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Autocatalytic Degradation and Stability of Obidoxime

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Abstract

The degradation of obidoxime chloride (toxogonin), a reactivator of inhibited cholinesterase in organophosphorus poisoning, in concentrated (250 mg mL^{-1}) acidic solutions was studied by HPLC at several temperatures to determine the degradation mechanism.

The degradation had an autocatalytic profile, which was found to result from the formation of formaldehyde during the degradation process. The activation energy of the hydrolysis was 26·2 kcal mol^{-1}. The shelf-life (t90, the time by which 10% of the drug has degraded) at 25°C was calculated by several methods and found to be more than 37 years. Autocatalysis, a mechanism found only rarely in the degradation of pharmaceuticals, has not been reported in previous studies of obidoxime hydrolysis.

Obidoxime chloride, *bis*(4-hydroxyiminomethyl-1-pyridiniomethyl) ether dichloride (toxogonin), is an oxime used as a reactivator of inhibited cholinesterase in organophosphorus poisoning. Because obidoxime is widely dispensed in most European armies during wartime for protection against the threat of chemical warfare attack, rather than as a consumable item, it is important to determine its shelf-life and the dependence of this on storage temperature. Christenson (1968a, b, 1972) conducted thorough investigations of the stability of this compound and its degradation products and concluded that in acidic solution initial degradation involves hydrolysis of the oxime groups to the corresponding aldehyde, *bis*(4-formyl-1-pyridinomethyl) ether dichloride, and hydroxylamine. The kinetics of the hydrolytic reaction were found to be first-order with regard to obidoxime until equilibrium was approached, post-equilibrium decomposition being zero-order. The mechanisms of hydrolytic reactions were found not to be affected by changes in temperature (Christenson 1968b). Commercial obidoxime ampoules contain 25% w/v obidoxime chloride (0·696 M), a concentration considerably higher than that studied by Christenson. Preliminary results indicated that the degradation of such concentrated solutions did not follow the mechanism suggested by Christenson (1968b). Consequently, a stability and mechanism study of concentrated obidoxime solutions was conducted; the results are presented below.

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Materials and Methods

A commercial obidoxime preparation (toxogonin ampoules) from Merck (batch number 26987) containing 250 mg mL^{-1} obidoxime chloride was used in all experiments. An old batch (K0757216, manufactured in 1982) was analysed to verify the predicted shelf-life. The preparation had a constant ionic strength of 2·08. All reagents were ACS grade and acetonitrile was HPLC grade.

Storage

The solutions described above were stored for up to 22 weeks at four different temperatures: 4, 67, 75 and 85°C. Six further solutions stored for up to 53 days at 85°C were also studied (see Results and Discussion). Refrigerated samples were used as zero-time controls. At different time intervals a sample was taken from each elevated temperature set and refrigerated until the end of the experiment, so that all samples could be analysed under uniform conditions. At the end of the storage period the tests described below were performed on each solution.

pH Measurement

Measurements were performed by means of a Metrohm 636 Titroprocessor fitted with a combined glass electrode, calibrated by means of two buffers (pH 4·01 and 7·0).

HPLC assay

The concentration of obidoxime in each sample was determined by high-performance liquid chromatography (HPLC), using a modification of the method proposed by Pohjola & Harpf (1994).

Chromatography was performed with a Hewlett-Packard HP 1050 liquid chromatograph equipped with an HP 3396A integrator and fitted with a 250 mm × 3·2 mm Spherisorb reversed-phase phenyl column, 10 μm packing (Phenomenex, USA). The mobile phase was a 1 : 3 mixture of acetonitrile and 3 mM heptanesulphonic acid (Sigma, MO) dissolved in phosphate buffer (pH 3·5) consisting of 30 mM sodium dihydrogen phosphate and 1·5 mM sodium hydrogen sulphate; the flow rate was 0·6 mL min^{-1}. The injection volume was 10 μL and the detector was set at 280 nm.

The test solutions were diluted to a final concentration of 20 μg mL^{-1} obidoxime chloride in mobile phase. A zero-time sample, kept at 4°C and diluted in the same manner, was used as a standard solution for each temperature series.

Results and Discussion

The main pathways of degradation of obidoxime are illustrated in Figure 1 (Christenson 1968a, b). Initially obidoxime degrades to the corresponding aldehyde and hydroxylamine and equilibrium is attained (Christenson 1968a). Although it is unlikely that both oxime groups are hydrolysed simultaneously, the dialdehyde is formed in preference to the monoaldehyde because of the kinetics of the reversible reactions. It has also been suggested that the hydrolytic reactions go to completion in very dilute ($\sim 10^{-5}$ M) solutions only (Christenson 1968b).

Interpretation of kinetic data

All obidoxime solutions stored at elevated temperatures developed a dark brown colour and complete degradation of obidoxime was achieved at all temperatures. Figures 2 and 3 show the per-

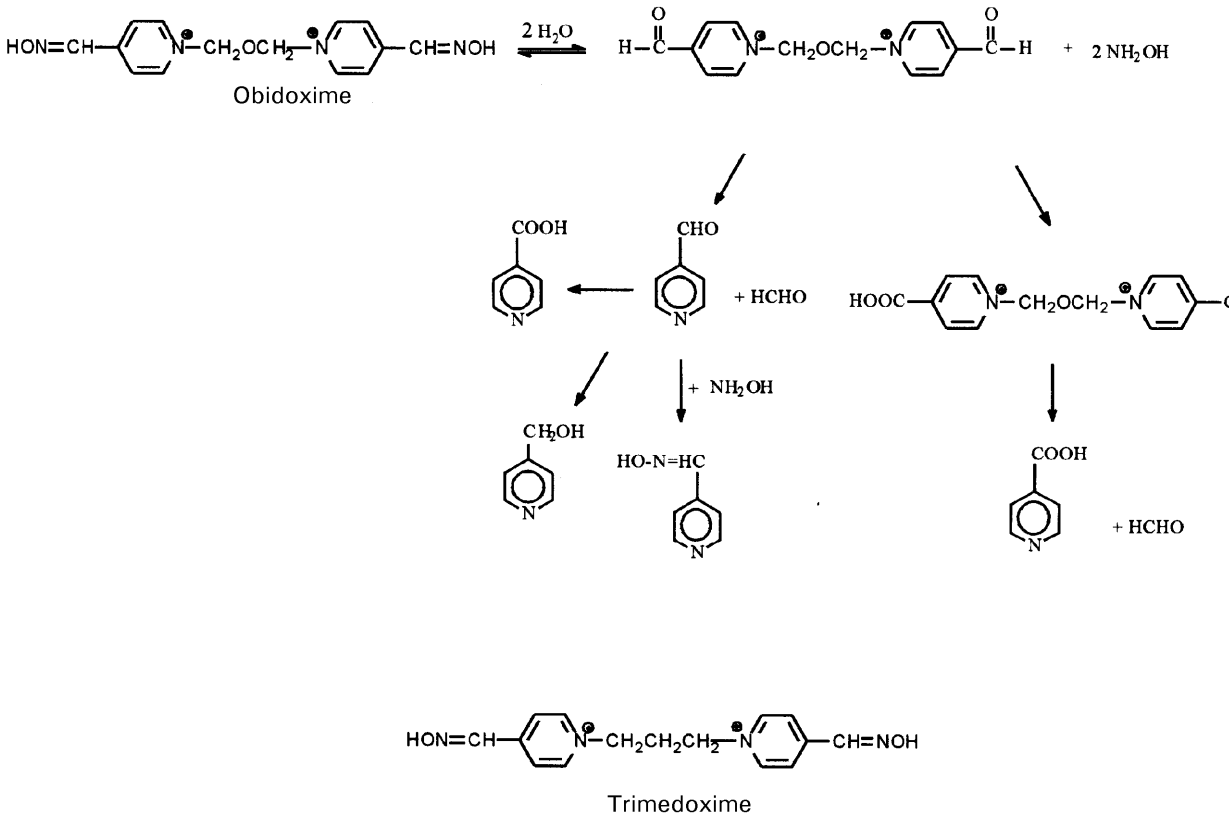

Figure 1. Proposed mechanism for the acid-catalysed degradation of obidoxime chloride.

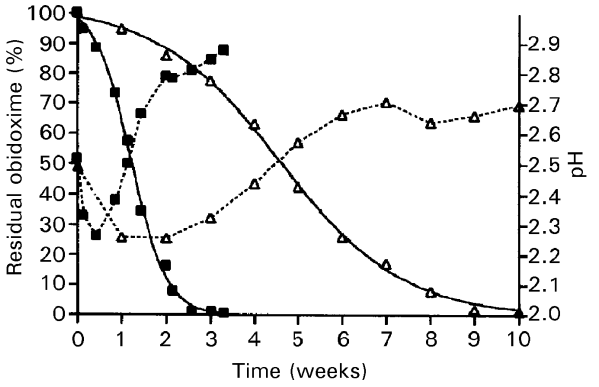

Figure 2. Obidoxime degradation (——) and at 75°C (△) and 85°C (■) pH (- - - -).

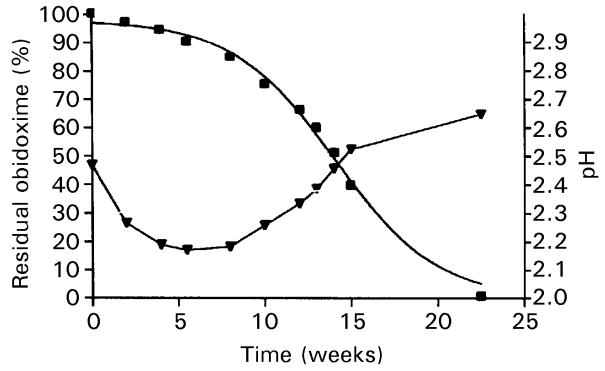

Figure 3. Obidoxime degradation at 67°C: ■ residual obidoxime (%); ▼ pH.

centage of residual obidoxime plotted against time and the pH of the solutions for three different temperatures (67, 75 and 85°C).

It is evident that in concentrated acidic solution the kinetics of obidoxime degradation are not first order pre-equilibrium and zero order post-equilibrium as reported by Christenson (1968b). It seems more appropriate to use an autocatalytic model, in which the degradation of obidoxime (A) depends on the formation of one of its degradation products (B), as expressed by equation 1:

$$-dA/dt = kAB \qquad (1)$$

In this model $A_0 - A = B - B_0$, where A_0 and B_0 are the initial concentrations. Integration yields equation 2 and solution of equation 2 for B as a function of t yields equation 3:

$$\ln (A_0B/B_0A)/(A_0 + B_0) = kt \qquad (2)$$

$$B = (A_0 + B_0)/[1 + (A_0/B_0)e^{-k(A_0+B_0)t}] \qquad (3)$$

Substituting α for A_0/B_0 and β for $A_0 + B_0$, equation 2 can be written:

$$A = (\alpha\beta e^{-kt\beta})/(1 + \alpha e^{-kt\beta}) \qquad (4)$$

Equation 4 was used to analyse the kinetic data shown in Figures 1 and 2 and to determine degradation rate constants (k) for each temperature. The experimental data was a good fit to the theoretical autocatalytic model at all temperatures (r^2 0·993). The values of the shelf-life t90 (the time by which 10% of the drug has degraded) were calculated by substituting A with 90% at each temperature studied. Table 1 summarizes the rate constants, t90 values and correlation coefficients obtained for 67, 75 and 85°C. The activation energy (E_a) and $k_{25°}$ were obtained by plotting log k against the reciprocal of the absolute temperature (in accordance with the Arrhenius equation) and found to be 26·2 kcal mol^{-1} and $1{\cdot}41 \times 10^{-5}$ $week^{-1}$, respectively ($r^2 = 0{\cdot}990$).

Table 1. Degradation rate constants (k) and shelf-life, t90, measured for obidoxime chloride at 67, 75 and 85°C.

Temperature (°C)	Correlation coefficient (r^2)	k ($week^{-1}$)	t90 (weeks)
67	0·993	$3{\cdot}44 \times 10^{-3}$	6·828
75	0·998	$6{\cdot}99 \times 10^{-3}$	1·794
85	0·997	$2{\cdot}41 \times 10^{-2}$	0·422

Determination of shelf-life (t90) at 25°C

By use of equation 4 and the value obtained for $k_{25°}$, the shelf-life (t90) at 25°C was calculated to be 37·7 years. B_0 was obtained from the extent of obidoxime degradation at the beginning of the experiment (compared with a standard solution).

Another convenient method for estimating the effects of temperature on reaction rates, discussed by Simonelli & Dresback (1972) and Connors et al (1979) and used by Bernasconi (1965), is to determine the factor by which the rate constant increases for a 10°C temperature increase (Q_{10}). This can be calculated from equation 5, if the activation energy is known.

$$Q_{10} = e^{-Ea/R[1/(T+10)-1/T]} \qquad (5)$$

Table 2. Calculated shelf-life (t90) values for obidoxime solution at 25°C, using the autocatalytic model and the Q_{10} method.

	Autocatalytic model	Q_{10} method		
		67 to 25°C	75 to 25°C	85 to 25°C
t90 (years)	37·7	68·7	59·5	62·2

Although Q_{10} changes with temperature, for calculations of approximate shelf-life it is considered to be essentially a constant and equal to its value within the interval 20 to 30°C. Thus, the value of Q_{10} was found to be 4·44. By use of equation 6, t90(25°C) (the shelf-life at 25°C) was calculated using data obtained at each of the three temperatures studied (Table 2).

$$t90(T2) = t90(T1)/Q_{10}(\Delta T/10) \qquad (6)$$

To verify the range of shelf-lives calculated, we tested an old batch of obidoxime ampoules manufactured 16 years ago. It was found to contain 95% of the labeled amount of obidoxime and 4% (approx.) of a degradation product, giving further support to the predicted t90. Although the degradation product was not directly identified, it was assumed to be the diacid (Figure 1); other possible stable degradation products such as isonicotinic acid and pyridine-4-aldoxime were eliminated by injection of reference standards.

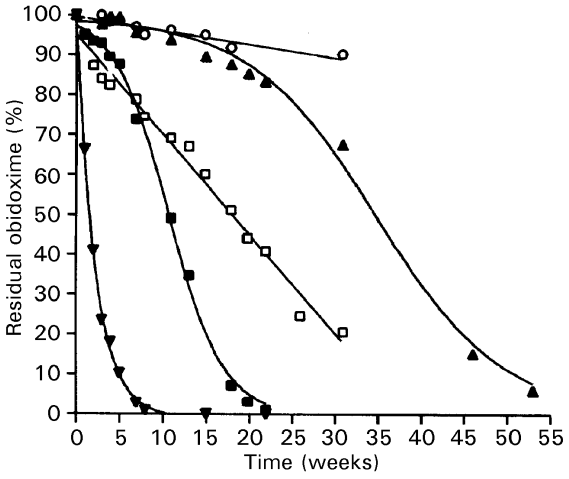

Figure 4. Degradation of trimedoxime and obidoxime at 85°C: ■ obidoxime (solution 1); ▲ obidoxime and hydroxylamine (solution 2); ▼ obidoxime and formaldehyde (solution 3); ○ trimedoxime (solution 5); □ trimedoxime and formaldehyde (solution 6).

Autocatalytic hydrolysis–mechanistic justification

The apparent autocatalytic degradation of obidoxime is unusual for a pharmaceutical compound. As mentioned above, the first products to be formed are the corresponding aldehyde and hydroxylamine (Figure 1). The reaction was found to be reversible and an initial equilibrium is attained (Christenson 1968b). This is shown in the current study as an initial lag period in the degradation process. Post-equilibrium degradation starts only after 10% (approx.) of the obidoxime has degraded.

Six other solutions were studied to determine which of the degradation products catalyses the hydrolysis of obidoxime: 250 mg mL^{-1} obidoxime chloride (0·696 M); 250 mg mL^{-1} obidoxime chloride, 12 mg mL^{-1} (0·174 M) hydroxylamine hydrochloride; 250 mg mL^{-1} obidoxime chloride, 14 μL mL^{-1} (0·174 M) 37% formaldehyde solution; 60 mg mL^{-1} obidoxime chloride (0·167 M), 123 mg mL^{-1} potassium equivalent to ionic strength 2·08; 200 mg mL^{-1} trimedoxime bromide (0·448 M); and 200 mg mL^{-1} trimedoxime bromide, 14 μL mL^{-1} 37% formaldehyde solution. The solutions were adjusted to pH 2·45 ± 0·05 (the pH of unmodified obidoxime ampoules) and sealed in dark ampoules.

Comparison of the results obtained from solutions 1 and 5, containing obidoxime and trimedoxime, respectively, (Figure 4) reveals that the degradation of obidoxime is faster than that of trimedoxime. The degradation of trimedoxime follows first-order kinetics with no autocatalysis (Rubnov et al 1999). The only difference between the two compounds is the aliphatic bridge. Whereas trimedoxime has three methylenic groups (Figure 1), the bridge in obidoxime contains ether oxygen, rendering the carbon atoms in the ether linkage more electrophilic and susceptible to nucleophilic attack. Therefore, in addition to hydrolysis and oxidation of the oxime groups other degradation pathways involving bridge cleavage are possible for obidoxime but not for trimedoxime (Figure 1). Thus, it can be concluded that bridge cleavage, which in acidic solution occurs only after hydrolysis of the oxime groups (Christenson 1968a), is the source of the autocatalytic nature of the degradation. It seems that the formaldehyde produced during this bridge fragmentation reacts with the hydroxylamine formed in the rate-determining reversible hydrolysis step, to produce the corresponding oxime. Thus, the initial equilibrium is disrupted and additional obidoxime undergoes hydrolysis.

The results of the degradation studies on the six solutions listed above supported this hypothesis. Figure 4 shows the results obtained for the degradation of obidoxime (solution 1) and with hydroxylamine (solution 2) and formaldehyde (solution 3) added in equal amounts. It is evident that the addition of formaldehyde resulted in much faster degradation of obidoxime compared with obidoxime alone. Moreover, the added formaldehyde overrides the lag-time in obidoxime hydrolysis such that degradation followed pseudo-first-order kinetics. In contrast, addition of hydroxylamine extended the lag-time considerably, supporting the

Table 3. Correlation coefficients (r^2) for the degradation profiles of different solutions (Figures 4 and 5).

Solution	r^2
Obidoxime chloride (250 mg mL^{-1}, 0·696 M)	0·998[a]
Obidoxime chloride (250 mg mL^{-1}) plus hydroxylamine hydrochloride (12 mg mL^{-1}, 0·174·M)	0·993[a]
Obidoxime chloride (250 mg mL^{-1}) plus 37% formaldehyde solution (14 μL mL^{-1}, 0·174 M)	0·998[b]
Obidoxime chloride (60 mg mL^{-1}, 0·167 M) plus potassium (123 mg mL^{-1}, equivalent to ionic strength 2·08)	0·996[a]
Trimedoxime bromide (200 mg mL^{-1}, 0·448 M)	0·997[b]
Trimedoxime bromide (200 mg mL^{-1}) plus 37% formaldehyde solution (14 μL mL^{-1})	0·983[c]

[a] Fitted to autocatalytic model (equation 4), [b] fitted to first-order kinetic equation, and [c] fitted to zero-order kinetic equation.

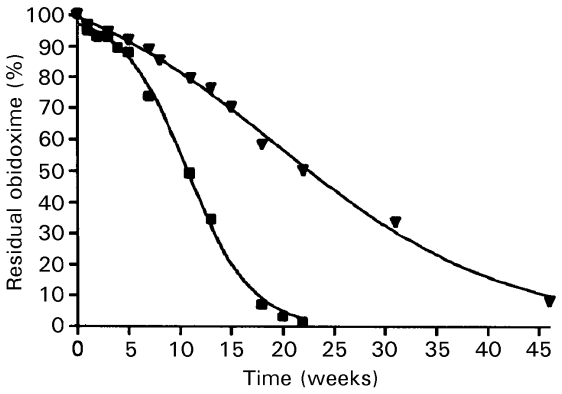

Figure 5. Degradation of obidoxime at 85°C: ■, 250 mg mL^{-1} (solution 1); ▼, 60 mg mL^{-1} (solution 4).

hypothesis that the rate-determining step in the degradation process is the initial hydrolysis of the oxime group. The results obtained from solutions 5 and 6 (Figure 4) give further support to the proposed mechanism of degradation. Addition of formaldehyde considerably increased the rate of degradation of trimedoxime.

Figure 5 compares the degradation profile of obidoxime in concentrated (250 mg mL^{-1}, solution 1) and dilute (60 mg mL^{-1}, solution 4) acidic solution. Autocatalysis is evident also in the more dilute solution, which is within the range of concentrations (up to 100 mg mL^{-1}) studied by Christenson (1968b). However, as the reaction between formaldehyde and hydroxylamine is second order, the rate of the reaction depends upon the concentration of both reactants and the autocatalysis is less noticeable in more dilute solutions of obidoxime.

Table 3 lists correlation coefficients for the degradation profiles of solutions 1–6, each fitted to the relevant kinetic equation. The pH profile of the obidoxime degradation (Figure 1) shows an initial decrease in pH coinciding with the lag period; this might be because of formation of the corresponding diacid. Subsequent bridge cleavage causes the pH to increase as non-quaternary pyridine compounds are formed. Formaldehyde produced at this stage leads to accelerated decomposition of obidoxime, which is accompanied by a further rise in pH.

Conclusions

The kinetics and mechanism of obidoxime degradation in concentrated acidic solutions was studied by HPLC analysis. It was shown that obidoxime undergoes autocatalytic degradation rather than the suggested (Christenson 1968b) mechanism involving pseudo-first-order pre-equilibrium degradation followed by zero-order post-equilibrium degradation. Formaldehyde formed during the degradation is the catalyst. Kinetic analysis of the data indicates that obidoxime ampoules can be stored at room temperature for more than 30 years before the content decreases by 10%.

Autocatalytic degradation of pharmaceuticals has seldom been reported in the literature. These findings suggest the possibility that autocatalysis might be involved in the degradation of other pharmaceuticals which do not fit simple first-order kinetics.

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