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Spectrophotometric Determination of Sodium Nitroprusside by Coupling with Diazotized p-Nitroaniline and Determination of Thermodynamic Parameters

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ABSTRACT

The coupling reaction of sodium nitroprusside (SNP) with diazotized p-nitroaniline (DPNA) in basic media has been studied using spectrophotometric method. The intense yellow water-soluble associated species which was formed, showed maximum absorbance at 278 and 396 nm, depending on the concentration of SNP. Two calibration curves have been obtained for the determination of SNP with DPNA. The first curve (λ_{max}=278 nm) obeys Beer's law over the range 25-300 µg/25 ml, with a regression equation of $A=0.0027C+ 0.0747$; $R^2 = 0.9853$. The second curve (λ_{max}=396 nm) obeys Beer's law over the range 300-5000 µg/25 ml, with a regression equation of $A= 0.0002C+ 0.0439$; $R^2=0.9943$. The stability constants (K) were determined at different temperatures (283, 288, 293, 298 and 303°K). Van't Hoof plot was obtained from which the thermodynamic parameters (free energy change ΔG, enthalpy change ΔH, and entropy change ΔS) were obtained.

Keywords: Sodium Nitroprusside, Diazotized p-Nitroaniline, Spectrophotometric Determination, Stability Constant, Thermodynamic Parameters.

التقدير الطيفي لنتروبروسايد الصوديوم باقترانه مع بارا- نايتروانيلين المؤزوت وقياس العوامل الثرموديناميكية

الملخص

لقد تمت دراسة تفاعل اقتران نتروبوسايد الصوديوم مع بارا- نايتروانيلين المؤزوت في وسط قاعدي باستخدام الطريقة الطيفية. إن المركب المتكون يذوب في الماء وذو لون اصفر ويعطي أعلى امتصاصية عند278 و396 نانومتر معتمدا على تركيزSNP. لقد حصلنا على علاقتين خطيتين لحساب النتروبروسايد. العلاقة الخطية الأولى عن الطول الموجي 278 نانوميتر تخضع لقانون بير ضمن المدى 25-300

مايكروغرام/25 مليلتر 12.0-1.0 جزء/المليون ولها معادلة انحسار $A=0.0027C+0.0747$ و $R^2=0.9853$. والعلاقة الخطية الثانية عند الطول الموجي 396 نانوميتر تخضع لقانون بير ضمن المدى 5000-300 مايكروغرام/25 مليلتر 200.0-12.0 جزء/المليون وبمعادلة انحسار $A= 0.0002C+0.0439$ و R^2 = 0.9943. كما وتم حساب ثابت الاستقرار K عند درجات الحرارة (283 و288 و293 و298 و303 درجة مطلقة). ومن رسم مخطط فانت هوف حصلنا على القيم الثرموداينامية (التغير في الطاقة الحرة، التغير في الانثالبي، والتغير في الانتروبي).

الكلمات الدالة: نايتروبروسايد الصوديوم، بارا-نايتروانيلين المؤزوت، التقدير الطيفي، ثابت الاستقرار، القيم الثرموديناميكية.

INTRODUCTION

Sodium nitroprusside (nipride), $Na_2[Fe(CN)_5NO]\cdot 2H_2O$, (SNP), has been known to inorganic chemists since 1849. It is used as a relaxant of muscles in the widening of the walls of the arteries due to its ability to generate free NO in situ (Ainscough and Brodie, 1995). This results in a widening of the arteries and reduction in blood flow. Interestingly, an aqueous solution of SNP under physiological conditions and in the dark is relatively stable and does not release nitric oxide (NO) appreciably(James *et al.,* 1995). Sodium nitroprusside is an excellent agent for lowering blood pressure in hypertensive emergencies, for producing controlled hypotension during anesthesia, and for treating acute myocardial infarction and chronic heart fails. Sodium nitroprusside is indicated for the immediate reduction of blood pressure of patients in hypertensive crises. Sodium nitroprusside is also indicated for the treatment of acute congestive heart failure. Sodium nitroprusside is often administered intravenously to patients who are experiencing a hypertensive emergency (Butler and Megson, 2002).

In general, chemical processes obey thermodynamic or kinetic control. If we know enthalpies, and equations of state of the chemical species involved, we can predict with accuracy the chemical composition of this final equilibrium state. However, thermodynamics is unable to say anything about the time required to attain equilibrium, or about the behavior, or about the composition of the system during the period of change. The stability constants and thermodynamic parameters of the interaction between ascorbic acid and sodium nitroprusside were determined at different temperatures (Rahawi, 2010). Smith and Dasgupta have carried out a detailed investigation on the kinetics and mechanism of the decomposition of SNP by L-ascorbic acid to afford NO in aqueous medium. Experiments were performed under an argon atmosphere and reaction rates were strongly pH dependent and increase with pH (Smith and Dasgupta, 2001).

EXPEREMINTAL

Apparatus

Spectral measurements were made on a Shimadzu UV-1601 recording spectrophotometer. All the pH measurements were done on Elico pH meter (LI-10T).

Reagents and Chemicals

All chemicals used were of highest purity.

Standard SNP solution (100 µg/ml). A 0.1000 g of SNP dissolved in 1L distilled water. This solution was prepared weekly.

Diazotized p-nitroaniline reagent solution, (5×10^{-3} M). A 0.0691g of p-nitroaniline (fluka) was dissolved in about 8 ml of distilled water. Then 1.6 ml of 5 M hydrochloric acid solution was added, the clear mixture was then transferred to a 100- ml volumetric flask and cooled to 0-5^{0}C in an ice bath. A 3.5 ml of 1% of sodium nitrite ($NaNO_2$) solution was added and the mixture was stirred vigorously. After 5 min., the solution was made up to volume in a 100- ml volumetric flask with cold distilled water. The solution was kept in brown bottle in refrigerator and was stable for five days at least(Ibraheem, 2011).

Hydrochloric acid solution (5 M). This solution was prepared by appropriate dilution of concentrated hydrochloric acid (fluka) with distilled water.

Basic solutions (2%). Sodium carbonate solution were prepared by appropriate dilution of concentrated ampoule solution (fluka) with distilled water and then transferred to a plastic bottle. The other base (sodium hydroxide, potassium hydroxide, sodium acetate, sodium bicarbonate, sodium formate) 2% solutions were prepared by dissolving 2.0000 g of base in 100 ml a volumetric flask with distilled water and then transferred to a plastic bottle.

Surfactant solutions (1×10^{-3} M). These solutions were prepared by dissolving 0.0289g of SDS and 0.0365 g of CTAB each in 20 ml distilled water and the volume was made to 100 ml in a volumetric flask with distilled water.

Triton X-100 (1%). This solution was prepared by dissolving 1.00 ml Triton X-100 in 100 ml volumetric flask with distilled water.

Procedure

Aliquots of aqueous sample solutions containing (10-300µg) of SNP were transferred into a series of 25-ml calibrated flasks. To each flask 1ml of DPNA reagent solution and 2ml of 2% NaOH solution were added. The solutions were mixed and diluted to the mark with distilled water. The absorbance of each colored solution at ~ 278 nm after 10 min. was measured against the reagent blank using 1-cm cells. The same procedure was repeated for sample solution containing (300-5000µg) of SNP to measure the absorbance of their colored solution at ~ 396 nm

SNP Coupled with p-NA

In this work, the reaction of SNP with DPNA, occurs in two steps: in the first step the reaction of p-nitroaniline with sodium nitrite occurs in an acid medium producing the diazo compound. In the second, the diazo compound in alkaline medium coupled with the sodium nitroprusside and produced associated compound. This is indicated by the appearance of the peak at ~ 278 nm and at 396 nm with low and high concentration of SNP, respectively.

Diazotized p-nitroaniline reagent had been selected in this study for the following reasons: (1) the strongest diazonium electrophile ever used . (2) the strongest color observed in its azo dye production. (3) the solution of the diazotized p-nitroaniline reagent is stable for long time (>1 week) if kept in cold and dark conditions, and (4) the color of the diazotized reagent is faint yellow thus giving lower blank values (Ibraheem, 2011, Al-Abbasi, 2009, Bashir and Mansour, 2007, Al-Kass and Younis, 2004, Othman, 2004, Rahim *et al*., 1986).

The reaction involving diazotized p-nitroaniline as the chromogenic reagent for the determination of sodium nitroprusside is shown below (nature of the dye). Diazotization and coupling reactions were found to be temperature dependent. Diazotization was carried out at 0-5 ºC and coupling reactions was carried out at room temperature 20-25 ºC**.**

Effect of Alkali

To produce the colored species with sodium nitroprusside upon coupling with diazotized p-nitroaniline reagent, only basic medium can form the colored compound with useful analytical properties. Therefore, different amounts (0.5-10.0 ml of 2% solutions) of various bases had been tried for the purpose of producing intense colored compound, and lower blank value. Reagent tested for the alkalization step, including sodium acetate, sodium bicarbonate, sodium carbonate، sodium hydroxide, ammonia, and potassium hydroxide. The color developed varied between yellow and red; sodium bicarbonate and sodium carbonate produced yellow blank more than its sample solutions, ammonia produced a yellowish red color in both the test and the blank solutions, and potassium hydroxide developed a less intense yellow complex than sodium hydroxide did. Consequently, 2.0 ml of 2% NaOH solution had been recommended for the subsequent experiments for DPNA reagent.

Effect of Solvent

Acetone gave less intense yellow color than methanol did. Whereas ethanol gave a higher intense yellow color compared with acetone and methanol. All previous solvent showed a peak at ~ 372 nm. Water was chosen because it gives an intense absorption and cheap.

Effect of Diazotized p-Nitroaniline Reagent Amount

The effect of the DPNA concentration on the color intensity of the maximum absorbance of the dye formed was studied using the proposed procedure and adding volumes from 0.5 - 5.0 ml of 5.0×10^{-3} M solutions of DPNA to a series of nipride solutions (25-5000 μg of SNP in final volume 25ml). The results showed that a 1.0 ml of 5.0×10^{-3} M of DPNA solution was sufficient for complete color development from both correlation as well as sensitivity points of view. Higher concentration did not enhance the absorbance further, and lower concentration did not give good results.

Effect of Surfactants

In order to verify the importance of the electrostatic attraction in the detection of surfactant aggregates, the effects of cationic surfactant CTAB, anionic surfactant SDS and nonionic surfactant Triton X-100 was studied on the UV–visible spectra of SNP. The addition of surfactants did not show a remarkable reduction or enhancement of the UV-visible absorption intensity of the longest wavelength absorption band of the dye formed between sodium nitroprusside and DPNA in the aqueous solution. Therefore, we excluded the surfactants from our experiments because of the small effect of surfactants on the absorption intensity maxima of the dye formed.

Nature of the Dye

To establish the composition (ratio of sodium nitroprusside to diazotized p-nitroaniline reagents) of the yellow associated compound formed, Job's method of continuous variations has been used (Harris,1988). The resulting data (Fig. 1) reveal that the compound (1) with the following suggested formula is formed due to the reaction of SNP with DPNA reagent in a 1:1 ratio.

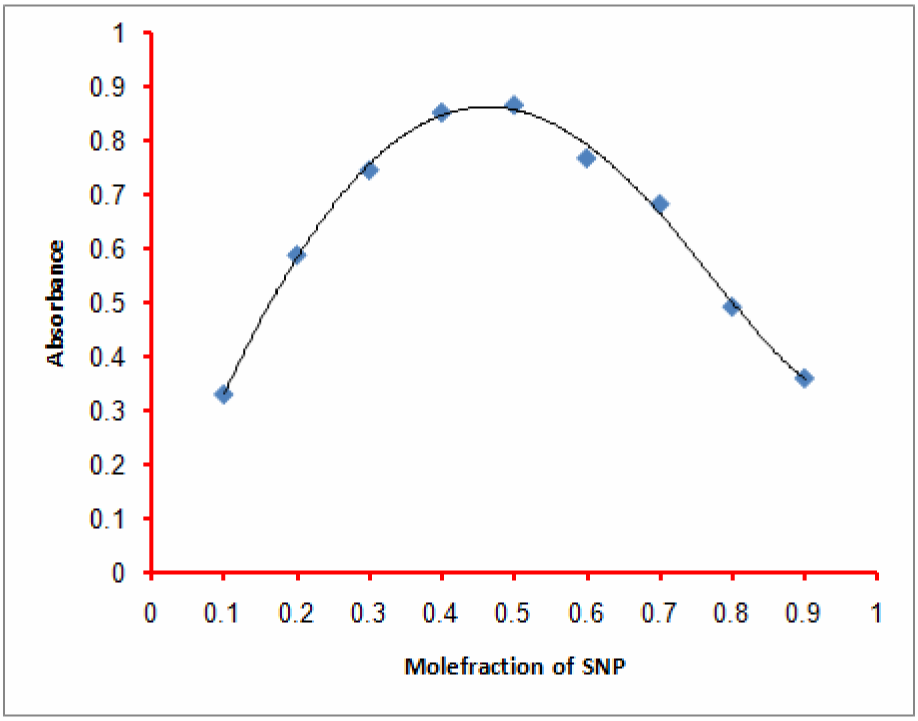
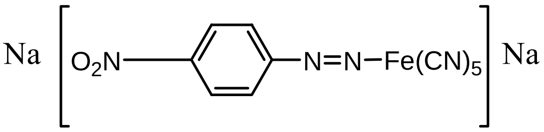


Fig. 1: Plot of job's method for SNP coupled with DPNA

The compound (1)



Order of Addition

The order of addition for reagent and corresponding volume of base to the sample solution had been examined. The order SNP + R + B for DPNA reagent was recommended due to their higher absorbance value since the order SNP + B + R gave very low absorbance which probably due to conversion of the active diazonium salt ($Ar-\overset{+}{N}\equiv N$) into inactive diazohydroxide species ($Ar-N=N-O^-$, in basic medium) (Ibraheem, 2011).

Final Absorption Spectra

When very dilute aqueous solutions of SNP and diazotized p-nitroaniline reagent solution are mixed in the presence of base, an intense yellow associated species forms depending on the concentration of nipride. At low concentrations of SNP, the intense yellow compound formed shows a maximum absorption at ~ 278 nm, while at high concentrations of SNP, another maximum absorption at ~ 396 nm appears in contrast to the reagent (DPNA) blank (Fig. 2).

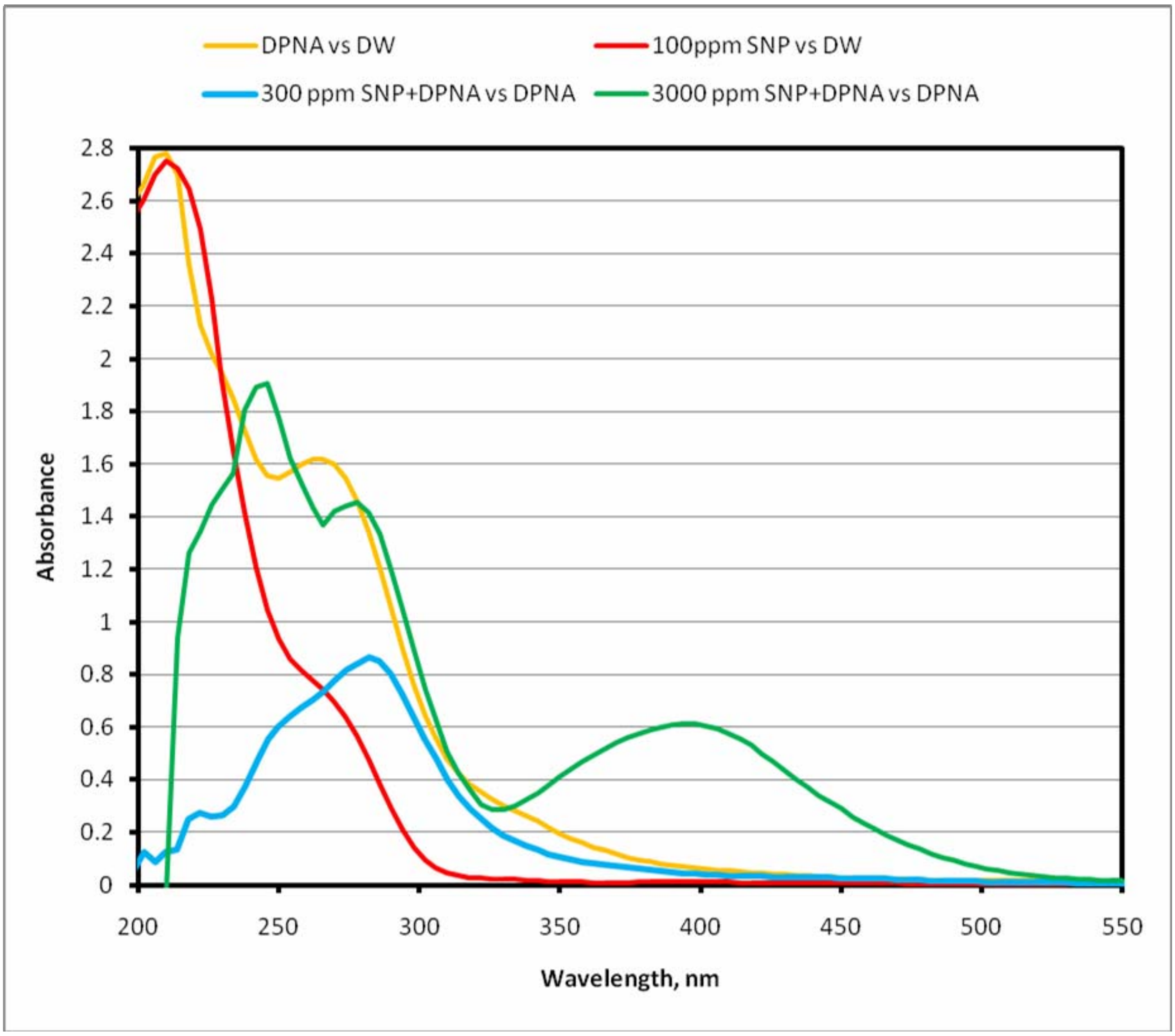


Fig. 2: Absorption spectra of 100 µg/25ml SNP versus distilled water; (300 and 3000) µg/25ml SNP treated as described under procedure and measured against a reagent blank; and reagent blank measured against distilled water.

Recommended Procedure and Validity of Beer's Law

The agreement of Beer’s law was studied by measuring the absorbance values of solutions and varying sodium nitroprusside amount. Two straight-line calibration curves were obtained, indicating that Beer's Law is obeyed over the low and high concentration ranges as shown in Fig. 3. The first one was over 25-300 µg of sodium nitroprusside in 25 ml final volume, i.e., 1-12 ppm while the second one was over high concentration 300-5000 µg/ml of SNP in 25 ml final volume, i.e.,12-200 ppm. The molar absorptivity values were 1.9991×10^{4}, 1.7082×10^{3} l mol^{-1} cm^{-1} and Sandell`s sensitivity index calculated as 0.014952, 0.17498 µg cm^{-2} for low and high concentration, respectively.

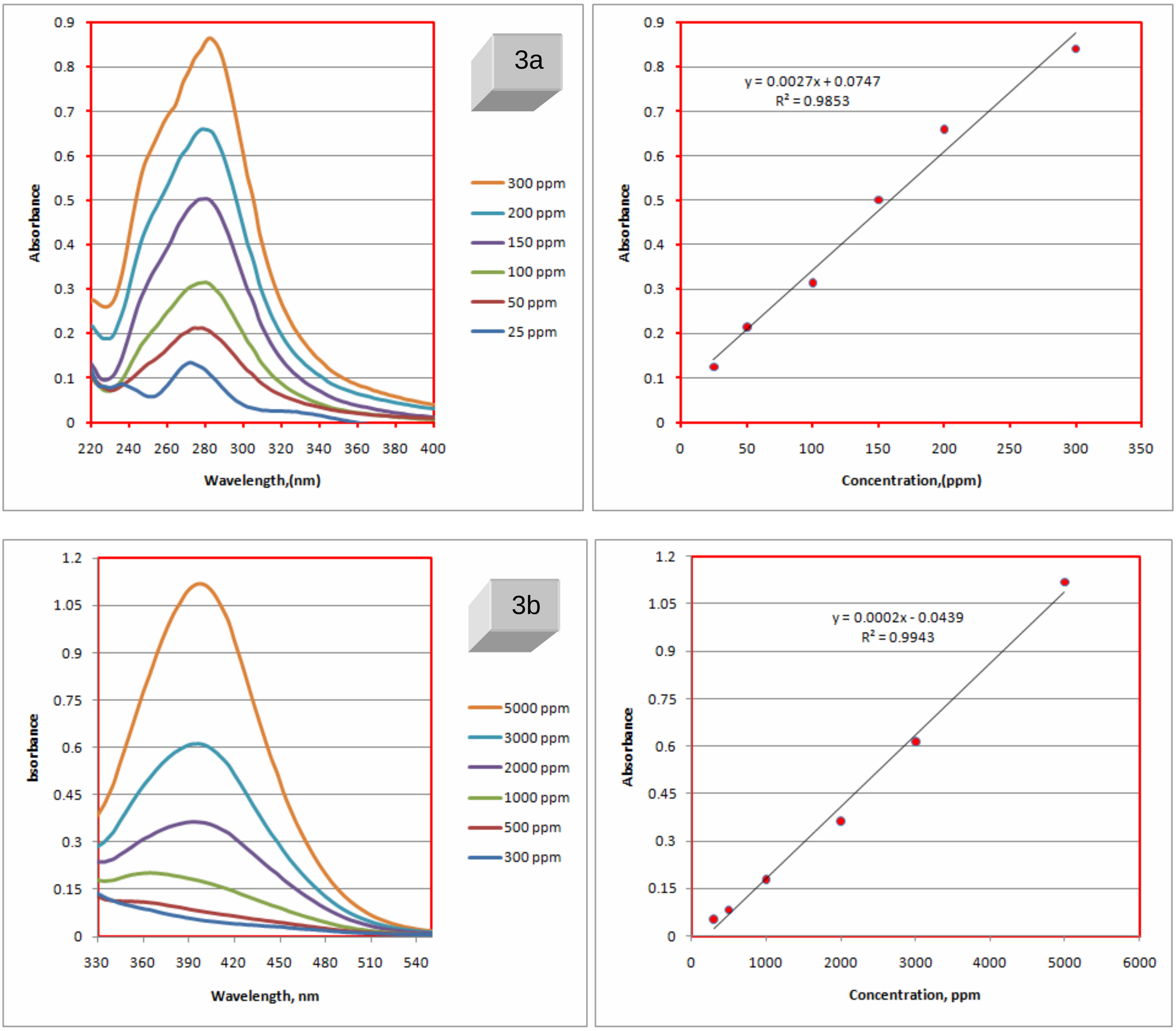


Fig. 3: Calibration curve for determination of different concentrations of SNP.

Two calibration curves have been obtained for the determination of SNP with DPNA:

The first curve obeys Beer's law over the range 25-300 µg/25 ml (λ_{max}=278 nm), with a regression equation of $A= 0.0027C + 0.0747$; $R^2 = 0.9853$ Fig (3a).

The second curve obeys Beer's law over the range 300-5000 µg/25 ml (λ_{max}=396 nm), with a regression equation of A= 0.0002C + 0.0439 ; R^2 = 0.9943 Fig (3b).

Stability Constant of the Complex

The formation of DA can be represented by the following reaction:

$$D + A \leftrightarrow DA \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

The apparent or conditional stability constant (K) of the complex of SNP with diazotized p-nitroaniline is evaluated (Harvey and Manning,1952), (Harris, 1988), and (Azzouz and Obed Agha, 2007). Solutions containing exactly one proportion of sodium nitroprusside to one of diazotized p-nitroaniline were prepared. Under this condition, the complex between them is appreciably dissociated and the absorbance, E_s was obtained. Similar solutions were then prepared containing the same amount of SNP but with excess amount of DPNA and its absorbance, E_m was obtained. The difference between the absorbance represents a measure of the degree of dissociation α of the interacted compound. The apparent stability constant of the dye formed had been calculated by using the formula (Harris, 1988).The estimation of stability constants of the dyes were tabulated in Table 1.

$$K= (1-\alpha)/(C\alpha^2) \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

K = Conditional stability constant of the dye.
C = Final concentration of the dye and equal to the molar concentration of sodium nitrprusside.

Thermodynamic Parameters

The Van 't Hoff equation (also known as the Van 't Hoff Isochore) (Atkins, 2006) in chemical thermodynamics relates the change in temperature (T) to the change in the stability constant (K) giving the standard enthalpy change (ΔH^θ) for the process. The equation was first derived by Jacobus Henricus Van 't Hoff .

$\frac{d\ln K}{dT} = \frac{\Delta H^\theta}{RT^2}$ This can also be written $\frac{d\ln K}{d\frac{1}{T}} = -\frac{\Delta H^\theta}{R}$

Since $\Delta G^\theta = \Delta H^\theta - T\Delta S^\theta$ And $\Delta G^\theta = -RT \ln K$, it follows that

$$\ln K = -\frac{\Delta H^\theta}{RT} + \frac{\Delta S^\theta}{R}$$

Therefore, a plot of the natural logarithm of the stability constant versus the reciprocal of temperature gives a straight line. The slope of the line is equal to minus the standard enthalpy change divided by the gas constant, $-\Delta H^\theta/R$ and the intercept is equal to the standard entropy change divided by the gas constant, $\Delta S^\theta/R$.

Thermodynamic Parameters at Different Temperatures

The enthalpy change (ΔH^{θ}_{f}) was calculated using Van't-Hoff equation (Table 1), by plotting ln K versus $\frac{1}{T}$; straight line was obtained (Figure 4) and from the slope = $-\Delta H^{\theta}_{f}/R$, ΔH^{θ} was obtained. The free energy changes, ΔG^{θ}_{f}, and the entropy changes, ΔS^{θ}_{f} , were calculated from the stability constants according to the equations $\Delta G^{\theta}_{f} = -RT \ln K$ and $\Delta S^{\theta}_{f} = (\Delta G^{\theta}_{f} - \Delta H^{\theta}_{f})/T$, respectively. All calculations were done on a personal laptop computer ASUS four GB microprocessor using Excel program.

Table 1: The stability constants and thermodynamic parameters for associated species at different temperatures.

Temp. K°	α	$\frac{1}{T}\times10^{3}$	$K\times10^{-4}$	Ln K	ΔG kJ/mole	ΔH kJ/mole	ΔS J/mol. K°
283	0.6850	3.5336	0.3356	8.1185	-19.102	129.557	-0.5253
288	0.4086	3.4722	1.7711	9.7819	-23.422	129.557	-0.5312
293	0.3763	3.4129	2.2016	9.9995	-24.359	129.557	-0.5253
298	0.2000	3.3557	10.0000	11.5129	-28.524	129.557	-0.5305
303	0.1771	3.3003	13.1211	11.7846	-29.687	129.557	-0.5256

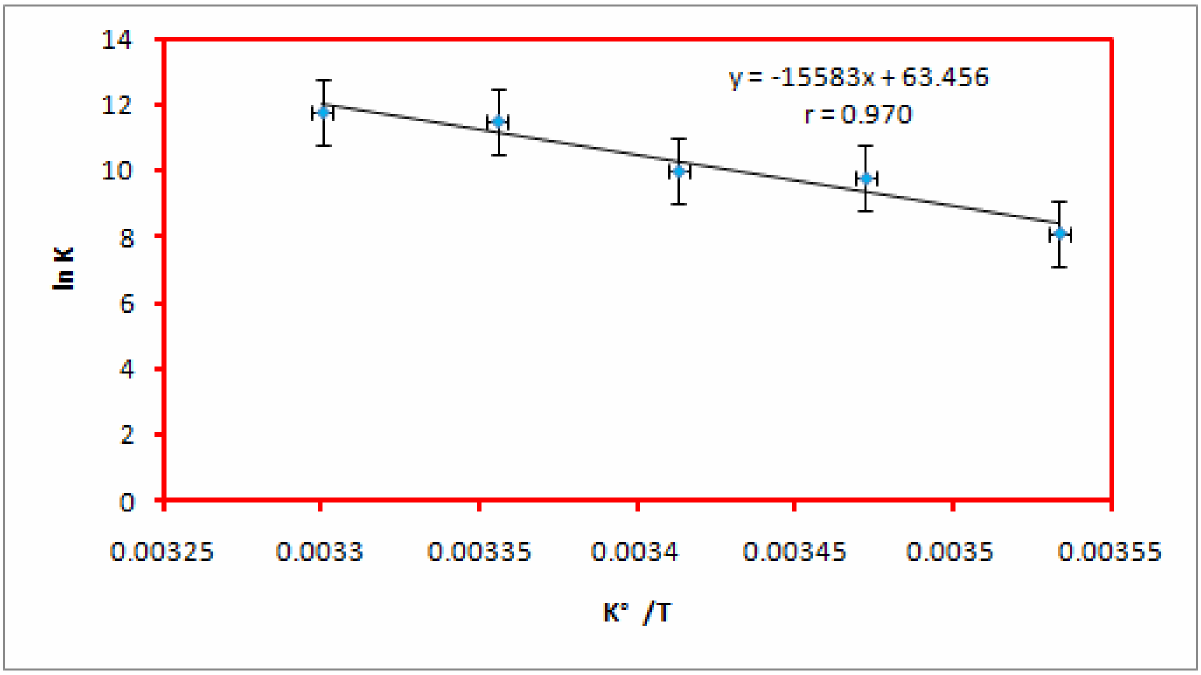


Fig. 4:Van't Hoff Plot of the natural logarithm of the stability constant versus the reciprocal temperature.

The stability constants of 1:1 SNP-DPNA increase with increasing temperature. Corresponding enthalpy changes are endothermic (129.557 kj.mol^{-1}) may be due to ion-ion interaction. The interaction was found to proceed spontaneously due to the negative free energy changes (-48.7713 to -51.7777 KJ.mol^{-1}). On the other hand the negative values of the entropy changes (-0.525 to -0.098 J.mol^{-1}.K^{-1}) indicate the interaction process to decrease the disorder (increase the order).

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