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Degradation kinetics of ifosfamide in aqueous solution

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Abstract

The degradation process of ifosfamide in aqueous solution at 50°C was investigated at different concentrations, different pH ranges (3.6–9.8), different molarity and constant ionic strength. The observed rate obtained by measuring the remaining intact ifosfamide was shown to observe first-order kinetics and was shown to be a specific acid and base catalysis. The pH rate profile in buffer solutions showed a plateau between pH 4 and 7.9. In order to determine the effect of temperature, the kinetic process at four temperatures (40, 50, 60 and 70°C) was studied. The apparent heat of activation for ifosfamide degradation in solution was found to be 118 kJ/mol and by application of the Arrhenius equation the stability at 25°C (k_{25}) and the shelf-life (t_{90}) has been predicted. The agreement between the thin-layer chromatography (TLC), nuclear magnetic resonance (NMR) and high performance liquid chromatography (HPLC) supports the hypothesis presented concerning the reactions involved in the degradation of ifosfamide solutions.

Keywords: Ifosfamide; Chemical stability; Degradation kinetics

Ifosfamide (chemical name: 3-(2-chloroethyl)-2-(2-chloroethylamino)tetrahydro 2*H*-1,2,3-oxazaphosphorine oxide) is an alkylating oxazaphosphorine with structural relation with cyclophosphamide. They both are used in the treatment of a wide variety of tumors including small cell lung cancer, soft tissue sarcoma, osteosarcoma and ovarian cancer (Brade et al., 1985; Zalupski and Baker, 1988). In numerous murine cancer models, ifosfamide has demonstrated equal or superior activity compared to cyclophosphamide. Some human studies suggest

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that ifosfamide is less myelosuppressive than cyclophosphamide (Brock, 1983; Elias et al., 1989; Lind et al., 1989). Data concerning the chemical stability of ifosfamide are scarce; however, some studies are available in the literature (Kaijser et al., 1992; Le Roux et al., 1995). In this work, we studied the kinetics of hydrolytic reactions of ifosfamide in aqueous solution by the HPLC method under different experimental conditions. The degradation accelerated method was used to calculate the observed rate constant at 25°C (k_{25}) and (t_{90}) by application of the Arrhenius equation. The degradation scheme was deduced by means of the NMR and TLC techniques.

Ifosfamide was gift from Funk Laboratories (Manlleu, Spain). All other chemicals were of analytical grade and were used as received from the various sources. For the general investigation the buffers used were: at pH 3.6, 5 and 5.6, acetic acid–sodium acetate; at pH 5.7, 6.5, 7.1, 7.5 and 7.9, monosodium phosphate–disodium phosphate and at pH 9.8, monosodium carbonate–disodium carbonate. A constant ionic strength of 0.5 was maintained for each buffer by adding an appropriate amount of potassium chloride. The solutions were freshly prepared and the pH was measured at 25°C by a research pH-meter and sc-glass electrodes. All assays were performed by HPLC at room temperature (20–25°C) according to the method described by Marginon et al. (1986). This procedure was carried out by liquid chromatograph with an isocratic pump and diode array ultraviolet (UV) light detector (model HP-1090, Hewlett Packard, Barcelona, Spain) coupled to an integrator (HP-3396 D, Hewlett Packard).

An ODS-Hypersil column (10 cm × 4.6 mm internal diameter and 5 μm particle size) was used as stationary phase (Hewlett Packard). The mobile phase had a flow rate of 1.5 ml/min under isocratic conditions of 30% acetonitrile and 70% water (double-distilled water was used after filtration in a Millipore system). The ultraviolet detector was set at 210 nm. Under these conditions, the retention time for the ifosfamide was 1.5 min. The linearity and reproducibility of the method were studied and the stability-indicating capability of the method was demonstrated by exposing the samples at forced conditions of pH and temperature (Muñoz et al., 1992). The decomposition product peaks were well resolved from the peak for the intact drug.

The ratio drug/standard peak area was used for quantitation. The initial concentration of ifosfamide was designated as 100% and all subsequent concentrations were expressed as percentages of the initial concentration.

For kinetics study, exactly 0.5 g of ifosfamide was reconstituted with appropriate buffer solutions and transferred to 100 ml volumetric flasks. They were stored in a constant temperature bath which was regulated by a thermostat with ± 0.1°C precision. Samples were taken at suitable intervals and stored at 4°C until analysis time. Previously the stability at 4°C was assayed and ifosfamide was found to be stable for more than 1 month.

The degradation process of ifosfamide in water solution was studied at 50°C and at various initial concentrations (0.5%, 0.6%, 0.7%, 0.8% and 0.9%) and was found to observe pseudo-first-order kinetics. The rate constants and their confidence interval were determined by the statistical method described in the literature (de Bolós et al., 1986). A mean value of $5.59 \pm 0.87 \times 10^{-2}$/day was found for them.

The pH rate profile was obtained by plotting the logarithm of the observed rate constants of ifosfamide degradation (initial concentration 0.5%)in aqueous solution against the pH values of the buffer (M = 0.1 and $\mu = 0.5$) at 50°C. A plateau rate at a pH of 4–7.9 was observed.

The observed rate in this pH apparent rate profile was actually a summation of a series of catalytic reaction rates induced by hydrogen and hydroxyl ions and water molecules.

The pH rate profile may be explained, assuming the following reactions to occur in the pH region studied:

$$\text{Ifosfamide(I)} + \text{H}^+ \xrightarrow{k_1/\text{H}_2\text{O}} \text{Products},$$

$$\text{Ifosfamide(I)} \xrightarrow{k_2/\text{H}_2\text{O}} \text{Products},$$

$$\text{Ifosfamide(I)} + \text{OH}^- \xrightarrow{k_3/\text{H}_2\text{O}} \text{Products},$$

where k_2 is the first-order rate constant for the spontaneous hydrolysis and k_1 and k_3 are the rate constants for specific acid–basic catalysis of ifosfamide.

The overall velocity is equal to the sum of the rate of these reactions:

$$-\mathrm{d[I]}/\mathrm{d}t = k_1[\mathrm{I}][\mathrm{H_3O^+}] + k_2[\mathrm{I}] + k_3[\mathrm{I}][\mathrm{OH^-}], \quad (1)$$

$$k_{obs} = k_1[\mathrm{H_3O^+}] + k_2 + k_3[\mathrm{OH^-}]. \quad (2)$$

The concentrations of $[\mathrm{OH^-}]$ may be calculated from the pH measurements. Harned and Harmer (1953) have shown that:

$$k_w = [\mathrm{H_3O^+}][\mathrm{OH^-}] = 5.476 \times 10^{-14} \quad (3)$$

in solutions of potassium chloride at 50°C and $\mu = 0.5$. Combining Eqs. (3) and (2) gave:

$$k_{obs} = k_1[\mathrm{H_3O^+}] + k_2 + k_3 \cdot 5.476 \times 10^{-14}/[\mathrm{H_3O^+}] \quad (4)$$

From applying experimental values to Eq. (4) by the multiple regression method, the values of k_1, k_2 and k_3 were obtained:

$k_1 = 455.3\ \mathrm{mol^{-1}\,l\,days^{-1}}$,

$k_2 = 8.91 \times 10^{-2}/\mathrm{day}$,

$k_3 = 452.9\ \mathrm{mol^{-1}\,l\,days^{-1}}$,

and the general equation:

$$k_{obs} = 455.3[\mathrm{H_3O^+}] + 0.0891 + (452.9/[\mathrm{H_3O^+}]) \quad (5)$$

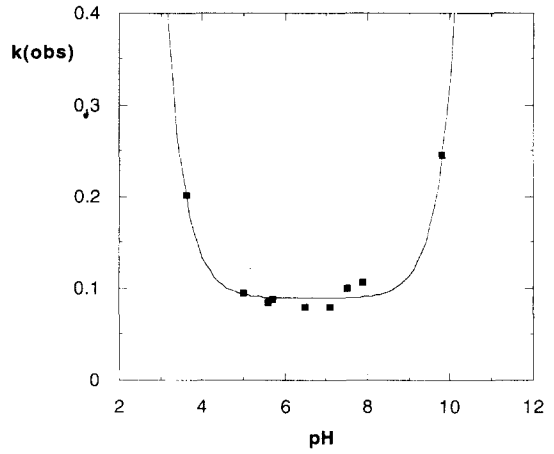


Fig. 1. pH rate profile from degradation process of ifosfamide in aqueous solution. Squares represent the experimental points and solid line the theoretical ones obtained from the proposed equation: $k_{obs} = 455.3[\mathrm{H_3O^+}] + 0.0891 + (452.9/[\mathrm{H_3O^+}])$.

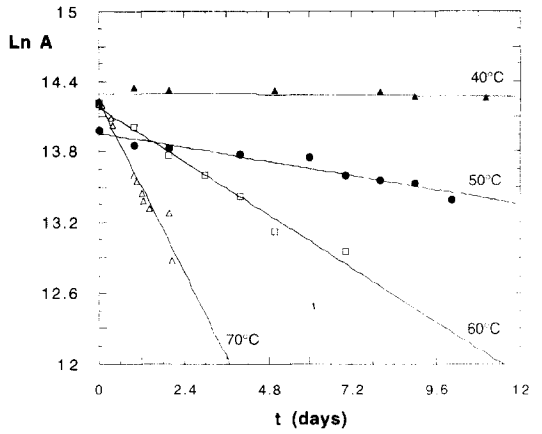


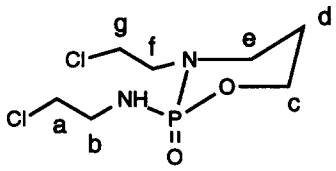
Fig. 2. The effect of temperature in degradation process of ifosfamide in acetate buffer solution at pH = 5.0, M = 0.1 and $\mu = 0.5$.

Fig. 1 shows the experimental results obtained and the theoretical ones. The relatively good agreement of the experimental data and the theoretical profile does not prove that the three reactions proposed are the correct ones. Other reactions could also lead to the same observed experimental dependence.

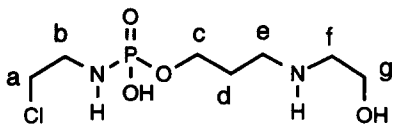
The catalytic effect of acetic/acetate buffer (0.10, 0.15, 0.20 and 0.25 M) and ionic strength influence (0.1, 0.2 and 0.5) were also studied at pH = 5 and at 50°C. No significant changes were observed in the values of the observed rate constants at different buffer molarities ($\mu = 0.5$) and at different ionic strengths (M = 0.1). These results confirm the non-ionic condition of ifosfamide at the pH studied as well as the fact that its degradation in acetic/acetate buffer is suitable for a specific acid–base catalytic model.

In order to apply the Arrhenius law to predict the stability of ifosfamide at 25°C the temperature dependence was studied at 0.5 initial ifosfamide concentration, in acetic/acetate buffer (pH = 5, M = 0.1 and $\mu = 0.5$). The first-order plots obtained and the observed rate constants at temperatures ranging from 40 to 70°C were shown in Fig. 2. The heat of activation was calculated and found to be 118 kJ/mol and the calculated rate constant at 25°C was $1.12 \pm 0.17 \times 10^{-3}$/day. From this value the $t_{0.90}$ was determined and found to be 94 ± 1.4 days.

Table 1
NMR spectral assignment of ifosfamide and compound A



Ifosfamide



Compound A

	^1H	^{13}C	^1H	^{13}C
(a)	3.54 t (J = 6 Hz)	52.5	3.7	47.7
(b)	3.19 m	53.8	3.3	48.2
(c)	4.23 dt (J = 12, 5.5 Hz)	75.6	3.9	69.8
(d)	1.88 quint (J = 5.5 Hz)	32.7	1.87	33.5
(e)	3.17 tdd (J = 10, 5.5, 2 Hz)	49.8	3.1	52.2
(f)	3.25 m	56.2	3.2	52.2
(g)	3.61 m	48.9	3.75	63.6

For characterization of the major hydrolytic product of ifosfamide, aqueous solutions of ifosfamide at 50°C were studied. Two sets of a solution of ifosfamide in unbuffered D_2O (10 mg/0.7 ml and 90 mg/0.7 ml) were kept at 50°C, and the change in the composition of the reaction system was monitored by ^1H and ^{13}C spectroscopy, respectively. The disappearance of ifosfamide occurs at 21 days. The major product formed, compound A, was identified. The degradation process at this temperature remains the same as at room temperature but is accelerated. The process was monitored using TLC, HPLC and NMR techniques.

Of the three spots observed by TLC, one corresponds to ifosfamide and two to final products that are more polar than the parent compound. The most polar degradation product (compound A) appears after 7 days and the other after 8–9 days.

Compound A and the free ifosfamide base were characterized by ^1H and ^{13}C NMR spectra (Table 1). The proton signals were assigned on the basis of distinct chemical shifts and coupling constants as well as the COSY spectra. The assignment of the carbon peaks was made from estimation of the substitution effects and using the DEPT pulse sequence technique. Moreover, the HMQC spectra are also registered. The ^1H and ^{13}C NMR chemical shifts and the assignments are presented in Table 1.

Compound A is an open chain product-3-[(2-hydroxyethyl)amino]propyl *N*-(2-chloroethyl)-phosphoramidate-. It can be formed by a hydrolytic cleavage of P(O)–N bond and substitution of one chlorine atom by a water molecule. Compound A does not show any methine carbon and shows a pattern very different to ifosfamide, thus indicating the disappearance of the ring. The maintenance of the coupling between the methylene linked at the oxygen atom with the ^{31}P ($J = 5.5$ Hz) involves the linkage of the subunit $P(O)-O-CH_2CH_2CH_2-N$ as reflected in the homocorrelated $^1H-^1H$ spectrum. The substitution at carbon *g* of the chlorine atom could be accounted for by the anchimeric assistance of the amine nitrogen generated after the cleavage of the heterocyclic ring.

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