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A preformulation study on the kinetics of HI-6 in concentrated solution

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Summary

The degradation kinetics of HI-6 have been investigated at various temperatures and at different pHs at a concentration of 200 mg/ml. Both aqueous and other hydrophilic solvents (glycols and glycerol–water mixtures) have been used. The degradation of HI-6 follows pseudo-first-order kinetics with respect to HI-6. The observed rate seems to depend on a hydroxyl ion-catalyzed reaction (k_{OH}) and an un/water catalyzed reaction (k_0) which show influence at pH below 3. The pH profile shows a positive slope less than one, which seems to depend on an error in the determination of the rate constants at higher pH. k_{OH} was determined to be $1 \cdot 10^9$ and k_0 to be $0 \cdot 011$ h^{-1} at 60 °C. The observed rate seems to be independent of the dielectric constant of the solvent. The activation energy k_0 has been determined to be 82.0 kJ mol^{-1}. Based upon these data the predicted shelf life ($t_{90\%}$) at pH 0 will not be longer than 13 days at 25 °C and 9 months at 0 °C. Thus, the systems studied seem to be unsuitable to formulate a stable intramuscular formulation of HI-6.

Introduction

HI-6 (1-(2-hydroxyiminomethyl-pyridinium)-2-(4-carboxyamidopyridinium)dimethylether dichloride) has been reported to be an efficient agent for the treatment of organophosphorus poisoning (Wolthuis and Kepner, 1978). The compound has currently received a widespread interest for use as an antidote against organophosphorus chemical warfare (CW) agents, e.g. Soman. To be effective it must be administered in a prophylactic dose or immediately after the exposure to the CW-agent. HI-6 is a quaternary ammonium compound and will thus be poorly absorbed from the gastrointestinal tract. The intramuscular route, however, shows 100% bioavailability compared to intravenous administration (Simons and Briggs, 1983). Prophylactic and acute administration must therefore be performed parenterally. Since oximes have a short biological half-life (Sidell, 1976; Simons and Briggs, 1983) and due to the need of rapid onset of the treatment, the major use of HI-6 will probably be as parenteral preparations for self administration in the field, i.e. autoinjectors. The volume of current autoinjectors is limited to 2–3 ml. The therapeutic dose may be 0.5 g or more, which implies that an autoinjector solution of HI-6 will be comparably concentrated.

The molecule HI-6 contains an oxime and a

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carboxamide function, both probably vulnerable to degradation. Several reports have been published on the kinetics of similar oximes such as obidoxime (Christensson, 1968a and b; Christensson, 1972a; Bernasconi, 1965), pralidoxime (Boeke, 1978; Christensson, 1974; Prue et al., 1983; Fyhr et al., 1986), Lü20 and Lü40 (Christensson, 1972c) and R1 and R53 (Christensson, 1972d). These authors report similar degradation mechanisms for all oximes. An acidic reaction leads to the corresponding aldehydes and hydroxylamine. The reaction attains equilibrium at higher concentrations of oxime. The other reaction is a hydroxyl ion-catalysed dehydration of the oxime. It yields the corresponding nitrile and is followed either by hydrolysis to carboxamide or to pyridone and cyanide ion. The carboxamide is subsequently hydrolysed to carboxylic acid, which finally might undergo decarboxylation (Fyhr et al., 1986). The observed rate constants of these reactions show a minimum in the pH range 3–4. At very high or low pH the ether link of bispyridinium oximes might break to form pyridine derivatives and formaldehyde. A similar mechanism and kinetics have been reported for HI-6 (Markov et al., 1984) and HS-6 (Christensson, 1972b). The structure of HS-6 differs from that of HI-6 only in that the carboxamide moiety is in meta-position. These data have been obtained from dilute solutions (less than 0.03 M). Concentrations to be considered for autoinjectors are not less than 0.3 M for bispyridinium oximes, and might even be as high as 2.5 M for pyridinium oximes. Such high concentrations may, as such, affect the degradation mechanism and studies on concentrated solutions of pralidoxime chloride have revealed no influence of acidic catalysis (Prue et al., 1983; Fyhr et al., 1986).

The purpose of this preformulation study was to evaluate the possibility of formulating HI-6 as a 200 mg/ml i.m. solution for use in an autoinjector.

Materials and Methods

Chemicals

HI-6 [1], sodium heptylsulphonate [2] used as obtained. HCl, NaOH, KCl, succinic acid, citric acid, Na_2HPO_4, acetic acid and acetonitrile were of analytical grade [3]. Polyethyleneglycol 400 [4], propyleneglycol [5] and glycerol [6] of pharmaceutical grade (Ph. Eur.).

pH profile

Solutions of 200 mg/ml HI-6 were prepared. The solutions were buffered with HCl to pH 1.5 and 1.80, with succinic acid 0.25 M + citric acid 0.25 M to pH 3.8 and 4.8, and with Na_2HPO_4 1 M to pH 6.90. Initial studies were performed in sealed 2-ml glass ampoules at 100 °C. During the experiments, pH was found to decrease rapidly to about 1, even when the solutions were buffered with 1 M Na_2HPO_4. The final studies were therefore performed in a sealed jacketed vessel, thermostated to 60 °C. Additional pH control was achieved by using a pH-stat [7] and 0.1 M NaOH. The observed degradation rate was therefore compensated for dilution by assuming that NaOH was added with first order rate. The dilution rate was calculated and subtracted from the observed rate. Due to the dilution the degradation was followed for less than one half-life; most of the rates have been calculated from 30% degradation or less.

Temperature dependence

Two ml glass ampoules with 200 mg/ml HI-6 at a pH of 1.5, were stored at 5, 10, 15, 25, 37, 50, 60, 80 and 100 °C. The pH did not change significantly over the temperature range studied and the rate constants were calculated from 20 to 72% degradation.

Hydrophilic solvent mixtures

HI-6 was dissolved in water with 25% w/w polyethyleneglycol 400, 25% w/w propyleneglycol and 27% w/w glycerol respectively to give solutions of approximately 150 mg/ml. pH was not adjusted. The solutions were filled into 1 ml glass

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[1] Raylo Chemicals, Edmonton, Alta., Canada

[2] Eastman Kodak Co, U.S.A.

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

[4] BP Kemi, Sweden

[5] Nafta Kemi, Sweden

[6] Plastkemi Unichema, Sweden

[7] pH-meter 632, Impulsomat 614, Dosimat E535, Metrohm, Schweiz

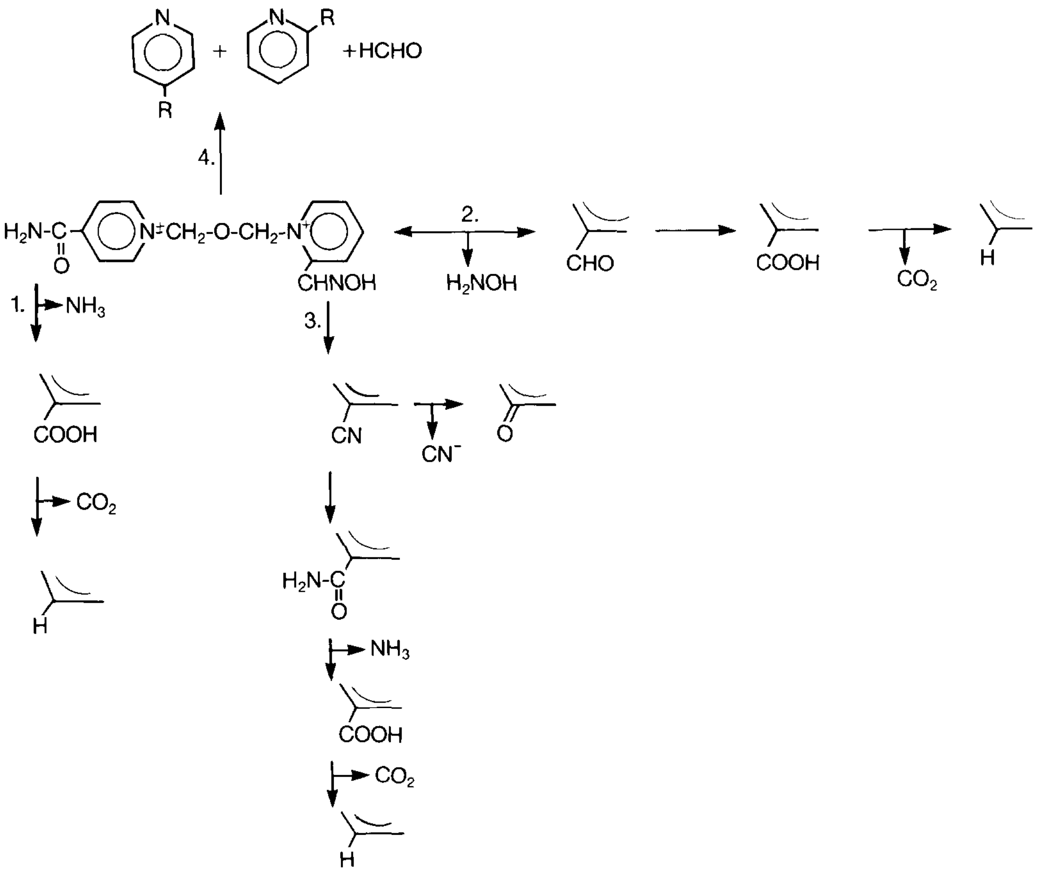

Fig. 1. Possible mechanisms for the degradation of HI-6.

ampoules and then stored at 100 °C in a water bath.

Analytical procedures

HI-6 was analysed using ion-pair reversed-phase HPLC (Brown et al., 1984). Column: μBondapak C-18 [8], 150 × 4 mm; mobile phase: sodium heptylsulphonate 0.01 M, acetic acid 0.025 M in 25% v/v acetonitrile/water; pump flow rate [9]: 1.5 ml/min; samples were injected with a sampling valve [10] fitted with a 20 μl loop. Detection was made with a variable wavelength UV-recorder [11] at 254 nm. Chromatograms were recorded using a computing integrator [12]. Samples were diluted with 0.001 M HCl to give an absorbance of approximately 0.03. Concentrations were calculated from standard curves established on the day of analysis.

Data evaluation

Linear and non-linear regression analysis were performed using a computer program [13].

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[8] Waters Ass., U.S.A.

[9] Constametric II, LDC, U.S.A.

[10] Rheodyne, U.S.A.

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

[12] Hewlett Packard 3392A, U.S.A.

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

Theory

Considering previous reports on the kinetics and mechanism of oximes, 8 reactions seem possible as shown in Fig. 1. Reactions are designated as hydrogen ion$_H$, hydroxyl ion$_{OH}$ and water or uncatalysed$_0$ reactions of the amide^A, oxime^O and ether link^E. The 8 reactions responsible for the disappearance of HI-6 are characterized by the rate constants: k_H^A, k_{OH}^A, k_0^A, k_H^O, k_{OH}^O, k_0^O, k_H^E and k_{OH}^E.

Thus, the degradation of HI-6 might be expressed by the following equation:

$$-\frac{d(HI)}{dt} = \big((k_H^A + k_H^O + k_H^E)(H) + (k_{OH}^A + k_{OH}^O + k_{OH}^E)(OH) + (k_0^A + k_0^O)\big)[HI] \quad (1)$$

The pH profile for the observed degradation of HI-6 (Fig. 2) indicates that the hydrogen ion catalysed paths does not influence the observed rate constant in concentrated solution. The different water/uncatalysed and hydroxyl-ion catalysed rate constants cannot be separated under the conditions studied which reduces Eqn. (1) to:

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

Since pH is maintained constant during the experiments [OH] becomes constant. Integration between $[HI]_0$ and [HI] and between t_0 and t thus gives:

$$\ln[HI] = \ln[HI]_0 - k_{obs}t \quad (3)$$

where k_{obs} contains:

$$k_{obs} = k_{oH}[OH] + k_0 = k_{OH}\frac{Kw}{[H]} + k_0 \quad (4)$$

During the pH dependence studies, part of the decrease in [HI] can be explained by dilution. It was assumed that the dilution followed first order rate kinetics according to the equation:

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

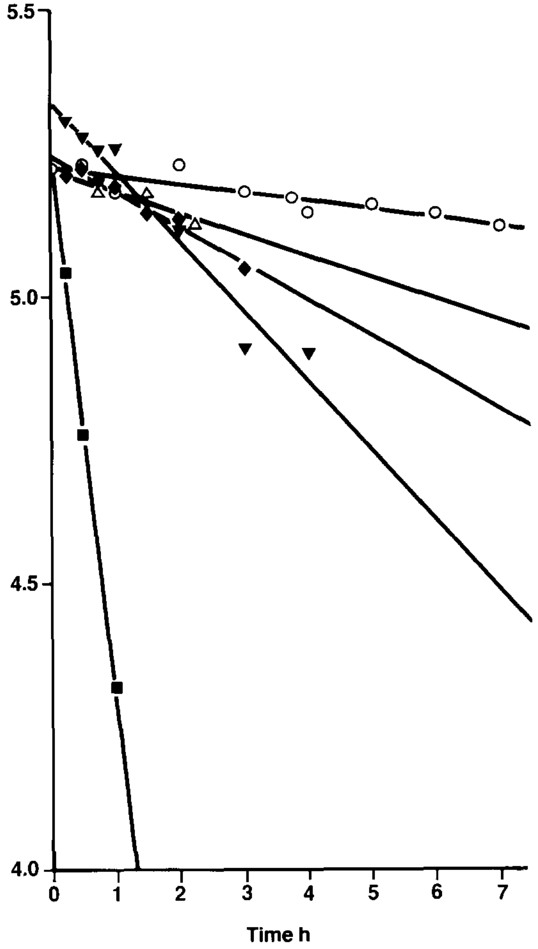

Fig. 2. pH dependence, concentration/time. ■, pH 6.9; ▼, pH 4.8; ◆, pH 3.8; △, pH 1.8; ○, pH 1.5.

where k_d is the rate of dilution calculated from the amount of NaOH solution added.

According to the "Operational pH Definition" (Linnet, 1970), pH is described as:

$$pH_x = pH_s + (E_x - E_s)\frac{F}{2.303RT} \quad (6)$$

where pH_x, pH_s designate the pH and E_x, E_s designate the emf of the measured and standard solutions respectively. The emf of a pH cell is expressed as:

$$E_{x,s} = (E_0 + E_j)\frac{F}{2.303RT} - \log a_{Hx,s} \quad (7)$$

where E_0 is the emf of the standard cell potential which is not affected by the composition of the solutions, and E_j is the liquid junction potential of the reference electrode (Linnet, 1970). E_j depends on the composition of the solutions and pH_x can only be correctly estimated if E_{jx} and E_{js} are equal. Thus, the composition of measured (x) and standard (s) solutions must be fairly similar. Since the ionic strengths of a 200 mg/ml HI-6 solution and standard buffer used were 1.67 and < 0.1, respectively, there will probably be an error in the estimation of pH in the HI-6 solutions. If $a_{Hx} = a_{Hs}$, introduction of Eqn. 7 into Eqn. 6 yields:

$$pH_x = pH_s + i \text{ or } (H)_x = (H)_s 10^{-i} \qquad (8)$$

where i is the difference in E_{js} and E_{Jx} due to different ionic strengths. Combining Eqn. 8 with Eqn. 4 then gives:

$$k_{obs} = k_{OH} \frac{Kw}{10^{-i}[H]} + k_0 \qquad (9)$$

The error, i, in Eqn. 9 represents a horizontal dislocation of the pH profile. This will not affect the value of k_0 but will cause an error in the determination of k_{OH}. The error i does not explain the deviation from a slope of +1. Another pH-dependent error, e, is therefore introduced into Eqn. 4 in order to allow mathematical treatment of such a deviation. After transformation

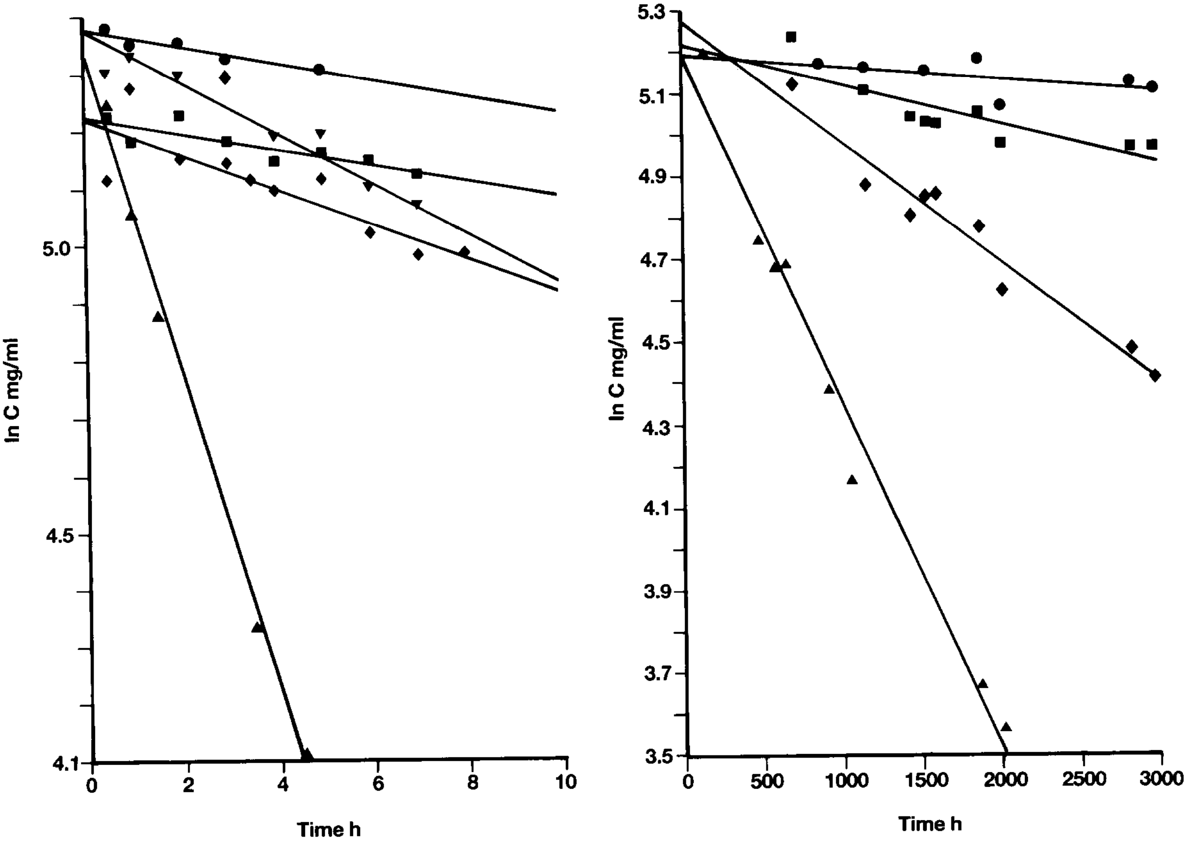

Fig. 3. Temperature dependence, concentration/time. A: ●, 50 °C; ■, 60 °C; ▼ ◇, 80 °C; ▲, 100 °C. B: ●, 5 °C; ■, 15 °C; ◆, 25 °C; ▲, 37 °C.

into logarithmic form the equation can be written:

$$\log k_{\mathrm{obs}} = \log\left(k_{\mathrm{OH}} \cdot 10^{\mathrm{pH} \cdot e - \mathrm{p}K_w} + k_0\right) \qquad (10)$$

Results and Discussion

The concentration–time plots used to establish the pH profile and the Arrhenius plot are presented in Figs. 2 and 3, respectively. Observed rate constants and regression data are given in Tables 1 and 2. An α-phase was observed during the preliminary studies. A similar behaviour has been reported for PAM in concentrated solutions (Fyhr et al., 1986), and zero values were therefore omitted from the regression analysis. The degradation of HI-6 seems to follow pseudo-first-order kinetics at all investigated pHs and temperatures.

According to Eqn. 4, the pH profile in Fig. 4 would depict a linear relationship with a slope of +1 and, depending on the value of k_0, approach a horizontal dependence at low pH. If $k_0 \ll k_{\mathrm{OH}}[\mathrm{OH}]$ down to pH 0, a linear pH profile would be expected, Eqn. 4. Linear regression gives:

$$k_{\mathrm{obs}} = -2.6(\pm 0.44) + 0.28(\pm 0.102) \cdot \mathrm{pH}$$

$$(\pm \mathrm{S.D.},\ n = 5,\ r^2 = 0.71).$$

It can therefore be concluded that log k_{obs} vs pH follows a non-linear relation and that a pH-independent reaction (k_0) gains influence at lower pH. A relation according to Eqn. 9 would still yield a slope of +1 for the hydroxyl-catalysed reaction. The value of the error i cannot be estimated unless pH is measured using techniques without liquid junctions (hydrogen electrode). By fitting Eqn. 10 to the data shown in Fig. 4 the following rate constants were obtained: $k_{\mathrm{OH}} = (1 \pm 3.3) \cdot 10^9$, $k_0 = 0.011 \pm 0.0047\ \mathrm{h}^{-1}$, $e = 0.68 \pm 0.224$ ($\pm$S.D., $n = 5$, $r^2 = 0.990$), where e is the slope of $k_{\mathrm{OH}}(\mathrm{OH})$. Thus, the highest stability will be obtained in the pH range 0–3. Similar kinetics was found for pralidoxime chloride in 43% solution, although the slope of $k_{\mathrm{OH}}[\mathrm{OH}]$ was close to +1 (Fyhr and Brodin, 1987). The deviation in this study might be explained by a larger dilution of the reaction solution the higher the pH, Table 1. The observed rate of PAM-Cl increases with concentration (Fyhr et al., 1986). Dilution of the reaction solution might therefore reduce the observed rate. Furthermore, the pH error (Eqns. 6–8) depends on the ionic strength which decreases through the dilution. The decreasing ionic strength might lead to changing "true" pH since E_j might be affected (Eqn. 7). Thus, the suggested pH-profile must be taken cautiously.

The activation energy of k_0 was calculated from observed rate constants (Table 2) according to the Arrhenius equation. The linear regression was found to be (Fig. 5):

$$\ln k_{\mathrm{obs}} = 25.1(\pm 2.01) - 9.9(\pm 0.64) \cdot \frac{1}{T}$$

$$(\pm \mathrm{S.D.},\ n = 9,\ r^2 = 0.971).$$

The activation energy was calculated to be 82.0 kJ mol^{-1}, which is close to the value normally found for hydrolytic reactions. The value is comparable to that found for pralidoxime in dilute solutions, 71 kJ mol^{-1} (Ellin et al., 1962). In concentrated

TABLE 1

Regression data — pH profile at 60 °C

pH	$k_{\mathrm{obs/d}}$ (h^{-1})	S.D.	i	S.D.	n	r^2	k_{d} (h^{-1})	k_{obs} (h^{-1})
1.5	0.014	0.0033	5.225	0.0137	8	0.758	0	0.014
1.8	0.037	0.0206	5.22	0.033	3	0.761	0.025	0.012
3.8	0.063	0.0054	5.245	0.0084	7	0.965	0.056	0.007
4.8	0.121	0.0119	5.341	0.0250	6	0.954	0.075	0.046
6.9	0.95	0.061	5.26	0.041	3	0.996	0.451	0.50

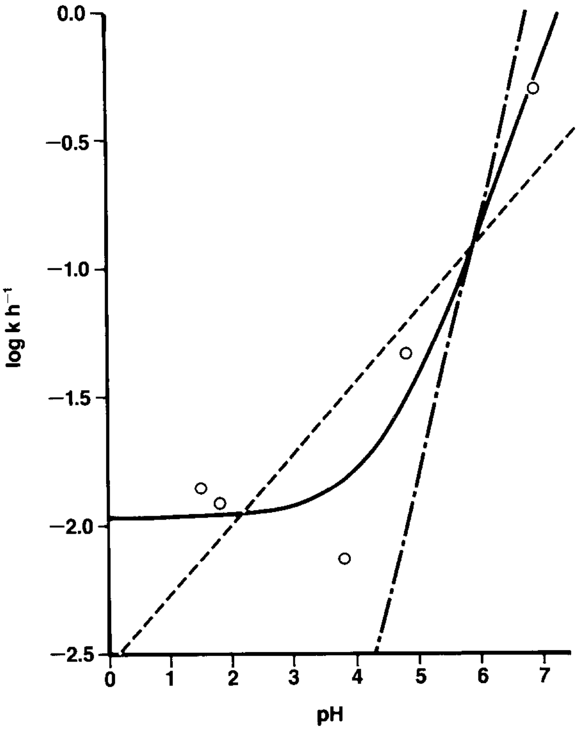

Fig. 4. pH profile. (———), Eqn. 10; (- - - - -), Linear regression; (· — · — ·), Slope = 1.

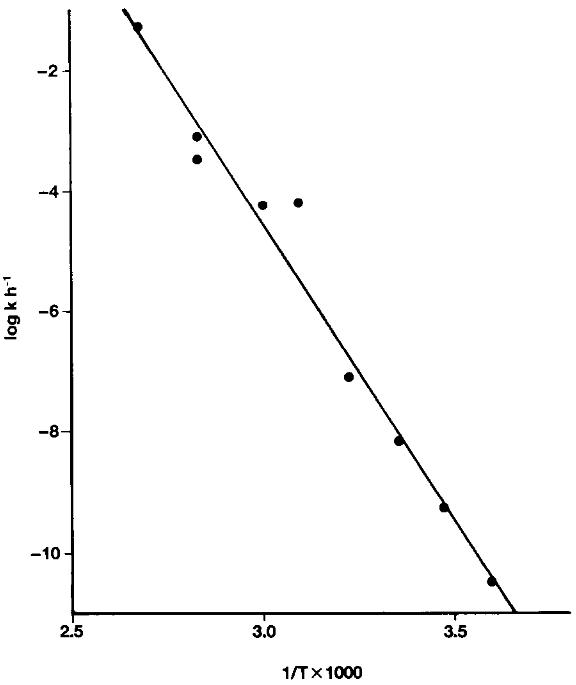

Fig. 5. Arrhenius plot.

solutions of PAM-Cl the corresponding values have been found to be 112 (Ellin, 1982) and 102 kJ mol^{-1} (Fyhr and Brodin, 1987).

Based upon an activation energy of 82 kJ mol^{-1} and the lowest possible degradation rate, i.e. k_0 at pH 0–3, the predicted shelf-life (90% labeled) will not be longer than 13 days and 9 months at storage temperatures of 25 and 0 °C, respectively.

The observed rate ought to be affected by the dielectric constant of the solvent (Florence and Attwood, 1981):

$$\log k_{\mathrm{obs}} = \log k_{\epsilon} - K \cdot \frac{z_{\mathrm{A}} \cdot z_{\mathrm{B}}}{\epsilon} \tag{11}$$

Table 2

Regression data — Arrhenius plot at pH 1.5

t (°C)	k (h)	S.D.	i	S.D.	n	r^2
5	2.8E-5	1.8E-5	5.19	0.037	7	0.31
15	9.6E-5	2.3E-5	5.22	0.044	9	0.71
25	2.9E-4	2.5E-5	5.27	0.048	9	0.95
37	0.00083	5.0E-5	5.19	0.058	8	0.98
50	0.015	0.0031	5.378	0.0087	5	0.88
60	0.014	0.0032	5.225	0.0137	8	0.76
80	0.031	0.0083	5.22	0.034	9	0.66
80	0.044	0.0046	5.377	0.0219	9	0.93
100	0.279	0.0120	5.34	0.032	5	0.99

TABLE 3

Rates of mixed solvent solutions

Solvent	% w/w	X_2	Observed rate (h^{-1})
Water	100		0.2791
PEG 400	25	0.0147	0.2867
PPG	25	0.0730	0.3804
Glycerol	27	0.0674	0.2215

Where k_ϵ is the rate constant at infinite dielectric constant, K is a constant depending on system and temperature and z_A, z_B are the charges of the reacting species. Thus, if the degradative reaction is hydroxyl ion-catalysed hydrolysis or dehydration of the cationic oxime, the observed rate will be expected to increase with a lower dielectric constant. As HI-6 is a quaternary ammonium compound, it is soluble only in polar solvents, and pure organic solvents cannot be used. Instead, polar organic solvent/water systems (PEG, PPG and glycerol) may be used to give saturated solutions of HI-6. The regression analysis reveals good fits to (pseudo) first-order kinetics with respect to HI-6. The following observed rate constants and intercepts for each solvent system were calculated by means of linear regression analysis, Eqn. 3:

PEG; $\ln[\mathrm{HI}] = 4.99(\pm 0.112) - 0.29(\pm 0.046) \cdot t(\pm \mathrm{S.D.}, n = 5, r^2 = 0.929)$.

Glycerol: $\ln[\mathrm{HI}] = 4.896(\pm 0.0255) - 0.221 (\pm 0.0058) \cdot t(\pm \mathrm{S.D.}, n = 5, r^2 = 0.998)$.

PPG; $\ln[\mathrm{HI}] = 5.035(\pm 0.0055) - 0.380(\pm 0.0224) \cdot t(\pm \mathrm{S.D.}, n = 5, r^2 = 0.990)$.

In Table 3, the observed rates are compared with the rates obtained in water. Although the data are incomplete and determinations at different dielectric constants for each solvent system are necessary, the results still indicate that the observed rate constants are unaffected by the solvent system. This might indicate that the dominating reaction is uncatalysed or catalysed by an uncharged species (e.g. water).

Conclusion

Based on the current preformulation data it seems difficult to formulate an i.m. 200 mg/ml solution of HI-6 with a sufficient shelf-life, i.e. at least 3 years, using water or hydrophilic solvent mixtures.

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