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# Voltammetric behavior and quantitative determination of ambazone concentrations in urine and in a pharmaceutical formulation

Research Article

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Abstract: The use of square wave adsorptive stripping voltammetry (SWAdSV) in conjunction with a cyclic renewable silver amalgam film electrode (Hg(Ag)FE) for the analytical determination of ambazone in urine samples and pharmaceutical formulations is described. A single reduction peak in Britton-Robinson buffer at pH 4.0 was detected at about -1.4 V *versus* Ag/AgCl. Mechanistic studies have shown that the compound can act as an electrocatalyst. The method was validated. The analytical curve was linear in the concentration range from  $1.0 \times 10^{-9}$  to  $1.0 \times 10^{-7}$  mol L<sup>-1</sup>. The detection and quantification limits were found to be  $3.0 \times 10^{-10}$  mol L<sup>-1</sup> and  $1.0 \times 10^{-9}$  mol L<sup>-1</sup>, respectively. The proposed method was successfully applied to ambazone determination in real samples.

Keywords: Voltammetry • Silver amalgam electrode • Ambazone drug analysis • Catalytic hydrogen evolution © Versita Sp. z o.o.

### 1. Introduction

Drug analysis is an important field of analytical chemistry [1,2]. Therefore, the development of fast and sensitive methods for quantitative determination of drugs is of great significance. Ambazone ([4-(2-(Diaminomethylidene)hydrazinyl)phenyl] iminothiourea) (AMB) (Fig. 1) is an oral antiseptic [3] with an antibacterial spectrum similar to sulfamides [4] and proven activity against various transplantable tumors [5].



Figure 1. Molecular structure of ambazone (AMB).

AMB is active against the bacteria Streptococcus Streptococcus pneumoniae, pyogenes, and Streptococcus viridans; exhibits low mutagenic activity; and has no gastrointestinal, metabolic or cardiovascular side-effects. Therefore, it is used in the treatment of pharyngitis, tonsillitis, and stomatitis at a concentration of 0.01-0.2% in the form of a solution or dusting powder, or internally in gastrointestinal tract infections. To our knowledge, only one method for quantitative determination of ambazone has been published to date [6]. In the current communication, we present a study of ambazone at a Hg(Ag)FE under conditions of square wave adsorptive stripping voltammetry. Squarewave voltammetry [7,8] is currently one of the most advanced pulse voltammetric techniques used in various types of research [9,10], mainly for analytical [11-14], mechanistic [15-17], and kinetic studies of electrode processes [18-20]. The properties of Hg(Ag)FE have been previously described in detail [21-23]. The cyclic renewable silver amalgam film

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electrode and other amalgam-based electrodes have been widely used in the analysis of metals [24-27], as well as organic [28-30] and inorganic compounds [31,32]. In the presented study, the electrode mechanism is analyzed in light of recent theories concerning guanidine compounds, which can act as electrocatalysts. Based on knowledge of the electrode reaction mechanism, a quantitative method using SWAdSV is proposed for the determination of AMB concentration.

# 2. Experimental procedure

### 2.1. Instrumentation

All voltammetric experiments were performed using a µAutolab with GPES software (Eco Chemie, Netherlands) and an M164 electrode stand (mtm-anko, Cracow, Poland). Experiments were performed in a three-electrode system consisting of Ag/AgCI (3 mol L<sup>-1</sup> KCI) as a reference electrode, Pt wire as a counter electrode, and a silver amalgam film electrode (mtm-anko, Cracow, Poland) as a working electrode. The construction and parameters of the Hg(Ag)FE used in our experiments were described previously [21-23]. The surface of a Hg(Ag)FE may be adjusted up to 12 mm<sup>2</sup>. In the present experiments, a surface of 12 mm<sup>2</sup> was used because the peak current was linearly dependent on the electrode surface. Silver amalgam film was prepared before each experiment. Measurements of pH were made using a CP-315M pH-meter (Elmetron, Poland) with a combined glass electrode.

### 2.2. Solutions and materials

All chemicals used were of analytical reagent grade and purchased from Sigma Aldrich. A fresh AMB stock solution of  $1.00 \times 10^{-3}$  mol L<sup>-1</sup> was prepared weekly by dissolving 5.92 mg of the compound in 25 mL of water. The supporting electrolytes were Britton-Robinson (BR), citrate, and acetate buffers. Distilled and deionized water was used throughout the experiments. Solutions were purged with pure argon for at least 10 min prior to each voltammetric scan, and argon was passed over the solutions during the measurements. All electrochemical measurements were carried out at the ambient temperature of the laboratory (20–22°C).

### 2.3. Voltammetric procedure

The general procedure used to obtain voltammograms was as follows: 10 mL of supporting electrolyte was transferred to the electrochemical

cell, deaerated by passing an argon stream for 10 min, and then a voltammogram was recorded under the inert atmosphere of the cell. When an initial blank was recorded, the required volumes of the compound were added by means of a micropipette. The voltammogram of the blank was subtracted from the voltammograms recorded for the compound.

### 2.4. Analysis of a pharmaceutical formulation

Faringosept tablets (Terapia), containing 10 mg of AMB each, were powdered, and amounts corresponding to 1.0×10-3 mol L-1 of AMB were weighed and dissolved in water. After sonication, working solutions were prepared by serial dilution. 5 mL of BR buffer (pH 4) and 5 mL of distilled water were placed in the voltammetric cell. After recording the initial blank, 50 µL of Faringosept solution  $(C_{AMB} = 1.0 \times 10^{-6} \text{ mol } \text{L}^{-1})$  was added by means of a micropipette and a voltammogram was recorded for the sample. In all the experiments, voltammograms were recorded under the same conditions as for pure AMB. Ambazone concentration in Faringosept was analyzed using the standard addition method. Each addition contained 50 µL of ambazone solution ( $C_{AMB} = 1.0 \times 10^{-6}$  mol L<sup>-1</sup>). Voltammograms were recorded after each addition. Recoveries were calculated after six replicate experiments.

### 2.5. Analysis of spiked urine samples

Morning mid-stream urine samples were collected daily from two volunteers. Spiked urine solutions were prepared as follows: 1.0×10-7 mol (for sample 1) or 1.0×10<sup>-8</sup> mol (for sample 2) of AMB was placed in a 10-mL flask and filled to volume with urine. Voltammograms of the blank were recorded after placing 10 µL of pure urine and 9.99 mL of supporting electrolyte in the voltammetric cell. Subsequently, 10 µL of spiked urine solution and 9.99 mL of supporting electrolyte were placed in the voltammetric cell, and a voltammogram was recorded for the sample. Urine was analyzed using the standard addition method. Each addition contained 10 µL of AMB solution ( $C_{AMB}$  = 1.0×10<sup>-6</sup> mol L<sup>-1</sup> – sample 1; 1.0×10<sup>-5</sup> mol L<sup>-1</sup> –sample 2). Recoveries were calculated after six replicate experiments.

## 3. Results and discussion

### 3.1. Influence of pH and square wave parameters

The choice of supporting electrolyte is an important stage in electrochemical studies. The composition of the supporting electrolyte affects the properties

of the solution and the solution-electrode interface, which influence the kinetics and thermodynamics of the charge transfer process [33]. Ambazone in a solution with pH between 2.0 and 9.0 yields one cathodic signal at a potential of about -1.4 V. This peak was the most sensitive in BR buffer solutions at pH 2.0-3.5, but, according to peak morphology, BR pH 4 was chosen as the supporting electrolyte. The influence of the ionic strength of the supporting electrolyte was also investigated. The best response was obtained when the supporting electrolyte was composed of 50% 0.04 mol L-1 BR buffer (pH 4) and 50% distilled water. Subsequently, the impact of instrumental parameters, such as amplitude  $E_{\text{SW.}}$  step potential  $\Delta E$ , frequency f, and accumulation time  $t_{acc}$  and potential  $E_{acc}$ , was investigated [7]. During optimization of the abovementioned parameters in 5×10-8 M AMB solution, one parameter was changed while the others were kept constant. For amplitudes of 10-80 mV, the peak current increased linearly. Amplitudes higher than 80 mV caused a non-linear growth of the peak (plateau), so 80 mV was applied in further work. The influence of frequency (8-700 Hz) on the peak current was studied. The best results were obtained for 50 Hz. The influence of frequency will be discussed in detail later. Step potential was studied in the range of 1-15 mV. The peak current increased linearly from 1 to 7 mV and was well-shaped. A step potential of 7 mV was chosen for further studies. To improve the detection limit and the sensitivity of the method, the influence of deposition potential  $(E_{acc})$ and deposition time  $(t_{acc})$  was studied for an AMB concentration of 5×10<sup>-9</sup> mol L<sup>-1</sup>. The observed AMB response was highly sensitive to the accumulation factor. The influence of the accumulation potential was studied in the potential range from 0 V to -2 V in steps of 0.1 V with  $t_{acc}$  = 15 s at each potential. The maximum current value for the AMB peak was observed at 0.0 V. Various accumulation times (5-150 s) were investigated. The AMB signal reached a maximum at a deposition time of 10 s. A further increase of accumulation time caused a significant decrease in the voltammetric response of the compound. Such behavior may suggest high affinity of AMB molecules for the electrode surface and/or an intermolecular reaction impeding electrode reaction. An accumulation time of 10 s was chosen for subsequent studies.

### 3.2. Electrochemical behavior of ambazone

Cyclic voltammograms of AMB in the selected supporting electrolyte showed one well-defined

reduction peak at -1.38 V at the first cathodic sweep from 0 to -2.0 V. On the reversal scan, no corresponding oxidation peak appeared, indicating that the electrode process of AMB is a totally irreversible one. To explain the nature of the process, the influence of the scan rate (v) on AMB signal was investigated. The relationship between the scan rate and the peak current was linear and can be described by the equation  $I = Av^x$ . The values of x were expected to be 1 and 0.5 for adsorption-controlled and diffusion-controlled reactions, respectively [34]. The regression of log *l* vs. log v gave a slope value of 0.94, indicating that the reduction current is of adsorptive nature. The CV method was also applied in order to find the number of electrons transferred in the electrode reaction. Using the equation  $[E_{p} - E_{p}/2] = 48/(\alpha n)$  mV, it was calculated that  $\alpha n$ is equal to 0.489. Given  $\alpha$  = 0.5, the number of electrons was found to be 1. Moreover, the presence of a guanidine group in the AMB structure (Fig. 1) and the peak potential characteristic of this type of mechanisms indicate a possibility of a catalytic hydrogen evolution (CHE) reaction. Experimental and theoretical studies of CHE in the presence of compounds with electrocatalytic properties are well known [35]. To confirm the electrocatalytic properties of the compound, frequency investigation might be used [17,35-37]. In this study, the influence of f on the 1×10<sup>-8</sup> M AMB peak current was investigated. The obtained parabolic, exponential and linear curves (Fig. 2) for  $I_{p} = f(f)$ ,  $I_{p}f^{-1} = f(f)$ ,  $\log(I_{n}f^{-1}) = \log(f^{-1})$ , respectively, are identical to theoretical ones. On the basis of the abovementioned mechanistic studies, it can be concluded that AMB acts as an electrocatalyst and the reaction proceeds according to the following equations:

1. Adsorption of the catalyst followed by protonation:  $AMB_{(ads)} + H^+_{(aa)} \longleftrightarrow AMBH^+_{(ads)}$ 

- 2. Reduction of the protonated form of the catalyst:
- $AMBH_{(ads)}^+ + e \longrightarrow AMBH_{(ads)}$

3. 
$$2AMBH_{(ads)} \longrightarrow 2AMB_{(ads)} + H_2$$

#### **3.3. Analytical application**

In order to develop an analytical method for the determination of AMB, square wave adsorptive stripping voltammetry at the Hg(Ag)FE was selected, as it guarantees effective and rapid determination at a low background current and low detection limits [38]. Quantitative measurements were performed

Concentration given [nmol L <sup>-1</sup> ]	Concentration found [nmol L <sup>-1</sup> ]	SD [× 10 <sup>-9</sup> ]	Precision CV [%]	Recovery <sup>b</sup> [%]	
1.000	$1.032 \pm 0.040^{\mathrm{a}}$	0.160	1.5	103.2	
3.000	$3.092 \pm 0.046$	0.186	0.6	103.0	
5.00	$5.19\pm0.16$	0.661	1.3	103.7	
7.00	$7.07\pm0.35$	1.41	2.0	100.1	
10.00	$10.21 \pm 0.55$	2.24	2.2	102.1	
30.0	28.8 ± 1.2	4.74	1.6	96.1	
50.0	$50.5 \pm 1.5$	5.94	1.2	101.0	
70.0	$69.2 \pm 3.6$	14.7	2.1	98.8	
100.0	100.5 ± 2.9	11.7	1.2	100.5	

<sup>a</sup> t(S/n<sup>1/2</sup>), p=95%, n=6

<sup>b</sup>Recovery = 100 % + [ (Found – Added) / Added] ´ 100 %



Figure 2. The influence of frequency (*f*) on net peak current  $(l_p)$  and the  $l_p/f$  ratio recorded at the Hg(Ag)FE. The inset shows the dependence of  $\log(l_p/f)$  on  $\log(1/f)$ . The experimental conditions were: BR buffer pH 4,  $c(AMB) = 1 \times 10^8$  mol L<sup>1</sup>, amplitude  $E_{sw} = 80$  mV, step potential  $\Delta E = 7$  mV.

using SWAdSV, BR buffer pH 4.0, and the best conditions for analytical application. Calibration curves for the SWAdSV technique were constructed for six consecutive measurements by plotting the peak currents against AMB concentrations. The cathodic peak current increases linearly with increasing concentration of AMB from  $1 \times 10^{-9}$  to  $1 \times 10^{-7}$  mol L<sup>-1</sup> (Fig. 3A) and is expressed by the equation  $I_p = a + b \times c$ (AMB) (correlation coefficient 0.9999), where the intercept  $a = 1.2 \times 10^{-7}$  A and slope b = 1099.4 A L mol<sup>-1</sup>. The limits of quantification

(LOQ) and detection (LOD) were calculated from the calibration curves as  $k \cdot SD/b$  (k = 10 for LOQ, k = 3 for LOD, SD = standard deviation of the intercept, b = slope of the calibration curve [39]) and were found to be  $1.0 \times 10^{-9}$  and  $3.0 \times 10^{-10}$ , respectively. The repeatability of the procedure was estimated with six measurements at the same AMB concentration. To check the validity of the method (Table 1), the precision and recovery of the method were also calculated for different concentrations in the linear range.

#### 3.4. Analysis of real samples

Initially, the standard addition method was used for the analysis of Faringosept tablets, and aliquots of AMB standard solution were added in order to evaluate the accuracy of the proposed method. Samples were prepared as described in section 3.2. The voltammograms recorded for AMB concentration determination in Faringosept are shown in Fig. 3B. As can be seen, other ingredients present in Faringosept tablets did not cause the appearance of any additional signals in the examined potential window, so it can be concluded that there are no interferences from the matrix. The calculated recoveries, ranging from 97.45 to 101.26 in six replicate experiments, are in good agreement with the labelled content (Table 2). Next, to check the practical applicability of the developed method, spiked human urine samples were analyzed with the standard addition method. Urine samples were collected from two volunteers of different ages (patient A – 29 and patient B – 57), as described in section 3.3. As is well known, human urine, being a complex matrix, is often difficult to analyze because

Declared amount [mg]		Found [mg]	Faringosept SD	Precision CV [%]		Recovery <sup>ь</sup> [%]
10.00		$9.99 \pm 0.52^{a}$	0.21	2.1		99.9
			Urine samples	i		
Sample	Patient	Added [µmol L <sup>-1</sup> ]	Found [µmol L <sup>-1</sup> ]	SD [× 10⁻³]	Precision CV [%]	Recovery [%]
1	А	1.00 ± 0.14	1.08	5.76	5.3	108.4
	В	$1.00\ \pm 0.13$	0.94	5.23	5.6	94.1
2	А	$10.00 \pm 0.77$	10.34	31.1	3.0	103.4
	В	$10.00 \pm 0.85$	10.15	34.3	3.4	101.5

 Table 2. Results of ambazone determination in Faringosept and urine samples by SWAdSV.

 $a t(S/n^{1/2}), p=95\%, n=6$ 

<sup>b</sup>Recovery = 100 % + [ (Found – Added) / Added] ´ 100 %





of the presence of uric acids, salts, and nitrogenous products of metabolism. In the presented study, urine samples were used without dilution, preseparation or pre-concentration steps. Each sample was spiked with a certain concentration of ambazone (concentration of ambazone in urine: sample  $1 - 1 \times 10^{-6}$  mol L<sup>-1</sup>, sample  $2 - 1 \times 10^{-5}$  mol L<sup>-1</sup>). It is clearly shown in Fig. 3C that the AMB signal is practically free from matrix interferences. Therefore, the proposed procedure can be successfully and easily used in the evaluation of recovery curves. The data for the recovery curves obtained for urine samples are listed in Table 2.

#### **3.5.** Interferences

The selectivity of the proposed method was evaluated by the addition of possible interferents – popular medicines for cold treatment (ascorbic acid, paracetamol, ibuprofen, acetylsalicylic acid, methimazole, caffeine, diclofenac). Interferents were added at concentration ratios of 1:1, 1:10, and 1:100 to  $5 \times 10^{-8}$  mol L<sup>-1</sup> AMB solution. The responses were compared with those obtained using only AMB standard solution. The presence of diclofenac shifted the AMB signal to more negative values, while paracetamol and methimazole caused deterioration of the AMB signal. Nonetheless,

none of the studied substances interfered with the quantitative determination of ambazone under the working conditions used (signal change <3%).

# 4. Conclusions

Both determination in real samples and the electrochemical behavior of ambazone were studied. The electrochemical behavior of ambazone at the renewable silver amalgam film electrode was studied for the first time. On the basis of frequency investigation, it was proved that the AMB signal is the result of a CHE reaction and that AMB can play the role of an electrocatalyst. A new, cheap, simple and precise square wave adsorptive stripping voltammetric method was optimized for the quantitative determination of AMB concentrations in bulk form, in a pharmaceutical formulation, and in urine samples. It was shown that direct analyses of real samples was possible without any time-consuming preparation steps. What is more, complex matrices, such as tablets or urine, do not interfere with ambazone determination on the Hg(Ag)FE electrode under the optimized conditions. Moreover, the main advantages of the proposed method are more pronounced in comparison with the procedures reported previously. The developed procedure based on the square wave technique is more environmentally friendly and has a lower detection limit.

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