Polarographic Study of the Reaction of Pyridoxal with Hydroxylamine

In the course of the study of biochemically important reactions, the condensation of pyridoxal with hydroxylamine was chosen as a model reaction. The difference in the heights of four-electron diffusion controlled polarographic wave of pyridoxaloxime and two electron kinetically controlled polarographic waves of pyridoxal enabled us to determine the increase of oxime concentration with time.

At pH >3 the equilibrium between pyridoxal and its oxime is shifted towards oxime so that the reaction occurs practically quantitatively already in stoichiometrical ratio. The reaction can thus be treated as irreversible. The reaction rate corresponds to a second order reaction, first order in pyridoxal and first in hydroxylamine. The rate constant is a function of pH (Figure). It is assumed that all three forms of pyridoxal, the protonized (PH_2^+), the neutral molecule or zwitter-ion (PH) and anionic form (P^-) undergo the reaction with constants $k_{PH_2^+}$, k_{PH} and k_{P^-}, and that only unprotonized form of hydroxylamine takes part in the reaction. Hence the measured rate constant k' follows equation (1):

$$k' = \frac{k_{PH_2^+}\, S_N}{\left(1 + \frac{[H^+]}{K_N}\right)\left(1 + \frac{K_{PH_2^+}}{[H^+]}\right)} +$$

$$\frac{k_{PH}\, S_N}{\left(1 + \frac{[H^+]}{K_N}\right)\left(1 + \frac{[H^+]}{K_{PH_2^+}} + \frac{K_{PH}}{[H^+]}\right)} +$$

$$\frac{k_{P^-}\, S_N}{\left(1 + \frac{[H^+]}{K_N}\right)\left(1 + \frac{[H^+]}{K_{PH}}\right)}$$

For $pK_{PH_2^+} = 4.23$, $pK_{PH} = 8.7$, $pK_N = 6.0$ and analytical concentration of hydroxylamine (S_N) the curve plotted in the Figure shows a good agreement with experimentally determined values for $k_{PH_2^+} = 500$ l mol^{-1} min^{-1}, $k_{PH} = 3.5$ l mol^{-1} min^{-1} and $k_{P^-} = 13.1$ l mol^{-1} min^{-1}. Hence all three forms of pyridoxal are reactive but show a marked difference in reactivity. Formation of a hydrated intermediate (which would probably be polarographically inactive) deduced[1] for reaction of hydroxylamine with benzaldehyde, fural etc., was not detected in the reaction with pyridoxal, as no deviations from second order kinetics were observed. General acid-base catalysis, demonstrated for reaction of pyridoxal with semicarbazide[2], practically does not affect the constant k_{PH}^+ and at higher pH-values causes only a minor perturbation of the observed constants. Hence, e.g. at pH 5.8, a twofold increase in acetate buffer concentration causes an increase in the value of rate constant of about 15%. The major factor affecting the pH-dependence of the rate of oxime formation is thus the antecedent acid-base equilibria changing the protonation of reactants.

At pH <3 equilibrium between protonized form of pyridoxal (PH_2^+) and unprotonized form of hydroxylamine was studied from the dependence of limiting currents of pyridoxaloxime on hydroxylamine concentration at various pH-values. For the reaction
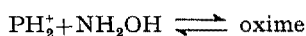
$$PH_2^+ + NH_2OH \rightleftharpoons oxime$$

for $pK_{NH_3OH}^+ = 6.0$, $pK_{PH_2^+} = 4.23$ and for $pK_{oxime} \gg 10$ the value of $K = [oxime]/[PH_2^+][NH_2OH] = 2.10^7$ was found.

Similarly the pH-dependence of the reaction rate of pyridoxal-5-phosphate was measured. Due to the additional dissociation steps resulting from the introduction of phosphoric acid part, the interpretation of the shape of this dependence is more complicated.

This work will be described in detail in Collection of Czechoslovak Chemical Communications.
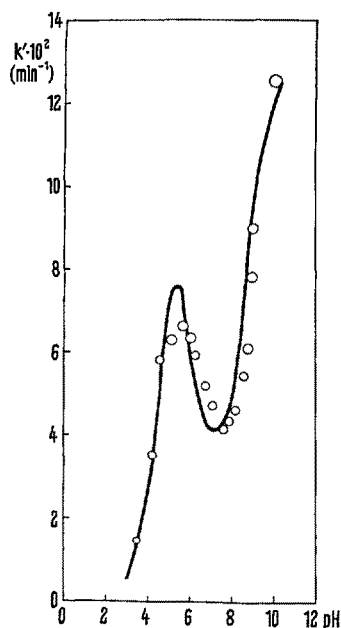
Theoretical curve of the pH-dependence of the rate constant for condensation of pyridoxal computed using equation (1). The circles denote experimental points. Buffers used: pH 3.6–5.6, 0.18 M acetate; pH 5.9–7.8, 0.18 M phosphate; pH 8.2, 0.09 M veronal; pH 8.6, 0.12 M veronal; pH 8.8–9.0, 0.18 M veronal; pH 10, 0.18 M phosphate; ionic strength $\mu = 0.5$ controlled by addition of sodium chloride. Concentration of hydroxylamine $S_N = 10^{-2} M$.

Zusammenfassung. Es wurde polarographisch die Kinetik der Reaktion des Pyridoxals mit Hydroxylamin verfolgt. Die pH-Abhängigkeit der Geschwindigkeitskonstante der Kondensierung konnte auf Grund verschiedener Reaktivität einzelner dissoziierter Formen des Pyridoxals mit nichtprotonisiertem Hydroxylamin erklärt werden.

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[1] W. P. Jencks, J. Amer. chem. Soc. *81*, 475 (1959).
[2] E. H. Cordes and W. P. Jencks, Biochemistry *1*, 773 (1962).