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Spectrophotometric Determination Stability Constant by Classical and Modified Varagas Equations for Procaine Penicillin G using Diazotization Reaction Depending On Stoichiometric Curves

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Abstract : A new sensitive spectrophotometric method has been suggested and developed for the estimation of procaine penicillin G in pure and vial injection. The method is based on the diazotization reaction of benzocain with procaine penicillin G to form a yellow azo dye, that has a molar absorpitivity of $1.732\times10^{+3}$ $L.mol^{-1}.cm^{-1}$, Sandell sensitivity of 0.339 $mg.cm^{-2}$ and limit of detection (LOD) 0.432 $mg.ml^{-1}$ with a maximum absorption at 420 nm and Beer's law obeyed over the concentration range (10-90) $mg.ml^{-1}$. The present work also describes classical equation and a modification of Varagas equation for the calculation of stability constant of Azo dye depending on the theoretical explanation of the stoichiometry, job's and Yoe-Jones'(mole ratio) methods. The results show there is no significant difference in stability constant values between the modified Varagas equation and classical equ.
Keyword: Spectrophotometric, Procaine penicillin G, Stability constant, Modification of Varagas equation, job's and Yoe and Jones' methods.

Introduction

Penicillin are a group of β-lactam antibiotics that have been widely used in human and treatment of animals medicine since their introduction in 1941. Penicillin G (PEGN) there is antibiotic can be classified at natural penicillin[1,2]. And it is the first antibiotic, which was used for the treatment of infections caused by most species of gram positive bacteria. various methods have been reported for the estimation of procaine penicillin G (fig. 1) in the pharmaceutical preparations. These methods are spectrophotometric[3], capillary electrophoresis[4,5], immune chromatography assay[6] and liquid chromatography[7].

The determination of the stability constants and the stoichiometrics of azo dye is an important matter in chemistry. Stability constant and the stoichemistry of azo dye are calculated from the treatment and measurement of analytical data using a many methods. Which can be expressed; job's, yoe and jones' (mole ratio), holme and langmyhr, harvy and manning, bent and French, rose and drago and diehl and lidstrom[8].

Of the above, job's and Yoe and Jones'(mole ratio) methods are the most used because of the assay of their experimental application and simplicity of their theoretical basis.

This papers describes an assay method for procaine penicillin determination in vial injection, the method based on coupling of the procaine penicillin with diazotized benzocain to form stable Azo dye product, this paper also describes two equations for calculating the stability constant of the A

Azo dye: the first one is classical equation, and the second is modified equation based on the principles of the Varagas equation.

Experimental

Equipment

All absorbance measurements and spectral had carried out on VARIAN UV –Visable with 1 cm path quartz cell.

Reagent

All analytical reagent grade chemicals and distilled water were applied thoroughly:

1. Hydrochloric acid (BDH-England) (1M): was prepared by dissolving 16.2 ml of 11.64 M of concentration HCl with distilled water in 200 ml volumetric flask.
2. Procaine penicillin stock standard solution 1000 mg.L^{-1} (3.46×10^{-3} M): was prepared by dissolving 0.1 gm of procaine penicillin in distilled water, then complete to 100 ml distilled water.
3. Procaine penicillin 100 mg.L^{-1}(3.46×10^{-4}M): was prepared by take 10 ml from procaine penicillin stock solution and then completed to the 100 ml in distilled water.
4. Diazonium salt reagent (1.55×10^{-2}M): was prepared by dissolving 0.256 gm benzocain (BDH-England) in 10 ml 1 M HCl, then in another beaker dissolving 0.107 gm $NaNO_2$ in 10 ml distilled water, and placed both beakers in ice bath for 15 min. Then diazonium salt which was obtained by transfer $NaNO_2$ solution by dropper to benzocain solution and shacked well, after 10 min, the volume was completed to 100 ml with distilled water, diazonium salt solution must be still in ice bath and prepared daily, and concentration of $NaNO_2$ was 1.55×10^{-2} M in equimolar solution of benzocain 1.55×10^{-2}M, so in this method we did not need to addition of sulfamic acid to get rid of excess $NaNO_2$.
5. Sodium hydroxide (0.1M): was prepared by dissolving 0.8 gm NaOH in 200 ml distilled water.
6. Procaine penicillin (1.55×10^{-2}M): was prepared by dissolving 0.447 gm in 100 ml distilled water.

Procedure

1. Calibration curve

An aliquot of sample solution containing 5 – 40 ml 0f 100 mg.L^{-1} procaine penicillin was transferred into a set of 20 ml calibration flask. To each flask, 2.5 ml of 0.1 M NaOH solution and 3.5 ml of diazonium salt reagent added then diluted to the mark with distilled water. After 15 min the absorbance of the yellow Azo dye was measured at 420 nm against reagent blank.

2. Method for assay of pharmaceutical preparation

Three procaine penicillin vial injection was diluted with small amount of distilled water then transfer into 100 ml volumetric flask and completed to the mark with the same solvent to obtain 100 mg.L^{-1} of procaine penicillin.

3. Job's method

In this method, an aliquot 0.5 – 7 ml of (1.55×10^{-2}M) procaine penicillin was added to a series of 20 ml volumetric flask, to each flask, 2.3 ml of 0.1M NaOH solution and 7.5 – 1 ml of (1.55×10^{-2} M) diazonium salt reagent were added then diluted to the mark with the distilled water and after 15 min the absorbance was measured at 420 nm. The ratio of mixture of procaine penicillin and diazonium salt reagent shown in table 1.

Table 1: Job method ratio of mixture of procaine penicillin and diazonium salt reagent.

Volume of procaine penicillin(ml).	0.5	1	2	3	4	5	6	7
Volume of diazonium salt reagent(ml).	7.5	7	6	5	4	3	2	1

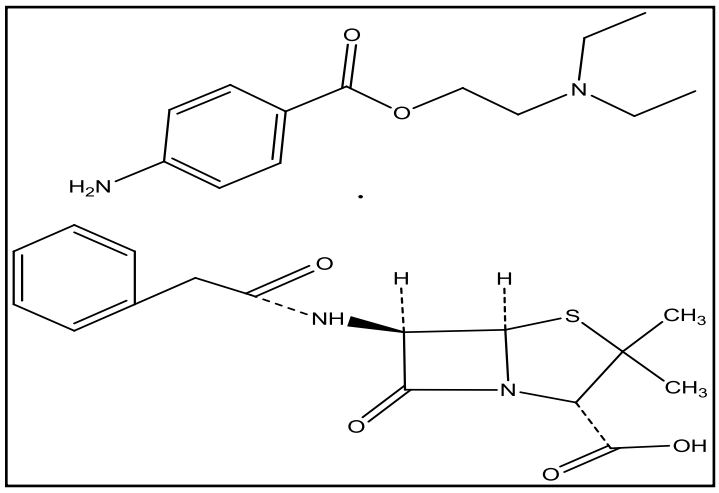


Fig. 1: Structure of Procaine Penicillin G.

4. **Yoe and Jones' method (mole ratio method**)

An a aliquot 1 ml of 1.55 $\times 10^{-2}$ M procaine penicillin solution was added to a series of 20 ml volumetric flask. Added to each flask 2.5 ml 0.1 M NaOH solution and (0.1, 0.25, 0.5, 1, 1.5, 2, 2.5 and 3 ml) of 1.55×10^{-2}M diazonium salt reagent were added then diluted to the mark with the distilled water and after 15 min the absorbance was measured at 420 nm.

Results and Discussion

Effect of hydrochloric acid

Acidic medium is very useful for the diazotization reaction. For that reason the effects of different acids solutions (1M) were studied such as nitric acid HNO_3, hydrochloric acid HCl, acetic acid CH_3COOH and sulfuric acid H_2SO_4. It was found that HCl is the most suitable acidic medium to produce a maximum absorbance under the reaction condition (Fig.2) which shows that maximum absorbance was reached when using 2.5 ml of 1M HCl.

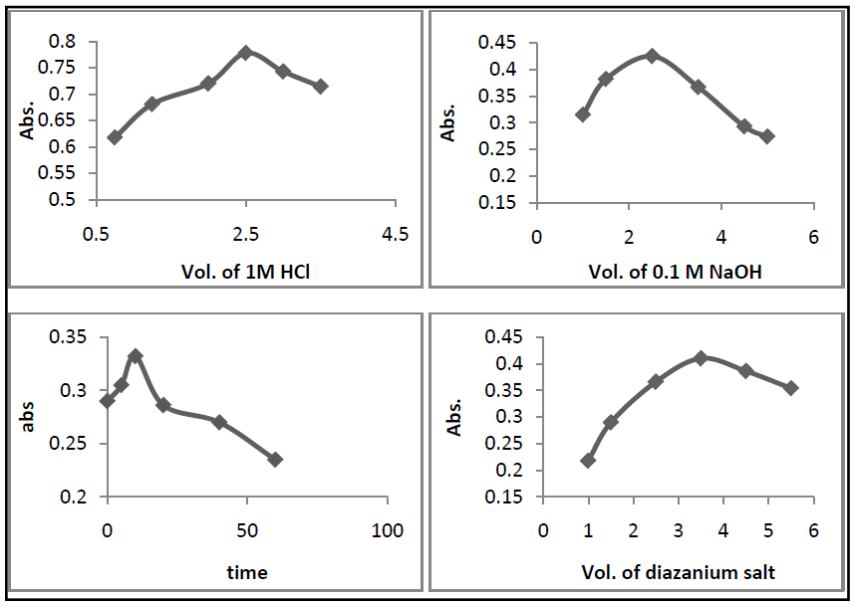


Fig. 2: Optimum condition for determination of Procaine Penicillin G.

Effect of NaOH

The effect of different alkaline medium such as, sodium carbonate Na_2CO_3 , ammonium hydroxide NH_4OH, sodium hydroxide NaOH and potassium hydroxide KOH were studied. It was found that 2.5 ml of 0.1M NaOH solution gave the highest absorbance for the Azo dye shown in (fig. 2)

Effect of reaction time

(Fig. 2) shows that the 10 min was a suitable time was chosen for the analytical procedure.

Effect of diazanium salt reagent

Different volumes of diaznium salt reagent 1.55×10^{-3} M was studed in the range (1-5 ml) with fixing the volumes of HCl and NaOH, the maximum absorbance was obtained with 3.5 ml of diazanium salt reagent shown in (fig. 2)

Order of additions of reagent

Different instructions of addition of reagent were studied and it was found that the Best absorption values was obtain by selecting the following sequence (drug+ NaOH+ diazanium salt) (fig. 3).

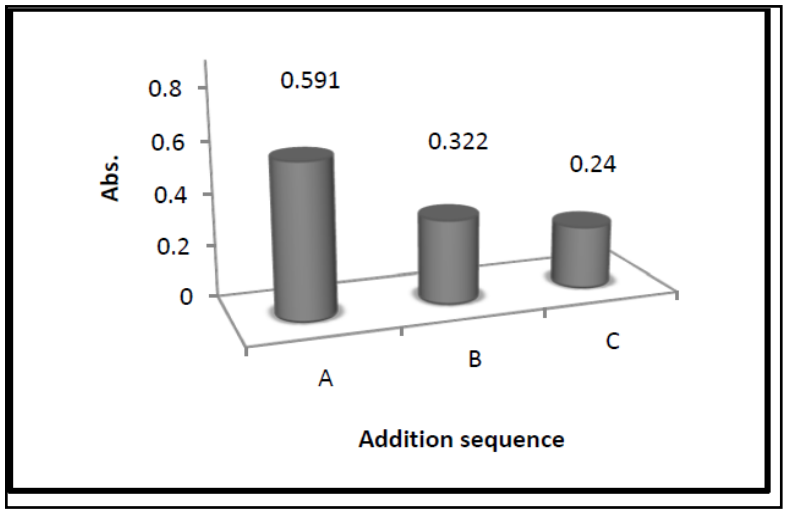


Fig. 3: Order of additions of reagent (A: drug+ NaOH+ diazanium salt), (B: diazanium salt+ NaOH+ drug) and (C: NaOH+ drug+ diazanium salt)

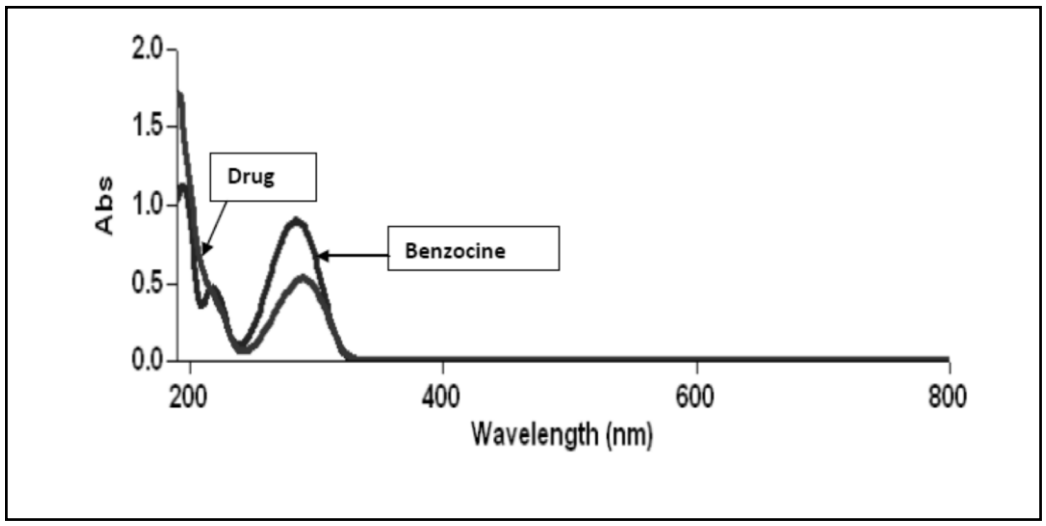


Fig. 4: Absorption spectra of 40 mg.L^{-1} benzocaine and 60 mg.L^{-1} Procaine Penicillin G drug against ethanol as reagent blank

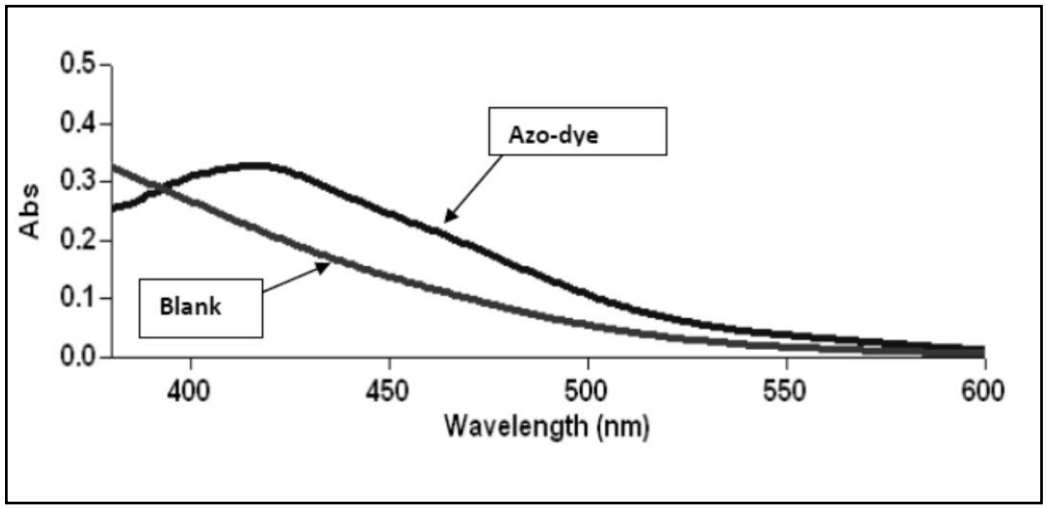
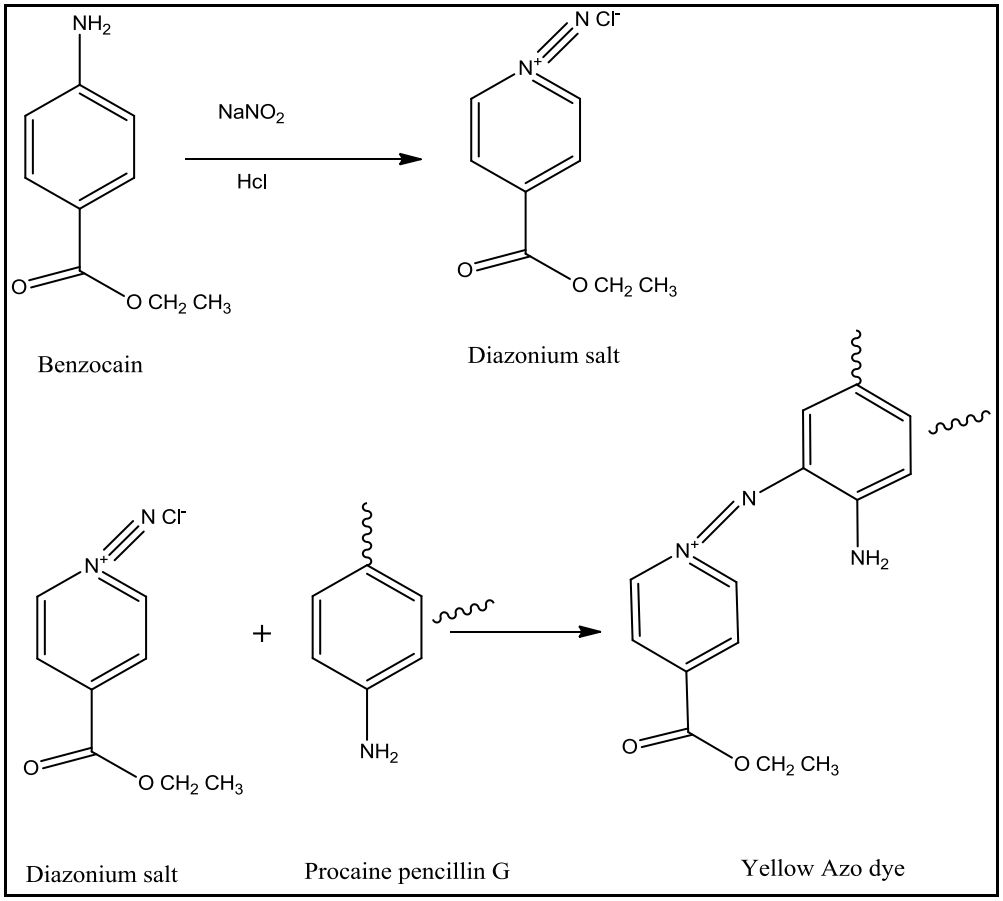


Fig. 5: Absorbance spectra of 45 mg.L^{-1} of Azo dye colored product measured versus blank and The blank versus distilled water .

Absorbance Spectra

This study depend on diazotization reaction of benzocaine with $NaNO_2$ in HCl medium; the formed diazonium salt is then coupled with procaine penicillin, a yellow Azo dye was obtain with maximum absorbance at 420 nm. (fig.4) show the normal spectrum of the procaine penicillin and benzocain were scanned between 190-800 nm in uv- visible spectrophotometer and (fig.5) show the spectrum of the Azo solution measured against reagent blank which has a negligible absorbance this wavelength.

The possible suggest reaction path might be written as follow:



Calibration Curve

A liner calibration curve for procaine penicillin G is obtain (fig.6) , which shows that correlation coefficient of 0.997, slop 0.009, intercept 0.047 and different parameters of the proposed method are a brief in Table 2 .

Table 2: the calibration curve and statistical parameters for estimation of procaine penicillin G

Parameter	**Value**
Linearity $mg.L^{-1}$	10-90
Regression equation	y= 0.009x-0.047
Correlation of determination R^2	0.997
Slop(b) $ml.mg^{-1}$	9×10^{-3}
Intercept (a)	47×10^{-3}
Conf. limit for slop $b\pm t_{sb}$	0.009±50.870
Conf. limit for intercept a $\pm t_{sa}$	0.047±(-4.364)
Molar absopitivity Є $L.mol^{-1}.cm^{-1}$	$1.732\times10^{+3}$
Sandell sensitivity S $mg.cm^{-1}$	0.339
Limit of detection (LOD) $mg.ml^{-1}$	0.432
Limit of quantification(LQD) $mg.ml^{-1}$	1.311

Table 3: analysis of variance of calibration curve.

source	**DF**	**SS**	**MS**	**F**
regression	1	0.590	0.590	2587.780
Residual error	7	0.002	0.0002	
total	8	0.592		

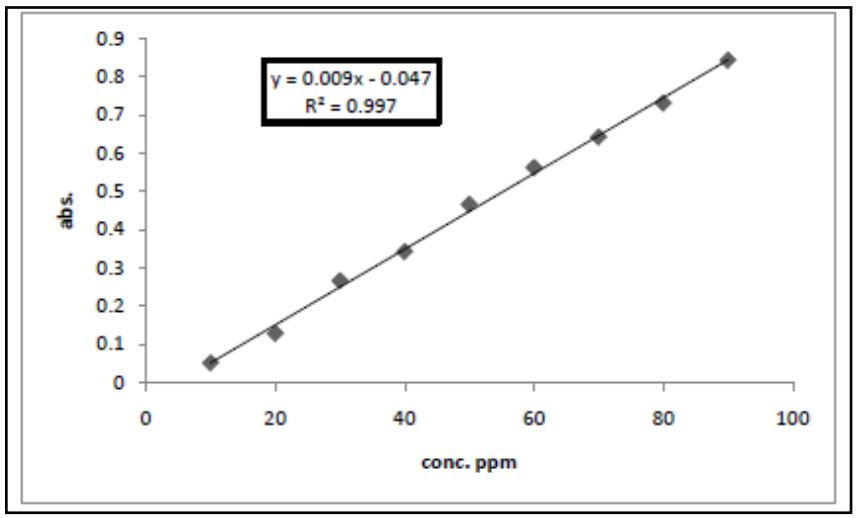


Fig. 6: Calibration graph for estimation of Procaine Penicillin G

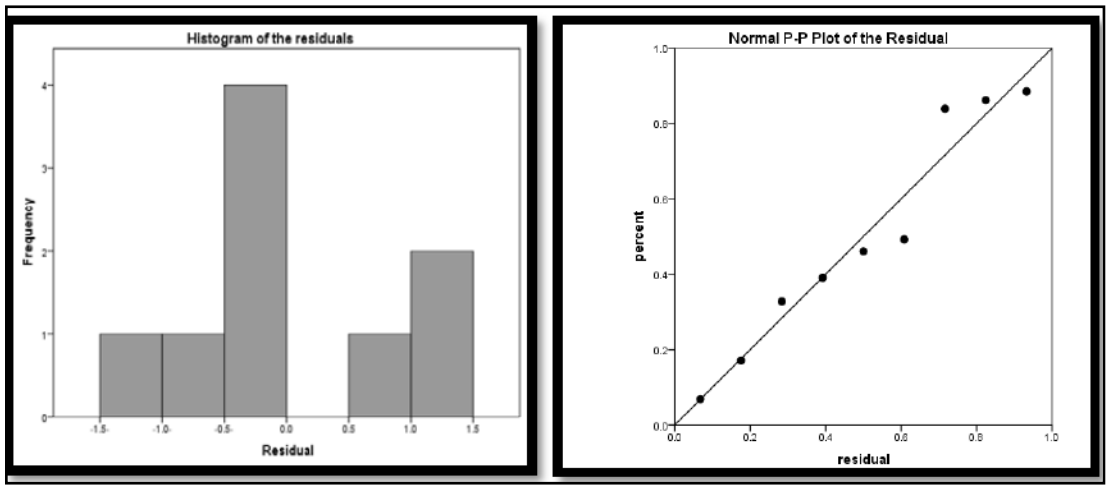


Fig. 7: Statistical analysis for Calibration curve showing residuals versus the amount of Procaine Penicillin G

ANOVA Table 3 and (fig. 7) is the analysis of variance was also prove the linearity of regression equation y= 0.009x-0.047 was statistically valid. This is because of the F ratio for the 1 and 7 degree of freedom is larger than the critical value ($F_{1.7}$=5.59 at 95%CL), indicating that the predication based on the regression line is satisfactory table 3 .

Theoretical and Application Section

I supposed the formation of azo dye reaction as the following:

$dD + rR \leftrightarrow D_dR_r$

where, D = represents the drug, R = is the reagent (primary amine) and (r & d) corresponding stoichiometric coefficients, respectively.

According to classical chemical equilibrium, a stability constant (K) can be expressed[10,11,12] :

$dD + rR \leftrightarrow D_dR_r$

c c 0 initial concentration

dαc rαc (1-α)c equilibrium concentration

$$K = \frac{[DdRr]}{[D]^d\,[R]^r}$$

$$K = \frac{(1-\alpha)C}{(d\alpha c)^d(r\alpha c)^r} = \frac{(1-\alpha)c}{d^d\, r^r\, (\alpha c)^{d+r}}$$

$$K = \frac{(1-\alpha)C}{d^d\, r^r\, (\alpha)^{d+r}\, (c)^{d+r}} \quad \ldots..(1)$$

Job's Method

The absorbance measured (Abs.) are drawn against molar fractions (x) of the reagent,

where $x = \frac{CR}{C}$ and C = CR + CD is a constant.

The value of molar fraction is obtain from a curve with a maximum absorbance, which can be expressed the stoichiometric molar fraction (SMF) by the following:

$$SMF = \frac{r}{r+d} \quad \ldots(2)$$

Which indicates the stoichiometry of Azo dye (r : d).

The bend of the empirical lines is because of the fact that the Azo dye formation reaction is not quantitative (the Azo dye in partial dissociation state), so the dye stability constant can be calculate from deviations of the theoretical straight lines.

The degree of the dissociation of the Azo dye (α) is used to calculate the stability constant (K), which can be expressed as the following:

$A\alpha = A_0 - A_{max}$ ….(3)

Where A_{max} = is the a maximum absorbance at Azo dye method curve that refer to the maximum quantity of the Azo dye which is created with a degree of dissociation.

A_0 = is a theoretical absorbance value which is estimated by intersect point of the theoretical straight lines and Aα is refer to absorbance of the part of dissociated concentration of Azo dye that represents the difference between A_0 and A_{max}

$$\alpha = \frac{A\alpha}{\epsilon\, bc} \ldots.(4)$$

where C = is a concentration of drug at stoichiometry point.

Є = is molar absorptivity and b = is a cell thickness (1cm).

By substituting the value of α from equation 4 in equation 1, it is possible to obtain a stability constant K .

I was running a whole application for the estimation of stability constant of reaction between benzocain and diazotized procaine penicillin the value of job method give the stoichiometry (1:1), the results were shown in (fig. 8) and Table 4.

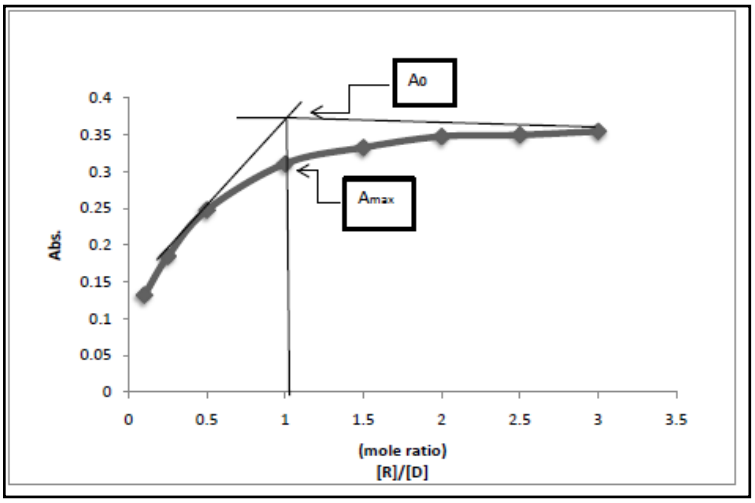


Fig. 8: Job's plot for diazotized benzocaine coupled with Procaine Penicillin G

Table 4: stability constant obtained from the purposed method.

	A_{max}	A_0	Aα	Є	$\alpha^{*}\times10^{-4}$	$\alpha^{**}\times10^{-7}$	C $mol.l^{-1}$	Classical equation		Modified Varagas equation	
								k	Ln k	k	Ln k
Job method	0.51	0.62	0.11	1732	635	635	10^{-3}	232178	12.3552	234125	12.3636
Yoe and jones method	0.31	0.37	0.06	1732	346	346	10^{-3}	804465	13.5979	804500	13.5979

α* = degree of dissociation calculate by classical equation = $\frac{A \propto}{\epsilon bc}$ and α** = degree of dissociation calculate by modified Varagas equation = $(A \propto) \notin$.

Yoe and Jones' Method (Mole ratio method)

In this method the absorbance (Abs.) measured are drawn against molar ratio (r), where r = $\frac{Cr}{Cd}$, and a curve is obtained.

The stoichiometry of the Azo dye is calculated from the point where this curve changes its slope and the stoichiometric molar fraction ratio (SMR) can be expressed:

SMF = $\frac{r}{d}$ …..(5)

Which indicates the stoichiometry of the Azo dye (r : d).

Similarly to jobs method, it is important to know the degree of the dissociation of Azo dye (α), which is obtained by the equations 3 and 4 where, A_{max} = is a maximum absorbance at Azo dye method curve that refer to the maximum quantity of the Azo dye that is formed with a degree of dissociation α

A_0 = is a theoretical value which is calculated by interest point of the theoretical straight lines..

and Aα = is refer to the part of dissociated concentration.

The parameters C, Є and b which have the same defined in the job method.

By substituting α from equation 4 in equation 1; it is possible to get a stability constant K. the reaction between benzocain and procaine penicillin by the yoe and jones method is give the stoichiometry (1:1), the results were shown in fig. 9 and table 4.

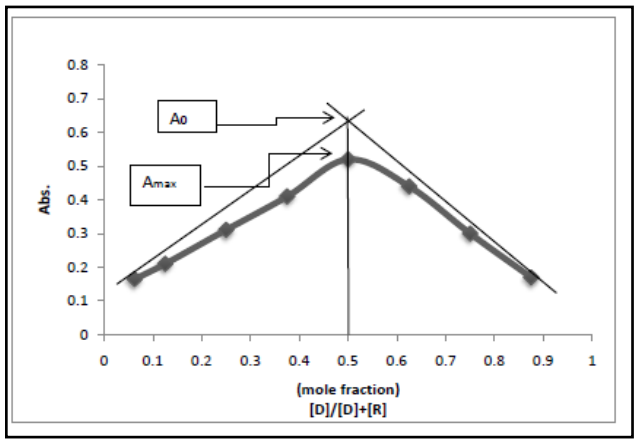


Fig. 9: Yoe and Jones' plot for diazotized benzocaine coupled with Procaine Penicillin G

Modified Varagas Equation

The stability consant equation K was proposed by Varagas as a suitable equation for fixing the stability constant of complex[9]. In this work, we have executed a whole application for the estimation of Varagas equation for stability constant of diazotized between benzocain and procaine penicillin.

The modified Varagas equation for stability constant is given by the expression:

$$K = \frac{c - (\alpha)}{r^r(\alpha)^{r+1}}$$

$$K = \frac{c - ((A\propto)\notin)}{r^r((A\propto)\notin)^{r+1}}$$

$$K = \frac{c - ((A0 - Amax)\notin)}{r^r\left((A0 - Amax)\notin\right)^{r+1}} \quad \ldots..(6)$$

Where α = degree of the dissociation of the Azo dye.

C = procaine penicillin drug concentration.

r = no. of benzocain reagent corresponding stoichiometric coefficients.

Є = molar absorptivity .

Aα = is refer to the part of dissociated concentration

A_0 = is the theoretical absorbance value which is estimation by intersect point of the theoretical straight lines.

And A_{max} = is a maximum absorbance at Azo dye method curve that refer to the maximum quantity of the formation Azo dye.

The modified Varagas equation for stability constant was calculated from reaction between procaine penicillin and benzocain using job method and yoe and Jones' method and the results shown in Table 4.

Tabe 5 showed the Gibbis free energy of the Azo dye reaction (△G) was calculated using the following equation[13]:

△G = -RT lnK

Where R = 1.987 cal / mol.k (gas constant) and T = 298.15 k (room temperature) .

The value of △G is found in table 5 which indicates that the reaction between benzocain and procaine penicillin is spontaneous.

Table 5: the Gibbis free energy of the purposed method.

	△G cal / mol.k	
	Classical equation	**Modified varagas equation**
Job's method	-7319.510	-7324.493
Yoe and jones' method	-8055.722	-8055.722

Table 6: the precision of stability constant for purposed methods.

	method	**mean**	**SD**	**RSD%**	**RSE%**
Classical equation	Job's	232160.333	295.950	0.127	0.064
	Yoe and jones'	803691.000	930.510	0.115	0.066
Varagas equation	Job's	234144.000	232.084	0.099	0.057
	Yoe and jones'	804301.340	644.393	0.080	0.046

The precision of the stability constant is calculated at three time using classical equation and modified Varagas equation. The result of mean , standard deviation (SD), relative standard deviation (RSD%) and relative standard error (RSE%) are illustrated in table indicate that the two equations is satisfactory.

Accuracy and Precission

At three different concentration of pure procaine penicillin was estimated, the value of relative error % , recovery % and average of recovery % are summarized in Table 7.

Table 7: Accuracy and precision for diazotized benzocaine coupled with procaine penicillin.

A mount of procaine penicillin (µg. ml^{-1})		**E.%**	**Rec*.%**	**Average of Rec.%**
taken	found			
25	24.660	-1.352	98.648	99.794
45	45.128	+0.284	100.284	
65	65.293	+0.450	100.450	

*Average of three determination, E% = $\frac{taken - found}{taken} \times 100$ and Rec.% =E.%+100

Table 8: Application of the suggest for determination of procaine penicillin G in vial injection.

Pharmaceutical vial injection	**Conc. of procaine penicillin mg.L^{-1}**		**E.%**	**Rec.%**	**Average of Rec.%**
Procaine penicillin vial Manufactured by xier kangtia pharmaceutical Co. Ltd. China	taken	found			
	15	15.100	+0.666	100.666	100.313
	35	34.930	-0.200	99.800	
	55	55.261	+0.474	100.474	

Application

One type of procaine penicillin vial injection have been analyzed using purposed method. three different concentration of procaine penicillin vial injection were analyzed five time to obtain statistical parameter are shown in Table 8 .

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