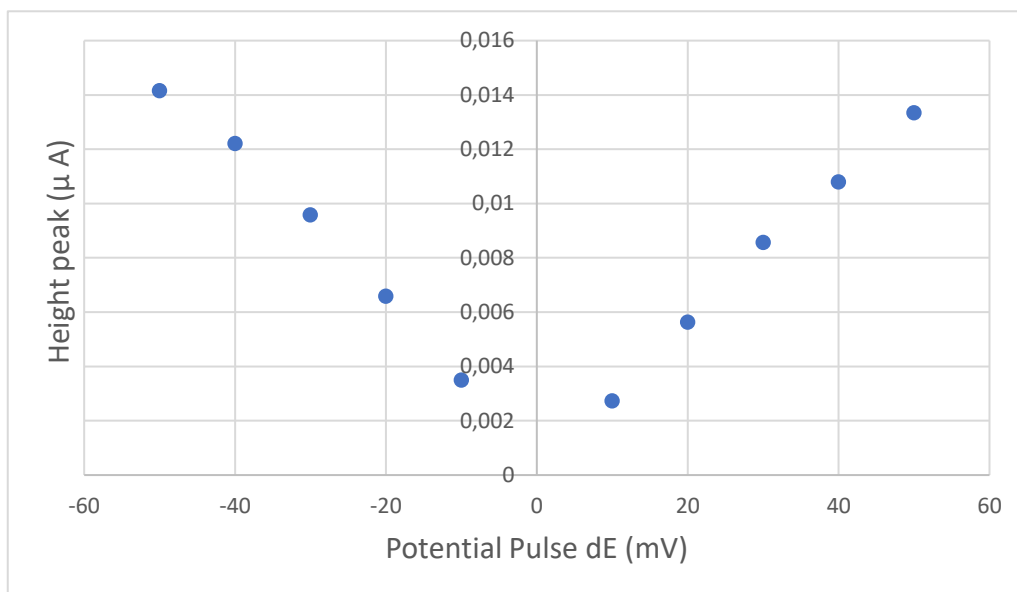
The fourth test concerns the potential pulse, the measurement parameters were: current range=1 $\mu$ A,  $E_p$ =-700 mV,  $E_k$ =-100 mV,  $E_{step}$ =3 mV,  $t_p$ =20 ms,  $t_w$ =20ms,  $t_d$ =500ms, break: 60 sec, accumulation potential: -700 mV. We varied the potential pulse ten times from -50 to 50 mV (Fig. 6) (Tab. 4).



**Figure 6a.** Set of voltammogram according to different potential pulses with 10  $\mu$ l of Tl standard stock solution ( $0.1 \text{ mol}\cdot\text{l}^{-1}$ ).

**Table 4.** Table of the results of the potential pulses optimization.

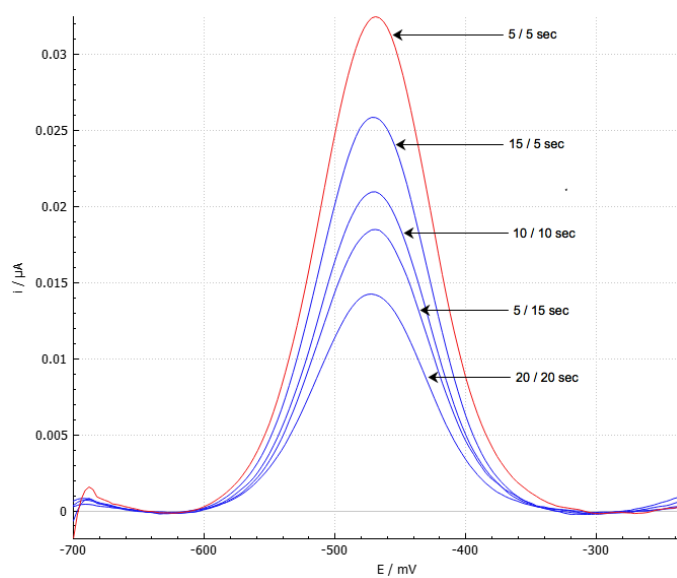
Potential Pulse dE (mV)	Height peak ( $\mu$ A)	SD ( $\mu$ A)	% RSD
-50	0.01416	0.000218	1.333148
-40	0.01221	0.000164	1.182854
-30	0.00958	0.000375	3.592912
-20	0.00659	0.000075	1.020238
-10	0.0035	0.000087	2.249844
10	0.00274	0.000072	2.336118
20	0.00563	0.000046	0.696440
30	0.00857	0.000114	1.149577
40	0.0108	0.000078	0.595359
<b>50</b>	<b>0.01335</b>	<b>0.000093</b>	<b>0.576281</b>



**Figure 6b.** Graph showing the correlation between potential pulse (in mV) and peak height (in  $\mu\text{A}$ ).

We chose a potential pulse of 50 mV because it was with this impulse that we obtained the highest peak and the %RSD was very small.

The fifth test concerns the sampling time and the waiting time, the measurement parameters were: current range=1 $\mu\text{A}$ ,  $E_p$ =-700 mV,  $E_k$ =-100 mV,  $dE$ =50 mV,  $E_{step}$ =3 mV,  $dE$ =50 mV,  $t_p$ =20 ms,  $t_w$ =20ms,  $t_d$ =500ms, break: 60 sec, accumulation potential: -700 mV. We vary the different combinations of sampling time and waiting time (**Fig. 7**) (**Tab. 5**).



**Figure 7.** Set of voltammograms according to different combinations of sampling time and waiting time with 10  $\mu\text{l}$  of Tl standard stock solution ( $0.1 \text{ mol}\cdot\text{l}^{-1}$ ).



**Table 5.** Table of the results of the different combinations of sampling time and waiting time.

tp (ms)	tw (ms)	Height peak ( $\mu\text{A}$ )	SD ( $\mu\text{A}$ )	% RSD
<b>5</b>	<b>5</b>	<b>0.03170</b>	<b>0.000273</b>	<b>0.832730</b>
5	15	0.01858	0.000056	0.292118
15	5	0.02631	0.000230	0.874357
10	10	0.02134	0.000548	2.562205
20	20	0.01431	0.000250	1.695190

We chose the 5-5 ms combination of sampling time and waiting time because it was with this combination that we got the highest peak and the %RSD was higher than the combination 5-15 ms but it remained low.

#### 4. Methodology for Validation

The validation of an analytical method is the process established by laboratory studies so that the performance characteristics of this method meet the application requirements. The analytical parameters that are required for the method validation are: selectivity, precision, linearity, detection limit, and accuracy [2].

**Table 6.** Table of validation methodology according to different criteria.

Settings	How to measure it	Acceptance criteria
Precision	The height of the peak is measured after each addition of 5 $\mu\text{l}$ of standard solution of Tl at 10 $\text{mg.l}^{-1}$ . This measurement is repeated three times. A concentration range of 10 $\mu\text{g.l}^{-1}$ to 100 $\mu\text{g.l}^{-1}$ is then obtained. For each concentration, we calculate the average height of peaks then the SD and finally the % RSD	The % RSD must be less than 5%
Linearity	The height of the peak is measured after each addition of 5 $\mu\text{l}$ of standard solution of Tl at 10 $\text{mg.l}^{-1}$ . The calibration line is then made to obtain the correlation coefficient.	The correlation coefficient must be greater than 0.995
Detection limit	The standard deviation of the baseline is calculated and multiplied by 3.3 and divided by the sensitivity (slope of the calibration line).	
Accuracy	We measured thallium at known concentrations added to Rudawa River water.	The goal is to find the same concentration by applying the method of adding standards.

#### 4.1. Selectivity

There is no problem with selectivity because in the potential region used, no other signal apart from Tl is observed. This remark confirms the selectivity of the method.

#### 4.2. Precision

Precision was evaluated through repeatability of the final determined concentration and repeatability of the signal (expressed by the Tl peak) recorded for the standard solution. Three replicates were realized for each measure. (Tab. 7).

Precision was expressed as standard deviations (SD) and relative standard deviations (%RSD) calculated for Tl peak heights.

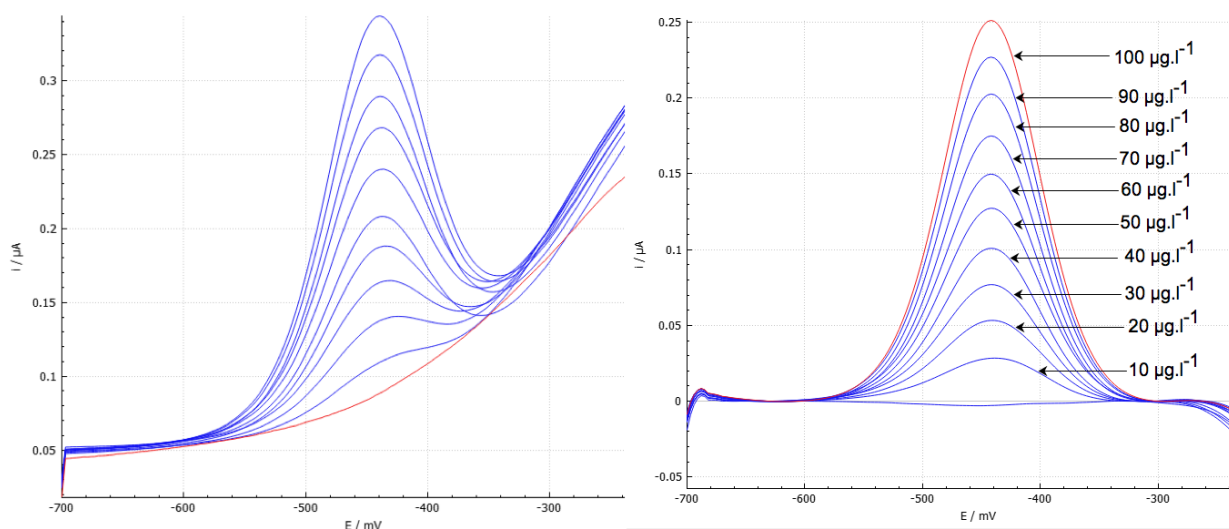
**Table 7.** Table of the different concentrations, the average of the three repetitions for each measure as well as their SD and % RSD.

C° Tl ( $\mu\text{g}\cdot\text{l}^{-1}$ )	Average peak height ( $\mu\text{A}$ )	SD ( $\mu\text{A}$ )	% RSD
10	0.02847	0.004368	16.496338
20	0.05336	0.001567	3.005598
30	0.0766	0.000341	0.449755
40	0.10079	0.001375	1.356712
50	0.12743	0.000178	0.140314
60	0.14955	0.003036	2.059573
70	0.17509	0.001424	0.816060
80	0.20287	0.003710	1.831915
90	0.22721	0.003303	1.462296
100	0.25145	0.001581	0.629133

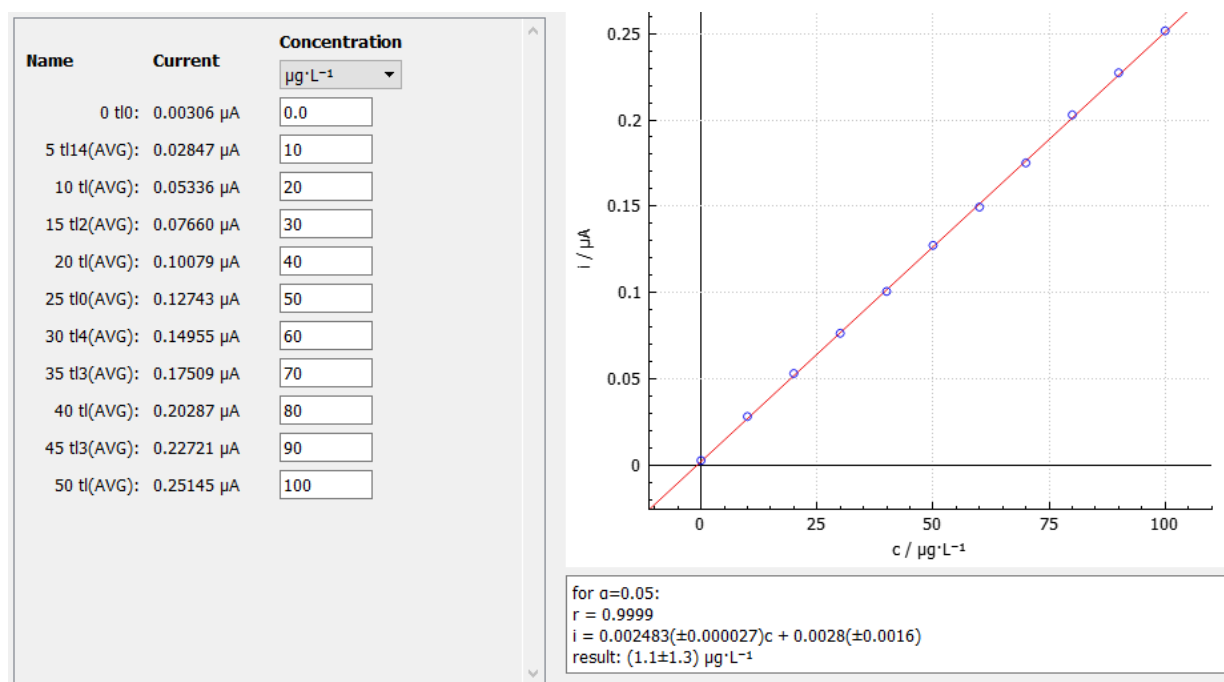
For all measurements except  $10 \mu\text{g}\cdot\text{l}^{-1}$  the %RSD was less than 5%. This method is precise however a handling error could interfere with the results of the measurement for  $10 \mu\text{g}\cdot\text{l}^{-1}$ .

#### 4.3. Linearity

To make the calibration line, we measured the height of the peak after additions of  $5 \mu\text{l}$  of standard solution of Tl at  $10 \text{mg}\cdot\text{l}^{-1}$ . The final Tl concentration range in the cell was  $10 \mu\text{g}\cdot\text{l}^{-1}$  to  $100 \mu\text{g}\cdot\text{l}^{-1}$ .



**Figure 8.** Two sets of voltammogram, left before adaptation of the background and right after adaptation of the background with ten additions of 5 µl of standard solution of 10 mg·l<sup>-1</sup> of TL.



**Figure 9.** The calibration line corresponding to the previous voltammograms (Fig. 8) with the different concentrations.

As it can be seen in **Fig. 9**, the coefficient of correlation  $r$  is 0.9999, so it is greater than 0.995. The method is therefore linear.

#### 4.4. Detection limit

We calculated the detection limit (LOD) which is 2.3 µg·l<sup>-1</sup>. We also calculated the limit of quantification (LOQ) which is 7.0 µg·l<sup>-1</sup>. The achieved LOD revealed the sensitivity of the described voltammetric method.

#### 4.5. Accuracy

To prove the accuracy of this method, we measured thallium at known concentrations added in Rudawa River water (**Tab. 8**). The goal is to find the same concentration by applying the method of adding standards.

**Table 8.** Standard addition voltammetry of thallium in Rudawa River water.

Added (mg·l <sup>-1</sup> )	Found (mg·l <sup>-1</sup> )	Recovery (%)	r
Supporting electrolyte (acetate buffer pH (5.61) + 0.5 ml of Rudawa River water)			
0.01	0.0091±0.0023	91.0	0.9996
0.05	0.0518±0.0092	96.5	0.9985
0.10	0.101±0.017	99.0	0.9976
Supporting electrolyte (acetate buffer pH (5.61) prepared in Rudawa River water)			
0.01	0.0097±0.0023	97.0	0.9996
0.05	0.0459±0.0093	91.8	0.9984
0.10	0.097±0.020	97.0	0.9966

The concentrations are quite similar and the percentage of similarity (recovery %) is always between 90 and 100%. Therefore, this method is accurate.

## 5. Conclusion

The main goal of this work was the optimization and validation of the anodic stripping voltammetry method for the determination of Tl. The optimal experimental variables as well as accumulation parameters were investigated, these were: preconcentration time of 60 seconds, accumulation potential of -700 mV, potential step of 3 mV, potential pulse of 50 mV and 5-5 ms combination of sampling time and waiting time using 0.5 ml of acetate buffer at 0.1 M pH 5.61 as a supporting electrolyte. The optimized method was validated and was found to be selective, precise, linear, sensitive and accurate. Precision expressed by % RSD not greater than 5% however a handling error could interfere with the results of one measurement. The linearity given by correlation coefficient not lower than 0.995 and the LOD was 2.3 µg·l<sup>-1</sup>.

## References

- [1] International Organization for Standardization (2005) General requirements for the competence of testing and calibration laboratories.
- [2] Opoka et al (2010) Development and validation of an anodic stripping voltammetric method for determination of Zn<sup>2+</sup> ions in brain microdialysate samples. *Bio Trace Elem Res* (2011) 142:671-682.
- [3] Chemicool Dictionary website, <https://www.chemicool.com>
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- [5] Elvar Theodorsson, EFLM European Federation of Clinical Chemistry and Laboratory Medicine, Limit of detection, limit of quantification and limit of blank.