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A preformulation study on the kinetics of pralidoxime chloride in concentrated acidic solution

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Summary

The degradation kinetics of pralidoxime (PAM) chloride have been investigated in various aqueous acidic solutions. A pH profile on solutions of 430 mg/ml have been established between pH 1 and 3.4 at 120 °C. The degradation of PAM follows pseudo-first order kinetics with respect to PAM. The observed rate constant seems to depend on a hydroxyl ion-catalyzed reaction (k_{OH}) and an un/water catalyzed reaction (k_0) that shows influence at a pH below 2.5. k_{OH} has been determined to $1.3 \cdot 10^{10}$ M^{-1} h^{-1} and k_0 was determined to 0.033 h^{-1}. The influence of concentration and ionic strength have been studied between 10 and 500 mg/ml at 120 °C, the observed rate constant shows a direct dependence on ionic strength. The temperature dependence was studied at a concentration of 480 mg/ml at pH 3, 3.5 and 4 in the temperature range 50–120 °C. The activation energy was determined to 102.2 kJ mol^{-1}.

Introduction

Pralidoxime (PAM^+, Cl^-, I^- and $CH_3SO_3^-$) was the first oxime antidote for treatment of organophosphate (nerve gas) poisoning (Ellin and Wills, 1964; Namba and Hiraki, 1958; Loomis, 1956; Karlog and Nimb, 1958). PAM and subsequent oximes (obidoxime, TMB-4, HS-6, HI-6 etc.) act through regeneration of the irreversibly inhibited enzyme acetylcholinesterase. Therapy with oximes against organophosphate poisoning must commence immediately after exposure since some organophosphates otherwise may cause permanent damage (ageing) to the enzyme (Briggs and Simons 1986). Since PAM is a quarternary ammonium compound it is poorly and slowly absorbed from the gastrointestinal tract. Together with a rapid renal elimination($t_{0.5} \sim 2$ h) the oral route will be less useful even for prophylaxis (Berglund et al., 1962; Lemanowicz et al., 1979; Sidell at al., 1969, 1972; Sundwall, 1960; Bhuta et al., 1980). Intramuscular administration shows high availability and rapid absorption. Thus, the parenteral route is the best if not the only way to administer PAM to a poisoned individual (Lemanowicz et al., 1979; Sidell and Groff, 1971; Sundwall, 1960). The need for rapid onset of treatment and parenteral administration require the utilization of a simple and

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easy to use device, an autoinjector. There are two constructions of autoinjectors available on the market, the ComboPen (Survival Technology, U.S.A.) and the Astra Autoinjector (Astra-Meditec, Sweden). The maximum volume of the ComboPen is 3 ml whereas the Astra injector accepts 2 ml. Since the therapeutic dose of PAM-Cl is 0.5–1.0 g, the solution will become very concentrated.

Two routes for the degradation of PAM have been reported. At pH values below 4, a hydrogen-ion catalyzed hydrolysis to give the corresponding aldehyde (2-formyl-1-methylpyridinium$^+$) and hydroxylamine takes place, the reaction attains equilibrium at higher concentrations of PAM-Cl (Ellin and Easterday, 1961; Ellin et al., 1962; Barkman et al., 1963; Ellin and Wills, 1964; Christensson, 1974). The aldehyde might subsequently undergo oxidation to the corresponding acid (2-carboxy-1-methylpyridinium$^+$) (Christensson, 1974).

Above pH 4, a hydroxyl-ion catalyzed dehydration to a nitrile (2-cyano-1-methylpyridinium$^+$) occurs and this material is subsequently hydrolyzed either to a pyridone (1-methylpyridone) and cyanide ion or to an amide (2-carboxamido-1-methylpyridinium$^+$) (Ellin et al., 1962; Barkman et al., 1963; Ellin and Wills, 1964; Ellin, 1958; Creasy and Green, 1959; Fan et al., 1964; Carter et al., 1968). PAM shows kinetics accordingly with a maximum stability at pH ~ 4 (Ellin et al., 1962; Fan et al., 1964; Carter et al., 1968). However, if at all reported, the concentration of PAM has been low in these studies. In more recently published papers (Prue et al., 1983; Fyhr et al., 1986), the degradation products have been identified in concentrated acidic solutions. Under these conditions there is no indication of an acidic catalysis, and different kinetics can be expected. The present studies were performed in order to preformulate PAM-Cl as a concentrated aqueous solution.

Materials and Methods

Chemicals

Pralidoxime chloride (PAM-Cl) [1], used as obtained. HCl, NaOH, $KHSO_4$, 2-propanol, methanol, alanine, acetic acid, citric acid, tartaric acid [2], tetramethylammonium chloride [3] and sodium octylsulphonate [4] of analytical grade.

Pralidoxime chloride reference

PAM-Cl contained particulate contamination and was purified by filtration through a 0.2 μm membrane filter, and 3 recrystallizations from 75% 2-propanol in 0.001 M hydrochloric acid, m.p. 232° C.

Analytical procedures

PAM was analyzed using reversed-phase ion-pair HPLC (Fyhr et al., 1986). The system consisted of : reversed phase column, RP-18 5 μm, 125 mm, i.d. 4 mm [5]; mobile phase consisted of 2 mM sodium octylsulfonate, 50 mM tetramethylammonium chloride, 20 mM acetic acid and, 10% methanol in distilled water; pump [6] set at 0.8 ml/min. Samples were injected using a injection valve with a 20 μl loop [7]. Detection was performed with a variable wavelength UV detector [8], the chromatograms were recorded with a computing integrator [9] and a pen recorder [10]. Peak heights were evaluated at the wavelength of their absorbance maximum.

pH was measured with a combined glass–calomel electrode [11], calibrated against standard buffers. The measured values were regarded as the true pH even though the ionic strength of the solutions was above 0.1.

pH Profile

43% w/v solutions of PAM-Cl of pH 1.05, 1.34, and 2.02 buffered with HCl; of pH 1.57, 1.70, and 2.02 buffered with potassium hydrogen sulfate 0.1 M and of pH 2.40, 2.80, and 3.20 buffered with citric acid 0.1 M and of pH 2.40, 2.80, 3.20

[1] Aldrich Co., Belgium

[2] E. Merck, Darmstad, F.R.G.

[3] Fluka A.G., F.R.G.

[4] Eastman Kodak, NY, U.S.A.

[5] Hibar Lichrosorb, E. Merck, Darmstad, F.R.G.

[6] LDC Constametric III, FL, U.S.A.

[7] Rheodyne 7120, CL, U.S.A.

[8] Perkin Elmer LC-75, U.S.A.

[9] Hewlett Packard 3390A, U.S.A.

[10] Philips PM8251, The Netherlands

[11] Metrohm, Schweiz

buffered with citric acid 0.1 M were filled into 2 ml glass ampoules that were treated at 120 °C (Fyhr et al, 1986). The observed rate constants were recalculated from the primary data reported previously. Data from 10 to 50% degradation of PAM-Cl were used. Regression data for the rate plots are given in Table 1. The pH–temperature dependence for each solution was established up to 80 °C, according to the Van't Hoff isochore by calibrating the electrode at the actual temperatures against buffers with known pH temperature dependence. The pH of PAM-Cl solutions were thereafter measured and the pH at 120 °C was calculated by plotting measured pH vs $1/T$.

Ionic strength / concentration

As previously reported, PAM-Cl solutions from 10 to 500 mg/ml were prepared and dispended into 2 ml glass ampoules that were treated at 120 °C (Fyhr et al., 1986). The solutions were not buffered and pH was not adjusted. Since the ionic strength of the solutions varied from 0.06 to 2.90, adjustment to the same pH would not yield solutions of the same hydrogen ion activity due to an error from the reference electrode junction potential. Initial pH ranged from 5.0 to 3.5 for 0.1% to 50% solutions, respectively. Final pH was 2.6–2.8 in all solutions. The observed rate constants were calculated from the primary data (Fyhr et al., 1986) in the interval of 10–25% decomposition of initial PAM-Cl; the regression data are given in Table 2.

Temperature dependence

Nine compositions with 480 mg/ml were used, buffered with D,L-alanine, citric acid and tartaric acid, 0.15 M, at pH 3.0, 3.5 and 4. The solutions were filtered through 0.2 μm membrane filter, dispensed in 10 ml vials and sealed using chlorobutyl stoppers [12]. The containers were divided into two groups of which one was autoclaved at 120 °C for 20 min prior to the study. The containers were stored at temperatures between 50 and 120 °C and the observed rates were calculated from 20–80% decomposition of initial PAM-Cl. The regression data are given in Table 3.

[12] Helwoet FM 50, F.R.G.

TABLE 1

Regression data for the pH profile

pH	k_{obs} $m^{-1} \cdot 10^3$	S.D. $\cdot 10^3$	I	S.D.	n	r^2
1.05	0.56	0.038	6.003	0.0010	3	0.996
1.34	0.63	0.070	6.002	0.0019	3	0.988
2.04	0.68	0.262	5.996	0.0198	4	0.771
2.36	0.77	0.200	6.011	0.0151	4	0.881
2.46	1.2	0.37	6.008	0.0275	4	0.835
2.46	1.5	0.45	6.01	0.034	4	0.842
2.82	1.8	0.56	6.01	0.042	4	0.840
2.98	2.4	0.79	6.01	0.060	4	0.826
3.26	3.9	0.89	6.00	0.068	4	0.903

TABLE 2

Regression data for the concentration profile ($n = 4$)

Conc. %w/v	k_{obs} $m^{-1} \cdot 10^3$	S.D. $\cdot 10^3$	I	S.D.	r^2
50	3.01	0.291	3.874	0.0148	0.982
43	2.75	0.222	3.704	0.0113	0.987
36	2.49	0.273	3.547	0.0139	0.976
29	2.28	0.226	3.333	0.0115	0.981
22	1.8	0.41	3.052	0.0206	0.905
15	1.5	0.31	2.676	0.0159	0.927
10	1.8	0.49	2.306	0.0251	0.867
5	1.2	0.54	1.602	0.0272	0.728
1	1.09	0.067	−0.019	0.0034	0.992
0.5	1.04	0.142	−0.720	0.0072	0.964
0.1	0.9	0.38	−2.362	0.0194	0.739

Data evaluation

Regression analysis and non-linear curve fittings were performed using a computer program [13]. Regression analysis of Arrhenius data were performed on an older program that does not report the standard deviation for the intercept [14].

Results and Discussion

The degradation of PAM-Cl follows pseudo-first-order kinetics with respect to PAM-Cl at all

[13] RS/1, BBN Research Systems, U.S.A.

[14] Regsta, Astra-program

TABLE 3

Regression data for the temperature profile

pH	t (°C)	Code 1	Code 2	Slope $\times 10^{-3}$	S.D.	Inter. $\times 10^{-3}$	n	r^2	Time in
3.5	50	A	A	1.946	0.241	1.935	7	0.929	days
		A	E	1.741	0.062	1.996	7	0.994	↓
		C	A	3.686	0.750	1.952	7	0.829	
		C	E	3.065	0.782	1.990	7	0.755	
		T	A	2.721	0.154	1.934	7	0.984	
		T	E	2.143	0.119	1.993	7	0.985	
4.0		A	E	2.471	0.205	1.985	7	0.948	
		C	E	7.047	0.091	2.005	7	0.999	
		T	E	5.543	0.121	2.001	7	0.996	
3.5	60	A	E	4.139	0.152	2.648	7	0.988	
		C	A	10.64	0.311	1.928	9	0.994	
		T	A	8.003	0.248	1.923	9	0.993	
4.0		A	E	7.100	0.349	1.971	12	0.977	
		C	E	14.90	1.018	1.951	12	0.955	
		T	E	12.60	0.735	1.960	12	0.967	
3.0	80	A	E	49.32	3.368	2.008	9	0.968	
		C	E	62.45	1.850	2.003	9	0.994	
		T	E	50.32	2.009	2.012	9	0.989	
3.5	80	A	E	2.448	0.081	1.681	9	0.992	hours
		C	A	2.831	0.099	1.913	10	0.990	↓
		T	A	2.655	0.086	1.909	10	0.992	
4.0		A	E	1.913	0.278	1.941	10	0.856	
		C	E	2.349	0.3468	1.887	10	0.852	
		T	E	2.423	0.331	1.901	10	0.870	
3.0	100	A	E	15.07	0.434	1.985	11	0.993	
		C	E	15.13	0.494	1.987	10	0.992	
		T	E	14.45	0.419	1.988	11	0.992	
3.5		A	E	23.98	0.020	1.683	14	0.971	
		C	A	20.83	0.246	1.940	17	0.998	
		T	A	20.16	0.106	1.933	17	0.996	
4.0		A	E	25.61	1.349	1.980	11	0.976	
		C	E	33.37	1.577	1.982	11	0.980	
		T	E	35.74	0.955	1.985	11	0.994	
3.0	110	A	E	0.655	0.053	1.977	11	0.944	minutes
		C	E	0.585	0.051	1.976	11	0.935	↓
		T	E	0.596	0.041	1.982	11	0.959	
3.5		C	A	0.935	0.044	1.937	10	0.983	
		C	E	0.775	0.080	1.976	10	0.922	
		T	A	0.910	0.041	1.932	10	0.984	
		T	E	0.953	0.057	1.979	10	0.981	
	120	A	E	1.799	0.025	1.650	9	0.999	

Code 1: A, alanine; C, citrate; T, tartrate. Code 2: A, autoclaved; E, untreated.

investigated pHs, concentrations and temperatures. A rapid α-phase, Fig. 1, appears at higher temperatures, which seems to be due to the formation of an unknown product in equilibrium with PAM (Fyhr et al., 1986). Zero values were therefore omitted from rate calculations. The regression data from the concentration–time plots for the pH profile, concentration dependence and temperature dependence are given in Tables 1–3.

Previous investigations have shown kinetics according to:

$$-\frac{d[P]}{dt} = (k_H[H] + k_{OH}[OH])[P] \quad (1)$$

where k_H and k_{OH} designate the rate constants of the hydrogen-ion and hydroxyl-ion catalyzed reactions, respectively. [P] = [PAM-Cl]. In the pH-profile, Fig. 2, the observed rate constants of PAM-Cl degradation shows an ascending slope from lower to higher pH. Therefore, it seems probable that $k_H \ll k_{OH}$ and that a water or uncatalyzed mechanism influences the reaction at low pH in concentrated solution. Thus, the degradation of PAM would obey the rate expression:

$$-\frac{d[P]}{dt} = (k_{OH}[OH] + k_0)[P] \quad (2)$$

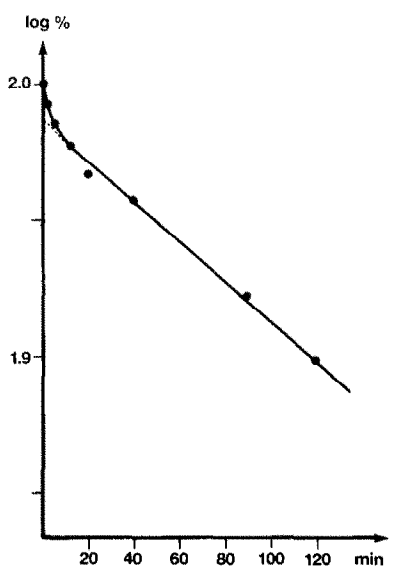

Fig. 1. Concentration–time plot, PAM-Cl 430 mg/ml, pH 3.5.

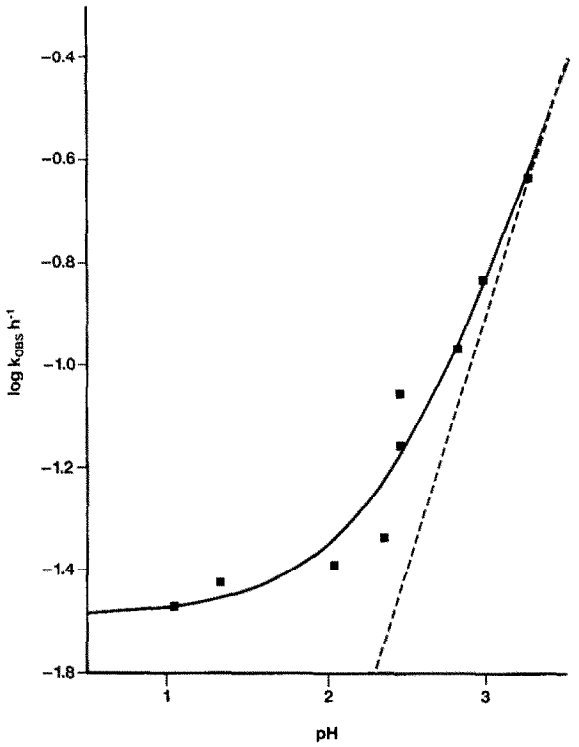

Fig. 2. pH profile PAM-Cl 430 mg/ml, 120 °C; - - - - - - slope = 1.

where k_0 designate the rate constant of the water/un catalyzed reaction. Since pH is constant during one rate determination, [OH] becomes constant and:

$$\int_{P_0}^{P} \frac{d[P]}{P} = -\int_{t_0}^{t} (k_{OH}[OH] + k_0)t \quad (3)$$

yields:

$$\ln [P] = \ln [P_0] - k_{obs}t \quad (4)$$

where:

$$k_{obs} = (k_{OH}[OH] + k_0) \quad (5)$$

Introducing:

$$[OH] = \frac{K_W}{[H]} \quad (6)$$

$$k_{obs} = \left(k_{OH} \frac{K_W}{[H]} + k_0 \right) \quad (7)$$

Thus, a plot of log k_{obs} vs pH would yield a line with a slope of zero when $k_0 > k_{OH}$ [OH], changing to +1 at higher pH when $k_0 < k_{OH}$ [OH]. The pH was measured conventionally by calibrating a combined glass electrode against a standard buffer according to the operational pH definition (Linnet, 1970):

$$pH_x = pH_s + (E_x - E_s) \frac{F}{2.3\,RT} \quad (8)$$

where E_x and E_s are the emf of the pH-cells for the measured and standard solutions, respectively. The emf of a pH-cell is expressed as:

$$E_{x,s} = (E_0 - E_j) \frac{F}{2.3\,RT} - \log a_{Hx,s} \quad (9)$$

where E_0 is the emf of the internal reference and E_j is the liquid junction potential of the reference electrode. E_j is affected by the composition, i.e the ionic strength, of the solutions measured. Thus, the pH of standard and measured solutions can only be compared if the compositions of both are fairly similar. The ionic strength of the buffer is < 0.1 whereas it is ~ 2.5 for the PAM-Cl solution. This difference will affect the liquid junction potential E_j, which probably will add an error to the estimated pH of PAM-Cl solutions. Setting $a_{Hx} = a_{Hs}$ introducing Eqn. 9, Eqn. 8 is reduced to:

$$pH_x = pH_s + i \text{ or } [H]_x = [H]_s 10^{-i} \quad (10)$$

where i is the error induced by the difference in E_{jx} and E_{js} due to different ionic strengths. Introducing i into Eqn. 7:

$$k_{obs} = \left(\frac{k_{OH} K_W}{10^{-i}[H]} + k_0 \right) \quad (11)$$

If $k_0 \ll k_{OH}$ [OH] a linear relationship with a slope of +1 will be expected:

$$\log k_{obs} = \log k_{OH} = pK_W - i + pH \quad (12)$$

and if $k_0 \gg k_{OH}$ [OH] a straight line with a slope of 0 will be expected:

$$\log k_{obs} = \log k_0 \quad (13)$$

Thus, the pH profile will give the specific rate constants from the intercepts. Due to the error i, the +1 line will be horizontally dislocated. Thus, the observed k_{OH} will contain the error i. The true value of k_{OH} can only be calculated if the error can be estimated through pH measurements without liquid junctions (hydrogen electrode). Non-linear regression fitting of the rates in Table 1 to Eqn. 11 yields (Fig. 2):

$k_{OH} \cdot i = (1.3 \pm 1.53)10^{10}$, $k_0 = 0.033 \pm 0.0048$,

slope of $k_{OH}[OH] = 0.98 \pm 0.166$

($\pm$S.D., $n = 9$, $r^2 = 0.998$).

Similar kinetics have been observed for another oxime, HI-6, in concentrated solution at an ionic strength of 1.67 (Fyhr et al., 1987). In previous studies on dilute solution, sharp to more diffuse minima at ~ pH 4 have been observed (Ellin et al., 1962, Carter et al., 1968, Fan et al., 1964). An uncatalyzed path at pH above 8 has also been observed (Ellin et al., 1962). From the data presented above it appears that the hydrogen ion-catalyzed path is exchanged for an uncatalyzed path at higher concentrations.

The problems with correct estimation of pH are accentuated in the study on the influence of concentration/ionic strength of k_{obs}. Since the ionic strength is between 2.9 and 0.006 it is impossible to state the true pH at each concentration/ionic strength. However, assuming similar pH at every determination, the observed rate will be expected to obey the modified Brönsted-Bjerrum equation (Florence and Attwood, 1981):

$$\log k_{obs} = \log k_I = 1.02 z_A z_B \frac{\sqrt{I}}{1 + \beta\sqrt{I}} \quad (14)$$

where k_I is the rate constant at infinite ionic strength, z_A and z_B the valence of the reacting species and β a constant depending on the ionic

diameter of the reacting species. Thus, for k_{OH} and k_0, slopes of -1 and 0 are expected. Whatever the value of β, the experimental data will not follow Eqn. 13, Fig. 3A. Only positive slopes are possible. Assuming $\beta = 1$ two parts with slopes of ~ 0.4 and ~ 2 appear. It appears probable that the mechanism changes between 5 and 15% PAM-Cl and that the hydrogen ion-catalyzed mechanism is active below 5%. The reaction might show dependence on ionic strength even if the uncatalyzed mechanism is dominating since a direct dependence on ionic strength sometimes is found for reactions between uncharged and charged species (Florence and Attwood, 1981):

$$\log k_{obs} = \log k_I + bI \qquad (15)$$

Fitting the rates for Eqn. 15 to 50%, Fig. 5, gives:

$$\log k_{obs} = -2.915 \pm 0.028 + 0.145 \pm 0.012$$

$$(\pm \text{S.D.}, n = 6, r^2 = 0.964)$$

Further conclusions can not be made without correction of the pH error.

Some inconsistency is observed among the rates in the Arrhenius study, Table 3. However, these differences can not be traced to pH or buffer substance. The cause might be unstable pH since the buffering capacity was insufficient. Regression analysis according to the Arrhenius equation gives, Fig. 4A–C:

pH 3.0: $\ln k = 33.6 \pm 0.77 - 12590 \pm 284 \cdot 1/T$

$(\pm \text{S.D.}, n = 9, r^2 = 0.997)$

pH 3.5: $\ln k = 34.7 \pm 0.75 + 12910 \pm 262 \cdot 1/T$

$(\pm \text{S.D.}, n = 20, r^2 = 0.993)$

pH 4.0: $\ln k = 32.3 \pm 2.50 - 12000 \pm 863 \cdot 1/T$

$(\pm \text{S.D.}, n = 12, r^2 = 0.951)$

There are no significant differences in activation energy depending on pH in the interval studied, and the activation energy for the hydroxyl ion catalyzed reaction can be determined using all available rates, Fig. 4D.

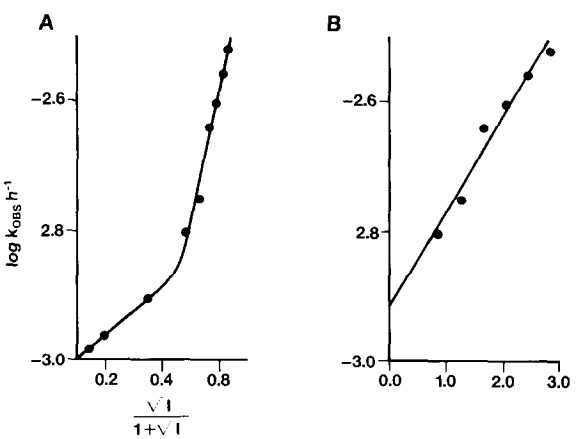

Fig. 3. Dependence on ionic strength. A: modified Brönsted Bjerrum equation. B: straight relation 15–50%.

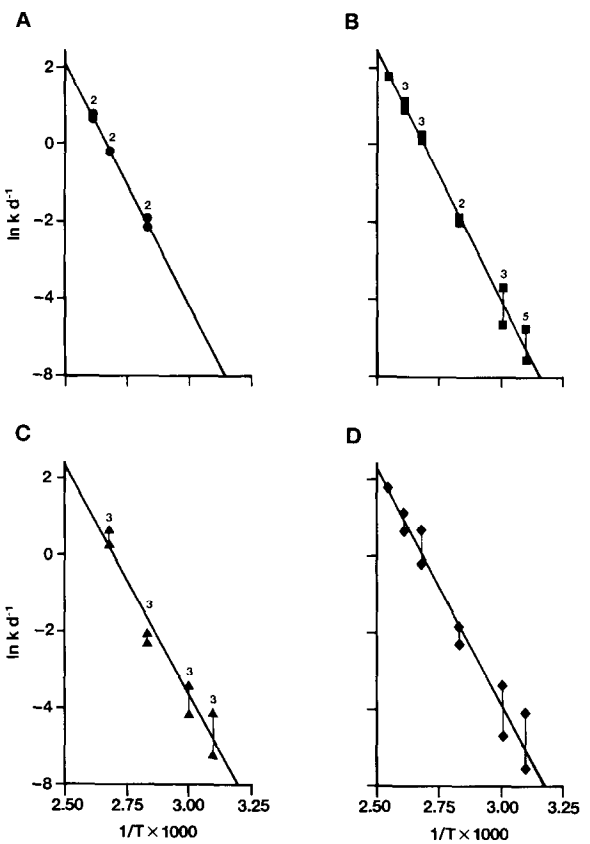

Fig. 4. Temperature dependence, PAM-Cl 480 mg/ml. A: pH 3. B: pH 3.5. C: pH 4, D; all.

All: $\ln k = 33.0 \pm 0.85 - 12290 \pm 297.1/T$

$(\pm \text{S.D.}, n = 41, r^2 = 0.978)$

Thus, the activation energy for k_{OH} is 102.2 ± 2.46 kJ mol^{-1}. The activation energy for k_{OH} has been determined to 71.1 kJ mol^{-1} in dilute solution (Ellin et al., 1962), and in 50% w/v solution to 111.9 kJ mol^{-1} under conditions similar to this study (Ellin, 1982). Since increased ionic strength reduces the attraction between ions of different charges, an increased activation energy seems logical.

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