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The Binding of Sodium Nitroprusside with Albumin Using Square Wave Voltammetry

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ABSTRACT

The voltammetric behavior of pure Sodium Nitroprusside was studied in a direct method in aqueous phosphate solution at (pH=7.0) by square wave voltammetry Sodium Nitroprusside gives a well defined square wave voltammetric peak at (-0.592) volt against the reference electrode (Ag/AgCl/SatKCl). The calibration curve is linear within the range of concentration [(0.398×10^{-6}) – (8.424×10^{-6})] molar with a correlation coefficient of (0.9991). The binding of Sodium Nitroprusside with Albumin has been studied, the binding constants (K) were calculated at different temperatures. Vant's hoff equation applied to calculate the thermodynamic variables (ΔG,ΔS,ΔH), and the results indicate that the binding of SNP with albumin is of the type (ion-ion).

Keywords: Binding, sodium Nitroprusside, albumin, square wave voltammetry.

الترابط لنيتروبروسيد الصوديوم مع الألبومين باستخدام فولتامتري الموجة المربعة

الملخص

تمت دراسة الخواص الفولتامترية للنيتروبروسيد الصوديوم (Sodium Nitroprusside) النقي بطريقة مباشرة في محلول الفوسفات المائي باستخدام تقنية فولتامتري الموجة المربعة (S.W.V) وقد أظهرت المادة موجة اختزال واضحة عند الجهد (0.592-) فولت ضد قطب المرجع (Ag/AgCl/SatKCl)، وتمت دراسة المنحنى القياسي للدواء في محلول الفوسفات المنظم ذي الرقم الهيدروجيني (pH=7.0) وكانت العلاقة خطية ضمن مدى التركيز [(0.398×10^{-6})-(8.424×10^{-6})] مولاري وكانت قيمة معامل الارتباط (0.9991).

كذلك تمت دراسة الترابطات للألبومين مع النيتروبروسيد الصوديوم وتم تقدير قيمة ثابت الارتباط (K) في درجات حرارية مختلفة (315,305,300,295) مطلقة، فضلا عن ذلك تم تطبيق معادلة (فانت هوف) لحساب القيم الثرموداينميكية (ΔG,ΔS,ΔH) ومنها تم الاستنتاج إن عملية الترابط كانت من نوع (أيون – أيون) وهي ضعيفة.

الكلمات الدالة: الترابط، نيتروبروسيد الصوديوم، الألبومين، فولتامتري الموجة المربعة.

INTRODUCTION

Sodium mitroprusside (SNP) is the chemical compound with the formula $Na_2[Fe(CN)_5NO].2H_2O$ as shown in Fig. 1. This salt serves as a source of nitric oxide, a potent peripheral vasodilator that affects both arterioles and venules (venules more than arterioles) (Butler, 2002). SNP is often administered intravenously to patient who are experiencing a hypertensive emergency. The principal pharmacological action of SNP is to relax a smooth muscle and consequent dilatation of peripheral arteries. Dilatation of the coronary arteries also occurs (Coppens, 2002). SNP has been suggested to cause cytotoxicity through either the release of cyanide and or nitro oxide, iron moiety of break down product of SNP could cause a long lasting oxidation stress,such as hydroxyl radical generation, lipid peroxidation and cytotoxicity (Navaza, 1989) (Rauhala, 2005). This drug can cause very large decreases in blood pressure. With out proper monitoring of serious injury, death could result. Nitroprusside, especial largerthan-recommended doses, might cause cyanide poisoning (Medicinenet, 2011) (Medicines, 2004).

Several methods are available for measuring SNP.The spectrophotometric method of analysis for SNP developed utilizing the molar absorptivity values at the maxima appearing in the electronic spectrum at 394 and 498 nm. These measurements appear to be indicative of the unaltered nitro ferricyanide complex. A reaction sequence is postulated for aqueous solutions of nitroprusside exposed to normal artificial light (Frank, 1976). A rapid high – performance chromatographic method for the determination of nitroprusside in commercial lyophilized product or in intravenous admixture solutions is described . Phenyl colum with a mobile phase acetonitrile-phosphate/tetrabutyl ammonium hydroxide buffer (pH=7.1) (30:70) and detection at 210 nm is worded out. Acoefficient of variation of better than 3.1%was achieved over the concentration range studied (10-50µg/ml) and the total analysis time was 9 minutes (Baaske, 1981). The electrochemical reduction of the nitroprusside ion (NP) was examined by (DPP) and (SWV) The effect of pH on the electro chemical behaviour of (NP) was studied and experimental evidence for the occurrence of adsorption of the product of the first one-electron reduction of (NP)is shown.Owing to the adsorption of these products, which are the reactants in the second reduction step of NP, the peak current of the second process is highly enhanced compared with that of the first process,especially at low concentration levels ($<1x10^{-5}$ mol/dm^{-3}). Square-wave cathodic voltammetry with adsorptive accumulation was used for the determination of NP. The detection limit depends on the square wave parameter and can be as low as (2.3 nmol/dm^{-3}) for a delay time of 60 sec (Helena, 1996).

The present work involves the use of square wave voltammetric method for trace determination of SNP and studying the molecular interaction with albumin.

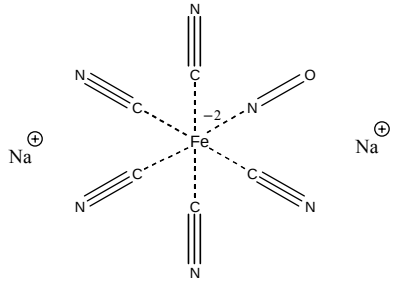

Fig. 1 :Chemical structure of sodium nitroprusside M. wt = 298 gm/mole.

EXPERIMENTAL

Apparatus

All experiments were performed using the EG and G PAR 384 B computerized polarographic analyser equipped with 303 A hanging mercury drop electrode and RE 0093 digital Plotter. A three electrode system was used. The working electrode was (HMDE); the reference electrode was Ag/AgCl, sat KCl electrode and the counter electrode was a Pt-wire electrode. pH measurement were made using PW 9421-Philips pH-meter. Temperature control was made by using Haake NK 22 water thermostate (±0.1 C).

Reagents

All the chemicals used were of analytical reagent grade. SNP which were obtained from Fluka. Solutions of 1.0x 10^{-3} M were prepared with deionised distilled water. Bovine serum albumin (BSA) was obtained from Merck, 0.1% solution was freshly prepared. Phosphate buffer was prepared by mixing certain amounts of 0.2 M of each of K_2HPO_4 and KH_2PO_4.

Procedure

The voltammetric cell was thermostated at 37 C°, the solution was de-aerated by passing through it a slow stream of purified nitrogen gas for 10 minutes for removing the dissolved oxygen.The square wave voltamogram was recorded on a degassed phosphate buffer solution at (pH=7.0) (5 ml). The blank current was recorded. Appropriate amount of SNP stock solution was added to this solution to yield the desired concentration and the current –voltage current was recorded again.The calibration curve was then constructed. The same procedure was also used to calculate the binding constant (K), using the optimum conditions in Table 1. For voltammetric measurements, the sample cell contained (5ml) of phosphate buffer at (pH=7.0) with a final concentration (9.9x10^{-6}) M of SNP. The square wave voltamogram was recorded to give the SNP peak.Then, appropriate amount of (10^{-5}) M of albumin was added to the cell and the square wave voltamogram were recorded at different temperatures in the range (295-315) K° in order to calculate the thermodynamic quantities ΔG, ΔS and ΔH.

RESULTS AND DISCUSSION

Typical square wave voltamogram of (7.578x10^{-6}) M SNP in phosphate buffer at (pH=7.0) is shown in Fig. 2.

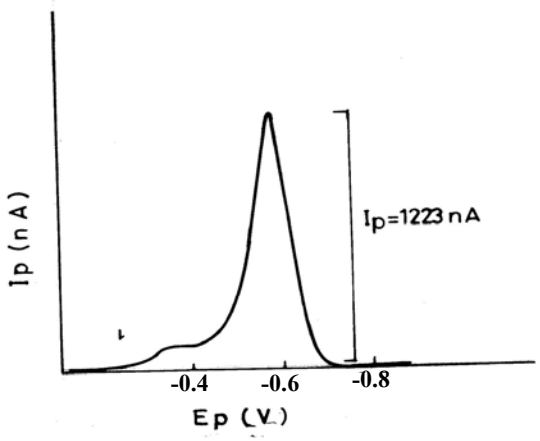

Fig. 2: Square wave voltamogram of (7.578x10^{-6}) M SNP at (pH=7.0).

It can be seen from Fig. 2, that a well-defined peak appeared at (-0.589 V) versus (Ag/AgCl,Sat KCl) electrode.

Optimum conditions

The SWV voltamogram of ($1.96x10^{-6}$) M of SNP was investigated in phosphate buffer (pH=7.0)by variation all the parameters of the measurements, the optimum values obtained are tabulated in Table 1.

Table 1: The optimum values obtained which give either the highest peak current or the best resolution of the peak.

Condition	Value	Condition	Value
initial pot.	- 0.1 V	frequency	120 Hz
final pot.	- 0.85 V	scan increment	2 mV/sec
deposition time	40 second	cond. potential	V0.000
condition time	10 second	pulse height	mV0.025
equilibrium time	5 second		

Effect of pH

The square wave voltammogram of ($2.152x10^{-6}$)M of SNP were investigated at different pH values (3-10) using the optimum conditions Table 1 in phosphate buffer. The peak current (Ip) and the peak potential (Ep) obtained are shown in Table 2.

Table 2: Effect of pH on SWV peak and peak current of ($2.152x\ 10^{-6}$) M of SNP

pH	Ep (v)	Ip (nA)
3	- 0.375	104.1
4	- 0.428	123.2
5	- 0.475	142.3
6	- 0.528	166.2
7	- 0.588	185.7
8	- 0.648	175.6
9	- 0.703	160.7
10	- 0.752	133.3

The peak current (Ip) is clearly dependent on pH. Maximum current response was found at (pH=7.0).On the other hand the peak potential (Ep)is found to be greatly dependent on pH and moves to more negative with increasing the pH values. Linear plot of Ep versus pH was obtained in Fig 3 with slope (-0.0547 V/pH) and (R=0.9995) which is very near to the theoretical value obtained by Hammett (0.059 V/pH) (Hammett, 1940).

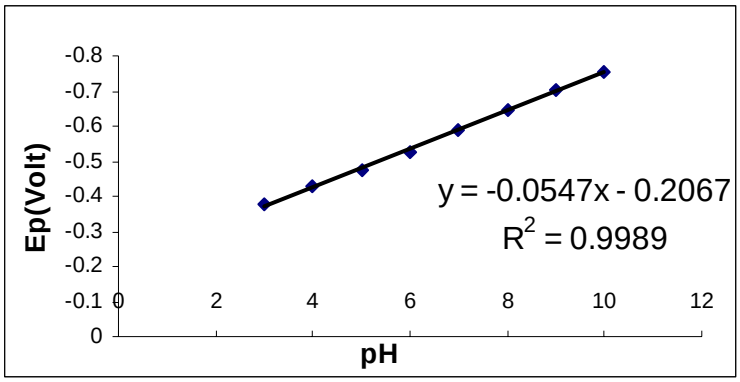

Fig. 3: The relation between Ep and pH of ($2.152x10^{-6}$) M of Sodium Nitroprusside.

Stability of SNP in aqueous phosphate buffer at (pH=7.0)

The square wave voltamogram of ($3.1x10^{-6}$)M of SNP were recorded at different time in phosphate buffer at (pH = 7). The results obtained are tabulated in table 3.

Table 3: Effect of time on SWV peak of ($3.1x\ 10^{-6}$) M of SNP at (pH=7.0) in aqueous solution.

Time (min)	Ip (nA)
3	250.0
6	252.1
9	254.0
12	253.1
15	256.4
18	260.7
21	253.2
24	249.1
27	263.4
30	264.3
33	255.2
36	257.0

It can be seen from the Table 3 that SNP is stable for more than 36 minute.

Analytical Consideration

Using The optimum conditions shown in Table 1, the calibration curve were constructed using a serial dilution of a standard SNP in aqueous-phosphate buffer (pH=7.0) (5ml). Some typical results are listed in Table 4. These solutions were prep-ared by adding appropriate aliquots of standard SNP to the phosphate buffer (5 ml) at (pH=7.0).

Table 4: Effect of concentration on peak current of [(0.398×10^{-6})–(8.424×10^{-6})] M of SNP at (pH=7.0) in aqueous solution at Ep = - 0.588 V.

Conc. (M) 10^{-6}	Ip (nA)
0.398	21.4
0.793	52.0
1.185	86.0
2.152	171.8
3.100	237.2
4.030	308.0
4.942	294.7
5.838	345.9
6.716	948.0
7.578	1330.0
8.424	1720.0

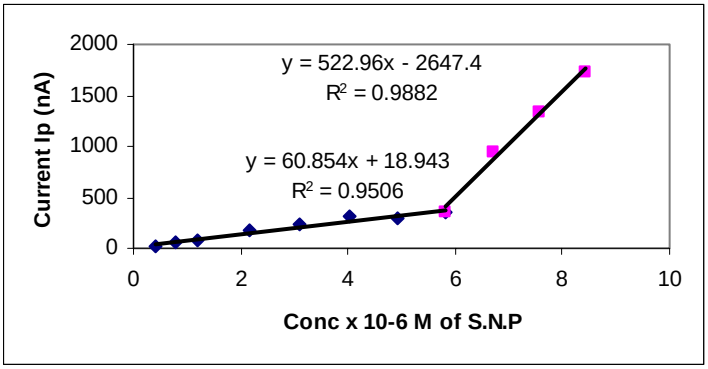

Fig. 4: The relation between peak current (Ip) and concentration of SNP at (pH = 7.0) phosphate buffer in aqueous solution.

The plot peak current Ip versus molar concentration of SNP is shown in Fig. 4. Regression analysis on standard indicated two straight lines, the first one with concentration range [$(3.98\text{x}10^{-7}$-$5.838\text{x}10^{-6})$] M with correlation coefficient (R=0.9751). The second with the concentration range [$(5.838\text{x } 10^{-6}$- $8.4\text{x}10^{-6})$] M with correlation coefficient (R=0.9941). This behavior maybe due to the change of rate of diffusion with concentration and increase gradually with increasing conc. It well known that the rat of discharge (reduction) depend mainly on the rate of diffusion. The lowest experimental determination limit was ($3.9\ \text{x}10^{-7}$).

Voltammetric Behavior of SNP in the Presence of Albumin

The square wave voltammogram of (9.9×10^{-6}) M of SNP in phosphate buffer at (pH = 7.0) was recorded at (295 K°). Successive amounts of albumin (10^{-5}) M were then added and the square wave voltammogram were recorded after each addition. The results are shown in Table 6. The peak current Ip of SNP were found to decrease gradually with the

addition of albumin. This behavior due to the interaction of SNP with albumin. The plot Ip/Ip_0 versus concentration of albumin added is shown in Fig. 5 where (Ip_0= 1447.12 nA) is the peak current of SNP only without albumin. From which the results of binding constant K of SNP with albumin was then calculated using quadratic equation where K obtained from the tangent.

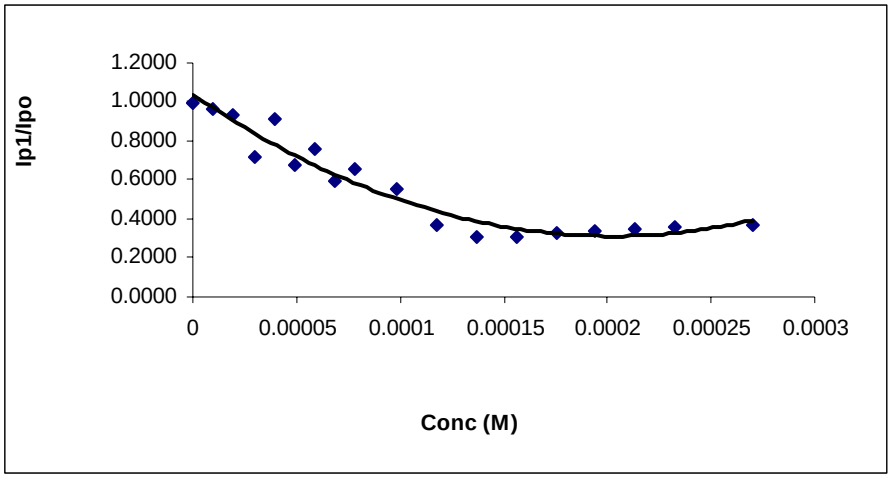

Fig . 5: The plot of Ip/Ip_0 versus the concentration of albumin added.

The effect of temperature on the interaction of Sodium Nitroprusside with albumin

The square wave voltammogram of (9.9×10^{-6}) M of SNP in the presence of albumin (successive addition) were recorded at different temperatures (295 – 315) K, using the optimum conditions shown in Table 5. The variation of peak current Ip at different temperature are shown in Tables (6-10). The binding constant K for the interaction of SNP with albumin was calculated and tabulated in Table 11. The Vant's hoff plot of log K versus 1/T gave a straight line Fig.6 from which the thermodynamic parameters were calculated as follows: ΔH from the relation slope = – Slope × R ; ΔG = – 2.303 R Log K and from the Gibbs equation ΔS was calculated ($\Delta G = \Delta H - T\Delta S$).The values of tend for ΔH, ΔG and ΔS are tabulated in Table 12.

Table 5: The optimum condition of binding SNP with albumin.

Conditions	Value
Deposition time	30 Second
Condition time	5 Second
Equilibrium time	5 Second
Frequency	120 Hz
Scan increment	2 mV/second
Condition potential	0.000 V
Pulse height	0.02 mV

Table 6: The peak current of (9.9×10^{-6})M SNP in the presence of different amount (10^{-5})M of albumin in phosphate buffer (pH=7.0), (Ip_0 = 1447.12 nA) at 295 K˚.

Conc (M) $\times 10^{-8}$	**Ip (nA)**	**Ip / Ip_0**
0.00	1447.12	1.0000
0.09	1390	0.9605
1.90	1340	0.9287
2.90	1044	0.7214
3.90	1330	0.9156
4.90	984	0.6800
5.90	1094	0.7560
6.80	868	0.5998
7.90	948	0.6551
9.80	798	0.5514
11.70	528	0.3649
13.60	442	0.3054
15.50	450	0.3110
17.50	472	0.3262
19.40	485	0.3351
21.30	503	0.3476
23.20	518	0.3580
26.90	537	0.3711

Table 7: The peak current of (9.9×10^{-6})M SNP in the presence of different amount (10^{-5})M of albumin in phosphate buffer (pH=7.0), (Ip_0 = 1223 nA) at 300 K˚.

Conc (M)$\times 10^{-8}$	**Ip (nA)**	**Ip/ Ip_0**
0.0	1223.0	1.0000
1.9	1127.0	0.9215
3.9	948.0	0.7751
5.9	743.0	0.6075
7.8	464.0	0.3794
9.8	326.3	0.2666
11.7	321.8	0.2631
13.6	366.9	0.3000
15.5	405.3	0.3314
17.5	426.0	0.3483
19.4	456.0	0.3729
21.3	476.0	0.3892

Table 8: The peak current of (9.9×10^{-6})M SNP in the presence of different amount (10^{-5})M of albumin in phosphate buffer (pH=7.0), (Ip_0 = 869.0 nA) at 305 K˚.

Conc (M)×10^{-8}	**Ip (nA)**	**Ip/ Ip_0**
00.0	869.0	1.0000
1.9	752.0	0.8654
3.9	476.0	0.5478
5.9	269.8	0.3105
7.8	239.7	0.2758
9.8	215.8	0.2483
11.7	210.5	0.2422
13.6	271.8	0.3128

Table 9: The peak current of (9.9×10^{-6})M SNP in the presence of different amount (10^{-5})M of albumin in phosphate buffer (pH=7.0), (Ip_0 = 778 nA) at 310 K˚.

Conc (M)×10^{-8}	**Ip (nA)**	**Ip/ Ip_0**
0.0	778	1.0000
1.9	660	0.8483
3.9	500	0.6427
5.9	398	0.5116
7.8	300	0.3856
9.8	280	0.3599
11.7	250	0.3213
13.6	186	0.2391
15.5	161	0.2071

Table 10 : The peak current of (9.9×10^{-6})M SNP in the presence of different amount (10^{-5})M of albumin in phosphate buffer (pH=7.0), (Ip_0 = 768.0 nA) at 315 K˚.

Conc (M)×10^{-8}	**Ip (nA)**	**Ip/ Ip_0**
0.0	768.0	1.0000
0.9	737.0	0.9596
1.9	447.0	0.5820
3.9	298.0	0.3880
5.9	262.8	0.3422
7.8	199.1	0.2592
9.8	170.0	0.2214
11.7	155.0	0.2018
13.6	135.0	0.1758
15.5	121.0	0.1569
19.4	99.2	0.1292

It's very clear from the results in tables (6-10) the peak current of SNP in all concentration decrease with the addition of albumin (due to the interaction), in the other words, the peak current of SNP in the absence of albumin found to decrease with increasing temperature due to the diffusion process of SNP. The binding constant K of the interaction between SNP and albumin at different temperature are shown in Table 11.

Table 11: The binding constant K of the interaction between SNP with albumin at different temperature.

Temp. (T) K	1/T	K	Log K
295	0.003389	0.3179	- 0.497709
300	0.003333	0.2755	- 0.559878
305	0.003278	0.2199	- 0.657774
315	0.003174	0.1110	- 0.954677

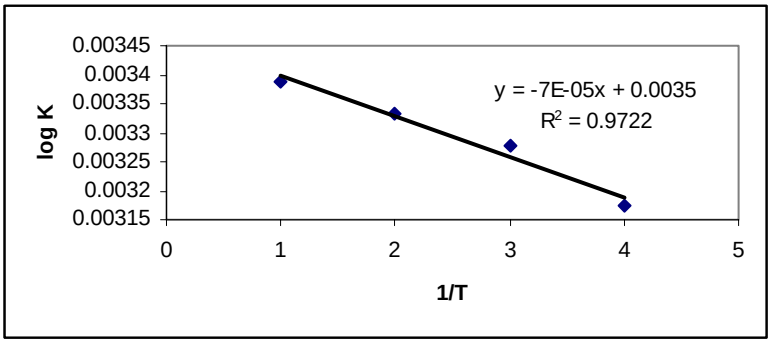

Fig. 6: The relation between Log K (binding constant) against 1/T.

From the results in the Table (11) It's found that the binding constant K decrease with increasing temperature. From which the thermodynamic quantities of the interaction of SNP with albumin can be calculated as shown in Table 12.

Table 12 : The thermodynamic quantities of the interaction of SNP with albumin.

Temp. (K°)	ΔH (KJ/mole)	ΔG (KJ/mole)	ΔS (KJ/mole.K°)
295	18.667 -	2.8112	- 0.0728
300	=	3.2160	- 0.0729
305	=	3.8413	- 0.0737
315	=	5.7579	- 0.0775

The negative values of ΔS indicate that the complexes formed after the interaction of SNP-albumin is more ordered. The negative values of ΔH indicate that the process of interaction is exothermic. The low and positive values of ΔG indicate that the interaction is of the type ion-ion (Bohinski, 1987).

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