

New Adsorptive Stripping Determination of ATP with Thorium(IV) on Renewable Silver Amalgam Film Electrode

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A new adsorptive stripping voltammetric method for the determination of trace ATP based on the adsorption of ATP-thorium(IV) complex on the renewable mercury film silver based electrode (*Hg(Ag)FE*) is presented. The effects of various factors such as: preconcentration potential and time, pulse height, step potential and supporting electrolyte composition are optimized. The calibration graph is linear from 2.5 nM ($1.27 \mu\text{g}\cdot\text{L}^{-1}$) to 130 nM ($65.9 \mu\text{g}\cdot\text{L}^{-1}$) for a preconcentration time of 30 s, with correlation coefficient of 0.999. For a *Hg(Ag)FE* with a surface area of 9.4 mm^2 the detection limit for a preconcentration time of 120 s is $81 \text{ ng}\cdot\text{L}^{-1}$. The repeatability of the method at a concentration level of the analyte as low as $2.5 \mu\text{g}\cdot\text{L}^{-1}$, expressed as RSD is 2.1% (n=5). The proposed method was successfully applied and validated by studying the natural samples with simultaneous recovery of ATP from spiked tablet samples.

Keywords: Adenosine-5'-triphosphate; Th(IV); Trace Analysis; Silver Amalgam Film Electrode; Adsorptive Stripping Voltammetry.

1. INTRODUCTION

Adenosine-5'-triphosphate (ATP) is a multifunctional nucleoside triphosphate, which is composed of an adenine ring, a ribose sugar and three phosphate groups. Because of its ubiquitous presence in living matter, ATP has been widely used as an index for biomass determinations in clinical microbiology, food quality control and environmental analyses. [1, 2]. Therefore, it is necessary to establish a sensitive method for ATP detection.

Many methods have been developed for the determination of ATP, including spectrophotometry [3], chromatography [4, 5, 6], chemiluminescence assays [7, 8], fluorescence [9], aptamer-based methods [10–12] and electrochemical methods [13–17].

Most voltammetric methods and techniques required mercury electrodes. The *HMDE* is the electrode of preference due to its high sensitivity, reproducibility and linearity. However, the toxicity of mercury limits the usage of the mercury electrodes in the analytical practice and excludes them from the out-of-laboratory applications. One of the solutions proposed by Baś and Kowalski greatly limits the amount of mercury in electrode construction thereby makes the use of this electrode more save [18]. Such mercury electrode (*Hg(Ag)FE*) was used for the determination of various compounds and organic elements in simple and complex matrix [19–33].

In this work differential pulse adsorptive stripping voltammetry (*DP AdSV*) is applied for the trace ATP determination in the presence of Th(IV) ions.

2. EXPERIMENTAL

2.1. Measuring apparatus and software

A multipurpose Electrochemical Analyzer M161 with the electrode stand M164 (both MTM-ANKO, Poland) were used for all voltammetric measurements. The classical three-electrode quartz cell, volume 20 mL, consisting of a homemade cylindrical silver based mercury film electrode (*Hg(Ag)FE*) [18], refreshed before each measurement and with a surface area of 1 – 12 mm², as the working electrode, a double junction reference electrode Ag/AgCl/KCl (3M) with replaceable outer junction (3 M KCl) and a platinum wire as an auxiliary electrode. pH measurements were performed with laboratory pH-meter. Stirring was performed using a magnetic bar rotating at approximately 500 rpm. All experiments were carried out at room temperature.

2.2. Chemicals and glassware

All reagents used were of analytical grade - CH₃COOH and CH₃COONa (Merck, Suprapur®), mercury GR for polarography (Merck). The 0.1 M standard stock solution of ATP was prepared by dissolving Adenosine 5'-triphosphate disodium salt hydrate (Aldrich) in water and was stored in fridge. Solutions with lower ATP concentrations were made daily by appropriate dilution of the stock solution. The 0.01 M solution of Th(IV) was prepared by dissolving Th(NO₃)₄·5H₂O (Merck) in water. The silver base for the film electrode was prepared from polycrystalline silver wire with a diameter of 0.5 mm, and of 99.99% purity (Goodfellow Science Park, England). Prior to use, glassware was cleaned by immersion in a 1:10 aqueous solution of HNO₃, followed by copious rinsing in distilled water.

2.3. Standard procedure of measurements

Quantitative measurements were performed using differential pulse adsorptive stripping voltammetry (*DP AdSV*) and the standard addition procedure. The procedure of refreshing the mercury

film $Hg(Ag)FE$ electrode was carried out before each measurement. A potential of -1.45 V was applied to condition the electrode after the refreshing step. The $Hg(Ag)FE$ electrode conditioned in this way was used to determine ATP in the supporting electrolyte: 0.04 M acetate buffer (pH 4.8) and 1 μM Th(IV) (total volume 10 ml) contained in a quartz voltammetric cell. The potential of the electrode was changed in the following sequence: conditioning potential -1.45 V for 5 s, preconcentration potential $E_{acc} = 0.05$ V for $t_{acc} = 30$ s and starting potential -0.3 V for 1 s. During the preconcentration step ATP was adsorbed while the solution was being stirred (ca. 500 rpm.) using a magnetic stirring bar. Then, after a rest period of 5 s a differential pulse voltammogram was recorded in the cathodic direction from -0.3 V to -1.45 V. The other experimental parameters were as follows: step potential, 5 mV; pulse potential, 50 mV; time step potential, 20 ms (10 ms waiting + 10 ms sampling time). The measurements were carried out from deaerated solutions.

2.4. Sample preparation

2.4.1. Tablets

For the determination of ATP in tablets three tablets containing 20 , 25 and 125 mg ATP per tablet were dissolved in 50 mL volumetric flask and additionally sonicated for 15 min.

3. RESULTS AND DISCUSSION

3.1. Influence of Th(IV) concentration on the ATP signal

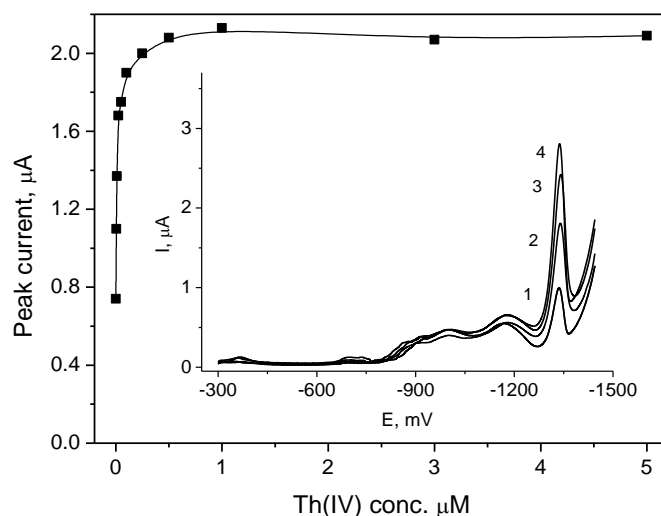


Figure 1. Dependence of the peak current on Th(IV) concentration in the range from 0 to 5 μM for 50 nM ATP in 0.04 M acetate buffer (pH of base electrolyte 4.8) and obtained voltammograms for (1) -0 , (2) -0.01 , (3) -0.05 , (4) -1 μM of Th(IV). The electrode area was 9.4 mm^2 . Instrumental parameters: $\Delta E = 50$ mV, $E_s = 5$ mV, $t_w, t_s = 10$ ms. Preconcentration potential $E_{acc} = 0.05$ V and time $t_{acc} = 30$ s. Stirring rate, 500 rpm.

Sensitive determination of ATP by *AdSV* method involves presence of thorium(IV) ions in the supporting electrolyte. The ATP peak current depend on the concentration of Th(IV) (Fig. 1). In the absence of Th(IV) in acetate buffer observed ATP peak current was 0.74 μA . The addition of Th(IV) to the base electrolyte (acetate buffer pH=4.8) is accompanied by increase of the ATP peak. The optimal concentration of Th(IV) is above 0.5 μM (the peak current for 50 nM of ATP reaching values about 2.1 μA). The concentration of Th(IV) practically had no influence on the peak potential. For further work, a concentration of 1 μM was used (the peak potential was -1340 mV and the peak half width was 36 mV). The obtained precision for $n=5$ and 50 nM of ATP was 2.2%.

3.2. Influence of DPV parameters on ATP signal

The important parameters of the *DPV* technique are pulse amplitude (ΔE), potential step amplitude (E_s), waiting time (t_w) and sampling time (t_s). Consequently, these parameters were investigated. To optimize the conditions for scandium measurements, the following instrumental parameters were systematically varied: ΔE in the range 5 – 100 mV (both positive and negative mode), E_s in the range 2 – 6 mV, t_w and t_s from 10 to 80 ms.

The best results were obtained for the pulse amplitude of 50 mV (the peak current was $\sim 2,1$ μA). Higher pulse amplitude (>50 mV) caused growth of the background current without significant growth of the ATP peak current. For further work, the pulse amplitude of 50 mV was applied.

Polarization rate measured as potential step height have an influence on the peak current and background current. The optimal step potential was 5 mV.

The waiting time and sampling time were changed in the range from 10 ms to 80 ms. The best results were obtained for waiting time and sampling time of 10 ms, and this was the value chosen for further work.

3.3. Influence of preconcentration potential and time on ATP signal

Influence of preconcentration potential and time are always important factors on the sensitivity and detection limit of the method. Optimal preconcentration potential for ATP determination in acetate buffer with Th(IV) (pH of base electrolyte 4.8) is in the range from -0.1 V to 0.1 V (Fig. 2). For preconcentration potentials lower than -0.1 V and higher than 0.1 V the ATP peak decreased. For further work, a 0.05 V preconcentration potential was applied.

The changes in magnitude of the ATP current vs. preconcentration time are presented in Figure 3. The peak current for 50 nM of ATP increased with the increase of the preconcentration time from 0.13 μA ($t_{acc} = 5$ s) to 11.1 μA ($t_{acc} = 240$ s). The ATP peak potential is practically independent on either the preconcentration potential and time.

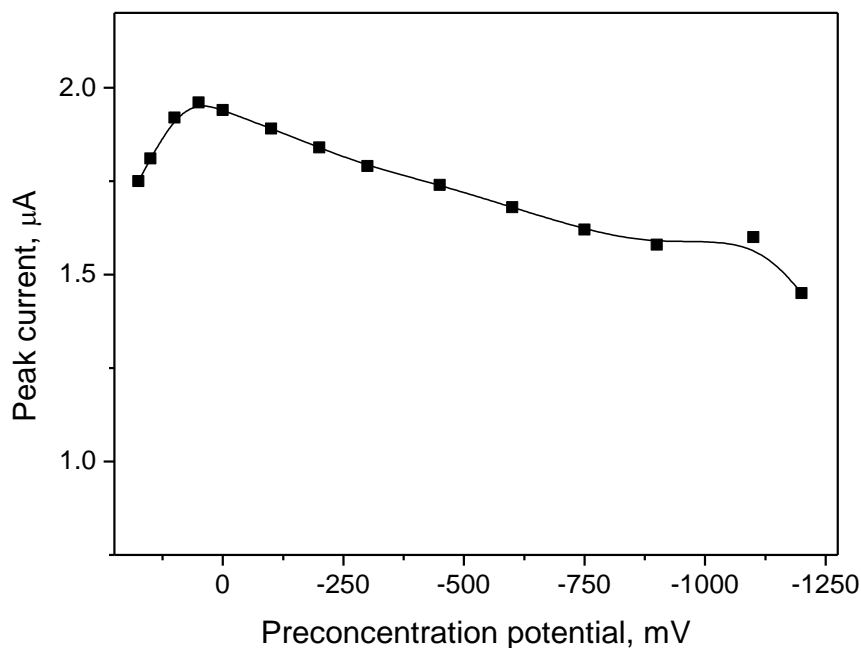


Figure 2. Dependence of the peak current on preconcentration potential in the range from 175 to -1250 mV for 50 nM ATP in 0.04 M acetate buffer and 1 μM Th(IV) (pH of base electrolyte 4.8). All other conditions as in Figure 1.

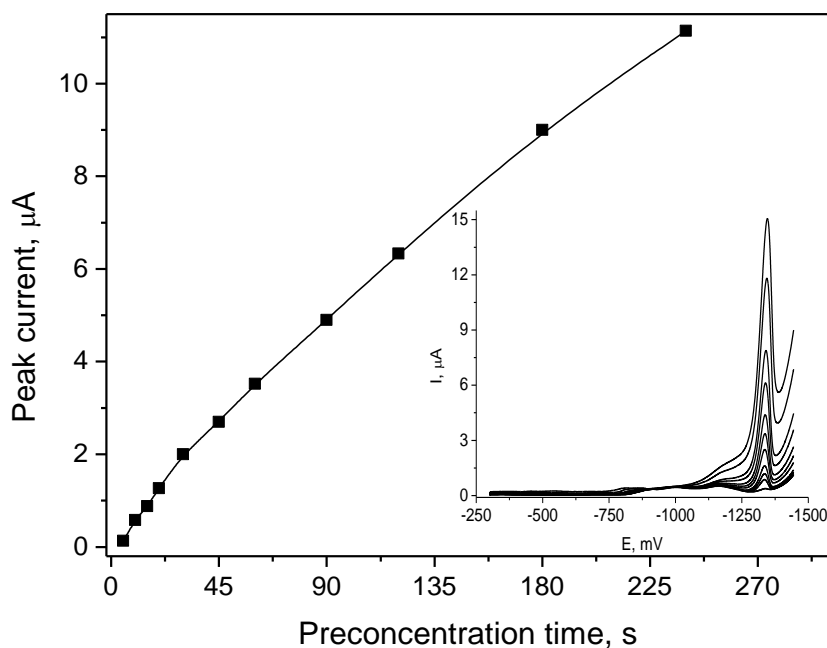


Figure 3. Dependence of the peak current on preconcentration time in the range from 0 to 240 s for 50 nM ATP in 0.04 M acetate buffer and 1 μM Th(IV) (pH of base electrolyte 4.8) and obtained voltammograms. All other conditions as in Figure 1.

3.4. Influence of pH on ATP signal

Determination of ATP in presence of Th(IV) requires a medium pH in order to obtain a ATP-thorium(IV) complex, which is adsorbed on the working electrode during the preconcentration. The peak current of ATP – complex depends on the pH. In Figure 4, the dependence of peak current and voltammograms on pH are presented. The optimal pH was in the range from 4.5 to 5 (with the peak current reaching values about 2.1 μA). More acidic and more alkaline conditions caused a decrease in the peak current. The pH also had an influence on the peak potential, which changed to positive values for lower pH values. For example, for a pH of 5.6 the peak potential was -1380 mV and for a pH of 2.8 the peak potential was -1230 mV. For further measurements, a pH of 4.8 was applied.

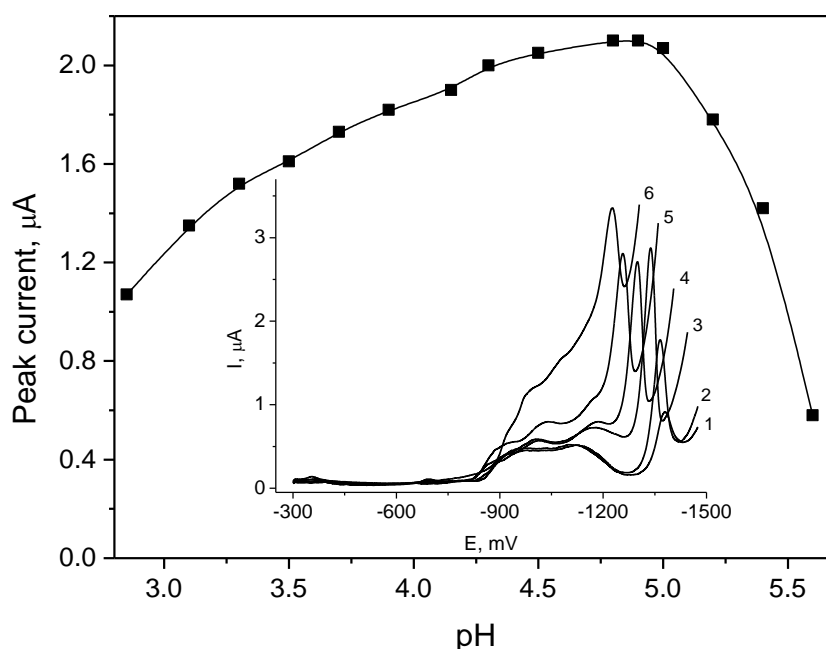


Figure 4. Dependence of the peak current on pH in the range from 2.85 to 5.6 for 50 nM ATP in 0.04 M acetate buffer and 1 μM Th(IV) (pH of base electrolyte 4.8) and obtained voltammograms for (1) – 5.6; (2) – 5.4; (3) – 4.8; (4) – 3.9; (5) – 3.3; (6) – 2.85 pH. All other conditions as in Figure 1.

3.5. Influence the surface of the Hg(Ag)FE electrode on ATP signal

The surfaces of solid electrodes are usually much larger than those of mercury drop electrodes. When using the Hg(Ag)FE electrode the surface of the working electrode may easily be varied in a wide range. The ATP peak grew linearly as the surface of the working electrode increased in size (Fig. 5). The parameters of the linear growth of peak current vs. surface of working electrode (50 nM ATP) are: slope, 0.233 ± 0.001 [$\mu\text{A} \cdot \text{mm}^{-2}$], intercept, 0.079 ± 0.039 [μA], and correlation coefficient $r = 0.998$. For further study, a 9.4 mm^2 surface area was applied.

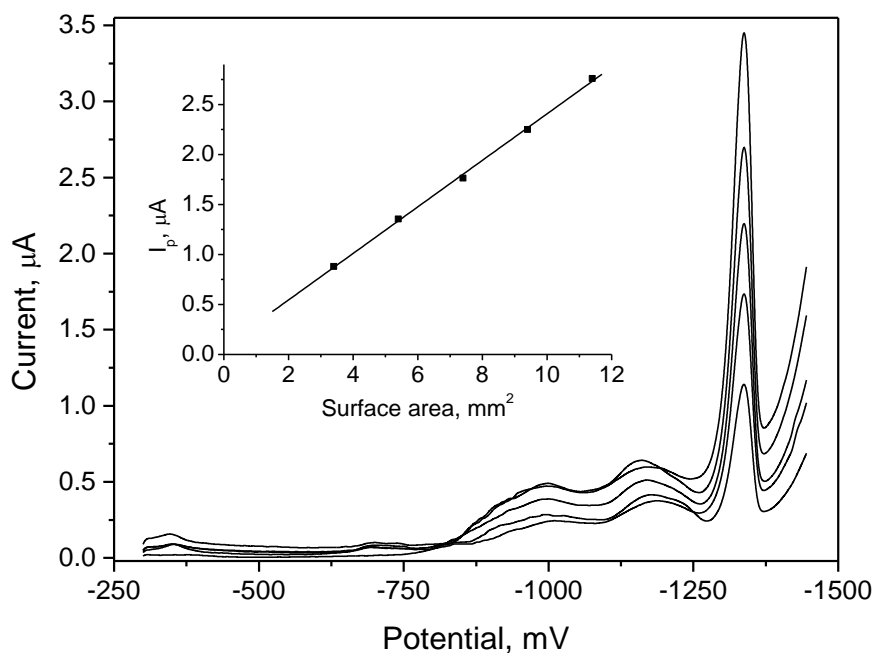


Figure 5. Voltammograms obtained for electrode surface area: 3.4, 5.4, 7.4, 9.4 and 11.4 mm² for 50 nM ATP in 0.04 M acetate buffer and 1 µM Th(IV) (pH of base electrolyte 4.8). All other conditions as in Figure 1.

3.6. Interferences

The examined ions, such as: Na(I), K(I), Ca(II), Mg(II), Fe(III), Mn(II) (1000-fold excess), Zn(II), Cu(II), Sn(II), Pb(II), Cd(II), Tl(I), Sc(III), La(III), Lu(III), U(VI), ascorbic acid in a 25-fold excess did not interfere. However, it was observed that for Mo(VI) ions in the same concentrations as ATP (50 nM), the ATP peak current decreased by 50% and for 5-fold excess the interpretation of ATP signal was very difficult (very high background current). Fortunately, the presence of Mo(VI) in real samples are rather very low. For citric acid in a 5-fold excess no interferences were observed and for 10-fold excess the ATP peak current decreased by 5% and 50-fold excess the peak current decreased by 30% and for 100-fold excess the peak current decreased by 40%. For glucose (10 mg·L⁻¹) no interferences were observed and for 100 mg·L⁻¹ the ATP signal decreased by 15%.

3.7. Analytical performance

The *DP AdSV* voltammograms of ATP for the 2.5-100 nM concentration range and preconcentration time of 30 s are presented in Figure 6. For the preconcentration time (30 s) the obtained detection limit, is 0.43 nM and the linearity is up to 100 nM (slope for regression line is 0.039 ± 0.0005 [µA·nM⁻¹], intercept 0.046 ± 0.018 µA, correlation coefficient 0.999). A longer preconcentration time results in a lower detection limit. For example, for a preconcentration time of

120 s the detection limit is 0.16 nM. The slope for regression lines is [$\mu\text{A}\cdot\text{nM}^{-1}$]: 0.202 ± 0.003 , intercept [μA]: 0.019 ± 0.006 and correlation coefficient 0.998.

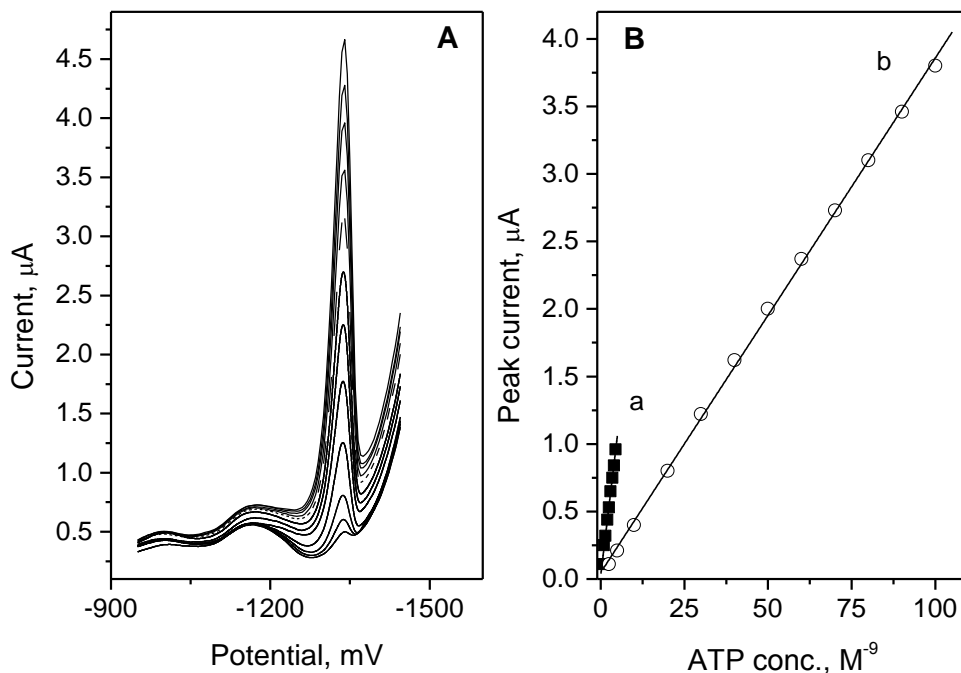


Figure 6. (A) – DP AdSV ATP calibration voltammograms obtained for preconcentration time 30 s. (B) –calibration curves for preconcentration times: (a) 120 s, (b) 30 s in 0.04 M acetate buffer and 1 μM Th(IV) (pH of base electrolyte 4.8). All other conditions as in Figure 1.

Precision and recovery were determined using three different samples spiked by 1.5, 3 and 10.5 nM of ATP (Tab.1).

Table 1. Recovery and precision of the determination of trace ATP.

Added [nM]	Found [nM]	Recovery [%]	RSD [%]
1.5	1.6	107	4.4
3.0	3.1	103	4.2
10.5	10.1	96	2.6

The tablet samples, spiked with ATP, were analyzed according to the described procedure using the $\text{Hg}(\text{Ag})\text{FE}$ electrode. Determinations of ATP were performed using the standard addition method. Results from ATP determination are presented in Table 2. The recovery of ATP ranged from 96–105%. The analytical usefulness, of the presented method for the determination of ATP in samples was confirmed.

Table 2. Results of ATP determination in tablet samples.

ATP added [mg]	ATP found $\bar{x} \pm s$ (recovery, %)		
	Sample ¹ [mg/tablet]	Sample ² [mg/tablet]	Sample ³ [mg/tablet]
0	22.1 ± 1.7	26.2 ± 1.9	123 ± 5
20	40.4 ± 2.2 (96)	48.5 ± 2.3 (105)	-
50	74.3 ± 4.3 (103)	77.7 ± 2.9 (102)	175 ± 5 (101)
100	-	-	216 ± 7 (97)

¹ – product declared 20 mg/tablet

² – product declared 25 mg/tablet

³ – product declared 123 mg/tablet

4. CONCLUSIONS

The presented *DP AdSV* method for the electrochemical determination of ATP with Th(IV) using a cylindrical silver based mercury film electrode (*Hg(Ag)FE*), refreshed before each measurement, allows to determine ATP at trace level, in concentrations as low as 0.16 nM (81 ng·L⁻¹), for a preconcentration time of 120 s. The reproducibility of the method is very good, i.e. when measured as RSD is 2.1% (with each measurement performed at a fresh surface of the working electrode). Acceptable recovery (96–105%) shows that the proposed method can be used for the determination of ATP in tablet samples. The new procedure was examined and successfully utilized for determination of low and high ATP concentration in tablet samples.

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