Polarographic Reduction of a Pyridoxal 5′-Phosphate Schiff's Base

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A Schiff's base of pyridoxal 5′-phosphate formed by reaction with n-hexylamine was reduced on a dropping mercury electrode. The polarographic reduction was studied as a function of pH and solvent composition in *NN*-dimethylformamide–water mixtures. Uptake of two protons before the rate-limiting step was observed in all cases.

We have recently studied the polarographic behaviour of pyridoxal 5′-phosphate in *NN*-dimethylformamide (DMF)–water mixtures.[1] The phosphate is bound to many proteins through a Schiff's base with the terminal amino-group of a lysine residue of the polypeptide chain.[2–5] Furthermore the pyridoxal group exists in some of these proteins in a hydrophobic environment.[5–9] This indicates the importance of a survey of the influence of solvent polarity on the properties of the Schiff's base. It was first necessary to study the reduction of pyridoxal 5′-phosphate itself due to the fact that the Schiff's base is dissociated to a certain extent in solution. In this paper some results obtained with the Schiff's bases of pyridoxal 5′-phosphate formed by reaction with primary amines are presented.

Several polarographic studies of pyridoxal 5′-phosphate (ref. 1 and references therein) and of its oxime [10,11] have been carried out, but no reference has been found to the polarographic reduction of the corresponding Schiff's bases. The investigation of solvent effects seemed to be important as solvent polarity has a large effect on the electronic spectra [6,7] which can be correlated [12] with solvent polarity parameters.

EXPERIMENTAL

Schiff's bases are formed by addition of an excess of a primary amine to known solutions of pyridoxal 5′-phosphate. Apparent equilibrium constants, *K*, were evaluated by equation (1) where i_b and i_a are the intensities of currents of

$$i_b/i_a = K[\text{amine}]_f \qquad (1)$$

the Schiff's base and the phosphate respectively. The free amine concentration $[\text{amine}]_f$ can be calculated by subtracting the Schiff's base concentrations (evaluated from their waveheights) from the total amine concentration. Solutions of pyridoxal 5′-phosphates (final concentration 0.57 mol m^{-3}) with varying amounts of n-hexylamine (0—0.02M) were adjusted to a given pH value, kept for *ca.* 15 h in the dark, and individual polarographic curves recorded.

Instruments, chemicals, and solutions were as described in ref. 1. Hexylamine from Merck was distilled before use. The transfer coefficients ($n_a\alpha$), half-wave potentials ($E_{1/2}$), and the number of protons transferred before the potential-determining step were determined as described previously.[1]

RESULTS AND DISCUSSION

Pyridoxal 5′-phosphate gives one or more waves depending on the pH and the solvent composition.[1] When a slight excess of amine is added to a solution of the phosphate a new wave appears at potentials more positive than that of the parent compounds (Figure 1B).

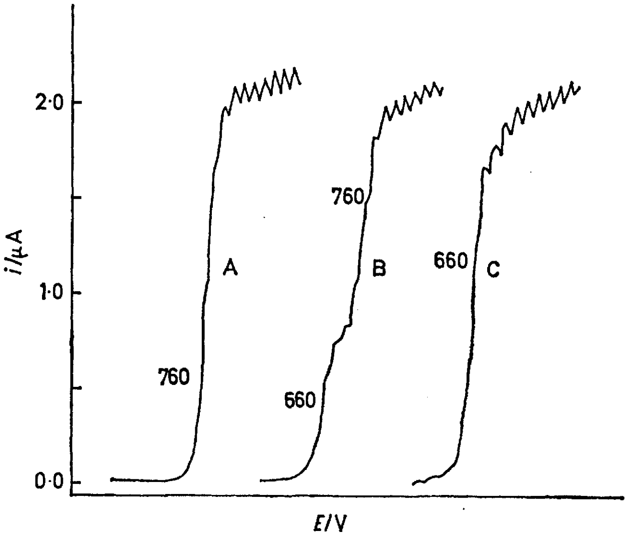

FIGURE 1 Polarographic curves for pyridoxal 5′-phosphate and its Schiff's base in aqueous solution: 0.2M-phosphate buffer, pH 7.3; 0.43 mol m^{-3} pyridoxal 5′-phosphate; concentration of added n-hexylamine: A 0; B 8.8; C 30 mol m^{-3}. Curves start at -0.5 V (s.c.e.); $E_{1/2}$ in mV (s.c.e.)

With a large excess of the amine a single wave at positive potentials is obtained (Figure 1C). This wave can be assigned to the reduction of the azomethine bond. The waveheight is proportional to the Schiff's base concentration; it has a diffusion character even when its height was lower than 10% of total limiting current. This indicates that the rate of establishment of the

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equilibrium yielding the Schiff's base [equation (2)] is slow when compared with the drop time (2.4 s).

$$\mathrm{R^1CHO} + \mathrm{R^2\overset{+}{N}H_3} \overset{K}{\rightleftharpoons} \mathrm{R^1CH{=}\overset{+}{N}HR^2} + \mathrm{H_2O} \quad (2)$$

The rate of formation of the Schiff's base was followed from the increase of the waveheight at −0.72 V (Figure 2A). The log plot of the difference $(i_\infty - i)$ as a function of time gives a straight line (Figure 2B) which corresponds to a pseudo-first-order reaction with an apparent rate constant of 3.4×10^{-3} s^{-1}, indicating a half-life of 198 s. This is of the same order of magnitude as values obtained for similar compounds;[13] nevertheless a more detailed study of the kinetics is necessary and will be carried out.

The total waveheight remains practically unchanged in the transition from pyridoxal 5′-phosphate to the imine by addition of increasing amounts of amine. Nevertheless a slight decrease of the total waveheight is observed at high amine concentration (Figure 1). The similarity of waveheights indicates that the number of electrons used in the reduction of the Schiff's base is the same as for pyridoxal 5′-phosphate (n 2),[1] as no substantial change of the diffusion constant can be expected.

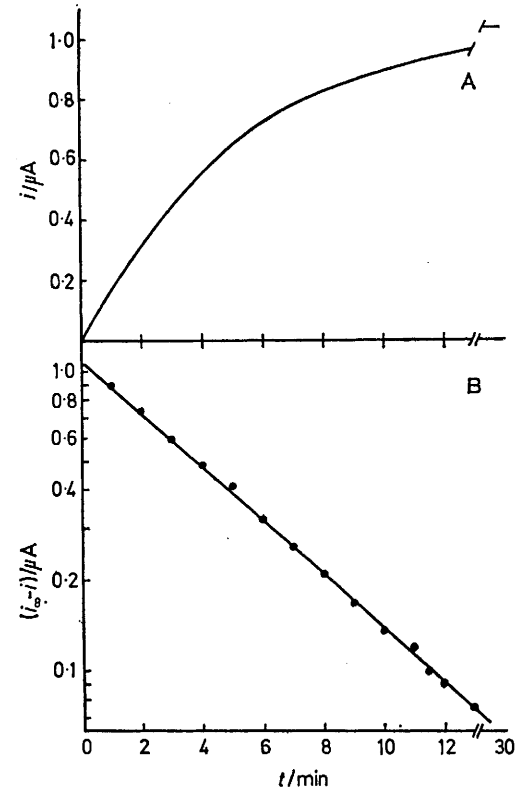

FIGURE 2 Kinetics of formation of pyridoxal 5′-phosphate Schiff's base: 0.2M-phosphate buffer, pH 7.3; 0.50 mol m^{-3} pyridoxal 5′-phosphate. Current (i) measured at −0.72 V (s.c.e.). A, $i = f(t)$; B, $\log(i_\infty - i) = f(t)$, $i_\infty = 1.035$ μA

The value of the apparent equilibrium constant was obtained from the plot of i_b/i_a *versus* $[\text{amine}]_f$ (Figure 3) (K 95 l mol^{-1} at pH 7.1). This value is a little greater than that obtained in the reaction of pyridine-4-carbaldehyde with various amino-acids[13] and the increase may be due both to the lower pK value of the amino-acids used compared with n-hexylamine and to the effect of the 3-hydroxy-group. Additional data are necessary to evaluate the individual contributions of these two factors.

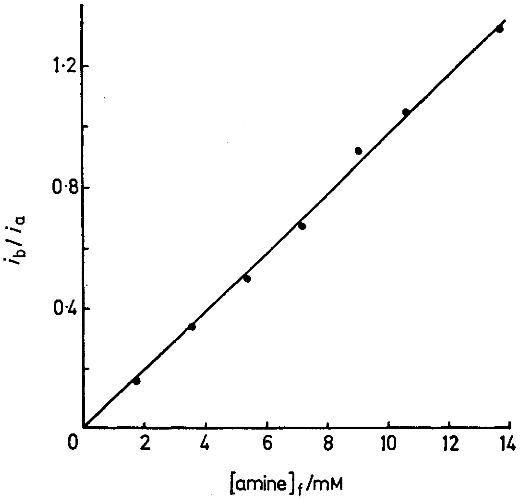

FIGURE 3 Equilibrium constant evaluation: 0.2M-phosphate buffer, pH 7.1; 0.57 mol m^{-3} pyridoxal 5′-phosphate; concentration of added n-hexylamine 0—0.02M. Reduction currents of the Schiff's base (i_b) and carbonyl form (i_a) measured after equilibration for 15 h

The results obtained in all DMF–water mixtures follow a similar pattern. The total limiting current decreases with increasing amounts of DMF; this has been shown[1] to be mainly due to variations in the viscosity of the media.

The dependence of half-wave potentials on pH for the Schiff's base shows a single straight line (Figure 4) with a slope corresponding to an uptake of two protons. Similar plots were obtained when n-hexylamine was replaced by n-butylamine or other primary aliphatic amines.

Simple buffer systems were used to overcome any solubility problems. Usually the highest possible concentration of the buffer in a given solvent mixture was used. Moreover, the change[14] in pK in mixed solutions was taken in consideration; this results in a pK value three or more units different than that in water. For instance, phosphate is an excellent buffer in the region 6—8 in water but is very poor in DMF–water solutions with a DMF content >50% in this pH region. This effect is shown in Figure 5 in which the slope of the line obtained with acetate–phosphate buffer is closer to the theoretical value than that obtained with phosphate alone. The pK of both substances increases with the proportion of apolar solvent.[15,16]

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The Schiff's base shows two absorption bands whose relative intensities change with the solvent used.[6,7,12]

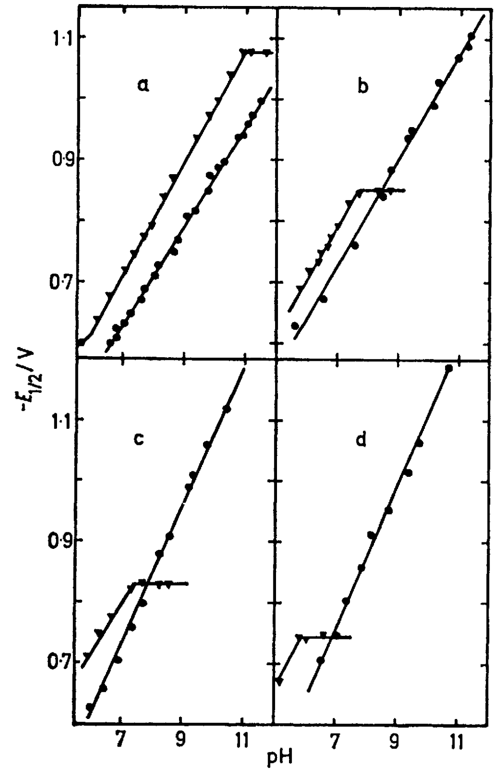


FIGURE 4 pH-dependences of the half-wave potentials for pyridoxal 5'-phosphate (▼) and its Schiff's base (●). The experimental points for pyridoxal 5'-phosphate were taken from ref. 1 and those for its Schiff's base were taken with a 100-fold excess of n-hexylamine (see Experimental section). Acetate phosphate, or carbonate buffers were used depending on the solvent and pH range. DMF content (% v/v): a, 0; b, 25; c, 50; d, 75

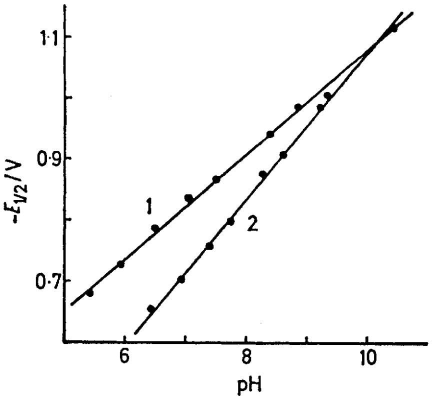
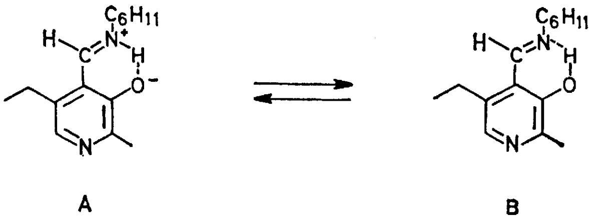


FIGURE 5 Effect of the buffer capacity on the $E_{1/2}$–pH profiles of pyridoxal 5'-phosphate Schiff's base in 50% v/v DMF–water: 0.10M-phosphate buffer plus 0.10M-tetramethylammonium bromide (curve 1). The pH values given in curve 2 were obtained by mixing two Schiff's base solutions (of equal concentration) containing 0.15M-HNa_2PO_4 and 0.20M-acetic acid respectively

The two bands, with maxima at 415 and 335 nm, were assigned to two tautomeric forms, A and B (Scheme).[12] Some Schiff's bases of 3-hydroxypyridine-4-carbaldehyde show absorption bands at 400—425 and 265—280 nm which have been assigned to the π_1 and π_2 transitions of a tautomeric 'enamine' similar to form A,[17] which has an absorption maximum at 415 nm. For these compounds, assignments of the 316—330 nm absorptions to the π_1 transition and of the 238—256 nm absorptions to the π_2 transition of an 'enol' form similar to B were made.[17] The equilibrium constant between these two forms changes with solvent polarity.[12,17]



SCHEME

We could not find any linear correlation of our polarographic results with the composition of the solvent, with the dielectric constant of the media, or even with the empirical Z parameter of Kosower.[18] A linear correlation was reported [12] between the Z values and the standard Gibbs free energy of the equilibrium in the Scheme. This result confirms that proton transfers are very fast in this system.

The half-wave potentials of the Schiff's base of pyridoxal 5'-phosphate were always more positive than those of the phosphate alone (*ca.* 100 mV) in the pH region in which the first wave of the phosphate depends on the pH. These differences are slightly lower than those observed for other Schiff's bases by Zuman.[19] The half-wave potentials of the Schiff's bases at a given pH are shifted to more negative values with increasing amounts of DMF. Since the half-wave potentials were measured against an aqueous saturated calomel electrode, these shifts include any change in the liquid-junction potential as well as the change in the true reduction potential. Similar shifts have been found for other systems [20] and their interpretation is difficult.

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