

## INTERACTION OF PYRIDOXAL WITH URANYL IONS IN AQUEOUS SOLUTION

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**Abstract** . The dissociation constants of pyridoxal hydrochloride were determined spectrophotometrically as well as potentiometrically at ionic strength of 0.50 M KCL and at 30°C. Interaction of uranyl ions with pyridoxal was studied spectrophotometrically. From the results it was drawn that only one complex was formed in solution in the pH range 3.00 - 4.70 with pK value of 7.71.

## INTRODUCTION

The vitamin B<sub>6</sub> compounds, pyridoxol, pyridoxal, and pyridoxamine undergo metal ion catalysed reactions with α - amino-acids, involving Schiff base intermediates, whose metal chelates have been studied by Eichorn and Davis (1954), Jencks and Cordes (1962), Martell (1962), Cennamo (1967) and Matsushima and Martell (1967). They are considered as model reactions for enzymic analogues. On the other hand, less research has been carried out on the metal ion complexes of pyridoxal, pyridoxol, and pyridoxamine. In an attempt to study the interaction of metal ions with biological molecules, it was decided to study the interaction of vitamin B<sub>6</sub> with uranium metal ions in vitro due to its hazardous effects on metabolic systems.

The dissociation constants of pyridoxal hydrochloride were determined potentiometrically as well as spectrophotometrically. The pK values so obtained were compared with those reported by Williams and Neilands (1954), Metzler and Snell (1955), and Leussing and Hug (1966). The interaction of uranyl ions with pyridoxal was followed spectrophotometrically and the instability constant of complex reaction was calculated.

## EXPERIMENTAL

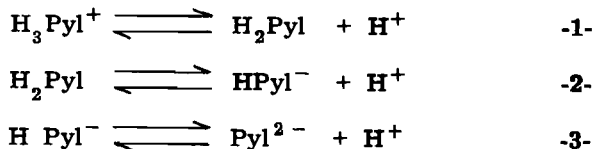
Pyridoxal hydrochloride (A. R. grade CID. Co. Cairo) was dried in vacuo over anhydrous phosphorus pentoxide. The freshly prepared solution for each series was standardised potentiometrically with standard carbonate-free sod-

ium hydroxide solution. The strength of the stock solution of the uranyl ions was checked gravimetrically as oxinate.

The spectrophotometric measurements were recorded using Unicam S.P. 800 and Carl Zeiss VSU-2P. The pH measurements were carried out on Radiometer pH meter type 28 provided with glass and calomel electrodes accurate to ± 0.05 pH units. The pH meter and the spectrophotometers were calibrated by standard buffers and potassium chromate solution, respectively. Both measurements were taken at (30 ± 0.1) °C and an ionic strength of 0.50 M KCl in pure nitrogen atmosphere.

## RESULTS AND DISCUSSION

Fig. 1 shows the deprotonation curve of pyridoxal hydrochloride (H<sub>3</sub>Pyl . Cl). Three equilibria are involved in aqueous solution:



It is impossible to calculate more than two dissociation constants for the above equilibria from potentiometric data since H Pyl<sup>-</sup> has a very weak acid character. The first two pK values were calculated as previously described by Osman *et al.* (1971) using the iterative methods.

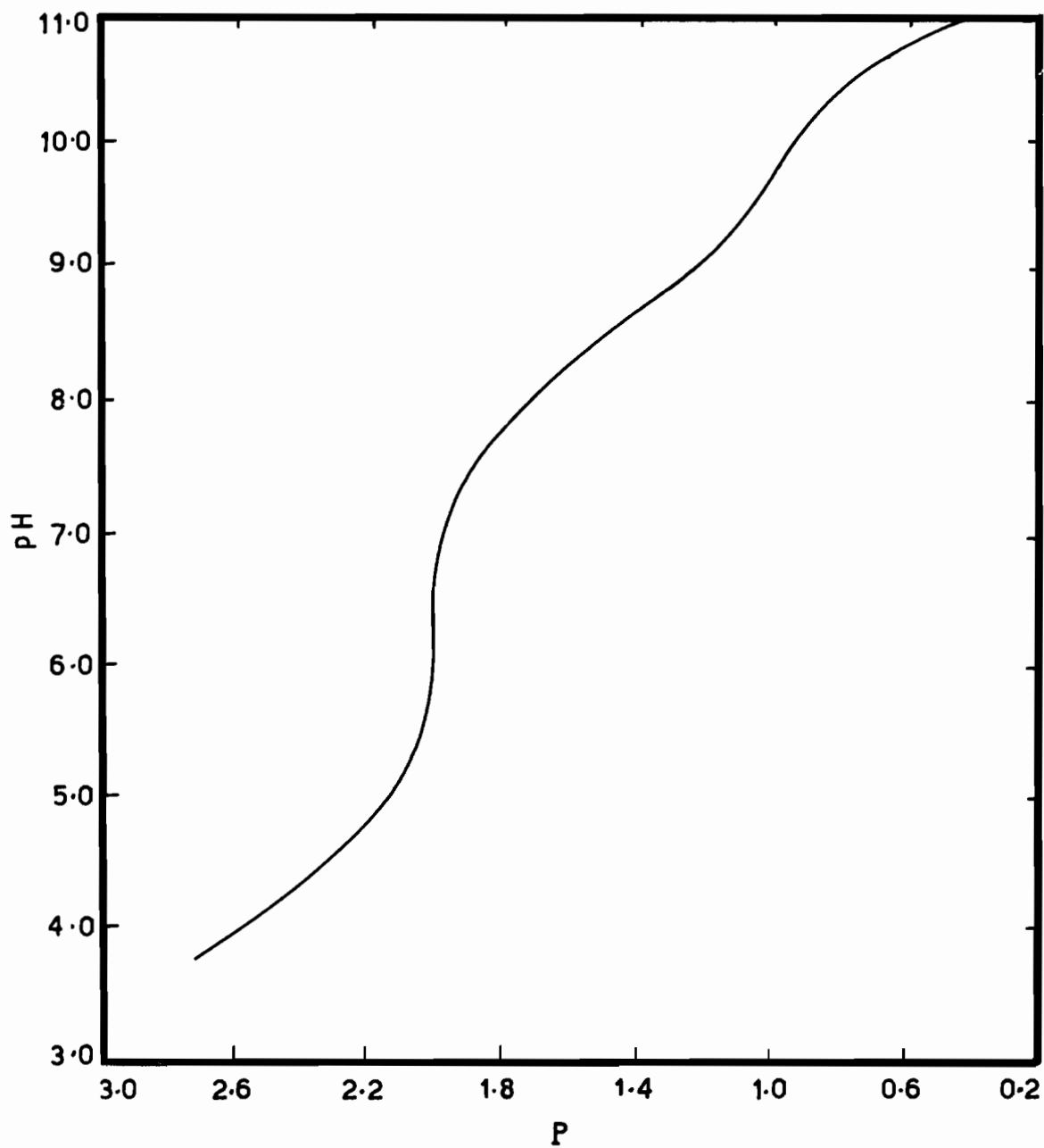


Fig. 1. Deprotonation curve of pyridoxal hydrochloride ( $1.0 \times 10^{-3} \text{ M}$ ) at  $\mu = 0.50 \text{ M KCL}$  and  $T = 30^\circ \text{ C}$ .

$$pK_1 = pH + \log \left( \frac{P - 2}{3 - P} \right) \quad -4-$$

$$pK_2 = pH + \log \left( \frac{P - 2}{2 - P} \right) \quad -5-$$

where

$$K_1 = \frac{[H^+] [H_2 \text{ Pyl}]}{[H_3 \text{ Pyl}^+]}$$

$$K_2 = \frac{[H^+] [H \text{ Pyl}^-]}{[H_2 \text{ Pyl}]}$$

P is the protonation degree which can be calculated as follows:

$$P = \frac{3L_T - C_{\text{NaOH}} - [H^+] + [OH^-]}{L_T}$$

where  $L_T$  is the total molar concentration of  $H_3 \text{ Pyl Cl}$  and  $C_{\text{NaOH}}$  is the calculated molar concentration of NaOH (0.10N), after each addition. Table (II) shows the values of pK's calculated according to equations 4 & 5.

Figure 2 shows the spectra of  $H_3 \text{ Pyl Cl}$  at different pH's. The spectrum at pH's > 3 shows three bands at 210 nm, 224 nm and 288 nm. In the pH range 3.0 - 7.0 another band is recorded at 254 nm. Further, the bands at 210 nm and 288 nm are red shifted to 220 nm and 317 nm, respectively. In the pH range 7.0 - 10.0 a new band appears at 392 nm, and the bands at 254 nm and 317 nm are blue shifted to 238 nm and 298 nm, respectively. On increasing the pH to > 10.0 the 220 nm band is slightly blue shifted with an increase in intensity. The bands at 210 nm and 288 nm are attributed to  $\pi-\pi^*$  transition as compared with those of pyridine, while the band at 224 nm is probably due to  $n \rightarrow \pi^*$  transition. The 392 nm band is of the charge transfer type<sup>†</sup>

Fig. 3 shows that possibly three stepwise equilibria exist. Two methods were used to calculate dissociation constants from spectrophotometric measurements. The first method employed the following equation :

$$\log \left( \frac{As_\eta - As_i}{As_i - As_{(\eta-m)}} \right) = pK_\eta - m \text{ pH}_i \quad -6-$$

where  $As_\eta$  and  $As_{\eta-m}$  are the absorbances of both  $H_\eta A$  and  $H_{\eta-m} A$  species respectively.  $As_i$  is the absorbance at a given  $\text{pH}_i$ , m is the number of dissociable hydrogen ion, and K is the dissociation constant.

The plot of  $\log \left( \frac{As_\eta - As_i}{As_i - As_{(\eta-m)}} \right)$  versus  $\text{pH}_i$  gives a straight line of slope-m and an intercept equal to  $pK_\eta$ . The slope was found to be equal to -1 in the pH range 3.0 - 5.0, 7.5 - 9.5 and 12.0 - 13.5. The second method utilizes the following equation derived by Coll  ter (1960).

$$K_\eta = \frac{[H^+]_2 - M [H^+]_3}{M - 1} \quad -7-$$

where  $M = \frac{As_3 - As_1}{As_2 - As_1} \cdot \frac{[H^+]_1 - [H^+]_2}{[H^+]_1 - [H^+]_3}$

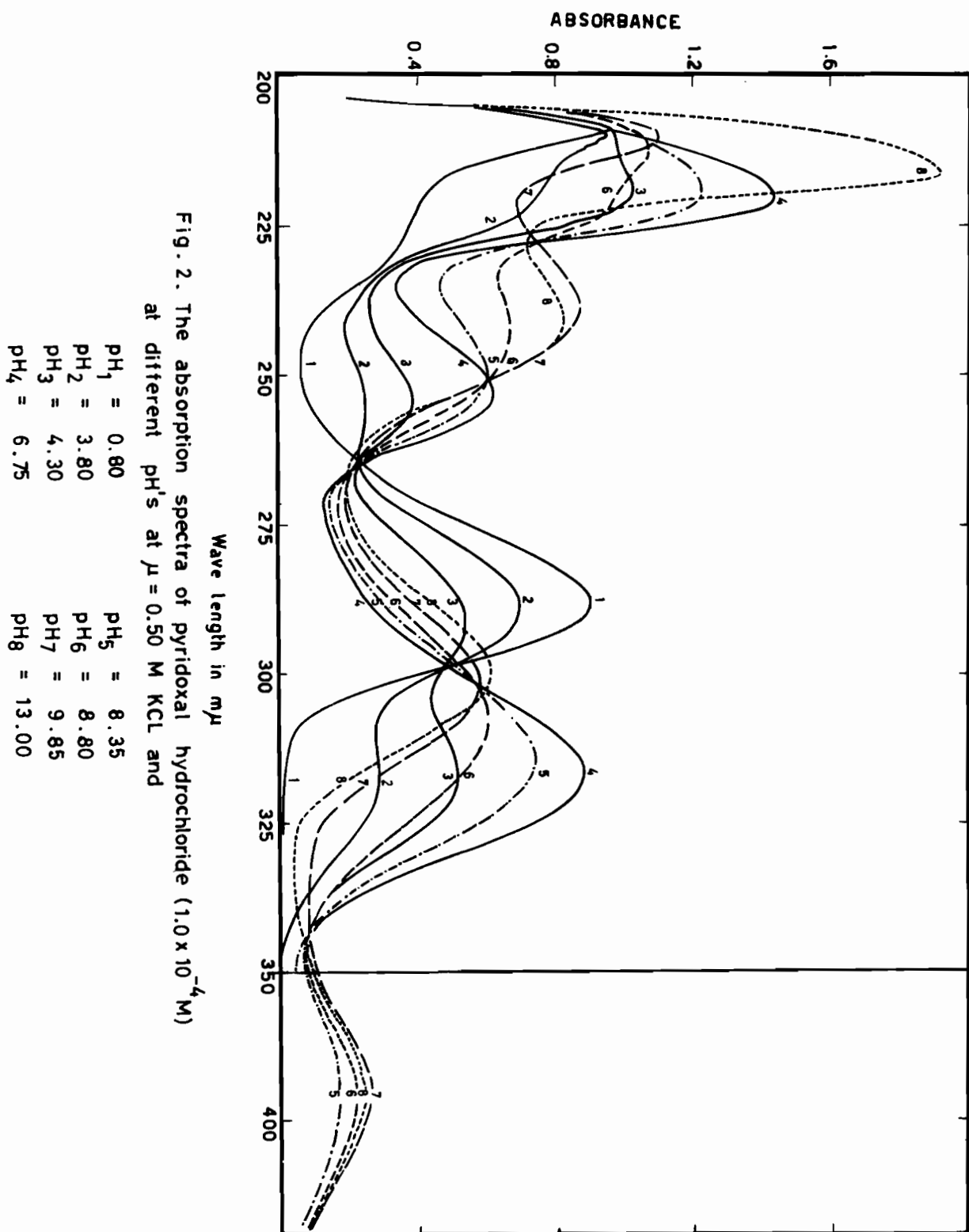
The molar absorptivities of both species are as follows

$$\epsilon_{\eta-1} = \frac{M As_2 - As_3}{M - I} \cdot \frac{I}{A} \quad -8-$$

$$\epsilon_\eta = \epsilon_{\eta-1} + \frac{[H^+]_2 - [H^+]_3}{[H^+]_2 - [H^+]_3} \cdot \frac{As_3 - \epsilon_{\eta-1} A}{A} \quad -9-$$

Table I depicts the pK values calculated by equations 6 and 7 and the molar absorptivities of different species as obtained according to equations 8 & 9. The pK values obtained previously by Williams and Neilands (1954), Metzler and Snell (1955) and Leussing and Hug (1966) were compared with the present values in Table II.

<sup>†</sup> A current research is undertaken in this Laboratory.



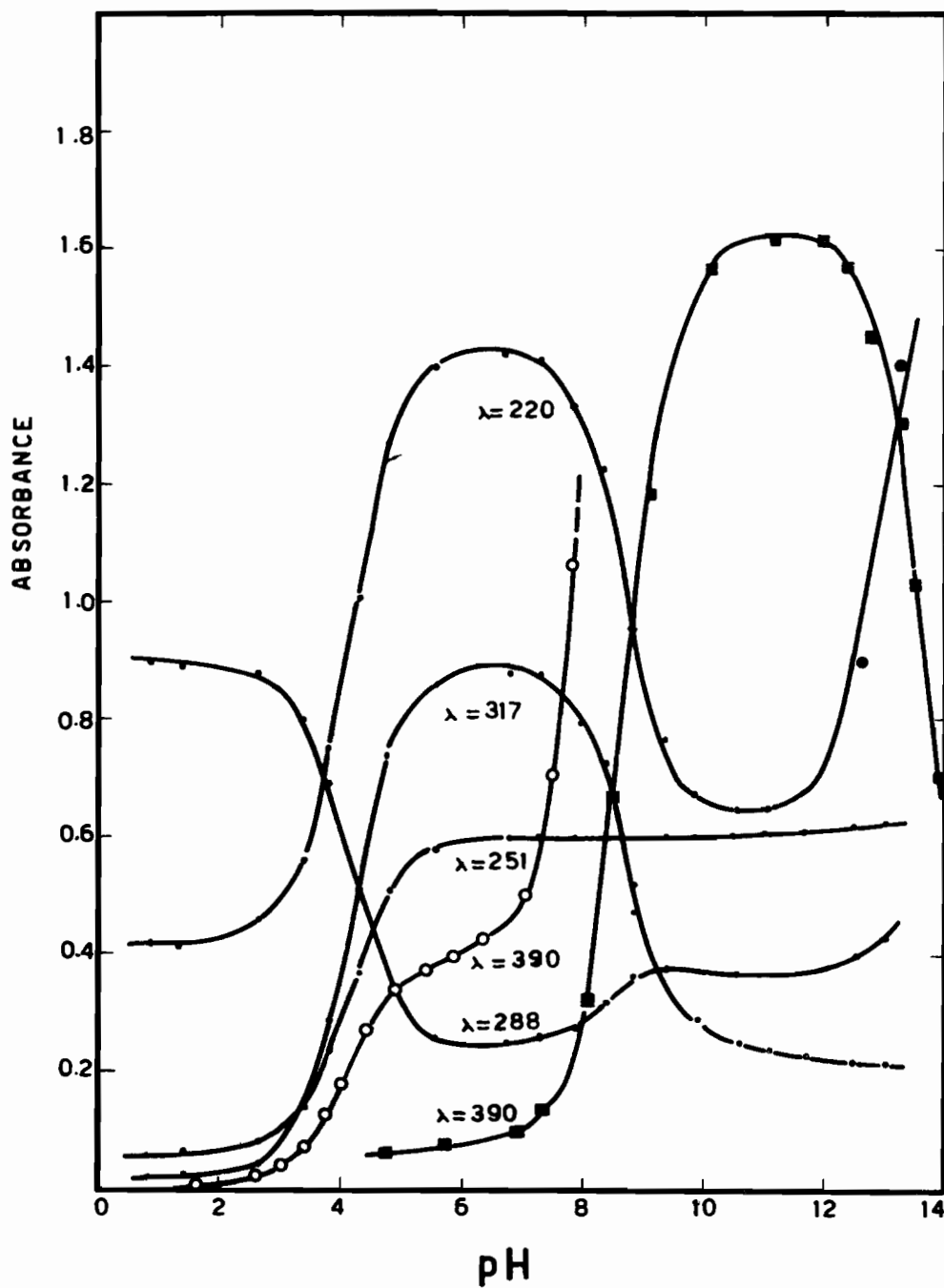


Fig. 3. Effect of pH on the absorbance of pyridoxal hydrochloride at different wavelengths,

for — · — · —  $1 \times 10^{-4}$  M  
 — ■ — ■ —  $1 \times 10^{-3}$  M  
 — ○ — ○ —  $5 \times 10^{-3}$  M

TABLE I: The pK values of (H<sub>3</sub>Pyl.Cl) using equations 6 and 7 and the molar absorptivities of different species using equations 8 and 9

Reaction	pK	Molar absorptivity
$H_3Pyl^+ = H_2Pyl + H^+$	4.15 ± 0.11 (6)	(4.06 ± 0.68) .10 <sup>3</sup> (220 nm) (8)
		(5.41 ± 1.11) .10 <sup>2</sup> (351 nm) (8)
	4.15 ± 0.06 (7)	(8.97 ± 0.25) .10 <sup>3</sup> (288 nm) (8)
		(1.80 ± 0.91) .10 <sup>2</sup> (317 nm) (8)
		0.00 (390 nm) (8)
$H_2Pyl = HPyl^- + H^+$	8.68 ± 0.09 (7)	(1.46 ± 0.04) .10 <sup>4</sup> (220 nm) (9)
		(5.87 ± 0.18) .10 <sup>3</sup> (251 nm) (9)
	8.67 ± 0.08 (7)	(2.68 ± 0.46) .10 <sup>3</sup> (288 nm) (9)
		(9.15 ± 0.32) .10 <sup>3</sup> (317 nm) (9)
		78 ± 8 (390 nm) (9)
$H Pyl^- = Pyl^{2-} + H^+$	—————	(5.83 ± 0.04) .10 <sup>3</sup> (220 nm) (9)
		(2.21 ± 0.35) .10 <sup>3</sup> (317 nm) (9)
		(1.55 ± 0.10) .10 <sup>3</sup> (390 nm) (9)
	13.05 ± 0.27 (7)	(1.42 ± 0.06) .10 <sup>3</sup> (220 nm) (9)
		2.50 . 10 <sup>2</sup> (390 nm) (9)

The pK's are the average of 5 measurements.

The molar absorptivities are the average of at least 3 measurements.

TABLE II : pK values of H<sub>3</sub>Pyl.Cl calculated by potentiometric and spectrophotometric methods

Reaction	pK				
	By potentiometric method		By spectrophotometric methods		
	μ = 0.50		μ = 0.50	μ variable	
	This work	Leussing & Hug	This work	Williams and Nellands	Snell
$H_3 Pyl^+ \rightleftharpoons H^+ + H_2 Pyl$	4.16 ± 0.01 (eq.4)	4.25	4.15	4.23	4.20
$H_2 Pyl^+ \rightleftharpoons H^+ + HPyl^-$	8.51 ± 0.02 (eq.5)	8.54	8.67	8.70	8.66
$HPyl^- \rightleftharpoons H^+ + Pyl^{2-}$	—————	—————	13.05	—————	13.00

Figure 4 shows the variation of absorbance with pH for the uranium VI-pyridoxal system at 360 nm. Beer's law is obeyed through the whole pH range when the ligand concentration is at least ten times the concentration of the metal ions.

In a given pH range the following equalities are valid if Beer's law is obeyed.

$$B = \sum_0^m B(H_\eta A)_i \quad -10-$$

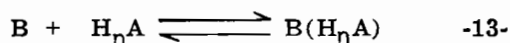
$$A = \sum_0^m H_j A \quad -11-$$

$$As_j = \sum_0^m \ell \epsilon_j B(H_\eta A)_i + \sum_0^m \ell \epsilon_j H_j A \quad -12-$$

where B is the total metal ion concentration, A the total ligand concentration, B(H<sub>η</sub>A)<sub>i</sub> is the

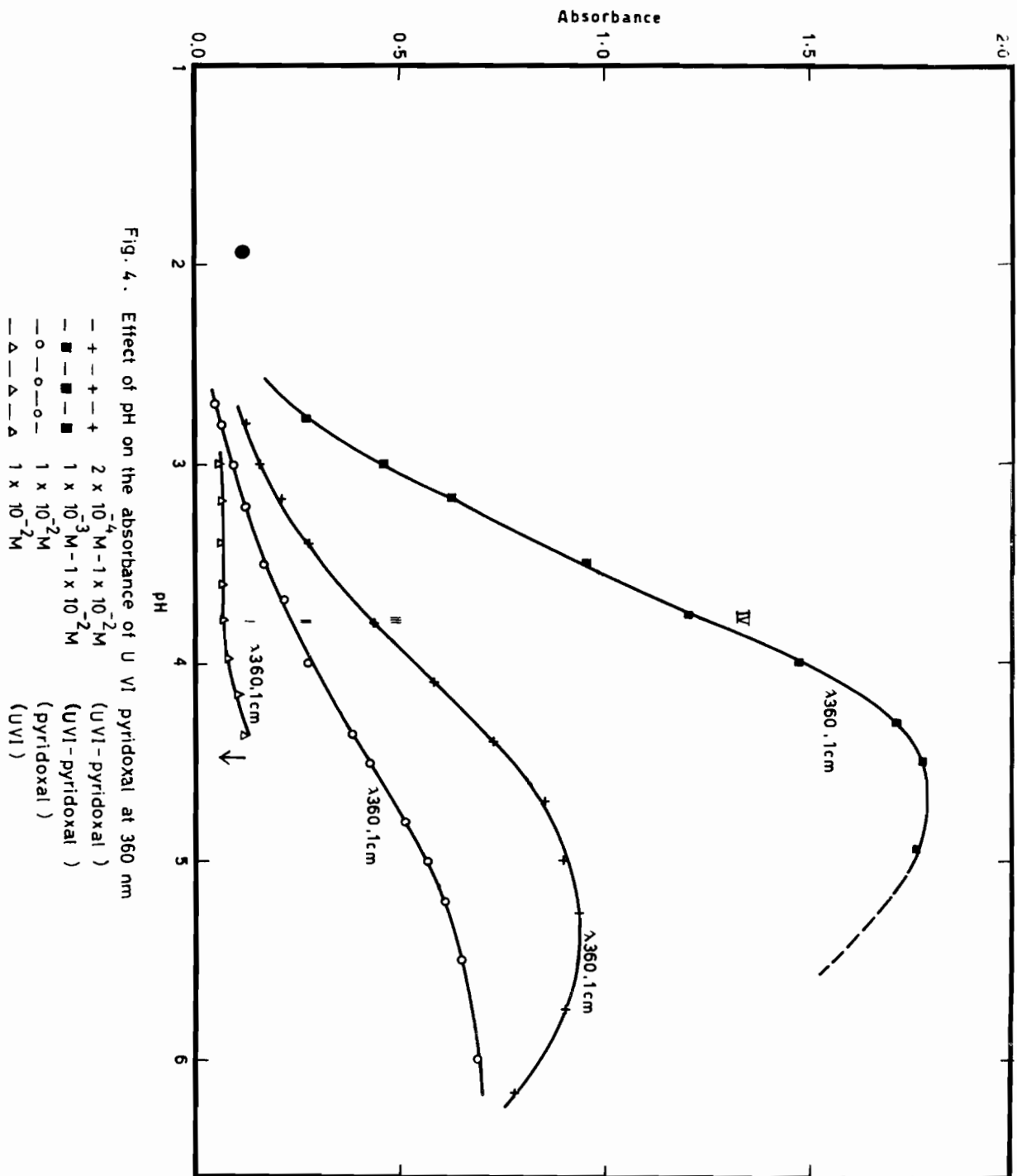
equilibrium concentration of complex species, H<sub>j</sub>A is the equilibrium concentration of the ligand species, As<sub>j</sub> is the absorbance at a given wavelength and pH and ε<sub>i</sub>, ε<sub>j</sub> are the molar absorptivities of different metal and ligand forms, respectively.

In the range of free ligand concentration where the metal ions and the first complex species are in equilibrium:



From equations 10, 11, 12 and 13 the following equation can be derived:

$$\frac{X_i AB}{\Delta As_i} + \frac{X_i As_i}{\ell^2 \Delta \epsilon_1^2} = \frac{K_i}{\ell \Delta \epsilon_1} + \frac{X_i (A+B)}{\ell \Delta \epsilon_1} \quad -14-$$



$$\text{where } X_1 = \frac{K_1 K_2 \dots K_n [H]^{n-1}}{[H]^n + K_1 [H]^{n-1} + \dots + K_1 K_2 \dots K_n}$$

$$\Delta As_1 = As_1 - \left( \ell \epsilon_o B + \frac{\ell AX_1}{Y_1} \right)$$

$$Y_1 = \frac{K K [H]}{\epsilon'_o [H]^n + \epsilon'_1 K_1 [H]^{n-1} + \dots + \epsilon'_n K_1 K_2 \dots K_n}$$

$$\Delta \epsilon_1 = \epsilon_1 - \left( \epsilon_o + \frac{X}{Y} \right), \text{ and } K_1 = \frac{[H_\eta A] [B]}{[B(H_\eta A)]}$$

If  $\epsilon_1$  is larger than  $(\epsilon_o + \frac{X}{Y})$ , then equation

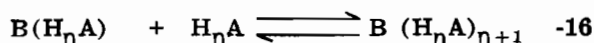
14 can be reduced to equation 15:

$$\frac{X_{1AB}}{\Delta As_1} = \frac{K_1}{\ell \epsilon_1} + \frac{X_1 (A+B)}{\ell \epsilon_1} \quad -15-$$

The plot of  $\frac{X_{1AB}}{\Delta As_1}$  versus  $X_1 (A+B)$  should give a straight line with slope  $\frac{1}{\ell \epsilon_1}$  and intercept  $\frac{K_1}{\ell \epsilon_1}$ . If  $\epsilon_1$  is not sufficiently larger than  $(\epsilon_o + \frac{X}{Y})$  as to render the latter negligible, the

constants obtained may be refined by successive approximation. Applying equation 15 satisfies UVI - pyridoxal systems in the pH range 3.0-4.7, when the total ligand concentration is at least ten times the total metal concentration where single negatively charged species,  $HPyI^-$ , is the complexing species. The pK so obtained is  $7.71 \pm 0.05$ .

In the range of free ligand concentration where the  $n^{\text{th}}$  complex is in equilibrium with the  $(n+1)^{\text{th}}$  complex.



An equation similar to equation 15 can be derived:

$$\frac{XB(A-B)}{\Delta As_1} = \frac{XA}{\ell \Delta \epsilon_{n+1}} + \frac{K_{n+1}}{\ell \Delta \epsilon_{n+1}} \quad -17-$$

$$\text{where } \Delta As_1 = As_1 - \left( \ell B \left( \epsilon_1 - \frac{X}{Y} \right) + \frac{\ell AX}{Y} \right)$$

$$\Delta \epsilon_{n+1} = \epsilon_{n+1} - \left( \frac{X}{Y} + \epsilon_n \right)$$

$$\text{and } K_{n+1} = \frac{[B(H_n A)] [H_n A]}{[B(H_{n+1} A)]}$$

Applying equation 17 on  $UO_2^{2+}$  - pyridoxal system does not give further indication of complex formation at higher pH' values.

The composition of U VI complex with pyridoxal can be further confirmed by using the following equation derived by El-Ezaby (1967)

$$\log \left\{ \frac{[H_n A] (\epsilon_n - \epsilon_{n-1})}{(As_1/\ell) - \epsilon_{n-1} B} \right\} = \log K_n - n \log \left\{ C_B \frac{(As_1/\ell) - \epsilon_{n-1} B}{\epsilon_n - \epsilon_{n-1}} \right\} \quad -18-$$

where  $\epsilon_n$  and  $\epsilon_{n-1}$  are the molar absorptivities of the  $n^{\text{th}}$  and  $(n-1)^{\text{th}}$  species, respectively, and  $K_n$  is the instability constant.

If  $n$  is assumed to be unity and the molar absorptivities are previously known then the plot of:

$$\log \left\{ \frac{[H_n A] (\epsilon_n - \epsilon_{n-1})}{(As_1/\ell) - \epsilon_{n-1} B} \right\} \text{ Vs. } \log \left\{ B - \frac{As_1/\ell - \epsilon_{n-1} B}{\epsilon_n - \epsilon_{n-1}} \right\}$$

should give a straight line. The slope is found to be nearly equal to one indicating the presence of 1:1 complex in case of U VI with pyridoxal.

From these results it was concluded that complex formation of U VI with pyridoxal is different from that with pyridoxol previously reported by Osman *et al.* (1971). U VI forms three soluble complexes with pyridoxol and only one with pyridoxal.



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دراسة تفاعل البيريدوكسال مع  
أيونات اليورانيل في المحاليل المائية

محمد سمير العزبي

قسم الكيمياء بجامعة الكويت

خلاصة

تتناول هذه الدراسة تعيين ثوابت التفكك الايوني لمركب البيريدوكسال هيدرو كلوريد بالطرق الطيفية والجهود القطبية في محاليل ثابتة القوة الايونية عند القيمة 0.5 جرام جزئي من كلوريد البوتاسيوم في اللتر . ويشمل البحث دراسة متراكبات اليورانيوم مع البيريدوكسال . ودلت النتائج على وجود متراكب تكوينه 1 : 1 في المحاليل ذات الاس الهيدروجيني 3.5 - 7.0 ، كما وجد أن ثابت تكوينه الاتزاني هو  $5.13 \times 10^4$