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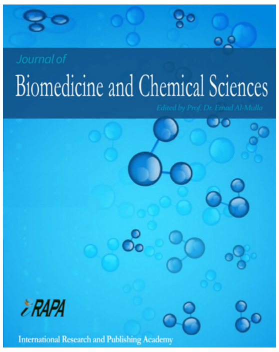

Indirect Estimation of Total Prostatic Specific Antigen (TPSA) on Glassy Carbon Electrode Through its Interaction with Zinc Acetate Using Differential Pulse Voltammetric Technique

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ABSTRACT

An indirect electrochemical behaviour of total prostatic specific antigen (TPSA) was studied using the differential pulse technique coupled with a three-electrode detection system, which consists of 2mm diameter glassy carbon as working electrode, 2mm diameter platinum wire as an auxiliary electrode and silver/silver chloride saturated potassium chloride as reference electrode (Ag/AgCl sat KCl). The method is based on monitoring the decrease in the zinc acetate reduction peak current, which is due to its interaction with TPSA. Acetate buffer solution (pH4) was used, the potential scanned between -1.4 V to -0.5V, and the optimum conditions were tested. The calibration curve for TPSA was constructed for a sequence addition of TPSA, and it was linear within the studied concentration range (2.4431- 24.4554) x10-9 M, with a correlation coefficient of 0.9763 and calibration equation y= 0.0308x + 1.287. The binding constant (k) and the thermodynamic parameters (ΔG, ΔH, and ΔS) for Zn2+-TPSA interaction were calculated.

INTRODUCTION

Prostate cancer (PCa) remains one of the most serious health threats affecting men over 50 years of age, primarily due to the absence of noticeable symptoms during its early development (Bray et al., 2018). As a result, significant research has been directed toward identifying reliable biomarkers for PCa and developing technologies for their clinical implementation. One of the earliest biomarkers explored for PCa detection and staging was prostatic acid phosphatase (PAP). However, the challenge in precisely measuring its prostate-specific isoenzyme limited its effectiveness, prompting researchers to search for more accurate diagnostic tools (Prensner et al., 2012; Ludwig & Weinstein, 2005). Subsequent findings revealed that prostate-specific antigen (PSA) could be measured in human serum, demonstrating that it is both cell-type specific and released into the bloodstream, allowing for minimally invasive detection (Mascini & Tombelli, 2008). It was later confirmed that serum PSA levels correlate with the progression of prostatic tumors, supporting its role in diagnosis staging and monitoring the disease after treatment (Healy et al., 2007). PSa was also identified in healthy males; however, a level above the threshold concentration of 4 ng ml−1 has been recognized as an indication of PCa.

Although PSA is one of the most validated biomarkers for clinical decision making in regards to PCa, it is not currently used for PCa screening due to the controversies raised in the literature regarding the specificity of PSA (Liu et al., 2008). Increased levels of PSA are also associated with benign prostatic Benign prostatic hyperplasia (BPH) can elevate PSA levels, often resulting in false-positive diagnoses of prostate

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cancer (PCa) and leading to unnecessary and invasive biopsies. This highlights the insufficient specificity of PSA as a standalone screening biomarker. Nevertheless, PSA blood tests remain widely used for tracking disease progression in patients undergoing treatment and, when combined with digital rectal examination (DRE), serve as a tool for early detection (Zheng et al., 2015). Regular PSA assessments are also crucial for evaluating therapeutic effectiveness in cancer patients, typically through monitoring PSA velocity or doubling time. PSA structure is shown in Figure 1.

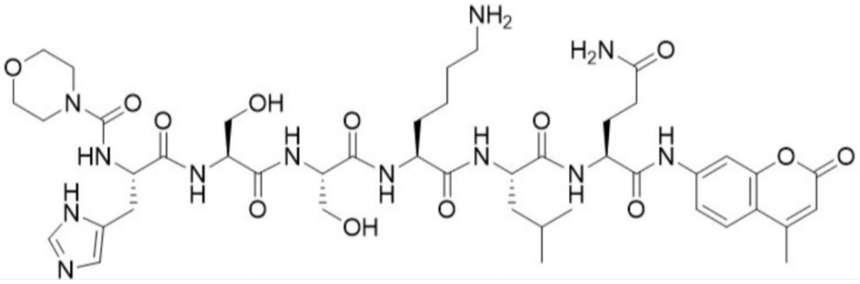

Fig. 1. shows the chemical structure of PSA

Different methods were used for determination of TPSA like biochemical methods (Parnsubsakul et al., 2017; Damborska et al., 2017) analytical methods (Li et al., 2022; Filik et al., 2021) electrochemical methods (Thakur & Kumar, 2023; Vanova et al., 2021) and clinical application (Tsoi, 2017; Mitrakas & Ochsenkühn-Petropoulou, 2021).

EXPERIMENTAL

Apparatus

All experiments were conducted system: the working electrode, which was a 2mm diameter glassy carbon electrode, the reference electrode Ag/AgCl sat. KCl, and 2mm diameter platinum wire as an auxiliary electrode. using voltammetric analyzer 797-AV Computrace device consisting of three electrode detection. A pH meter from Philips was used for pH measurements, and a thermostatic water bath of type haake NK 22 was used to control the temperature.

Reagents

The TPSA pure material was supplied by France Drug Industry (biomerieux). 0.1 g/L of TPSA solution was prepared by diluting 0.1 ml of pure TPSA in 0.9 ml of distilled water. The acetate buffer solution was prepared by mixing 41ml of 0.2 M acetic acid with 9 ml of 0.2 M sodium acetate in 100 ml volumetric flask and brought the volume up to 100 ml by distilled water.

Procedure

A differential pulse voltammetry was used by scanning the potential between -1.4 V to -0.5 V, with scan rate of 0.025V/s. Firstly, the voltammogram was recorded for a 10 ml of a acetate buffer (pH 4) solution, then the voltammogram of $2.63x10^{-5}$ M of Zn^{2+}ion was done, then a sequence additions of TPSA were added and the voltammogram was recorded for each addition under the measured optimum conditions A calibration curve was constructed under the optimum conditions for a sequence additions of TPSA. The binding constant was calculated at different temperatures (293, 298, 303 and 308) K using thermostatic water bath with a voltammetric jacket cell and the thermodynamics parameters were calculated.

Pretreatment of Glassy Carbon Electrode

Glassy carbon electrode was cleaned ultrasonically sonicated in water for five minutes after being polished by aluminum oxide powder (0.05 µm) (Al-Hyali & Al-Taee, 2021; Morrin et al., 2021).

RESULTS & DISCUSSION

Indirect Estimation of TPSA through its Interaction with Zinc Cation

Indirect estimation of TPSA was carried out through its interaction with zinc cation on a glassy carbon electrode using acetate buffer solution (pH 4) by following the decrease in the zinc cation reduction peak current which is appeared at -1.01 V as a result of TPSA added. For this purpose, a differential pulse voltammogram of $2.63x10^{-5}$ M zinc cation solution was recorded under the initial default conditions table 1, zinc cation shows a well-defined reduction peak at -1.01 V versus Ag/AgCl saturated KCl reference electrode Figure 2.

Table 1
The initial default conditions for zinc ion reduction

Parameters	Values
S. p. (V)	-1.4
E. p. (V)	-0.5
P. a. (V)	0.01
P. t. (s)	0.04
V. s. (V)	0.005951
V. s. t. (s)	0.4
No. c.	1
C. p. (V)	-0.1
C. t. (s)	0
D. p. (V)	-0.9
D. t. (s)	60
E. t. (s)	5
S. r. (V/s)	0.0149

S.p.: Start potential, E.p.: End potential, P.a.: Pulse amplitude, P. t.: Pulse time, V. s.: Voltage step, V. s. t.: Voltage step time, No. c.: No. of cycles, C.p.: Cleaning potential, C.t.: Cleaning time, D. p.: Deposition potential, D. t.: Deposition time, E. t.: Equilibrium time, S. r.: Sweep rate.

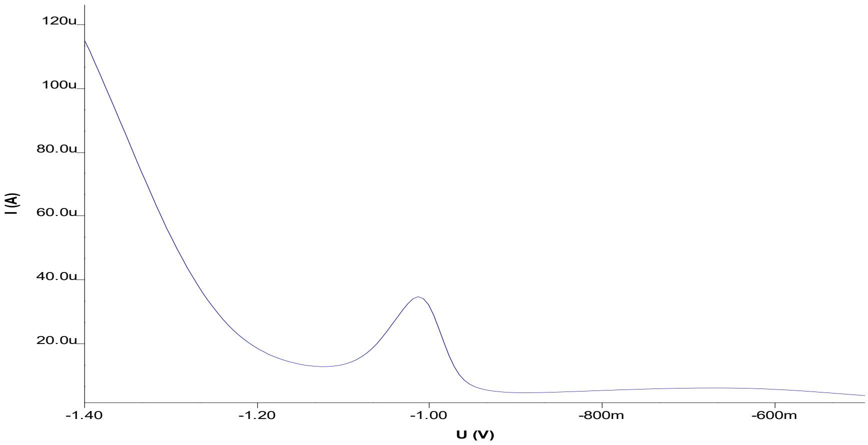

Fig. 2. The reduction peak of zinc acetate under the default conditions of instrument

Optimum Conditions

To examined the optimum conditions for measurement, 9.9x10^{-5} M zinc acetate solution was used. Many experimental and instrumental conditions that effect on the reduction process of zinc acetate were optimized. including the effects of pulse amplitude, pulse time, voltage step, number of cycles, cleaning potential, cleaning time, deposition potential, deposition time, and equilibrium time. Table 2 shows the obtained optimum conditions.

Table 2
The measured optimum conditions for direct determination of zinc acetate

Parameters	Values
S. p. (V)	-1.4
E. p. (V)	-0.5
P. a. (V)	0.2
P. t. (s)	0.0025
V. s. (V)	0.01
V. s. t. (s)	0.4
No. c.	1
C. p. (V)	-0.1
C. t. (s)	0
D. p. (V)	-0.4
D. t. (s)	90
E. t. (s)	7
S. r. (V/s)	0.025

Figure 3 shows the recorded voltammograms for 9.9x10^{-5}M of zinc acetate under the instrument default conditions (a) and under the measured optimum conditions (b), it's clear that the reduction current measured under the optimum condition was higher than that measured at the instrument default conditions.

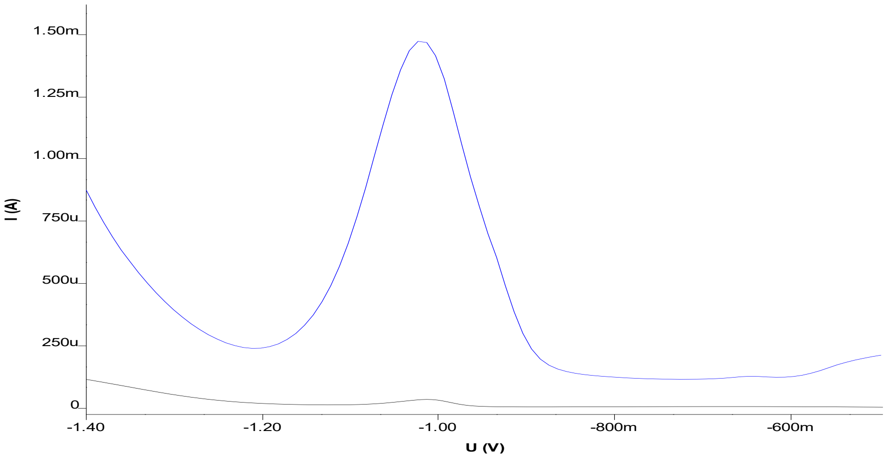

Fig. 3. Recorded voltamogram for 9.9x10^{-5} M a/ after and b/ before optimum condition

Stability

The effect of time on the of Zn-TPSA peak was studied by recording the differential pulse voltammogram for various durations time under the optimum measured conditions. The voltammogram was recorded for addition of 2.44x10^{-9} TPSA to 9.9x10^{-5}M Zn. solution every five minutes for a total duration time of (40) minutes. It was observed that the current value remained almost constant within the studied time. The results are shown in the table 3.

Table 3
Stability of the reduction peak current

Time (min.)	E p(V)	I p*10^{-4} A
0	-1.01	1.60
5	-1.01	1.64
10	-1.01	1.65
15	-1.01	1.67
20	-1.01	1.68
25	-1.01	1.66
30	-1.01	1.65
35	-1.01	1.67
40	-1.01	1.64

Effect of Zinc Ion Concentration

To examine the amount of zinc required to make a bigger change in its reduction peak current, different concentration of zinc was added to the electrolysis cell and the voltammogram was recorded for each concentration after the addition of 2.44 X10^{-9} M TPSA, the results obtained are summarized in table 4. The best zinc concentration was 9.900x10^{-5}M which was adopted in construction of calibration curve.

Table 4
Different concentration from zinc ion

Conc of Zn^{2+}x10^{-5} A	Ep(V)	IpX10^{-4} A	IpX10^{-4} A	ΔIpX10^{-4}A
4.975	-0.993	1.44	1.40	0.04
9.900	-0.954	1.65	1.35	0.30
14.778	-0.954	1.80	1.56	0.24
19.607	-0.954	1.87	1.61	0.26

Effect of Varying pH Levels

The effect of the pH on the reduction peak potential and peak current of the reduction wave of zinc was studied in the presence of (9.9x10^{-5}) M of TPSA, using acetate buffer solution for a range of pH (2-5). The results obtained are tabulated in table 5.

Table 5
Different pH from zinc ion

pH	Ep (v)	Ipx10^{-5}A
2	-0.924	8.94
3	-0.994	8.73
4	-0.954	16.00
5	-1.00	1.32

It is noted that there is a decrease in the diffusion current with an increasing of the supporting electrolyte acidity, except at pH4, which show a significant increase in the current value and gave the best reduction wave shape. Therefore, it was chosen as an ideal pH for studying the electrochemical behaviour of TPSA in the presence of the zinc ion Figure 4.

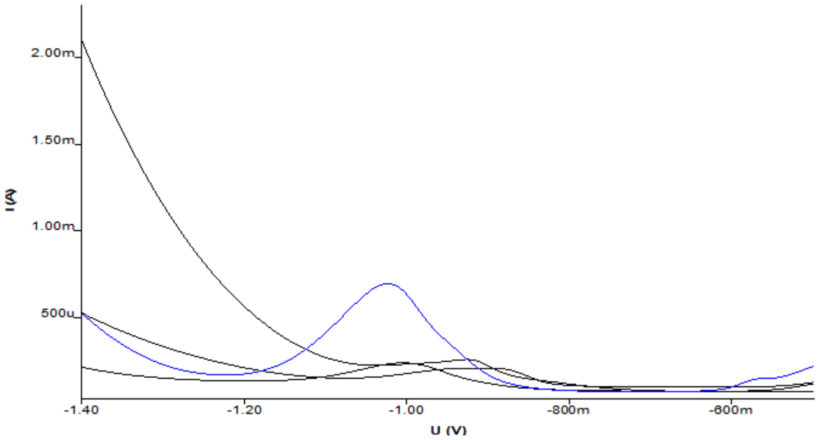

Fig. 4. Different pH from zinc ion

Calibration Curve

The calibration curve was constructed by adding sequence additions of a 2.47x 10^{-6}M TPSA in the presence of 100 µL of 10^{-2} M zinc ion in acetate buffer solution (pH4). The voltammogram was recoded under the measured optimum conditions for each addition of TPSA Figure 5.

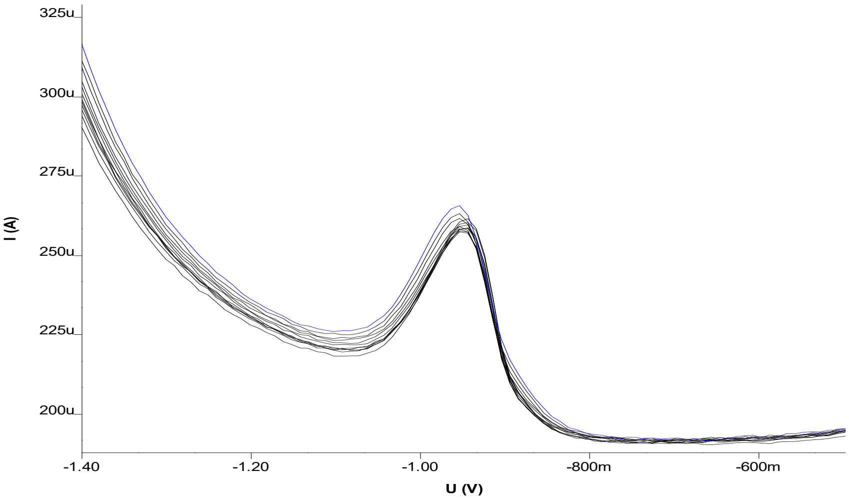

Fig. 5. Differential pulse voltammograms of (2.47x 10^{-6}) M zinc ion in the presence of different amount of TPSA

The plot of reduction peak current versus concentration gives straight line figure 6. in the concentration range (2.4431 – 24.4554) x10^{-9} M with a calibration equation y=0.0308x+1.2873 and correlation coefficient equal to 0.9763.

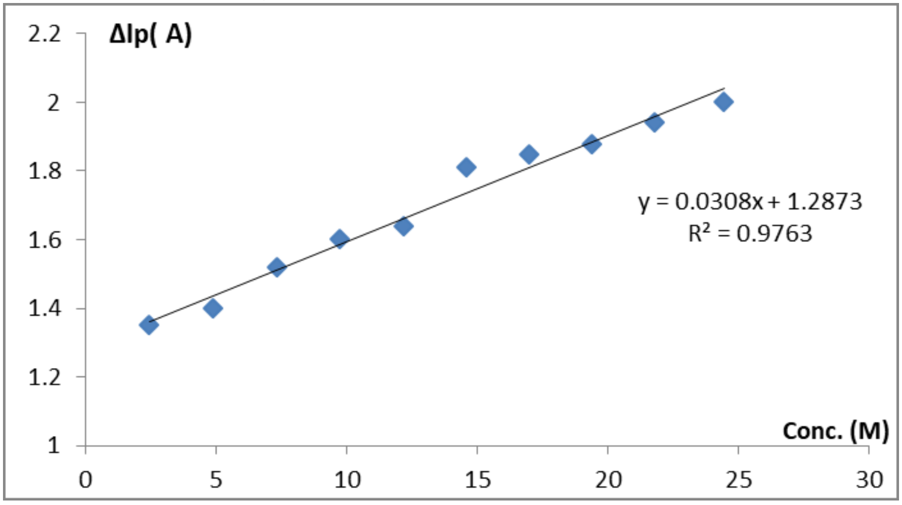

Fig. 6. Calibration curve of TPSA under the measured optimum conditions

Thermodynamic Calculations

The binding constant of TPSA–zinc ion interaction was calculated according to the equation (1).

$$\ln (Ip/(Ip^{o} - Ip)) = \ln (1/[Conc.(M)]) - \ln (K_b) \ldots\ldots\ldots\ldots. (1)$$

Where Ip^{o} is the reduction current of zinc ion alone, Ip is the reduction current of TPSA – zinc complex, Conc. is the molar concentration of zinc ion (10^{-2} M), and Kb is the binding constant of TPSA – zinc complex. The binding constant was calculated at different temperatures (293, 298, 303 and 308) K, and the results obtained are shown in table 6.

Table6

The binding constant of TPSA – zinc complex at different temperatures

Temp(K)	**1/T**	**Ln K_b**
293	0.0034129	18.586
298	0.0033557	14.588
303	0.0033003	12.444
308	0.0032467	10.120

Thermodynamic parameters were calculated using Van't Hoff equation (equation 2)

$$\mathrm{LnK} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \text{.......2}$$

Enthalpy(ΔH) was calculated from the slope Van't Hoff equation

$$\Delta H = - \text{Slope} \times R \text{ .....3} \quad (R= 8.314 \text{ J. mole-1 . K-1})$$

The free energy (ΔG) was calculated from equation 4

$$\Delta G = - R \times T \times \ln K \text{ .....4}$$

Entropy (ΔS) was calculated from the intercept of the Van't Hoff equation (equation 5)

$$\Delta S = \text{Intercept} \times R \text{ .....5}$$

The plot of lnK versus 1/T gives straight line with R^2 equal to 0.9804, Figure 7.

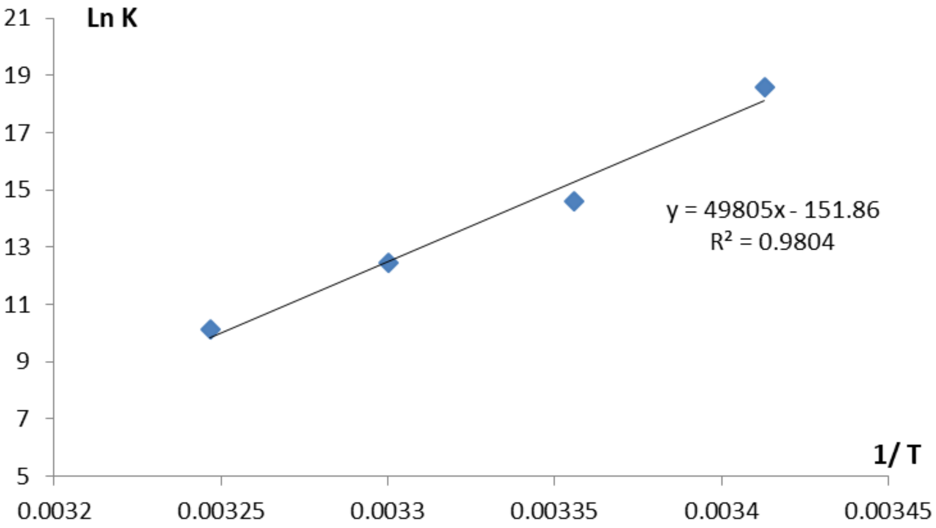

Fig. 7. Relation between ln k and 1/T

Thermodynamic values for TPSA– zinc interaction were tabulated in Table 7

Table 7
Thermodynamic values for TPSA – zinc interaction

Temp(K)	ΔH(KJ/mol)	ΔG(KJ/mol)	ΔS(J/mol.K)
293	-414.078	-45.231	-1262.564
298		-36.142	
303		-31.348	
308		-25.914	

The negative value of ΔH indicates that the interaction between TPSA and zinc ion was exothermic then the value of the binding constant decrease with increasing temperature (Al-Taee & Al-Hafidh, 2021). Also ΔG becomes more positive with increasing temperature means the spontaneously of the binding decrease as a result of temperature increasing, whereas the negative value of ΔS indicates that the system became more ordered (Al-Hasany et al., 2019). The negative ΔH and ΔS values for the interaction of TPSA and zinc ion indicates that the binding is mainly enthalpy and entropy driven, and the interaction may involve coordination bond between zinc and TPSA (Hasan et al., 2024; Al-Imam & Al-Abady, 2022).

CONCLUSION

The electrochemical behaviour of total prostatic specific antigen TPSA can be done indirectly, in acetate buffer solution pH4, by following the decrease in zinc reduction peak current which is appeared at -1.01 V as a result of TPSA added. The binding constant was calculated and thermodynamic values were examined. The results of thermodynamic study indicates that the binding is mainly enthalpy and entropy driven, and the interaction may involve coordination bond between zinc and TPSA.

Competing Interest

The authors had no competing interests.

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