Anal. Bioanal. Electrochem., Vol. 11, No. 9, 2019, 1148-1165

Analytical & Bioanalytical Electrochemistry

Full Paper

The Validation of Novel Voltammetric Study for Direct Determination of Biogenic Polyamines with Hanging Mercury Dropping Electrode

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Received: 17 May 2019 / Received in revised form: 28 August 2019 / Accepted: 31 August 2019 / Published online: 30 September 2019

Abstract- Polyamines have a pivotal role in many biological functions. The goal of this study was to assess the validity of a voltammetric technique for the evaluation of the behavior of some biogenic amines. A square wave voltammetric (SWV) method was applied for direct assessment of Spermine (SPM) and Spermidine (SPD). Then, the voltammetric peak of SPM was used to find the Polyamine oxidase (PAO) activity in some animals' milk and to determine the SPD concentration. Only SPM and SPD generated an adsorption peak at -0.446 V and -0.576 V vs Ag/AgCl, respectively. The proposed method was specific and sensitive. The LOD was found at 0.7×10^{-8} M and LOQ was computed as 2.1×10^{-8} M. The standard deviation (SD) and the relative standard deviation (RSD) for SPM and SPD sensitivity (n=6) was ±1.5 (3.4%) and ±0.5 (4.5%), respectively. The suggested method showed a good reproducibility, the relative standard deviation RSD (n =6) was 4.1% and 4.3%; whereas, the accuracy was between 86-92% and 108–111% for SPM and SPD, respectively. The proposed method was valid for determination of polyamine oxidase (PAO) activity in sheep and cow milk. Furthermore, SPD concentration can be assessed in the same sources, using this method.

Keywords- Square Wave Voltammetry, Spermine, Spermidine, Polyamine oxidase, Milk

1. INTRODUCTION

Nowadays, some voltammetric techniques, display more sensitive pulse approaches, however the Square Wave Polarography (SWP), with a hanging mercury drop electrode

(HMDE), particularly Square Wave Voltammetry (SWV), used new possibilities in trace analysis. SWV is an efficient technique in determining the adsorption characters of a wide range of compounds, using an electrode surface [1,2].

The method is simple to use, cost- and time-effective, has a low detection limit, high accuracy, and applicable to colored and turbid solutions [3,4].

Polyamines (PAs) compounds like putrescine (PUT), cadaverine (CAD), spermidine (SPD), and spermine (SPM) (Figure 1), found in living organisms, are simple protonated aliphatic amines in normal physiological conditions [5,6]. They have a pivotal rule in many biological functions; among them are the transcription and translation of nucleic acid, and monitoring cell growth [7-9].

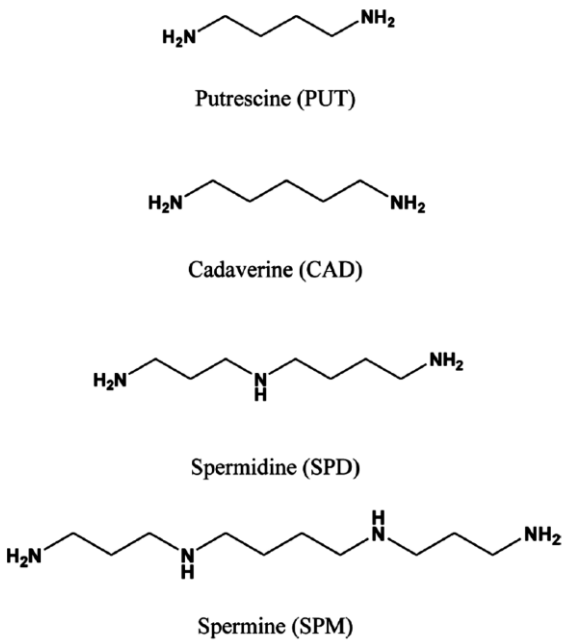


Fig. 1. Polyamine structures

High level of PAs could cause cellular toxicity and death [10], which is manifested as headache, nausea, and palpitation [11].

Many approaches exist in literatures to determine PAs, like thin layer chromatography, HPLC, capillary electrophoresis, LC−MS, GC, and electroanalytical method. However, these methods are costly, need considerable time for preparing the sample, specific skills for handling, and are mostly time-consuming [12-15]. On the other hand, voltammetric techniques, using solid electrode could address some of these problems [16,17].

The concentration of PAs could be maintained by polyamine oxidase (PAO; E C 1.5.3.11); an enzyme which is available in biological fluids and in all vertebrate tissues. PAOs catabolize either SPM or SPD (and their acetylated byproduct in animals) or transform them into corresponding lower amines (SPD and PUT, respectively) [18].

The direct detection of PAs is difficult, as they do not have original adsorption and fluorescence in an identical wavelength to interfere [11,19]. In fact, the species to be determined at mercury electrodes must be electroactive (i.e., must undergo electro-reduction or electro-oxidation within the available potential) or must react with Hg ions, or must be catalytically active and adsorbed on mercury [2]. Thus, there is a possibility to determine electro-inactive compound like polyamine, which have a special structure with 2 or more aliphatic amine, considered as catalytic groups to be adsorbed on the mercury drop electrode [20].

Therefore, in the previous study a simple approach was developed for direct SPM compound determination [21], using a voltammetric technique. Electrochemical investigation of SPM and SPD from proposed method may be attributed to the adsorption phenomenon. The linear increase in current with concentration creates the possibility of valid determination of polyamine oxidase (PAO) activity in sheep and cow milk. Furthermore, SPD concentration can be assessed in the same sources, using this method.

The aim of this study was to validate the previous study. In addition, the PAO activity was estimated depending on the SPM voltammetric adsorption peak current.

2.MATERIALS AND METHODS

2.1. Chemicals and Instrumentation

All SWV assessments were carried out, using 797 VA Computrace stand (Metrohm AG, Switzerland), connected to a PC and administered by the control software, VA Computrace 2.0. The voltammetric assessments were performed in a glass cell (working volume=5–10 ml). The following three-electrode system was applied: The working electrode was a glass capillary, mercury droplets with a surface of 0.15 mm^2–0.6 mm^2, formed at the end of the capillary tube. During the tests, the electrodes and the solution were retained stationary and rigorously deoxygenated by bubbling high purity nitrogen for 10 min. In order to control the temperature, thermo-stating circulation water bath, HAAKE F3 (manufactured in Germany) was applied. The reference electrode was an Ag/Ag Cl in 3 M KCl with platinum tip, as auxiliary electrode.

A spectrophotometric method was applied to measure the PAO activity, using UV–VIS NIR spectrophotometer (Model Varian Cary 5000, USA), and Shimadzo UV–VIS recording spectrophotometer. Hanna pH 211 (manufactured in Romania) was applied for pH adjustment. For removing the fat layer and preparing the milk samples for further analysis, Sorvell^{TM} centrifuge was used.

The following compounds and enzyme were purchased from Sigma-Aldrich Chemical Company: enzyme substrates spermine tetrahydrochloride, spermidine, putrescin dihydrochloride, cadaverine dihydrochlorid and 1,3 diamine propan dihydrochloride. Stock amine solutions with a concentration of 1×10^{-2} M, was newly made in deionized water and kept

in a dark room. Working solutions of 1×10^{-3} M to 1×10^{-4} M were also made, by deionized water. Potassium phosphate dibasic, potassium phosphate monobasic, sodium carbonate, sodium hydroxide, Tris buffer and sodium bicarbonate were acquired from Fluka Chemicals. Perchloric acid, sodium hydroxide ampoules and hydrochloric acid were obtained from BDH. All other chemicals were applied without further purification and were of analytical and reagent grade. Deionized water was supplied by the State Company for Drugs Industry and Medical Appliances (N.D.I) Ninavah /Iraq, which offers a water resistance of ≈0.5 Ohm.

2.2. Voltammetric Analysis and Procedure

2.2.1. Square wave Voltammetry for Polyamines

The process of acquiring SWV for polyamines was as follows: 10 ml of liquid carbonate buffer (supporting electrolyte) was transferred in a dry and clean voltammetric cell, at a desired pH. Afterward, the electrodes were placed in the solution through which pure nitrogen gas was applied for 5 min prior to acquiring the voltammograms. First, the voltammogram of the blank solution was recorded and then, a precise volume of polyamine solution was applied to the supporting electrolyte, and deoxygenation was performed for another 30 s.

2.3. Voltammetric Procedure

The peak intensities are determined entirely by the SWV parameters. After immersion of HMDE as a working electrode (WE) in the electrolytic buffer solution, containing 10 ml of 40 mM bicarbonate (pH 8.0) at 20 ºC, in order to obtain the maximum amount of square wave adsorption peak for SPM and SPD, the instrument parameters have been optimized as presented in Table 1. The SW with HDME procedure resulted in an acceptable reproducibility. The relative standard deviation RSD % (n=6) at a concentration of 9.99×10^{-7} M SPM and SPD were calculated as 4.1% and 4.3%, respectively.

Table 1. The optimal conditions for SPM and SPD

Parameter	**Optimal value (SPM)**	**Optimal value (SPD)**
Voltage step (V)	0.01	0.01
Amplitude (V)	0.05	0.05
Deposition Potential (V)	-1.0	- 1.0
Depositon Time (S)	60	60
Equilibrium time (S)	10	15
Frequency (Hz)	50	55

2.4. Applications for SPM and SPD SWV Peak

2.4.1. Determination of PAO activity in the cow and sheep milk

Fifteen milk samples from cow and sheep were gathered locally for the assessment of PAO activity. The study was approved by the Ethics Committee of University of Mosul.

2.4.2. Isolations of lipids from milk samples

The fatty layer of milk (10 ml) was separated by centrifugation for 30 min at 4000 rpm, in a cold centrifuge. Then, the milk sample was kept in a freezer at -18 ºC, for further analysis.

2.4.3. Electrochemical assessment of PAO activity

After optimization of SPM adsorption peak, the PAO activity was evaluated, using the following procedure: 100 µL of 1.0×10^{-3} M SPM solution were added to a polarographic cell, having 10 ml of 0.1 M carbonate buffer (pH 8.0). Then, the SW voltammogram was recorded (peak current=Ip_1), after degassing the solution for 30 s. Afterwards, the reaction started by adding 10 µl of milk sample (diluted and kept for 10 min at 35 ºC). The voltammogram was documented again (peak current=Ip_2), and the amount of SPM consumed, corresponding to the PAO activity, was read from the difference between the two peaks (ΔIp) , as $Ip_1–Ip_2$ [21]. A comparison of the acquired data was made with that taken from the spectroscopic method.

2.4.4. Spectrophotometric Quantification of PAO activity

PAO activity in the milk samples was determined, using a spectrophotometer, with minor adjustments, compared to what was previously reported [23], as clearly described in our previous study [22].

2.5. Determination of Spermidine (SPD) Concentration in Cow and Sheep colostrum

The colostrum sample was prepared to measure the SPD concentration, but before the dilution step, 5 ml of the sample (free from fat) was suspended in 5 ml of 0.6 M perchloric acid. Then, the resulted solution was centrifuged at 4000 rpm for 15 min. The top layer was collected, and then diluted ten times with distilled water, which was then used for voltammtric measurements.

SWV measurements were used under the optimum conditions of SPD, after the immersion of WE in the electrolytic buffer solution, containing 10 ml of 40 mM bicarbonate (pH 8.0) at 20 ºC. The solution was de-aerated for 5 min by the passage of nitrogen gas. A voltammogram was recorded (the peak current belongs to the blank; Ip_{blank}). Then, 40 µl of the sample was prepared as shown (under the section of 2.2.1), and degassing was performed for 30 seconds. The SW voltammogram was then documented again. Subsequently, in the same cell, a known

volume of the SPD standard solution was added, and the voltammogram was documented again (peak current belongs to SPD standard solution; $Ip_{standard}$).
The concentration of SPD in the unknown sample ($C_{unknown}$) is obtained from equation (1):

$$\text{Concentration of SPD in sample} = \frac{Ip_{unknown}}{Ip_{standard}} * Conc.of\ standard * dilution\ factor \quad ...(1)$$

2.6. Theoretical Study

In order to explain the electrochemical behaviors of SPM, SPD, CAD and PUT, some physical properties such as the lowest unoccupied molecular orbital (LUMO) energy level, the highest of occupied molecular orbital (HOMO), and formation heat, were computed by ChemOffice package, using semi-empirical AM1 calculations.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Activity of SPM and SPD using Hanging Mercury Dropping Electrode (HMDE)

Unexpected data were obtained by SWV, while determining the PAO activity, after adding SPM at -0.446 V (versus Ag/AgCl), as a well-defined peak shape in the other separated experiment, SPD showed also a specific peak at -0.575 V (versus Ag/AgCl), this is due to the presence of catalytic wave because of catalytic center (nitrogen atoms), and to the high ability adsorption for these species [20].

Under the same conditions, no noticeable peak was found for CAD and PUT, this may be due to the smaller number of nitrogen atoms, as two nitrogen atoms are present in each compound, On the other words, this may be the absence of secondary amine, SPM has two secondary amine and SPD has one.

These interesting results for SPM and SPD, urged us to examine further the cause. In this process, an organic compound has been used, which can be adsorbed on mercury electrodes [2,3,24]. Therefore, the unexpected peaks can be due to non-faradic operation, causing a physical alteration on the electrode surface, which may be due to the adsorption. For further validation, the following procedures were conducted.

3.2. Effect of Different Buffers on the Stability of Peak Current of SPM and SPD

Since the adsorptive phenomenon of polyamine on the HMDE was suggested for voltammetric investigation, it was crucial to characterize various variables and experimental conditions that affected the engaged adsorption process. In fact, the sensitivity of the square wave voltammetry with HMDE procedure for a particular analytes is usually significantly influenced by the composition of the supporting buffer. Consequently, different media such as (Na_2CO_3-$NaHCO_3$, $NaHCO_3$-NaOH and $NaHCO_3$-HCl buffer solutions (pH 8.0)) were used in order to study the voltammetric behavior of polyamine. All assessments were performed in the

presence of 9.99×10^{-7} M SPM and SPD, at ambient temperature of 24±2 ºC. The data demonstrated that the polyamine peak in Na_2CO_3–$NaHCO_3$ buffers is time dependent, as it was steady for only five minutes. Moreover, in $NaHCO_3$-NaOH buffer, the peak current was not steady at all; in these buffers, one of the compounds that can be formed is carbonic acid, $O=C(OH)_2$, which is not a stable compound. The formal replacement of one OH group in the formula of carbonic acid with NH_2, leads to the formation of carbamic acid H_2N-C(=O)-OH [25], and this may cause SPM and SPD sorption from HMDE that lead to peak depression and instability with time. Although the peak current was less in $NaHCO_3$-HCl, compared to the other buffer solutions, the data demonstrated that the SPM and SPD peak were more steady (about 12 and 15 min), respectively; thus, it was selected as supporting electrolyte for SPM and SPD. This may due to HCl being an acid, which reacts with the weak base sodium bicarbonate, $NaHCO_3$ in a simple acid base reaction. The bicarbonate will release carbon dioxide (CO_2) and the liquid will be a salt of sodium chloride (NaCl), as a result no carbonic acid in the solution[26].

Table 2. Effect of different supporting electrolyte types on the peak current of 9.99×10^{-7} M SPM and SPD

Types of supporting electrolyte (0.1M, pH 8.0)	**$E_{p\ SPM}$ (V)**	**I_p correct (nA)**	**$E_{p\ SPD}$ (V)**	**I_p correct (nA)**
Potassium phosphate buffer	No peak	-	No peak	-
Tris-HCl	No peak	-	No peak	-
Na_2CO_3-$NaHCO_3$	-0.464	8.00	No peak	-
$NaHCO_3$-NaOH	-0.446	15.70	-0.592	10.7
$NaHCO_3$-HCl	-0.446	5.21	-0.575	3.0

After carrying out a set of experiment, the best adsorption peak for SPM and SPD were observed at 0.04 M bicarbonate buffer. A decrease in solubility of the organic substance was the reason behind this outcome, indicating high hydrophobic – hydrophobic interactions at the surface of the electrode [27].

3.3. Effect of pH

It is well known that the reduction peak potential shift to negative values as the medium become more basic, and with decreasing pH to more positive value, as a result a decrease in electron density, indicating that there is a proton in electrode reaction.

Predominantly, the adsorption peak was mostly pH-independent, since the monitored voltammetric signal for SPM was only altered by the current. As the voltammetric peak potential of SPM was not moved by the change of pH, in the range used in this study, these data further clarify that the voltammetric peak fit in a SWV adsorption (Table 3). Luckily, the

maximum current was obtained at pH 8.0. The increase in the peak current at pH 8 is due to fully protonation of SPM above pH 7.4. The same results were obtained when the SPD was added.

The experimental optimal conditions for SWV was assessed after evaluating the suitable supporting electrolyte, pH and peak position.

The adsorption process has a significant effect in a basic medium [27] and this can be observed from the peak current of SPM and SPD and less effect in acidic media, also supposedly in strong acidity, the compound was adsorbed from the electrode surface.

Table 3. Effect of pH on peak potential and current for 9.99×10^{-7} M solution of SPM and SPD in 0.04 M $NaHCO_3$-HCl buffer

pH	E_p SPM (V)	I_p (nA)	E_p SPD (V)	I_p (nA)
6	-0.446	9.57	-0.552	7.5
7	-0.444	13.04	-0.552	10.0
8	-0.446	18.47	-0.575	13.7
9	-0.464	8.95	-0.575	9.2
10	-0.467	5.83	Irregular shape	_

3.4. Analytical Performance

After finding the optimal instrumental parameters and chemical conditions for the SWV determination of SPM, using the optimized conditions, the validity of the SWV method was assessed as a function of the concentration and as an analytical technique for the evaluation of bulk SPM. As can be seen in Figure 2(A), even the first small additive of SPM leads to a shift of the hydrogen discharge half wave potential toward positive values, at a small significant change in the limiting current, we speculate that catalyzes the evolution of hydrogen and that this process involves adsorbed protonated SPM species. A complete coverage of the electrode surface takes place in a narrow concentration range, which relates to a high ability of SPM that is adsorbed on the mercury electrode due to large organic molecule(SPM) with catalytic center (4 nitrogen atoms; Figure 1). This circumstance hinders the use of the shift of the halfwave potential of the catalytic hydrogen evolution for the quantitative determination of SPM, in a wide range of solution concentrations as a linear relation over the concentration range of 4.99×10^{-8} to 7.98×10^{-7} M was obtained with a correlation coefficient of R=0.994, as demonstrated in Figure 2 (B and C).

However, the relation between peak current and concentration, at high concentration was no more a linear function, suggesting the concerning minimum substrate concentration in the analytical range of 4.99×10^{-8} M, the peak current was 1.38 nA for SPM. The above steps were also applied for SPD, Figure 3(A), and its behavior was similar to SPM, so SPD did not exceed the 4.99×10^{-7} M, and the linear range started from and 9.99×10^{-8} with a peak current of 1.6 nA,

as shown in Figure3(B and C) .This probably is due to it small structure (with 3 nitrogens only, Figure 1), so it adsorption is weaker than SPM.

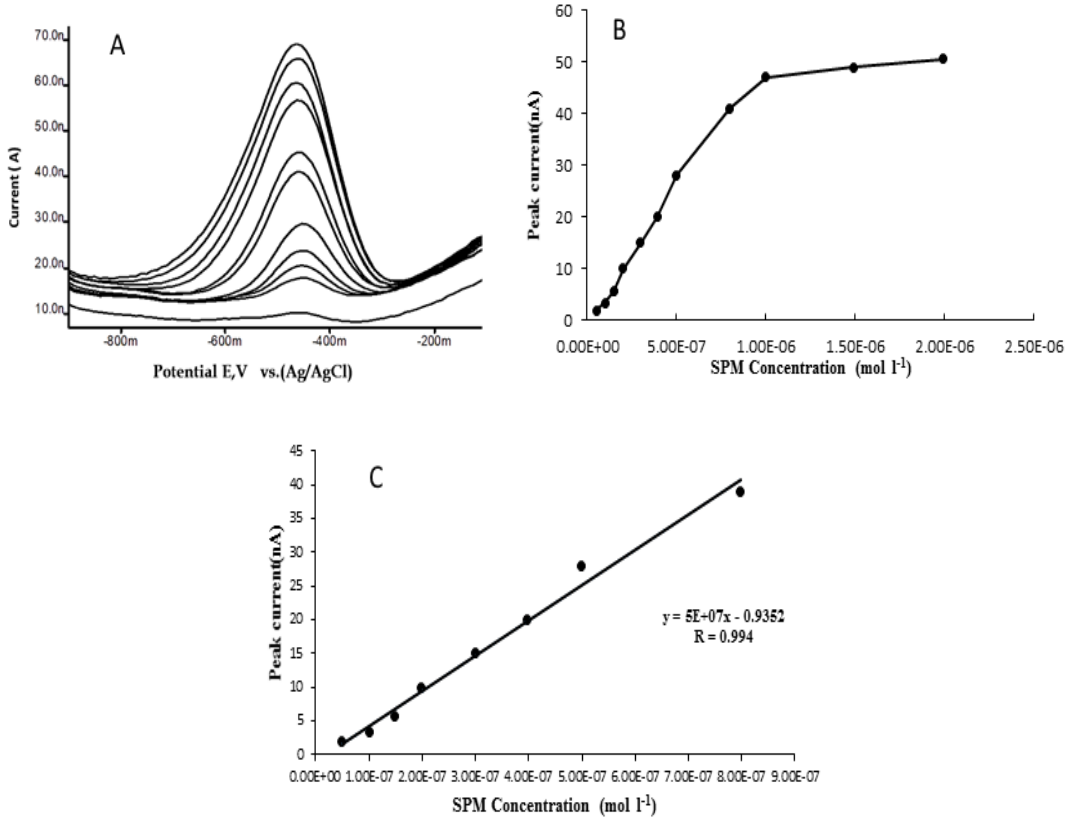


Fig. 2. A) SW voltammogram for a linear range of SPM adsorption peak from 4.99×10^{-8} to 7.98×10^{-7} M in buffer $NaHCO_3$-HCl buffer solution (pH 8.0), Voltage step 0.01 V; Amplitude 0.05 V; Deposition. Potential -0.1 V; Deposition. 60 s; Equilibrium time 10 s; Frequency 50 Hz; **B)** Calibration graph s for the polarographic determination of SPM until saturation reaches from 4.99×10^{-8} to 1.99×10^{-7} M; **C)** Calibration graph for the polarographic linear range determination of SPM 4.99×10^{-8} to 7.98×10^{-7}M; with linear equation y=5E+07x-0.9352, R=0.994

Several processes are presented in the ICH guideline to assess the LOQ (limit of quantification) and LOD (limit of detection). LOQ and LOD were computed from the equations of LOQ=10 $_{\varrho}$ /S and LOD=3.3$_{\varrho}$ /S [28,29], using the calibration curve and standard deviation of response (S). The LOD was detected at 0.7×10^{-8} M, and LOQ was calculated at 2.1×10^{-8} M for the suggested method.

3.5. Sensitivity, Precision and Accuracy

The sensitivity of developed SW with HDME procedure evaluated from six measurements (n=6) of 9.99×10^{-7} M SPM and SPD, respectively. The standard deviation (SD) and the relative standard deviation (RSD) of SPM and SPD sensitivity, was ±1.5 nA (3.4%) and ±0.5 nA

(4.5%), respectively. The reproducibility was also determined from six repeated measurements of 9.99×10^{-7} M SPM and SPD, respectively. The precision of the method, in terms of the relative standard deviation (RSD%) of SPM and SPD was 4.1% and 4.3% nA, respectively. In addition, the accuracy of the proposed electrochemical technique was determined through finding the recovery of known amounts of SPM and SPD, 9.99×10^{-7} M, in the buffer solution and analyzed by optimized procedure. Recovery values of six measurements were between 86-92% and 108–111% for SPM and SPD, respectively.

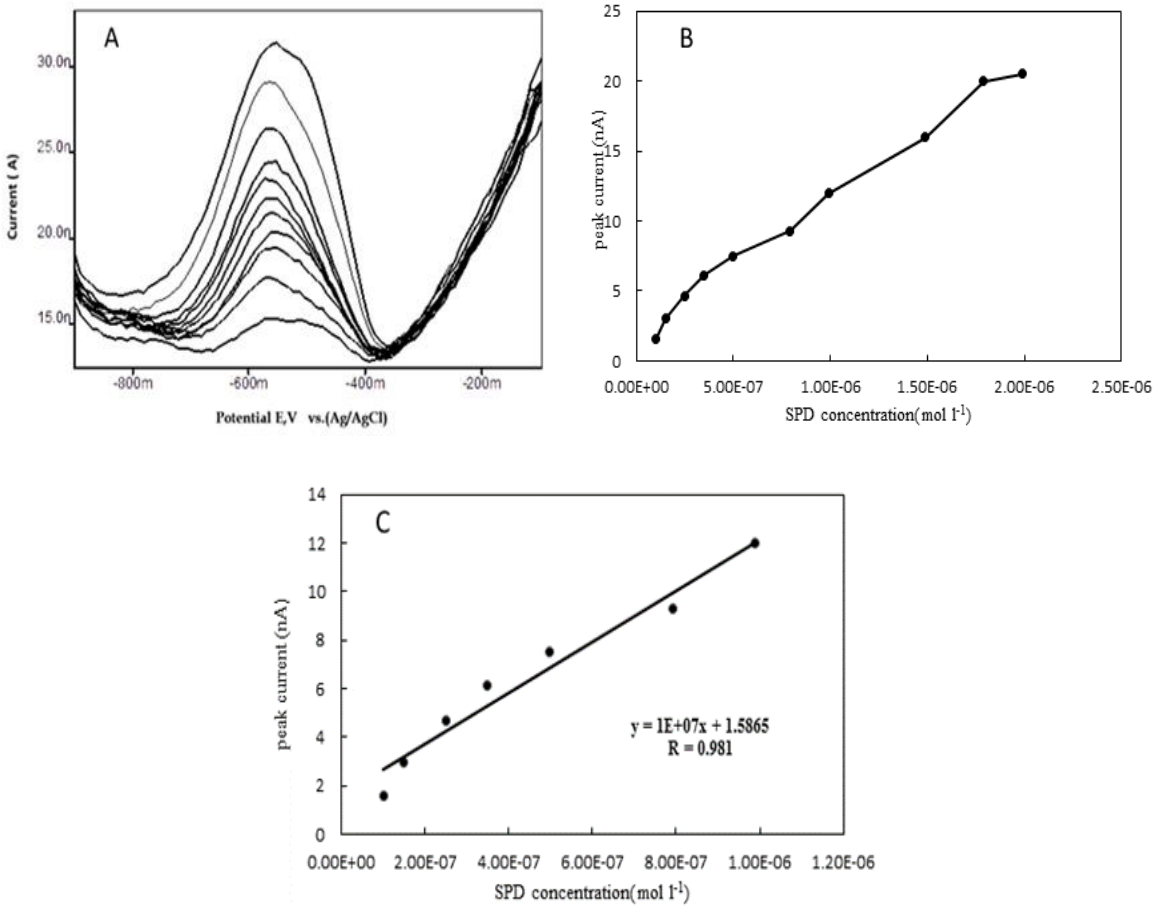


Fig. 3. A) SW voltammogram for a linear range of SPD adsorption peak from 9.99×10^{-8} to 4.99×10^{-7} M in $NaHCO_3$-HCl buffer solution (pH 8.0), Voltage step 0.01 V; Amplitude 0.05 V; Deposition Potential -0.1 V; Deposition time 60 s ; Equilibrium time 15 s; Frequency 55 Hz; **B)** Calibration graph for the polarographic determination of SPD until saturation reaches from 9.910^{-8} to 1.99×10^{-7}M; **C)** Calibration graph for the polarographic linear range determination of SPD 9.99×10^{-8} to 4.99×10^{-7} M; with linear equation y=1E+07x +1.5865, R= 0.981

Table 3 and 4 show the comparison of the applied method with other methods. The SWV method was easy and very quick, and the SPM and SPD could be assessed with small

experimental concentration. However, in high concentration no detection was observed due to saturation.

Table 4. Comparison of the proposed method for SPM with other methods

Techniques used	Linearity (µM)	R [a]	Ref.
HPLC MS-MS [b]	0.04-4.9	0.9970	[30]
LC-ELCD [c]	9.39	0.9985	[31]
PAO biosensor [d]	3-300	0.9927	[32]
(AHNSA)/GCE [e]	1.49– 19.6	0.9918.	[17]
Ag-Au/AgCl nanohybrid [f]	0.115-0.854	0.97	[33]
SWV	0.04-0.79	0.9940	Present study

[a]Correlation coefficient; High performance liquid chromatography tandem mass spectroscopy; [c]liquid chromatographic with evaporative light scattering detection; [d]polyamine oxidase biosensor; [e]thin film of 4-Amino-3-Hydroxynaphthalene sulphonic acid (AHNSA) was electropolymerized on a glassy carbon electrode (GCE), using square wave voltammetry; [f]silver-gold/silver chloride nanozymes

Table 5. Comparison of the proposed method of SPD with other methods

Techniques used	Linearity (µM)	R [a]	Ref.
HPLC MS-MS [b]	0.03 -13.7	0.9914	[30]
LC-ELCD [c]	9.63	0.9987	[31]
PAO biosensor [d]	10-400	0.9946	[32]
CE-ECL [e]	0.06-68.8	0.9961	[34]
Terphenyl probe [f]	0.046	N.R	[35]
SWV	0.099-0.99	0.9821	Present study

[a]Correlation coefficient; [b]High performance liquid chromatography tandem mass spectroscopy; [c]Liquid chromatographic with evaporative light scattering detection; [d]Polyamine oxidase biosensor; [e]Capillary electrophoresis with electrochemiluminescence; [f]Terphenyl derivative probe

3.6. Theoretical Study

To further explore this behavior of SPM and SPD, theoretical study was carried out, as presented in Table 5.

Considering the heat formation values, the increase in the number of CH_2 group stabilizes the polyamine compound, while NH group destabilizes this compound. According to this fact, it is clear that CAD is the most stable compound. Although SPM has more CH_2 group, but the presence of two NH groups decreases its stability. This is the reason behind the adsorption peak of SPM and SPD.

In addition, it can be seen that the oxidation potential of these compounds is almost constant. In the meantime, HOMO energy values are also constant within the calculation error.

Table 6. Theoretical study of Biogenic Amines (A Comparative study between practical and theoretical values of the electrochemical behaviors of biogenic amines)

Polyamines	**E_p reduction (V)**	**HOMO**	**$E_{1/2}$ * Oxidation (V)**	**LUMO**	**Heat of formation (Kcal)**
SPM	-0.464	-8.660	+ 0.89	1.241	-28.34
SPD	-0.563	-8.653	+ 0.91	1.396	-25.80
CAD	_	-9.073	+ 0.88	1.978	-29.01
PUT	_	-9.075	+ 0.88	1.992	-22.75

* oxidation potential of biogenic amines at diamond film electrode using cyclic Voltammetry in pH 10 carbonate buffer [36]

3.7. Application

3.7.1. Analytical Applications

For the assessment of the validity of this method, sheep and cow milk samples have been utilized for evaluation of PAO activity, due to accessibility and high activity of PAO in milk [37,38]; as other substances or drugs, harboring SPM could not be provided.

3.7.2. Determination of PAO Activity of Partially Purified Cow milk

Partial purification of PAO from cow milk was explained in the previous study [22]. Thus, it was applied to assess its activity by the SWV method.

Usually, enzyme activity is calculated based on moles of substrate converted per unit time (the amount of substrate consumed is correlated with the amount of product produced). Here, the ΔI was calculated by subtracting the current observed after the addition of the sample (Ip) from the magnitude of SPM (Ip_0), and the PAO activity was assessed by evaluation of the difference between the two currents (ΔI).

$$\Delta I = I_{p0} - I_p$$

The calibration graph of SPM was applied to calculate the concentration of SPM consumed, which is equal to the PAO activity. Subsequently, the results acquired by SWV were compared with the spectroscopic technique. As described in the section of 2.4.4, the PAO activity was 705 U/min, using electrochemical method, and 670 U/min in spectrophotometric method. Our data suggest that the suggested technique is potent for evaluating the enzyme activity, using SWV.

3.7.3. Determination of PAO activity in milk samples

After acquiring promising result, the SWV technique was used to assess the PAO activity in fifteen sheep and cow milk samples. A comparison was made with that acquired from the spectroscopic method, and performed two times. The data are presented in Table 6. A good linear correlation between the two methods was acquired by comparing the SWV electrochemical and spectroscopic method, using fifteen milk samples. The student t-test was applied, but no significant difference was found between these two techniques.

The graph shown in Figure 6presented a linear equation of y=1.1119x–39.883; where y is the PAO activity in milk samples, acquired from the spectroscopic method, with a correlation coefficient of R=0.9881 and x is the PAO activity in milk samples, obtained from the SWV electrochemical method applied, in U/min;

The data acquired in this study suggest that the proposed method, which depends on the SWV of SPM, is more valid, as no toxic compound was applied, and small amount of sample and material were needed, in comparison with the other methods.

Table 7. A comparison between SWV and spectroscopic methods for the assessment of PAO activity (U/ min), from 1-8 cow milk and 9-14 sheep milk

Sample No.	**Spectroscopic method (U*/ min)**	**Dilution factor**	**E_p(V)**	**ΔI_p (nA)**	**SWV method (U/min.)**
1	1706	10	-0.464	8.8	1817
2	1300	10	-0.464	9.7	1384
3	853	10	-0.464	6.6	936
4	853	10	-0.464	6.9	979
5	1950	10	-0.464	13.9	1990
6	1584	10	-0.464	10.9	1557
7	934	10	-0.464	8.4	1183
8	1029	10	-0.464	7.6	1081
9	1950	20	-0.464	6.9	1959
10	2113	20	-0.464	8.4	2392
11	5038	10	-0.464	17.0	5216
12	4063	14	-0.464	25.1	5051
13	2166	20	-0.464	8.4	2392
14	1463	20	-0.464	5.4	1529

*= One unit of PAO is defined as the amount of enzyme catalyzes the oxidation of one nmol of SPM per minute

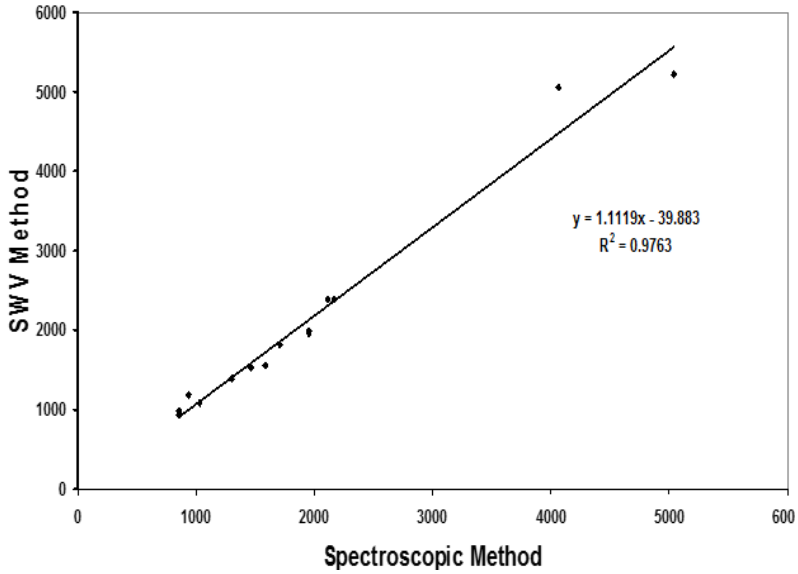


Fig. 4. The linear relationship between SWV and spectroscopic method for determination of PAO activity (U/ min)

3.7.4. Determination of SPD Concentration in the Colostrum

Mammalian milk contains considerable amount of polyamine, mostly SPD and SPM, and low amount of putrescine diamine [37].

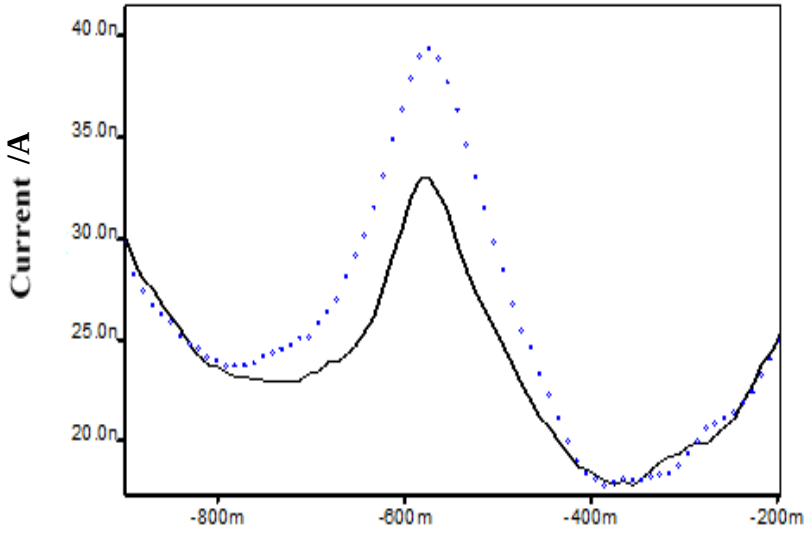
Table 8. Different additions of diluted colostrum samples (the first and second day after cow delivery)

Amount added of colostrum (µl)	**First day after delivery**		**Second day after delivery**	
	E_p (V)	**I_p (nA)**	**E_p (V)**	**I_p (nA)**
10	-0.573	3.3	_	_
20	-0.573	6.6	-0.573	2.3
40	-0.573	18.3	-0.573	10.5
60	-0.573	24.0	-0.573	14.2
80	-0.555	27.0	-0.555	22.3
100	-0.525	32.0	-0.555	26.0

These compounds have been evaluated by high performance liquid chromatography or two-dimensional gel chromatography [37,38]. The proposed SWV method was applied here for examining the biogenic amine (SPM and SPD) contents of colostrum samples. As described in section of 2.5.

Different amount of diluted colostrum samples (first and second day after cow delivery) was added, after protein precipitated by $HClO_4$ (a supernatant diluted ten times), and the results are depicted in Table 7.

Since the Ep of polyamine in the colostrum at - 0.573 V is very close to SPD adsorption peak at -0.56 V, so this result demonstrates the presence of SPD mainly in these samples. From another point of view, no peak appeared at -0.464 V for SPM, this is may be due to high SPM degradation and as a result of the high activity of PAO in milk and colostrum. These results were in agreement with other studies [39,40].



[a] —— 40 µL milk (diluted 10 times), b º º º º a+ 3.98×10^{-7} M SPD (authentic)

Fig. 5. The SPD peak in the milk sample with the addition of authentic SPD solution of 3.98×10^{-7} M in in voltammetric cell containing 0.04 M $NaHCO_3$-HCl buffer pH 8

As shown in Table 9, the concentration of SPD was measured in a number of colostrum milk samples.

Table 9. Concentration of SPD in the milk, using SWV

Sample No.	Milk types From different animals	Dilution factor	Addition volume (µL)	E_p (V)	ΔI_p (nA)	Conc. of SPD (µM)
1	First day after sheep delivery (colostrum)(1)	10	30	-0.555	24.30	53.46
2	Third day after sheep delivery (colostrum)(1)	10	30	-0.555	9.90	21.70
3	Second day after cow delivery (colostrum)(1)	10	30	-0.555	4.63	4.30
4	Third day after cow delivery (colostrum)(1)	10	30	-0.555	3.70	3.44
5	1st day after cow delivery (colostrum)(2)	10	20	-0.555	4.59	6.48
6	2nd day after cow delivery (colostrum)(2)	-	5	-0.555	32.00	5.70

It is very clear from the Table 7 that the peak current of SPD in the colostrum of the first day was greater than that of the second day. This indicated that colostrum contains a higher concentration of SPD, in the first day.

To confirm that this peak belongs to SPD, standard has to be used during the measurement, as shown section 2.5. The following experiments were performed. The SWV of 40 µl of milk sample was recorded in the presence of supporting electrolyte 0.04 M bicarbonate (pH 8.0) at 20 ºC, and then 3.98×10^{-7} M of authentic sample of SPD was added and applied the equation as written in methodology ***(e)*** to determine the SPD concentration in milk samples**.** Figure 7 shows the application of the above method to find the concentration of the sample of SPD in one of the milk colostrum samples.

4. CONCLUSION

To the best of our knowledge, in this study for the first time, a well-defined adsorption peak of the major polyamine compounds (SPM and SPD) was found directly without modification, using SWV, as it was clearly discussed and demonstrated. Furthermore, the new voltammetric peak current has a potential to be applied to the evaluation of PAO activity. Clearly, for evaluation of the PAO activity, an easy, quick and cost-effective SWV technique was compared with spectrophotometric method. Nevertheless, the use of the suggested technique for evaluation of PAO activity in other biological fluids, needs validation, using the more extended future study and a larger sample size. Moreover, the high concentration of SPD was detected directly in the colostrum of cow and sheep; the measurement can carry out successfully through the SPD adsorption peak.

Funding: No funding has been provided from any funding agencies such as commercial, public, or non-profit organizations.

Declarations of interest: None

REFERENCES

[1] J.Wang, Analytical Electrochemistry, 2nd ed. New York: Wiley-VCH (2002).
[2] V. Vyskočil, and J. Barek, Critical Rev. Anal. Chem. 39 (2009) 173.
[3] A. F. Al-Ghamdi, and M. M. Hefnawy, Arabian J. Chem. 5 (2012) 383.
[4] U. T. Yilmaz, and D. Inan, J. Food Sci. Technol. 52 (2015) 6671.
[5] Morgan, M. L. David (Ed.) Polyamine Protocols. Totowa, NJ: Humana Press (1998).
[6] M. Y. Khuhawar, and G. A. Qureshi, J. Chromatogr. B 764 (2001) 385.
[7] H. Tabor, and C. W. Tabor, Pharmacol Rev. 16 (1964) 245.
[8] A. Kanavarioti, E. E. Baird, and P. J. Smith, J. Org. Chem. 60 (1995) 4873.
[9] U. Bachrach, Plant Physiol. Biochem. 48 (2010) 490.
[10] P. H. Hoet, and B. Nemery, Am. J. Physiol. Lung. Cell. Mol. Physiol. 278 (2000) L417.

[11] D. Teti, M. Visalli, and H. McNair, J Chromatogr B 781 (2002) 107.
[12] L. Elisa, F. Baldini, A. Giannetti, C. Trono, and T. Carofiglio, Chem. Commun. (Camb) 46 (2010) 3678.
[13] A. Plenis, I. Oledzka, P. Kowalski, N. Miekus, and T. Baczek, J. Clin. Med. 8 (2019) 640.
[14] J. J. Learey, S. Crawford-Clark, B. J. Bowen, C. J. Barrow, and J. L. Adcock, J. Sep. Sci. 41 (2018) 4430.
[15] L. He, J. Ren, Z. Shi, and Z. Xu, J. Chromatogr. Sci. 54 (2016) 1428.
[16] M. T. Alaa, Ö. T. Al Layla , S. Kurbanoglu, S. T. Sulaiman, K. A. Al-Flayeh, and L. Toppare, J. Macromol. Sci. Part A 50 (2013) 914.
[17] A. M. Tayeb, A. M. Al-Layla, H. A. Abdalla, and S. T. Sulaiman. Int. J. Enh. Res. Sci. Technol. Eng. 3 (2014) 300.
[18] W. Wuddineh, R. Minocha, and S. C. Minocha, Methods Mol. Biol. 1694 (2018) 1.
[19] S. K. Sadain, and J. A. Koropchak, J. Chromatogr. A 844 (19991) 111.
[20] S. G. Mairanovskii, Catalytic and Kinetic Waves in Polarography, New York, Plenum, (1968).
[21] M. T. Alaa, S. T. S. Al-Layla, and K. A. Al-Flayeh, Eurasian J. Anal. Chem. 13 (2018) em63.
[22] A. M. T A-L. Electro - biochemical Study of Some Biogenic Amines and Amine Oxidases Using Different Modified Electrodes-Construction of Nano Composite Biosensor. Iraq: Coll Sci Mosul University (2011).
[23] K. A. Flayeh, Clin. Chem. 34 (1988) 401.
[24] P. Zuman, Acta Chim. Slov. 56 (2009) 18.
[25] C. MS. Applications to Health and Environmental Issues, Pyrolysis of Derivatives of Carbonic Acid With Nitrogenous Functionalities. 2nd ed Elsevier Science (2019) Subchapter 15 697.
[26] C. M. Buffers, A guide for the preparation and use of buffers in biological systems. In: Calbiochem, editor. Germany (2003).
[27] RJ ZP. Polographic Studies of Adsorption at Mercury Electrode Surfaces. In Encyclopedia of Surface and Colloid Science: Marcel Dekker Inc.; 2002.
[28] Christopher M. Riley TWR. Development and Validation of Analytical Methods. 1st ed (1996).
[29] Swartz ME K, I.S. Analytical Development and Validation. New York: Marcel Dekker Inc; (1997).
[30] F. Gosetti, E. Mazzucco, M. C. Gennaro, and E. Marengo, Anal. Bioanal. Chem. 405 (20132) 907.
[31] D. Restuccia, U. G. Spizzirri, F. Puoci, G. Cirillo, M. Curcio, O. I. Parisi, et al. Talanta 85 (2011) 363.

[32] A. Boffi, G. Favero, R. Federico, A. Macone, R. Antiochia, C. Tortolini, G. Sanzó, and F. Mazzei, Anal. Bioanal. Chem. 407 (2015) 1131.
[33] P. C. Kuo, C. W. Lien, J. Y. Mao, B. Unnikrishnan, H. T. Chang, H. J. Lin, and C. C. Huang, Anal. Chim. Acta 1009 (2018) 89.
[34] D. An, Z. Chen, J. Zheng, S. Chen, L. Wang, Z. Huang, and L. Weng, Food Chem. 168 (2015) 1.
[35] R. Tejpal, M. Kumar, and V. Bhalla, Sens. Actuators B 258 (2018) 841.
[36] M. D. Koppang, M. W. John Blau, and G. M. Swai, Anal. Chem. 71 (1999) 1188.
[37] P. F. Pollack, O. Koldovsky, and K. Nishioka, Am. J. Clin. Nutr. 56 (1992) 371.
[38] J. Sanguansermsri, P. Gyorgy, and F. Zilliken, Am. J. Clin. Nutr. 27 (1974) 859.
[39] C. Loser, Br J. Nutr. 84 (2000) S55.
[40] S. Novella-Rodriguez, M. T. Veciana-Nogues, and M. C. Vidal-Carou, J. Agric. Food. Chem. 48 (2000) 5117.