International Journal of Pharmaceutics, 37 (1987) 257–264
Elsevier

IJP 01269

Hydrolysis of L-phenylalanine mustard (melphalan). II. Further observations on the effects of pH, chloride ions and buffers on the rate of reaction

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(Received 15 September 1986)
(Modified version received and accepted 31 January 1987)

Key words: L-Phenylalanine mustard; Melphalan; Phosphoramide mustard; Stability; Hydrolysis kinetics; pH effect; Chloride effect; Buffer effect

Summary

The effects of pH (3.7–13), ionic strength, buffer composition (acetate, phosphate and borate) and buffer concentration (15–200 mM) on the rate of degradation of melphalan in the presence 0.3 M chloride at 50 ± 0.1° C were investigated using high-performance liquid chromatography. In addition, the data published in the literature for the degradation of phosphoramide mustard have been compared with those of melphalan, placing emphasis on mechanisms of hydrolysis and the effects of pH and chloride. In the presence of chloride, the degradation rate of melphalan was influenced by pH and buffer composition but not by ionic strength. These effects were not seen in the absence of added chloride and have been explained in terms of competition between chloride and other nucleophiles such as the hydroxide ion, water and buffer components for the active intermediate of the alkylating agent. These results help to explain differences in reported values for the rates of hydrolysis of various alkylating agents in the presence of chloride.

Introduction

Melphalan (Fig. 1) and cyclophosphamide (Fig. 2) are alkylating agents with demonstrated activity against a number of neoplastic diseases (Wasserman et al., 1975; Alexanian et al., 1968). Phosphoramide mustard (Fig. 2) is an active metabolite of cyclophosphamide (Colvin et al., 1976) produced by chemical hydrolysis of 4-hydroxycyclophosphamide which is a product of hepatic metabolism of cyclophosphamide (Fig. 2). The hydrolysis of alkylating agents takes place via a cyclic ethyleneimmonium ion intermediate (Figs. 1 and 2) which is then hydroxylated by nucleophilic attack by water (Golumbic et al., 1946; Fruton and Bergman, 1946; Bartlett et al., 1947; Chang et al., 1978, 1979; Flora et al., 1979; Stout and Riley, 1985). Watson et al. (1985) have shown that a change in the mechanism of hydrolysis of phosphoramide mustard takes place when the hydroxyl group is protonated at low pH. In this case, the electron-withdrawing effect of the phosphoramide group prevents formation of the ethyleneimmonium ion but favors dephosphorylation of phosphoramide mustard (Fig. 2). Watson et al. (1985) and Stout and Riley (1985) have published the pH–rate profiles of phosphoramide mustard and melphalan, respectively; however, the former

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pH–rate profile was not fitted to a kinetic model. Consequently, one of the purposes of the present study was to evaluate the data for phosphoramide in terms of a model similar to that described for melphalan (Stout and Riley, 1985) and to compare and contrast the microscopic rate constants and dissociation constants obtained. The second purpose of the present study was to evaluate the effects of pH, ionic strength, buffer composition and buffer concentration on the hydrolysis of melphalan in the presence of chloride.

Watson et al. (1985) have reported a linear relationship between the half-life for the hydrolysis of phosphoramide mustard and the concentration of added chloride. This implies a linear relationship between the reciprocal of the pseudo-first order-rate constant (k_{obs}) and the concentration of added chloride which has been reported previously for melphalan (Stout and Riley, 1985). The stabilizing influence of added chloride may be explained in terms of competition between the chloride ions and water for the cyclic intermediate (Fig. 1). Differences in literature values for the rate of hydrolysis of melphalan in the presence of chloride (Tabibi and Craddock, 1984; Stout and Riley, 1985) have led to the present hypothesis that the hydrolysis of alkylating agents in the presence of chloride may be influenced by pH, buffer components and concentration, and ionic strength making it difficult to compare kinetic data reported in the literature.

Experimental

Chemicals and reagents

Melphalan was obtained from Sigma Chemicals, St. Louis, MO and was used as received. HPLC grade methanol and ACS grade buffer components were obtained from Fisher Scientific, Fair Lawn, NJ. Distilled deionized water was used throughout, after filtration through 0.45 μm Nylon-66 membranes (Rainin Instruments, Woburn, MA).

Buffer solutions

For solutions of pH 3.7 and 4.7, acetic acid/sodium acetate buffers were used. For solutions of pH 5.8–7.7 KH_2PO_4/Na_2HPO_4, buffers were used. For solutions of pH 8.66–10.0, boric acid/NaOH buffers were used. Above pH 10, NaOH was used. Unless otherwise stated, the ionic strength of the solutions was adjusted to 0.6 with $NaNO_3$. The pH of each solution was measured at the temperature of the experiments with a Fisher MicroProbe Combination electrode and a digital pH millivolt meter model 611 (Orion Research, Cambridge, MA).

Liquid chromatography

The HPLC conditions used for the determination of melphalan were as described previously (Stout and Riley, 1985). The liquid chromatograph consisted of a Constametric IIIG pump (LDC, Riviera Beach, FL), a Spectromonitor D detector (LDC) operated at 260 nm and a Micromeritics 728 autosampler (Micromeritics, Norcross, GA) used with a Valco RC6U injection valve fitted with a 20 μl loop (Valco Instruments, Houston, TX). The peak areas, which were proportional to concentration injected, were measured using an HP 3392A integrator (Hewlett-Packard Company, Avondale, PA).

Kinetic studies

The influence of pH (3.7–13.0) and buffer composition (acetate, phosphate and borate) and buffer concentration (15–200 mM) on the rate of degradation of melphalan at $50 \pm 0.1°C$ was investigated, using the methods described previously (Stout and Riley, 1985). Duplicate solutions were prepared and analyzed for each set of conditions and sampling was continued for at least 4 half-lives.

Results and Discussion

Effects of pH – comparison of melphalan with phosphoramide mustard

In the absence of added chloride, the rate of hydrolysis of melphalan is determined by the production of the cyclic intermediate (Fig. 1) and is influenced by the overall charge on the molecule (Stout and Riley, 1985). Increasing protonation of the alkylating agent decreases its ability to form

the cyclic intermediate which is subjected to nucleophilic attack by water, forming hydroxymelphalan. The phosphoramide group in phosphoramide mustard is electron-withdrawing and prohibits the formation of the cyclic intermediate. Colvin et al. (1976) have suggested that once the hydroxyl group is deprotonated the negative charge is distributed over the entire molecule allowing for the formation of the ethyleneimmonium ion. Hydrolysis of phosphoramide mustard then proceeds in a manner analogous to that of melphalan (Figs. 1 and 2). Watson et al. (1985) have shown that at low pH, where the phosphoramide group is protonated, phosphoramide mustard is hydrolyzed to yield nornitrogen mustard.

In the absence of added chloride the hydrolysis of melphalan and phosphoramide mustard is pseudo-first-order and k_{obs} is given by:

$$k_{obs} = \sum f(n) \cdot k_1^{(n)} \qquad (1)$$

where n refers to the charge on the molecule, $f(n)$ is the fraction of the species present with charge n at any pH and k_1 is the first-order rate constant for the conversion of that species to its corresponding ethyleneimmonium ion which will have a charge of $n+1$. Melphalan has 3 ionizable functional groups (a tertiary amine, a primary amine and a carboxylate) and may exist as a

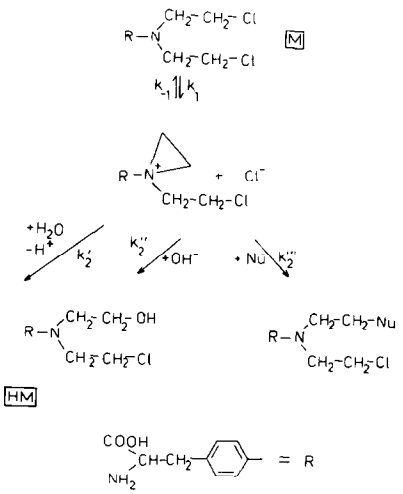

Fig. 1. Proposed scheme for the hydrolysis of melphalan (M) to hydroxymelphalan (HM).

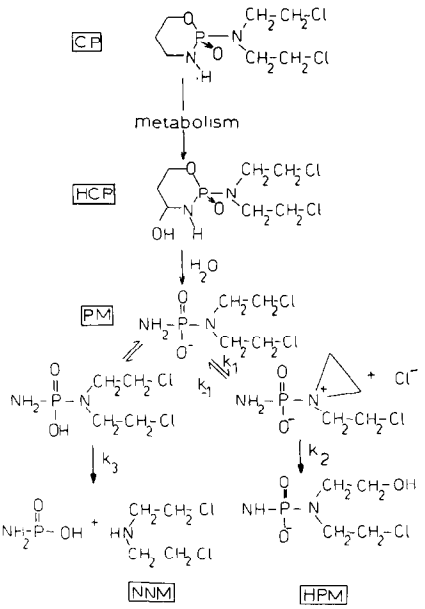

Fig. 2. Proposed scheme for the conversion of cyclophosphamide (CP) to hydroxycyclophosphamide (HCP), phosphorphosphoramide mustard (PM), hydroxyphosphoramide mustard (HPM) and nornitrogen mustard (NNM).

dication ($n = +2$), a cation ($n = +1$), a zwitterion ($n = 0$) and an anion ($n = -1$). It follows from Eqn. 1 that the relationship between k_{obs} and the hydrogen ion concentration is given by Eqn. 2, for melphalan:

$$k_{obs} = \left\{ k_1^{(+2)}[H^+]^3 + k_1^{(+1)}[H^+]^2 K_{a_1} + k_1^{(0)}[H^+] K_{a_1} K_{a_2} + k_1^{(-1)} K_{a_1} K_{a_2} K_{a_3} \right\} \times \left\{ [H^+]^3 + [H^+]^2 K_{a_1} + [H^+] K_{a_1} K_{a_2} + K_{a_1} K_{a_2} K_{a_3} \right\}^{-1} \qquad (2)$$

where K_{a_1}, K_{a_2} and K_{a_3} are the dissociation constants for the tertiary amine, the carboxylic acid and the primary amine, respectively. The superscripts refer to the charges on the individual species of melphalan. The kinetic data for the hydrolysis of melphalan (Stout and Riley, 1985) have been fitted to Eqn. 2 by non-linear regression and the appropriate rate constants and pK_as are

TABLE 1

Microscopic rate constants for the hydrolysis of melphalan (37 °C) and phosphoramide mustard (38 °C) and their kinetically determined pK_as

Parameter [a]	Melphalan [b]	Phosphoramide mustard [c]
$k_1^{(+2)}$ (h^{-1})	0.00	na
$k_1^{(+1)}$ (h^{-1})	0.74	cd
$k_1^{(0)}$ (h^{-1})	0.98	0.64
$k_1^{(-1)}$ (h^{-1})	2.02	3.30
pK_{a_1}	1.42	cd
pK_{a_2}	2.75	3.58
pK_{a_3}	9.17	na
k_{-1}/k_2 (M^{-1})	8.01	1.26

[a] Superscripts refer to charge on the molecule.
[b] Eqn. 2.
[c] Eqn. 3.
na = not applicable; cd = could not be determined.

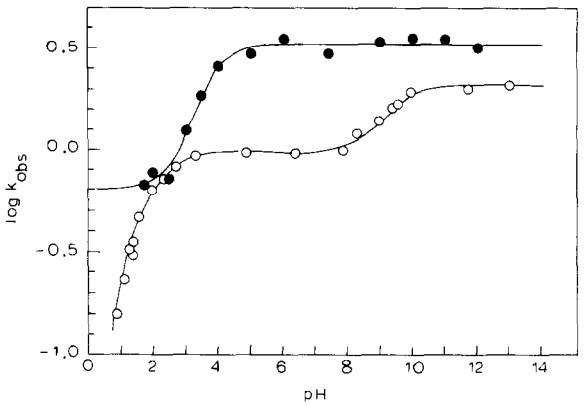

Fig. 3. Log k_{obs}–pH profiles for the hydrolysis of melphalan (○) and phosphoramide mustard (●). The data have been taken from the works of Stout and Riley (1985) for melphalan and Watson et al. (1985) for phosphoramide mustard. The lines have been simulated from Eqn. 2 for melphalan and Eqn. 3 for phosphoramide mustard, using the constants in Table 1.

given in Table 1. The data could only be fitted successfully when a value of zero was ascribed to $k_1^{(+2)}$ which is the rate constant for the conversion of the fully protonated species to its corresponding ethyleneimmonium ion.

Phosphoramide mustard has two ionizable functional groups (a tertiary amine and a hydroxyl); however, the data of Watson et al. (1985) could not be fitted to an equation containing two dissociation constants and 3 rate constants. Instead, the data was satisfied by Eqn. 3 which contains two rate constants and only one dissociation constant.

$$k_{obs} = \frac{k_1^{(0)}[H^+] + k_1^{(-1)}K_{a_2}}{[H^+] + K_{a_2}} \quad (3)$$

The pH profile for phosphoramide mustard is shown in Fig. 3 in which the line has been simulated using Eqn. 3 and the values for the various constants given in Table 1. The pH profile for phosphoramide mustard shows an inflection point around pH 3.58 which corresponds to the pK_a of the hydroxyl group (Table 1). This region of the pH profile also corresponds to a change in the mechanism of hydrolysis (Watson et al., 1985) of phosphoramide mustard which is converted to hydroxyphosphoramide mustard at higher pH and nornitrogen mustard at lower pH. For comparison, the pH profile for melphalan is also shown in Fig. 3. The decrease in the observed rate constant for melphalan at low pH arises from protonation of the tertiary amine which stabilizes the molecule. Since $k_1^{(+2)}$ is zero for melphalan, Eqn. 2 predicts that k_{obs} will decrease continuously with decreasing pH. In contrast, Eqn. 3 predicts that the pH profile of phosphoramide mustard will have a plateau region below pH 1.5 since $k_1^{(0)}$ for phosphoramide mustard has a finite value (Table 1).

Effect of chloride and competing nucleophiles

Several workers (Chang et al., 1979; Tabibi and Craddock, 1984; Watson et al., 1985; Stout and Riley, 1985) have shown that alkylating agents may be stabilized by the addition of chloride. This has been attributed to the competition between the chloride ions and water for the ethyleneimmonium ion (Figs. 1 and 2), thereby introducing a significant contribution from the reverse reaction (k_{-1}). The effect of chloride on the rate of hydrolysis of alkylating agents has been previously derived (Stout and Riley, 1985) and may be described quantitatively by:

$$\frac{1}{k_{obs}} = \frac{1}{k_1} + \frac{k_{-1} \cdot [Cl^-]}{k_1 \cdot k_2} \quad (4)$$

where k_{-1}/k_2 is the competition factor describing the relative affinities of chloride and water for the ethyleneimmonium ion (Figs. 1 and 2).

Analysis of the data by Watson et al. (1985) for phosphoramide mustard according to Eqn. 4, gives a value for the rate constant ratio of 1.26 M^{-1} (Table 1) at 38 °C and pH 7.4. This compares with a value of 8.01 M^{-1} for melphalan at 37 °C and pH 6.0, suggesting a difference in the relative affinities of chloride and water for the cyclic intermediate of phosphoramide mustard (Fig. 2) compared with that of melphalan (Fig. 1). It should be noted that these two studies (Watson et al., 1985; Stout and Riley, 1985) were conducted in buffers of different composition, pH and ionic strength. It is unlikely that the 1 °C difference in temperature would account for the difference. In addition, Tabibi and Craddock (1985) have reported a value of 0.20 h^{-1} for the first-order disappearance of melphalan in 0.15 M NaCl at 37 °C and pH 4.2. Substitution of this value into Eqn. 4 gives a value 24.0 M^{-1} for k_{-1}/k_2 for melphalan. These 3 studies indicate that the competition between chloride and water for the ethyleneimmonium ion may be influenced by pH, ionic strength and buffer composition. Consequently, these effects were investigated using melphalan as a model compound.

Fig. 4 shows that the hydrolysis of melphalan at pH 6.55 is independent of ionic strength but strongly dependent on the concentration of phosphate buffer in the presence of 0.3 M Cl^-. The latter may be explained in terms of the additional contribution of the competition between the phosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) and the chloride ions for the ethyleneimmonium ion. Thus k_2 (Fig. 1 and Eqn. 4) must be expanded to take into account other nucleophiles, such as buffer components, and the hydroxide ion as follows:

$$k_2 = k_2' + k_2''[OH^-] + k_2'''[Nu^-] \qquad (5)$$

where k_2', k_2'' and k_2''' are the rate constants for the reaction of water, OH^- and a nucleophile (Nu), respectively, with the ethyleneimmonium ion. Additional terms could be added to Eqn. 5 for each additional nucleophile present in the system. Substitution of Eqn. 4 into Eqn. 5 gives, after

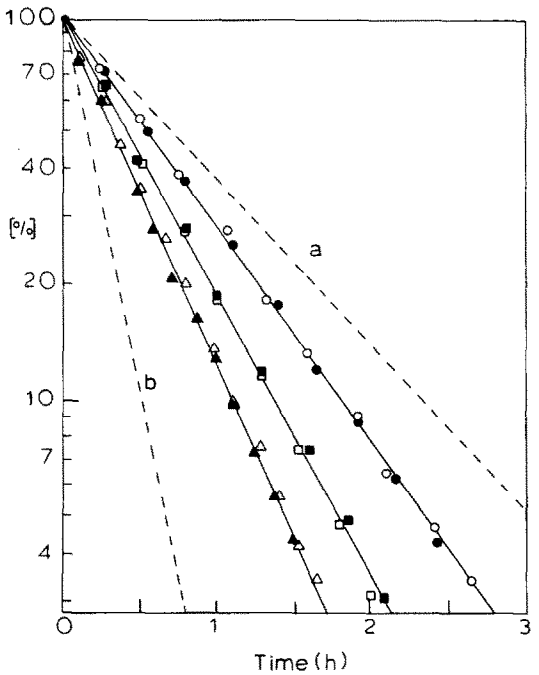

Fig. 4. Representative first-order plots of log % remaining against time (50 °C, pH 6.55), showing the effects of ionic strength and phosphate concentration on the hydrolysis of melphalan in the presence of 0.3 M Cl^-. The concentrations of phosphate buffer were 25 mM (●), 50 mM (■) and 100 mM (▲). The open symbols represent data from solutions in which the ionic strength was adjusted to 0.6. For the closed symbols the ionic strength was not adjusted. The dotted lines have been simulated from $\% = 100 \cdot \exp(-k_{obs} \cdot t)$ using: (a) $k_{obs} = 0.28$ h^{-1} when [phosphate] = 0 (Table 2); and (b) $k_{obs} = 4.72$ h^{-1} when $[Cl^-] = 0$ (Table 2).

rearrangement:

$$\frac{k_{obs}}{(k_1 - k_{obs})} = \frac{k_2^*}{k_{-1}[Cl^-]} + \frac{k_2'''[Nu]}{k_{-1}[Cl^{-1}]} \qquad (6)$$

where

$$k_2^* = k_2' + k_2''[OH^-] \qquad (7)$$

Eqns. 6 and 7 predict that the rate of hydrolysis of melphalan in the presence of chloride will be accelerated by increases in pH and the concentration of nucleophilic substances, such as buffer components, in the solution. To test this hypothesis, the effects of pH and buffer composition on the rate of degradation of melphalan were studied

at 50 °C and an ionic strength of 0.6, in the presence of 0.3 M Cl^-. The results of these experiments are shown in Table 2 and it can be seen that k_{obs} is significantly influenced by pH and by the compositions of the buffers used. The data in Table 2 were plotted according to Eqn. 6 and the results are shown in Fig. 5. In the case of phosphate buffers, the slopes of these plots ($k_2'/k_{-1}[Cl^-]$) increase with increasing pH, indicating that HPO_4^{2-} competes more strongly than $H_2PO_4^-$ for the ethyleneimmonium ion. The values of k_{obs} (and hence $k_{obs}/(k_1 - k_{obs})$) were much less dependent on the concentration of borate and acetate (Fig. 5), indicating the weak nucleophilicity of

TABLE 2

Kinetic data for the hydrolysis of melphalan showing the effects of pH and buffer composition in the presence of 0.3 M sodium chloride (50 °C)

Buffer [a]		k_1 [b] (h^{-1})	k_{obs} [c] (h^{-1})	$\frac{k_{obs}}{k_1 - k_{obs}}$	$\frac{k_2^*}{k_{-1}[Cl^-]}$ [d]
Concentration (mM)	pH				
Acetate					
25	3.70	4.61	1.05	0.295	0.280
100	3.70	4.61	1.06	0.299	
200	3.70	4.61	1.06	0.299	
25	4.60	4.71	1.11	0.308	0.290
100	4.60	4.71	1.30	0.381	
200	4.60	4.71	1.41	0.427	
Phosphate					
25	5.80	4.71	1.11	0.308	0.273
50	5.80	4.71	1.25	0.361	
100	5.80	4.71	1.52	0.476	
25	6.55	4.72	1.17	0.331	0.275
50	6.55	4.72	1.64	0.532	
100	6.55	4.72	2.10	0.801	
25 *	6.55	4.72	1.23	0.364	0.280
50 *	6.55	4.72	1.64	0.532	
100 *	6.55	4.72	2.03	0.755	
25	7.70	4.85	1.73	0.554	0.332
50	7.70	4.85	2.18	0.816	
100	7.70	4.85	2.72	1.28	
Borate					
15	8.66	5.76	1.89	0.488	0.462
25	8.66	5.76	1.90	0.492	
50	8.66	5.76	2.02	0.540	
15	10.0	9.02	3.20	0.550	0.532
25	10.0	9.02	3.21	0.552	
50	10.0	9.02	3.37	0.596	
Sodium hydroxide					
10	12.0	9.70	4.41	0.834	0.834
100	13.0	9.70	7.90	4.40	4.40

[a] $\mu = 0.6$ except where indicated by *.
[b] Stout and Riley (1985).
[c] Mean of two determinations.
[d] Intercept of Eqn. 6.

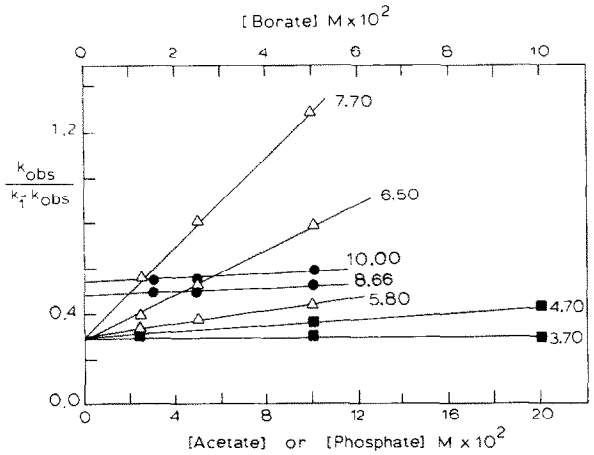

Fig. 5. Relationship between k_{obs} for melphalan and the buffer concentration, plotted according to Eqn. 6 ($\mu = 0.6$, 50 °C). The numbers on the figures refer to the pH of the solutions containing these buffers: phosphate (△), borate (●) and acetate (■).

these ions compared with phosphate. The intercepts of these relationships ($k_2^*/(k_{-1}[Cl^-])$, Eqn. 6) were independent of pH over the range 3.7–7.7 but increased with increasing pH above 7.7 (Table 2 and Fig. 5). To define the relationship between k_2^* and pH further, the degradation of melphalan

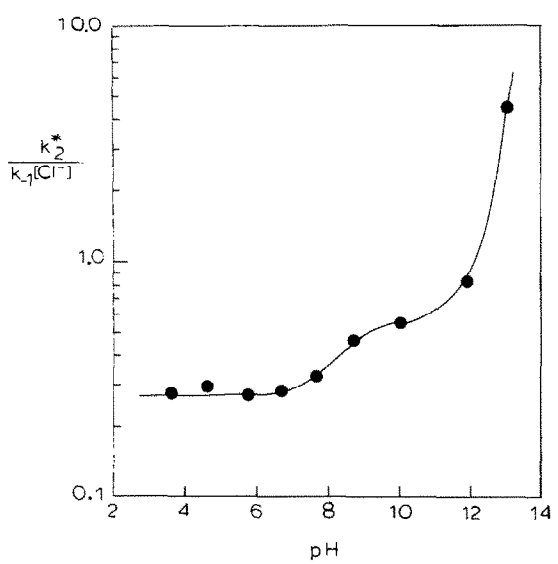

Fig. 6. Relationship between $k_2^*/k_{-1}[Cl^-]$ (Eqn. 6) and pH for melphalan. See text for further explanation.

was also monitored at pH 12 and 13 ($[Cl^-] = 0.3$ M, $\mu = 0.6$, 50 °C) and the complete profile is shown in Fig. 6.

Fig. 6 illustrates that the competition between Cl^-, water and the hydroxide ion for the ethyleneimmonium ion changes with pH. As the pH increases, the function $k_2^*/k_{-1}[Cl^-]$ remains constant up to pH 7.7. This is followed by an inflection point around pH 9.2 and a continuous rise in $k_2^*/k_{-1}[Cl^-]$ above pH 10. The inflection point coincides with the change in protonation of the primary amine which takes place around this pH ($pK_a = 9.17$; Stout and Riley, 1985). Between pH 3.7 and 7.7 the the only competition which significantly influences the hydrolysis of melphalan (excluding buffer effects) is that between water and Cl^- for the ethyleneimmonium ion and the value of k_{-1}/k_2' (Eqn. 7) is 11.9 M^{-1}. The value of k_1/k_2' decreases to 5.56 M^{-1} when the drug is anionic ($n = -1$). Above pH 11, competition between OH^- and Cl^- dominates and the value of k_{-1}/k_2'' was calculated to be 0.086. This indicates that OH^- is a stronger nucleophile than Cl^- which is consistent with published indices of nucleophilicity (Edwards, 1956; Belluco et al., 1965).

Conclusions

Phosphoramide mustard is considerably less stable than melphalan over the pH range of 4–8 which is the most acceptable for intravenous administration of drugs. However, it is interesting to note that the pseudo-first-order rate constants for the two drugs are similar at higher pHs when the charge on the two molecules is the same ($n = -1$). Since chemical hydrolysis is believed to be a major route by which alkylating agents are cleared from plasma (Chang et al., 1978, 1979), it is to be expected that the half-life of phosphoramide mustard in vivo will be much less than that of melphalan. Phosphoramide mustard suffers from an additional disadvantage that it cannot be stabilized completely by lowering the pH since its mechanism of degradation changes at low pH. This has important implications for the preparation of stable stock solutions for in vitro investiga-

tions and the preparation of biological fluids for analysis.

Alkylating agents which degrade via an ethyleneimmonium ion in aqueous solutions may be stabilized by the addition of chloride. However, the effects of chloride may be diminished by the presence of other nucleophiles such as buffer salts. If alkylating agents are formulated for intravenous administration in the presence of other drugs or additives such as antioxidants, then the stability of the drug should be ascertained. This is supported by Tabibi and Craddock (1984) who found that the rate of degradation of melphalan in Lactated Ringer's Injection was greater than that which was found in aqueous solutions containing the same concentration of chloride. It is important to appreciate that the situation with alkylating agents is not as potentially serious as that reported recently by Garren and Repta (1985) who found that the chloride in cisplatin is rapidly replaced by bisulfite. Displacement of chloride from cisplatin takes place via direct Sn_2 nucleophilic substitution. In aqueous solution, the replacement of chloride in alkylating agents such as melphalan takes place via Sn_1 nucleophilic substitution and the rate of degradation in the presence of chloride can never exceed that found in the absence of added chloride.

Acknowledgements

The technical assistance of Ms. Virgina Tomat is gratefully acknowledged.

References

Alexanian, A., Bersagel, D.E., Migliore, P.J., Vaugh, W.K. and Howe, C.D., Melphalan therapy for plasma cell myeloma. *Blood*, 31 (1968) 1–10.

Bartlett, P.D., Ross, S.D. and Swain, C.G., Kinetics and mechanisms of the reactions of tertiary β-chloroethylamines in solution. I. Methyl-bis-β-chloroethylamine. *J. Am. Chem. Soc.*, 69 (1947) 2971–2977.

Belluco, U., Cattalini, L., Basolo, F., Pearson, R.G. and Turco, A., Nucleophilic constants and substrate discrimination. Factors for substitution reactions of platinum (II) complexes. *J. Am. Chem. Soc.*, 87 (1965) 241–246.

Chang, S.Y., Alberts, D.S., Farquhar, D., Melnick, L.R., Walson, P.D. and Salmon, S.E., Hydrolysis and protein binding of melphalan. *J. Pharm. Sci.*, 67 (1978) 682–684.

Chang, S.Y., Evans, T.L. and Alberts, D.S., The stability of melphalan in the presence of chloride ions. *J. Pharm. Pharmacol.*, 31 (1979) 853–854.

Colvin, M., Brundrett, R.B., Kan, M.-N., Jardine, I. and Fenselau, C., Alkylating properties of phosphoramide mustard. *Cancer Res.*, 36 (1976) 1121–1126.

Edwards, J.O., Polarizability, basicity and nucleophilic character. *J. Am. Chem. Soc.*, 78 (1956) 1819–1820.

Flora, K.P., Smith, S.L. and Craddock, J.C., Applications of a simple high performance liquid chromatographic method for the determination of melphalan in the presence of its hydrolysis products. *J. Chromatogr.*, 177 (1979) 91–97.

Fruton, J.S. and Bergmann, M., Chemical reactions of the nitrogen mustard gases. III. The transformation of ethyl-bis(β-chloroethyl)amine in water. *J. Org. Chem.*, 11 (1946) 543–549.

Garren, K.W. and Repta, A.J., Incompatibility of cisplatin and Reglan injectable. *Int. J. Pharm.*, 24 (1985) 91–99.

Golumbic, C., Fruton, J.S. and Bergmann, M., Chemical reactions of the nitrogen mustard gases. I. The transformation of methyl-bis(β-chloroethyl)amine in water. *J. Org. Chem.*, 11 (1946) 518–535.

Stout, S.A. and Riley, C.M., The hydrolysis of L-phenylalanine mustard (melphalan). *Int. J. Pharm.*, 24 (1985) 193–208.

Tabibi, S.E. and Craddock, J.C., Stability of melphalan in infusion fluids. *Am. J. Hosp. Pharm.*, 41 (1984) 1380–1382.

Wasserman, T.H., Cormis, R.L., Goldsmith, M., Handelsman, H., Penta, J.S., Slavik, M., Soper, W.T. and Carter, S.K., Tabular analysis of the clinical chemotherapy of solid tumors. *Cancer Chemother. Rep.*, Part 3, 6 (1975) 399–419.

Watson, E., Dea, P. and Chan, K.K., Kinetics of phosphoramide mustard hydrolysis in aqueous solution. *J. Pharm. Sci.*, 74 (1985) 1283–1292.