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The hydrolysis of L-phenylalanine mustard (melphalan)

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Summary

The chemical stability of L-phenylalanine mustard (melphalan) was studied in aqueous solution using an HPLC procedure. The kinetics of degradation was investigated as a function of pH (1–13), ionic strength (0.12–1.0 M), temperature (25–50°C) and chloride concentration (0–0.5 M). In addition, the kinetics of the production of chloride ions arising from the degradation of melphalan was studied using an ion-specific electrode. No evidence of specific acid/base or general acid/base catalysis was found and the results were explained in terms of the conversion of melphalan to hydroxymelphalan occurring via a unimolecular, rate-determining reaction in which the drug is converted to an ethyleneimmonium ion. The effect of pH on stability was explained in terms of the degree of protonation of the various ionic species of melphalan (MH_3^{2+}, MH_2^{+}, MH and M^{-}) present in solution and their abilities to take on an additional positive charge and form the corresponding ethyleneimmonium ions (MH_3^{3+}, MH_2^{2+}, MH^{+}, M). The kinetically determined pK_a for the tertiary amine of melphalan was found to be 1.42. The pK_as for the carboxylate and amino functional groups were found to be 2.75 and 9.17, respectively, which compare well with the corresponding literature values of 2.59 and 9.25 for the respective groups of phenylalanine.

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Introduction

L-Phenylalanine mustard (4-di-(2-chloroethyl)aminophenylalanine, melphalan, L-PAM) was first synthesized in 1954 by Bergel and Stock in a search for alkylating agents with greater selectivity. Melphalan is active against multiple myeloma (Alexanian et al., 1968) and cancers of the breast, ovary and testes (Wasserman et al., 1975), when administered orally or by bolus intravenous injection. Recently, high dose melphalan (120–225 mg/m^2, over 3 days) given by slow intravenous infusion has been used for the successful treatment of various refractory cancers (Lazarus et al., 1983).

The hydrolysis of melphalan has been studied under simulated physiological conditions in both plasma (Furner et al. 1976) and buffer at pH 7.4 (Flora et al., 1979; Chang et al., 1978a and b), since this reaction is believed to be a major source of biotransformation of the drug (Alberts et al., 1979). It has been reported that the stability of melphalan is improved by decreasing the pH (Flora et al., 1979), decreasing the temperature (Flora et al., 1979) and by the addition of chloride (Chang et al., 1979). However, detailed information on these effects and the mechanism of hydrolysis of melphalan is limited. Consequently, the present study was undertaken to obtain detailed knowledge on the kinetics of melphalan in aqueous solutions as a function of pH, chloride ion concentration, ionic strength and temperature.

Materials and Methods

Chemicals and reagents

Melphalan was obtained from Sigma Chemicals, St. Louis, MO and was used as received. HPLC grade methanol and buffer constituents were obtained from Fisher Scientific, Fair Lawn, NJ. Sodium hydroxide (1 M) was prepared from Dilut-it Analytical Concentrate supplied by J.T. Baker Chemicals, Phillipsburg, NJ. De-ionized water was filtered through 0.45 μm nylon filters (Rainin Instruments, Woburn, MA) before use.

Buffer solutions

For solutions of pH 2.2–8.0 McIlvaine buffers were used as described by Elving et al. (1956). Below pH 2.2, nitric acid was used. Above pH 8.0, boric acid–sodium hydroxide buffers were prepared as described by Perrin and Dempsey (1974) and above pH 10.2 sodium hydroxide was used. Unless otherwise stated, the ionic strength of the solutions was adjusted to 0.5 M with $NaNO_3$.

Liquid chromatography

The loss of melphalan from aqueous solutions was monitored by reversed-phase high-performance liquid chromatography using an ODS Hypersil column (HETP, Sutton, UK) (150 mm × 4.6 mm, i.d.) prepared as described by Bristow et al. (1977), and a mobile phase of 50% methanol in acetate buffer (pH 4.7, 0.1 M). A flow rate

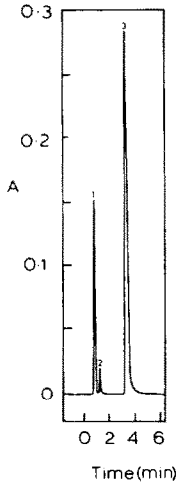


Fig. 1. Separation of melphalan from its degradation products in aqueous solution. Column: ODS Hypersil (15 cm×4.6 mm i.d.); mobile phase: methanol/acetate buffer (pH 4.7, 0.1 M) (50/50); flow rate: 1.8 ml/min; temperature: ambient. Peaks: 1, buffer components and degradation products; 2, attributed to hydroxymelphalan: 3, melphalan.

of 1.8 ml/min and ambient temperature (22 ± 1°C) were used throughout. Under these conditions melphalan eluted with a retention volume of 6.7 ml. With the exception of peak 2 (Fig. 1) attributed to hydroxymelphalan (MOH, Scheme 1) all the other degradation products (Scheme 1) and buffer components eluted at the solvent front.

The liquid chromatograph consisted of a Constametric IIG pump (LDC, Riviera Beach, FL), a Negretti model 194 injector (20-μl sample loop) (HPLC Technology, Palos Verdes Estates, CA) and Spectromonitor D variable wavelength detector (LDC) operated at 260 nm. Peak areas were measured using a data system consisting of an HP87 microcomputer (Hewlett Packard, Palo Alto, CA), a Nelson Analytical Intelligent Interface (model 761, Nelson Analytical, Cupertino, CA), a Nelson Analytical Software model 366, version 2.2, and a Hewlett Packard dual $3\frac{1}{2}''$ disc drive (model 9121). The chromatograms were displayed on the CRT of the HP87 and on a Recordall 5000 strip chart recorder (Fisher Scientific, Fair Lawn, NJ). The relative standard deviation (n = 6) of the HPLC methodology for the analysis of melphalan was 2.03% and the lower limit of detection was 100 ng/ml.

Chloride determinations

A combination chloride electrode model 96-17B and a digital pH/millivolt meter model 611 (both Orion Research, Cambridge, MA) were used to monitor the production of chloride due to the degradation of melphalan (Scheme 1). A Haake Circulator (Saddle Brook, NJ) was used for temperature control (±0.1°C). Standard solutions were prepared in the pre-determined linear range of 4.4×10^{-4} to 10^{-1} M Cl^- in phosphate buffer (pH 6.0). Standard curves of voltage (E) vs log [Cl^-] were constructed at each temperature under study. Melphalan (50 mg) was dissolved in

ethanol (1.5 ml) and nitric acid (20 μl). This solution was immediately transferred to a 100 ml beaker containing 48.5 ml of buffer (pH 6.0, $\mu = 0.50$), equilibrated to temperature. Voltage readings were taken as a function of time (up to 6 h) and compared with the calibration curve to obtain the chloride concentration. Readings were taken after 48 hours to obtain an accurate value of $[M]_0$ ($[M]_0 = [Cl]_\infty/2$) (Scheme 1).

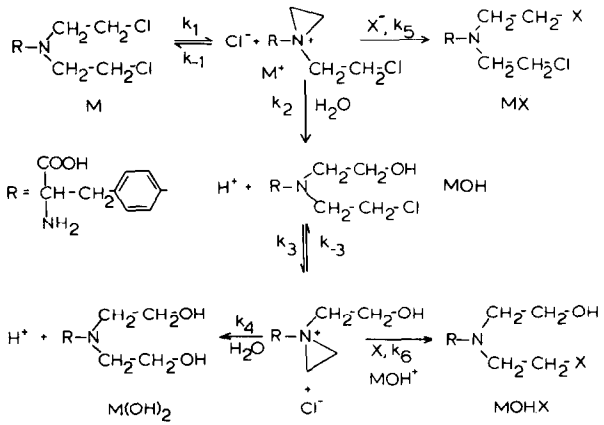
Kinetic studies

The influence of pH (1–13, $\mu = 0.5$, 37°C), temperature (25–50°C, pH 2, 6, 9 and 11, $\mu = 0.5$) and ionic strength (0.15–1.00, pH 6, 37°C) on the degradation of melphalan was investigated. Melphalan (100 μg/ml, 3.28×10^{-4} M) was dissolved in the appropriate buffer with the aid of sonification and placed in a thermostated (±0.1°C) water bath. The solution was allowed to equilibrate to temperature for 10 min before the first sample (100 μl) was taken and injected (20 μl) directly onto the HPLC column. The first injection was taken as $t = 0$ since in all cases the loss of melphalan was first-order and independent of its initial concentration. Subsequent samples were injected at appropriate intervals and the peak areas found for melphalan were recorded as a percentage of that found at $t = 0$. These values were taken as a percentage of concentration at $t = 0$, since the within-day relative standard deviation of the peak areas of analytical standards was 2.03% and the peak areas of these standards were linearly related to the concentration injected (0.10–200 μg/ml). Single determinations were made for each set of concentrations and sampling was continued for at least 4 half-lives.

Theory

The degradation of melphalan (Scheme 1) has been reasonably assumed (Chang

Scheme 1. Proposed pathway for the hydrolysis of melphalan in aqueous solution (after Bartlett et al., 1949).



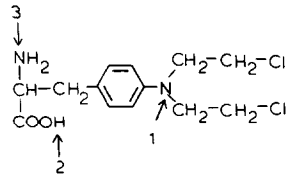


Fig. 2. Structures of melphalan showing three ionizable functional groups.

et al., 1978a and b; Flora et al., 1977) to procede via the mechanism proposed earlier (Golumbic et al., 1946; Fruton and Bergmann, 1946; Bartlett et al., 1947) for the transformation of methyldi(2-chloroethyl)amine in water. The rate-limiting step for the transformation of 2-chloroethylamines is the cyclization reaction to give the corresponding ethyleneimmonium ions with the loss of chloride (Bartlett and Swain, 1949). Ethyleneimmonium ions are highly susceptible to substitution by nucleophiles which in the case of water results in hydroxylation (Scheme 1). The hydroxylated analogues of bifunctional alkylating agents such as melphalan may react further to produce the corresponding dihydroxy-analogues. Side reactions with nucleophiles (X) other than water may occur giving rise to MX, MXCl and MX_2 (Scheme 1) (Chang et al., 1979).

Scheme 1 shows the generalized mechanism of the nucleophilic substitution of melphalan in aqueous solution. However, melphalan has three ionizable functional groups (Fig. 2) and up to four species may exist in aqueous solution such that

$$[M] = [MH_3^{2+}] + [MH_2^+] + [MH] + [M^-] \quad (1)$$

The appropriate dissociation constants for melphalan are given by Eqns. 2–4, respectively.

$$K_{a,1} = [H^+][MH_2^+]/[MH_3^{2+}] \quad (2)$$

$$K_{a,2} = [H^+][MH]/[MH_2^+] \quad (3)$$

$$K_{a,3} = [H^+][M^-]/[MH] \quad (4)$$

The fractions of each species present in aqueous solution may be given by Eqns. 5–8.

$$f_{(MH_3^{2+})} = [H^+]^3/B \quad (5)$$

$$f_{(MH_2^+)} = [H^+]^2 K_{a,1}/B \quad (6)$$

$$f_{(MH)} = [H^+] K_{a,1} K_{a,2}/B \quad (7)$$

$$f_{(M^-)} = K_{a,1} K_{a,2} K_{a,3}/B \quad (8)$$

where

$$B = [H^+]^3 + [H^+]^2 K_{a,1} + [H^+] K_{a,1} K_{a,2} + K_{a,1} K_{a,2} K_{a,3} \quad (9)$$

Results and Discussion

At constant pH, ionic strength and temperature the overall loss of melphalan was first-order over at least 4 half-lives consistent with previous observations (Flora et al., 1979; Chang et al., 1978a and b, 1979). The pseudo-first-order rate constants (k_{obs}) were calculated by least-squares linear regression from the slope of linear plots of the logarithms of the percentage of melphalan remaining against time. Representative first-order plots are shown in Fig. 3.

Influence of pH

The influence of pH on the degradation of melphalan was investigated over the range 0.91–13.00 at 37°C and an ionic strength of 0.5. There was no evidence of any general acid/base or specific acid/base catalysis. Instead the data (Fig. 4) were consistent with a unimolecular reaction of $M \rightarrow M^+$ (Scheme 1) being the rate-determining step. The form of the log k_{obs}–pH profile (Fig. 4) was consistent with the different rates at which the four ionic species of melphalan (MH_3^{2+}, MH_2^+, MH, M^-) are converted to their corresponding ethyleneimmonium ions. The respective micro rate constants for these reactions are $k_1^{(MH_3^{2+})}$, $k_1^{(MH_2^+)}$, $k_1^{(MH)}$ and $k_1^{(M^-)}$. It follows that k_{obs} is given by

$$k_{obs} = k_1^{(MH_3^{2+})} f_{(MH_3^{2+})} + k_1^{(MH_2^+)} f_{(MH_2^+)} + k_1^{(MH)} f_{(MH)} + k_1^{(M^-)} f_{(M^-)} \quad (10)$$

Substitution of Eqns. 5–9 into Eqn. 10 gives:

$$k_{obs} = \frac{k_1^{(MH_3^{2+})}[H^+]^3 + k_1^{(MH_2^+)}[H^+]^2 K_{a,1} + k^{(MH)}[H^+] K_{a,1} K_{a,2} + k_1^{(M^-)} K_{a,1} K_{a,2} K_{a,3}}{[H^+]^3 + [H^+]^2 K_{a,1} + [H^+] K_{a,1} K_{a,2} + K_{a,1} K_{a,2} K_{a,3}} \quad (11)$$

The kinetic data were fitted to Eqn. 11 using using non-linear least-squares regression (SASNLIN, version 82.4 SAS Institute, Cary, NC) to obtain the values for the rate constants and the dissociation constants (Eqn. 11). An initial fitting of the data to Eqn. 11 gave a value of 10^{-9} for $k^{(MH_3^{2+})}$ indicating that the contribution of the reaction shown in Eqn. 11 is negligible. This is not surprising since a protonated 2-chloroethylamine is unlikely to form an ethyleneimmonium ion. The kinetic data (Fig. 4) were reanalyzed by non-linear least-squares regression (SASNLIN) with $k_1^{(MH_3^{2+})} = 0$ and the estimates for the rate constants and pK_a values are given in Table 1. The pK_a values of 2.75 and 9.17 ($\mu = 0.5$, 37°C) compare well with the thermodynamic pK_as (Merck Index, 1983) of 2.59 and 9.25 for the carboxylic acid

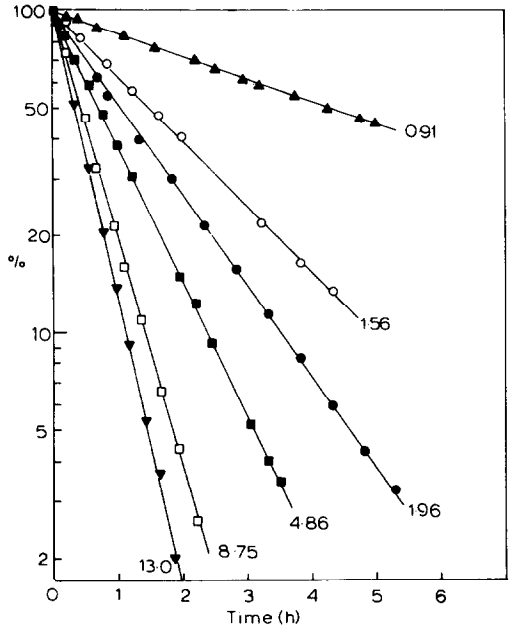


Fig. 3. Representative first-order plots of log % remaining against time ($\mu = 0.5$ M, 37°C, $[M]_0 = 100$ μg/ml; the numbers on the figure refer to the pHs of the solutions).

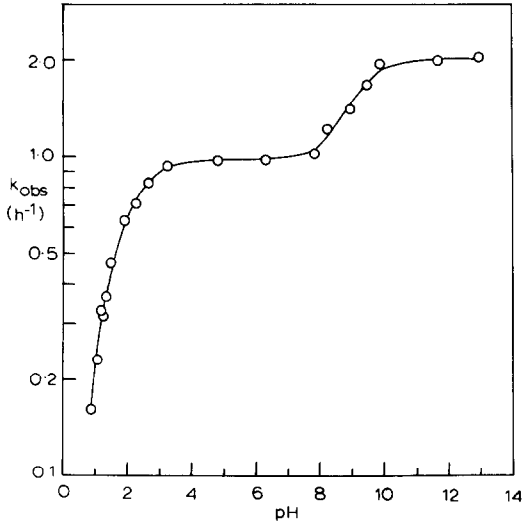


Fig. 4. Log k_{obs}–pH profile for melphalan at 37°C ($\mu = 0.5$). The line has been simulated from Eqn. 11 using the constants in Table 1.

and amino groups of phenylalanine, respectively. Thus the pK_as of 1.42, 2.75 and 9.17 may be ascribed to positions 1, 2 and 3 in Fig. 2, respectively.

The values of k_1 (Table 1) increase with decreasing protonation of the melphalan species such that:

$$k_1^{(MH_3^{2+})} < k_1^{(MH_2^{+})} < k_1^{(MH)} < k_1^{(M^-)}$$

This order reflects the magnitude of the positive charge on the various species and the tendency of the species to take on an additional positive charge and form the corresponding ethyleneimmonium ion.

Influence of chloride

Chang et al. (1979) have shown that the degradation of melphalan is inhibited by the addition of sodium chloride. This may be attributed to the competition between the chloride ions and water for the ethyleneimmonium ion (M^+, Scheme 1) thereby introducing a significant contribution from the reverse reaction (k_{-1}). The effect of chloride concentration (0–0.49 M) on the loss of melphalan was studied at pH 6.00 ($\mu = 0.5$) and 50°C. At this pH the zwitterionic form (MH) of melphalan predominates and the contributions from the reactions of MH_3^{2+}, MH_2^+ and M^- to the overall loss of melphalan may be considered negligible (i.e. [M] = [MH]). Thus, in this section the nomenclature has been simplified by eliminating the superscripts (i.e. $k_1 = k_1^{(MH)}$). The total loss of melphalan at pH 6 may be described by:

$$-\frac{d[M + M^+]}{dt} = -\frac{d[M]}{dt} - \frac{d[M^+]}{dt} \tag{12}$$

where

$$-\frac{d[M]}{dt} = k_1[M] - k_{-1}[M^+][Cl^-] \tag{13}$$

TABLE 1

MICROSCOPIC RATE CONSTANTS FOR THE HYDROLYSIS OF MELPHALAN AND KINETICALLY DETERMINED pK_a VALUES AT 37°C ($\mu = 0.5$)

Parameter	Value
$k_1^{(MH_3^{2+})}$	0.00 h^{-1}
$k_1^{(MH_2^{+})}$	0.74 h^{-1}
$k_1^{(MH)}$	0.98 h^{-1}
k_1^{M-}	2.02 h^{-1}
$pK_{a,1}$	1.42
$pK_{a,2}$	2.75
$pK_{a,3}$	9.17

and

$$-\frac{d[M^+]}{dt} = k_2[M^+] - k_1[M] + k_{-1}[M^+][Cl^-] \tag{14}$$

At steady-state it may be assumed that:

$$-\frac{d[M^+]}{dt} = k_2[M^+] - k_1[M] + k_{-1}[M^+][Cl^-] = 0 \tag{15}$$

and an expression (Eqn. 16) to describe the concentration of the intermediate ($[M^+]$) may be derived.

$$[M^+] = \frac{k_1[M]}{k_{-1}[Cl^-] + k_2} \tag{16}$$

Substituting Eqns. 13, 14 and 16 into Eqn. 12 gives

$$-\frac{d[M]_{Total}}{dt} = \frac{k_1 k_2[M]}{k_{-1}[Cl^-] + k_2} \tag{17}$$

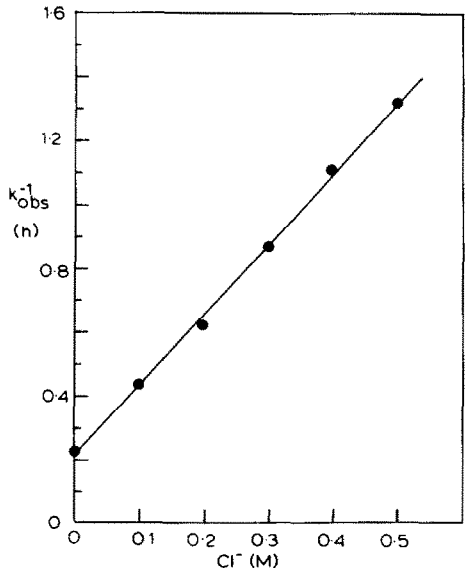


Fig. 5. Graph of the reciprocal pseudo-first-order rate constant for the degradation of melphalan against concentration of chloride added (pH = 6.0, μ = 0.5, 50°C).

which may be solved to give Eqn. 18 relating the pseudo-first-order rate constant (k_{obs}) to the chloride concentration.

$$\frac{1}{k_{obs}} = \frac{k_{-1}[Cl^-]}{k_1 k_2} + \frac{1}{k_1} \tag{18}$$

Fig. 5 shows the linear relationship between $1/k_{obs}$ and $[Cl^-]$ (Eqn. 18). The slope (k_{-1}/k_1k_2) and intercept ($1/k_1$) coefficients of this relationship (Fig. 5) were calculated by linear regression to obtain values of 4.74 h^{-1} and 10.47 M^{-1} for k_1 and the ratio, k_{-1}/k_2, respectively. The ratio, k_{-1}/k_2, may be taken as the competition factor describing the relative reactivity of the intermediate (M^+) with chloride compared with water. Values of k_1 and k_{-1}/k_2 were also determined at 25 and 37°C (Eqn. 18) from the values of k_{obs} in the presence of 0 and 0.40 M sodium chloride. The value of the competition factor (k_{-1}/k_2) was found to increase with increasing temperature (Table 2).

The production of chloride arising from the hydrolysis of melphalan (Scheme 1) was also studied using an ion specific electrode at 25, 37 and 50°C and pH 6.0 ($\mu = 0.5$). With no added chloride and a low initial concentration of melphalan ($[M]_0 = [Cl^-]_\infty/2 = 3.28 \times 10^{-4}$ M) the contributions from the reverse reactions may be considered negligible and the rate equation for the production chloride is given by:

$$\frac{d[Cl^-]}{dt} = k_1[M] + k_3[MOH] \tag{19}$$

The solutions of Eqn. 19 may be derived as follows. Since the loss of melphalan is first-order we may write:

$$[M] = [M]_0 e^{-k_1 t} \tag{20}$$

The rate equation for the production of MOH is:

$$\frac{d[MOH]}{dt} = k_2[M^+] - k_3[MOH] \tag{21}$$

TABLE 2

RATE CONSTANTS FOR THE HYDROLYSIS OF MELPHALAN AT pH 6.0 ($\mu = 0.5$)

Rate constant	25°C	37°C	50°C
k_1 (h^{-1})	0.19	0.92	4.74
k_3 (h^{-1})	0.13	0.39	2.38
k_{-1}/k_2 (M^{-1})	6.38	8.01	10.47
k_{-1}/k'_2 [a]	(354.2)	(444.6)	(581.2)

[a] Values in parentheses have been corrected for the concentration of water (i.e. $k_{-1}/k'_2 = 55.51 \times k_{-1}/k_2$).

and the steady-state concentration of the ethyleneimmonium ion (M^+) is given by:

$$[M^+] = \frac{k_1}{k_2}[M]_0 e^{-k_1 t} \tag{22}$$

Substitution of Eqn. 22 into 21 gives an expression which may be integrated to give Eqn. 23.

$$[MOH] = \frac{k_1[M]_0}{(k_3 - k_1)}(e^{-k_1 t} - e^{-k_3 t}) \tag{23}$$

Substituting Eqn. 23 into 19 gives an expression which may be integrated to give Eqn. 24.

$$\frac{[Cl^-]}{[M]_0} = 2 + \frac{(k_1 - 2k_3)}{(k_3 - k_1)} e^{-k_1 t} + \frac{k_1}{(k_3 - k_1)} e^{-k_3 t} \tag{24}$$

Eqn. 24 is consistent with two unimolecular reactions in which the 2-chloroethylamino functional groups of M and MOH are transformed to their respective ethyleneimmonium ions (M^+ and MOH^+) with the loss of two chloride ions. Fig. 6 shows that the ratio, $[Cl^-]_t/[M]_0$ approaches a value of 2 with time, consistent with Eqn. 24. Using previously determined values of k_1 the data shown in Fig. 6 were fitted to Eqn. 24 by least-squares non-linear regression (SASNLIN) to obtain the values of k_3 at 25, 37 and 50°C. The values of the various rate constants obtained by studying the role of chloride in the hydrolysis of melphalan are summarized in Table 2.

Influence of temperature

The influence of temperature on the degradation of melphalan was studied at three temperatures (25, 37 and 50°C), four pHs (2.0, 6.0, 9.0 and 11.0) and constant ionic strength ($\mu = 0.5$) (Fig. 7). The values of k_{obs} were fitted to the Arrhenius equation (Eqn. 25) by linear regression to obtain the activation energies (E_a) at the chosen pHs (Table 3)

$$\log k_{obs} = \log A - \frac{E_a}{2.3RT} \tag{25}$$

These pH values were chosen so that the individual activation energies for the reactive species (MH_2^+, MH and M^-) could be determined from the appropriate simultaneous equations. Fortunately, however, it was found that the activation energies were independent of pH over the range 2–11 (Table 3) as indicated by the parallel Arrhenius plots (Fig. 7). The constancy of E_a indicates that the nature of the rate-determining step (k_1, $M \rightarrow M^+$) does not change with changing pH (Jencks, 1969). Additionally, the microscopic rate constants ($k_1^{(MH_2^+)}$, $k_1^{(MH)}$, $k_1^{(M^-)}$) could be

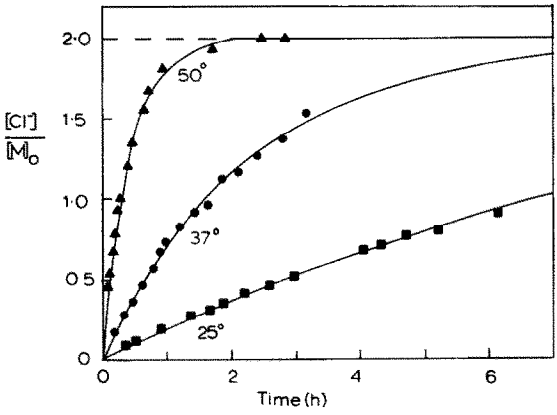


Fig. 6. Graph of the ratio of the chloride ion concentration to the initial concentration of melphalan against time at 25, 37 and 50°C ($\mu = 0.5$). The line has been simulated using Eqn. 24 and the rate constants in Table 2.

simply estimated at any temperature from Eqn. 26 using a mean activation energy (Table 3) of 24.0 kcal/mol.

$$\log \frac{k_1^{(2)}}{k_1^{(1)}} = \frac{E_a}{2.3R} \cdot \frac{(T^{(2)} - T^{(1)})}{T^{(2)}T^{(1)}} \tag{26}$$

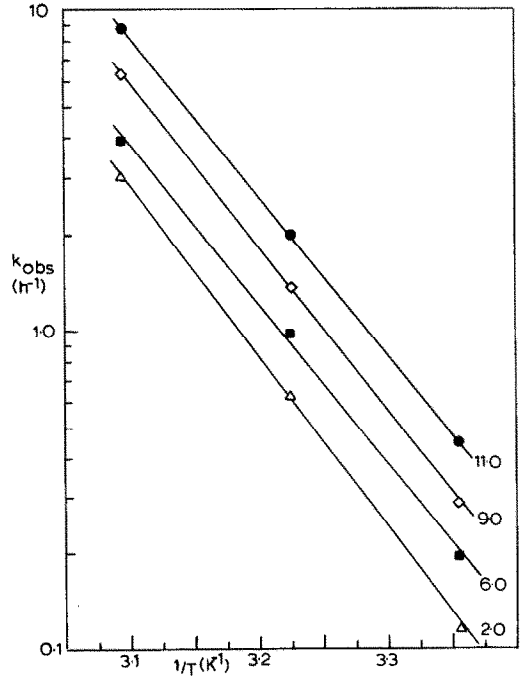


Fig. 7. Arrhenius plots of log k_{obs} against reciprocal absolute temperature for the hydrolysis of melphalan at pH 2.0, 6.0, 9.0 and 11.0 ($\mu = 0.5$).

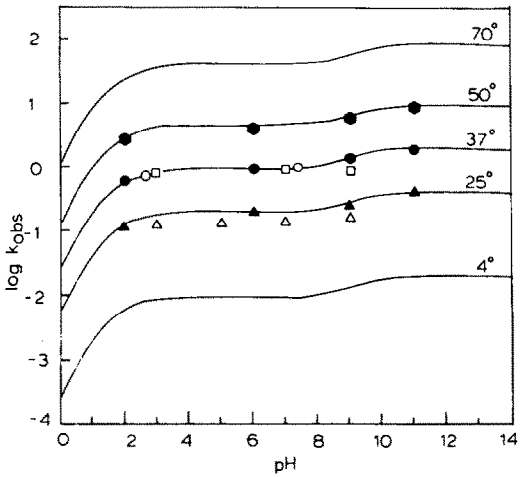


Fig. 8. Simulated log k_{obs}–pH profiles for the hydrolysis of melphalan at various temperatures ($\mu = 0.5$). The closed symbols are from this study. Open symbols are literature values: Δ (25°C) and □ (41°C) Flora et al. (1979); and ○ (37°C) Chang et al. (1979).

The log k_{obs}–pH profiles for the hydrolysis of melphalan have been simulated (Fig. 8) at 70, 50, 37, 25 and 4°C using Eqns. 10 and 26 and the microscopic rate constants shown in Table 1. The errors due to the changes in the dissociation constants with changing temperature can be considered negligible, since these changes appear to be small compared with changes in the rate constants (Fig. 7). The values of k_{obs} at various pHs and temperatures reported previously (Chang et al., 1979; Flora et al. 1979) are also shown in Fig. 8. Whereas there is excellent agreement between the literature values of k_{obs} and those obtained here at 37°C, the literature values at 25°C and 41°C (Flora et al., 1979) are somewhat lower than expected. These differences are probably due to differences in ionic strength which was not controlled in the study of Flora et al. (1979).

Influence of ionic strength

The influence of ionic strength ($\mu = 0.12$–1.00, adjusted with $NaNO_3$) on the degradation of melphalan (Fig. 9) was studied at 37°C and pH 6.0 and the data

TABLE 3

ACTIVATION ENERGIES AT VARIOUS pHs ($\mu = 0.5$)

pH	E_a (kcal/mol)
2.0	24.96
6.0	24.39
9.0	23.84
11.0	22.81

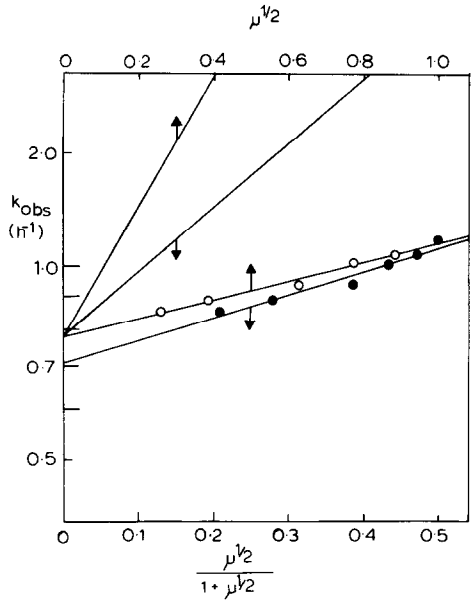


Fig. 9. Relationships between k_{obs} and ionic strength (μ) for the hydrolysis of melphalan at pH 6.0, plotted according to Eqns. 27a and b. The solid lines are simulations of Eqns. 27a and b drawn with slopes of 1.0 for a and a'.

fitted by least-squares linear regression to Eqns. 27a and b:

$$\log k_{obs} = a\sqrt{\mu} + \log k_0 \tag{27a}$$

$$\log k_{obs} = a'\sqrt{\mu}/(1 + \sqrt{\mu}) + \log k_0 \tag{27b}$$

where k_o is the pseudo-first-order rate constant at $\mu = 0$, a and a' are the slopes. Although Eqns. 27a and 27b have been derived for dilute solutions (Martin et al., 1983) they did prove useful as an empirical method of describing the effect of ionic strength on the degradation of melphalan. The logarithm of k_{obs} was found to be linearly related to $\mu^{\frac{1}{2}}$ and the data was therefore best described by Eqn. 27a. When the data was plotted according to Eqn. 27b significant curvature was observed and the correlation coefficient was lower ($r = 0.971$ using Eqn. 27b compared with $r = 0.989$ using Eqn. 27a). Overall, the effect of ionic strength on the hydrolysis of melphalan was small (a = 0.15) (Eqn. 27a), consistent with the rate-determining step ($M \rightarrow M^+$) being unimolecular (Eqns. 11–14, Scheme 1).

Conclusion

The degradation of melphalan in aqueous solution is influenced by pH, temperature and chloride concentration. However, the effect of ionic strength on the loss of the drug was found to be minimal. The kinetic data indicate that the rate-determin-

ing step for the loss of melphalan involves its conversion to an ethyleneimmonium ion. The ethyleneimmonium ion undergoes attack by a nucleophile which in the case of water gives rise to hydroxymelphalan. The addition of chloride has a stabilizing effect because it can compete with the water for the ethyleneimmonium ion. However, at low concentration (3.3×10^{-4} M) in the absence of added chloride the contribution of the reverse reaction to the overall loss of melphalan is negligible.

The results of the present study should prove useful in the prediction of melphalan stability in pharmaceutical formulations and biological fluids. For example, since the drug is more stable in acidic media and in the presence of added chloride (pH < 2.5) it would appear advantageous to acidify biological samples containing melphalan by the addition of hydrochloric acid. Unfortunately the pH necessary to achieve a significant reduction in the loss of melphalan is too low to be considered useful in drug formulation. On the other hand, 0.9% sodium chloride would appear to the best diluent for the intravenous infusion of melphalan.

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