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The effects of 2-hydroxypropyl-β-cyclodextrin on the solubility and stability of chlorambucil and melphalan in aqueous solution

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Summary

Chlorambucil and melphalan were shown to form inclusion complexes with 2-hydroxypropyl-β-cyclodextrin (2-HPCD) in aqueous solution. The phase solubility diagram (0–25% (w/v) 2-HPCD) of chlorambucil can be classified as type A_P and that of melphalan as type A_L. The kinetics of chlorambucil and melphalan degradation in aqueous solution was investigated as a function of pH (1.3–11.7), 2-HPCD concentration (0–20% (w/v)) and temperature (40–70 °C). The aqueous degradation has been shown to follow first-order kinetics and introduction of up to 20% (w/v) of 2-HPCD to the reaction medium did not change this. Also, no evidence of specific acid/base or general acid/base catalysis was found in aqueous 2-HPCD solutions and the results were explained in terms of a unimolecular reaction being the rate-determining step and ionization of the drug molecule. The rate constant was in all cases larger for the free drug than for the complex. The pseudo-first-order rate constant for the degradation of the drug in the complex (k_c) and the 1:1 stability constant of the inclusion complex (K_c) were determined by analyzing the 2-HPCD concentration dependancy of the degradation rate (k_{obs}). The values of k_c and K_c were affected by changes in pH.

Introduction

Chlorambucil and melphalan are aromatic nitrogen mustard type bifunctional alkylating agents, i.e. they have a di-(2-chloroethyl) amino group connected to an aromatic residue, and are used as biological alkylating agents in the control of certain types of cancer. The alkylation proceeds through a cyclic ethyleneimmonium ion or carbonium ion intermediate which is susceptible to nucleophilic attack by components in the double helix of DNA (Stenlake, 1979; Calabresi and Parks, 1985). Unfortunately this intermediate, which is essential for the pharmacological activity, is also readily formed in aqueous solutions where it is attacked by nucleophiles such as water. Thus, nitrogen mustards generally have short shelf-life in aqueous solution, e.g. chlorambucil 12 minutes (Ehrsson et al., 1980) and melphalan about 25 min (Stout and Riley, 1985) both at pH 7.4 and 25 °C. Also, many nitrogen mustards, such as chlorambucil and melphalan, are practically insoluble in water which makes parenteral administration of these drugs difficult.

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2-Hydroxypropyl-β-cyclodextrin (2-HPCD) forms inclusion complexes with a number of drugs, provided their structure (or part of it) fits sufficiently in the 2-HPCD cavity (Müller and Brauns, 1985; Pitha et al., 1986; Brewster et al., 1988; Loftsson and Bodor, 1989). Formation of an inclusion complex usually involves occupation of the 2-HPCD cavity by a single guest molecule without formation of any covalent bonds and this type of complexation is frequently referred to as molecular encapsulation (Szejtli, 1982; Szejtli et al., 1987). Encapsulation of a molecule will affect many of its physicochemical properties such as chemical stability and aqueous solubility (Lin and Yang, 1984; Duchêne et al., 1985; Brewster et al., 1988; Yoshida et al., 1988). It is also quite possible that many side effects associated with drugs, for example mucosal damage in the gastrointestinal tract, can be reduced by this type of molecular encapsulation.

The purpose of this study was to investigate the effects of 2-HPCD on solubility and stability of chlorambucil and melphalan in aqueous solutions.

Materials and Methods

Materials

2-HPCD, with 5.1 degree of average substitution was supplied by the courtesy of Pharmatec Inc. (Alachua, FL). Chlorambucil and melphalan were supplied by the courtesy of the Wellcome Foundation Ltd. (U.K.). All other chemicals were commercially available products of special reagent grade.

Chromatographic conditions

The quantitative determinations of chlorambucil or melphalan were performed on a high-performance liquid chromatographic (HPLC) equipment consisting of a Rheodyne 7125 injector, a LKB 2150 pump, a LKB Lichrosorb RP18 10-μm column (4 × 250 mm) and a LKB Uvicord S fixed wave length detector operated at 254 nm. The mobile phase used for the quantitative determination of chlorambucil consisted of acetonitrile/acetic acid/water (55 : 1 : 44) and the retention time was 4.4 min at 2.00 ml/min flow rate. The mobile phase used for quantitative determination of melphalan consisted of methanol/0.1 M pH 4.7 acetate buffer (50 : 50) and the retention time was 9.2 min at 1.00 ml/min flow rate (Stout and Riley, 1985).

Buffers

McIlvaine buffers (pH 2.2–8.3) were prepared according to Elving et al. (1956), nitric acid solutions were used at pH below 2.2, and sodium hydroxide solutions were used at pH above 8.3.

The ionic strength of the buffer solutions was not adjusted and addition of chloride ion was avoided. The water used for the buffer preparation was distilled in all-glass apparatus.

Solubility studies

Solubilities were measured by adding an excess amount of a drug to be tested to aqueous solutions containing various concentrations of 2-HPLC. The suspensions formed were sonicated in an ultrasonic bath (Kerry, U.K.) for up to 3 h and placed in a 30.0 ± 0.1° C constant-temperature water bath [Tempette (Techne), U.K.]. After equilibrium was attained (after up to 24 h), an aliquot was filtered through a 0.45-μm membrane filter unit [Millex-HV (Millipore), U.S.A.], diluted with a mixture of acetonitrile and water (80 : 20) and analyzed by HPLC.

Kinetic studies

Kinetic studies were carried out by adding stock solution of chlorambucil or melphalan in methanol to 5.0 ml of an aqueous buffer 2-HPCD solution, previously equilibrated at the desired temperature in a water bath, and mixed thoroughly. The initial chlorambucil concentration in the final solution was 1.1×10^{-4} M, the initial melphalan concentration was 3.3×10^{-5} M, and the 2-HPCD concentration ranged from 0 to 20%, w/v. The methanol concentration in the final reaction mixture was 0.5%. The pH of the final reaction mixture was determined at the end of each experiment with a pH-meter [PW 9420 (Philips), U.K.] standardized at appropriate temperature. All reactions were run under pseudo-first-order conditions. Aliquots (20 μl) were injected into the column at

various intervals, and the pseudo-first-order rate constants were determined from the disappearance of the drug by linear regression of natural logarithm of the peak height vs. time plots. The half-life and the correlation coefficient were calculated for each run.

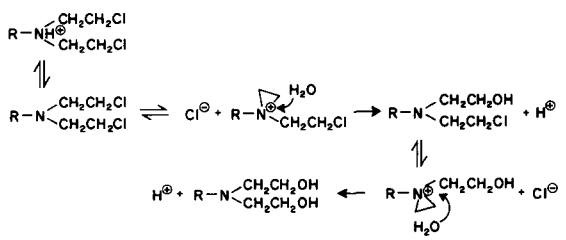
Theory

The degradation of both chlorambucil (Ehrsson et al., 1980) and melphalan (Stout and Riley, 1985) is believed to go through a cyclic ethyleneimmonium ion which is formed by intramolecular displacement of chloride by nitrogen (Bartlett et al., 1947) (see Scheme 1). The ethyleneimmonium ions are highly susceptible to substitution by nucleophiles such as water and, thus, the main degradation products in aqueous solutions are the dihydroxyderivatives (Scheme 1). Since the rate constants for degradation of both compounds have been shown to be directly proportional to the degree of unprotonated amine present, only this form of the drugs appears to undergo degradation in aqueous solutions. The ethyleneimmonium ion formation is a reversible reaction and addition of chloride ion to the solution results in slower degradation (Chatterji et al., 1982; Stout and Riley, 1985). Both chlorambucil and melphalan have more than one ionizable group and can exist in several ionized forms in aqueous solution (Schemes 2 and 3).

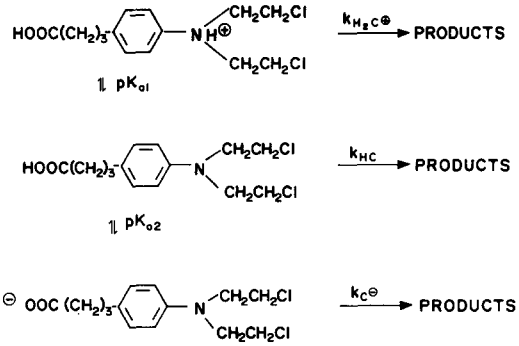
The pseudo-first-order rate constant (k_{obs}) for overall loss of chlorambucil can be defined by Eqn. 1 (Chatterji et al., 1982):

$$k_{obs} = k_{H2C^{\oplus}} f_{H2C^{\oplus}} + k_{HC} f_{H} + k_{C^{\ominus}} f_{C^{\ominus}} \quad (1)$$

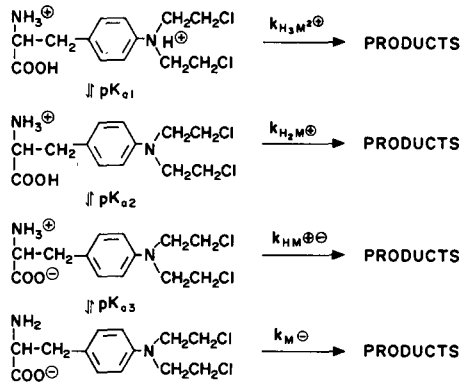
where $k_{H2C^{\oplus}}$, k_{HC} and $k_{C^{\ominus}}$ are the rate constants



Scheme 1. The degradation pathway of nitrogen mustards in aqueous solution.



Scheme 2. Ionic species of chlorambucil.



Scheme 3. Ionic species of melphalan.

for the degradation and $f_{H2C^{\oplus}}$, f_{HC} and $f_{C^{\ominus}}$ are the fractions of each species indicated in Scheme 2. The value of $k_{H2C^{\oplus}}$ has been shown to be negligible. Substitution of the fraction of each species by appropriate equation and making $k_{H2C^{\oplus}}$ equal to zero gives:

$$k_{obs} = \frac{k_{HC}[H^{\oplus}]Ka_1 + K_{c^{\ominus}}Ka_1Ka_2}{[H^{\oplus}]^2 + [H^{\oplus}]Ka_1 + Ka_1Ka_2} \quad (2)$$

where Ka_1 and Ka_2 are the dissociation constants of the species. The pseudo-first-order rate constant (k_{obs}) for the overall loss of melphalan can be defined by Eqn. 3 (Stout and Riley, 1985):

$$K_{obs} = k_{H3M^{2\oplus}} f_{H3M^{2\oplus}} + k_{H2M^{\oplus}} f_{H2M^{\oplus}} + k_{HM^{\oplus\ominus}} f_{HM^{\oplus\ominus}} + k_{M^{\ominus}} f_{M^{\ominus}} \quad (3)$$

where $k_{H3M^{2\oplus}}$, $k_{H2M^{\oplus}}$, $k_{HM^{\oplus\ominus}}$ and $k_{M^{\ominus}}$ are the rate

constants for the degradation of the ionized species of melphalan shown in Scheme 3 and $f_{H3M^{2\oplus}}$, $f_{H2M^{\oplus}}$, $f_{HM^{\oplus\theta}}$ and $f_{M^{\theta}}$ are the fractions of each species. The value of $k_{H3M^{2\oplus}}$ has been shown to be negligible.

Substitution of Eqns. 6–8 into Eqn. 5 and making $K_{H3M^{2\oplus}}$ equal to zero results Eqn. 4:

$$k_{obs} = \left\{ k_{H2M^{\oplus}}[H^{\oplus}]^2 Ka_1 + k_{HM^{\oplus\theta}}[H^{\oplus}] Ka_1 Ka_2 + k_{M^{\theta}} Ka_1 Ka_2 Ka_3 \right\} \times \left\{ [H^{\oplus}]^3 + [H^{\oplus}]^2 Ka_1 + [H^{\oplus}] Ka_1 Ka_2 + Ka_1 Ka_2 Ka_3 \right\}^{-1} \quad (4)$$

where Ka_1, Ka_2 and Ka_3 are the dissociation constants of the species $H_3M^{2\oplus}$, $H_2M^{\oplus}$ and $HM^{\oplus\theta}$, respectively.

Results and Discussion

Solubility studies

Due to their instability it was not possible to determine the solubility of the nitrogen mustards in aqueous solutions by the methods of Higuchi and Connors (1965). Instead we formed a supersaturated solution of the drug to be tested in an aqueous 2-HPCD solution by sonicating for a short period of time, a suspension of the drug in this medium and then allowing it to precipitate at 30 °C. Each experiment was repeated several times and the results reported are the average values, i.e. each point in Fig. 1 is the average value of at least two determinations.

Figure 1 shows the phase solubility diagrams or chlorambucil and melphalan in aqueous 2-HPCD solutions (0–25%, w/v) at 30 °C. The solubility of both drugs increased as a function of the 2-HPCD concentration and the melphalan solubility curve can be classified as type A_L, i.e. the complex formed has a first-order dependance on the 2-HPCD concentration, while the chlorambucil solubility curve appears to be of type Ap (Higuchi and Connors, 1965; Repta, 1981). Although the Ap type curve indicates that the complex contains

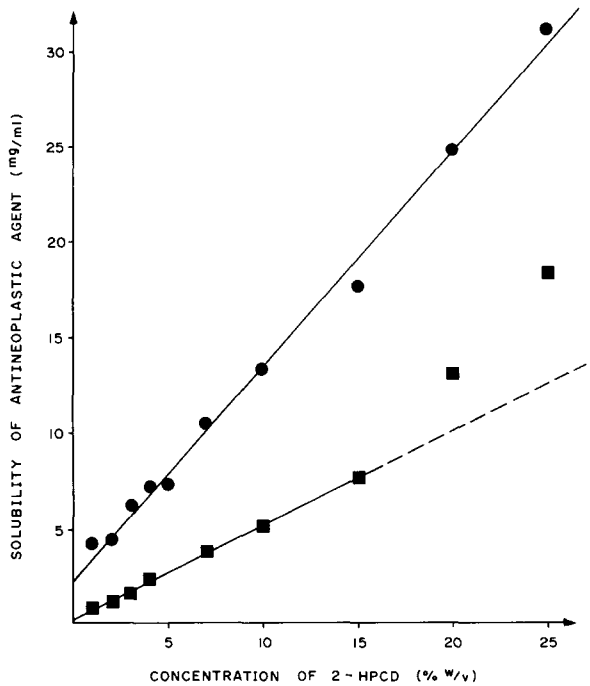


Fig. 1. Phase-solubility diagram of chlorambucil (■) and melphalan (●) in pure aqueous 2-HPCD solutions at 30 °C.

more than one molecule of 2-HPCD it was assumed throughout this study that the stoichiometry of both the chlorambucil and the melphalan 2-HPCD complex was 1 : 1 (drug : 2-HPCD molar ratio) at low ($\leq 20\%$, w/v) 2-HPCD concentrations.

Influence of pH

The degradation of both chlorambucil (Ehrsson et al., 1980) and melphalan (Stout and Riley, 1985) has been shown to follow first-order kinetics in aqueous buffer solutions at constant pH and temperature. Introduction of up to 20% (w/v) 2-HPCD to the reaction medium did not affect this kinetic behaviour, as linear relationship was in all cases obtained between the logarithms of the percent of drug remaining and time.

The influence of pH on the degradation of chlorambucil and melphalan in aqueous 2-HPCD buffer solutions was investigated over the pH-range of 1–12. The ionic strength of the buffer solutions was not constant but ranged from about 0.01–0.6 (Elving et al., 1956). As observed by

other investigators our data gave no evidence of any general acid/base or specific acid/base catalysis, but was consistent with a unimolecular reaction being the rate determining step (Ehrsson et al., 1980; Chatterji et al., 1982; Stout and Riley, 1985). Presence of up to 10% (w/v) 2-HPCD in the reaction medium did not appear to affect this mechanism.

The pH-rate profiles for the observed first-order degradation of chlorambucil in aqueous solutions containing 0 and 5% (w/v) of 2-HPCD at 40 °C are shown in Fig. 2. Both profiles display a sharp inflection between pH 2 and 3 and a plateau region at pH from about 5 (3 when 2-HPCD is present) to 12. These profiles are consistent with the different rates at which the three ionic forms of chlorambucil, i.e. $H_2C^{\oplus}$, HC and C^{θ}, are converted to their corresponding unstable ethyleneimmonium intermediate. Since only the unprotonated nitrogen mustard is capable of forming this intermediate the sharp inflection is attributable to the ionization of the nitrogen mustard group (Scheme 1). The ionization of the carboxylic group (Scheme 2) is thought to have some long-range

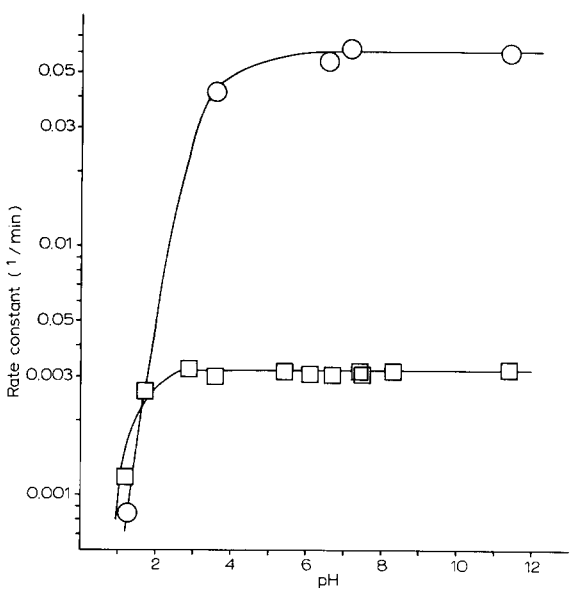


Fig. 2. The pH-rate profiles for the observed first-order degradation of chlorambucil in aqueous 2-HPCD buffer solutions at 40 °C: ○, 0% (w/v) and □, 5% (w/v) 2-HPCD. The solid lines are calculated using Eqn. 2 and the constants in Table 1.

TABLE 1

Apparent microscopic rate constants and the acidity constants for the degradation of chlorambucil in aqueous 2-HPCD buffer solutions at 40 °C

Parameter	Value	
	0% (w/v) 2-HPCD	5% (w/v) 2-HPCD
k_{HC}	5.0×10^{-2} min^{-1}	3.2×10^{-3} min^{-1}
$k_{c^{\theta}}$	6.0×10^{-2} min^{-1}	3.2×10^{-3} min^{-1}
pKa_1	3.0	1.3
pKa_2	5.1	–

electrodonating inductive effect on the nitrogen mustard, making C^{θ} slightly more reactive than HC (Chatterji et al., 1982). The solid lines in Fig. 2 are calculated using Eqn. 2 and the constants in Table 1. The pKa values of 3.0 ($H_2C^{\oplus}$) and 5.1 (HC), obtained from the pH-rate profile at 40 °C when no 2-HPCD is present in the buffer solutions, are comparable to 2.4 and 4.9 obtained from a pH-rate profile at 0.1 M ionic strength and 37 °C (Chatterji et al., 1982). The observed pKa-value ($H_2C^{\oplus}$) is 1.3 when 5% (w/v) 2-HPCD is present in the buffer solutions and no difference in reactivity of HC and C^{θ} could be observed under these conditions. Introduction of 5% (w/v) 2-HPCD to the buffer solutions results in about 20-fold increased stability at the plateau region.

The pH-rate profiles for the observed first-order degradation of melphalan in aqueous solutions containing 0, 5 and 10% (w/v) 2-HPCD at 60 °C are shown in Fig. 3. The shape of the pH-rate profile, obtained in aqueous solutions containing no 2-HPCD, can be explained by the different reaction rates of the four ionic species of melphalan ($H_3M^{2\oplus}$, $H_2M^{\oplus}$, $HM^{\oplus\theta}$, M^{θ}). As before only the unprotonated nitrogen mustard is capable of forming the unstable ethyleneimmonium intermediate and a sharp inflection is observed on the pH-rate profile at the pKa of this group. In general, the values of the microscopic rate constants increase with decreasing protonation of the melphalan molecule. The solid lines in Fig. 3 are calculated using Eqn. 4 and the values in Table 2. The pKa values of 1.2 ($H_3M^{2\oplus}$), 2.7 ($H_2M^{\oplus}$) and 8.5 ($HM^{\oplus\theta}$), obtained from the pH-rate profile at 60 °C when no 2-HPCD is present in the buffer

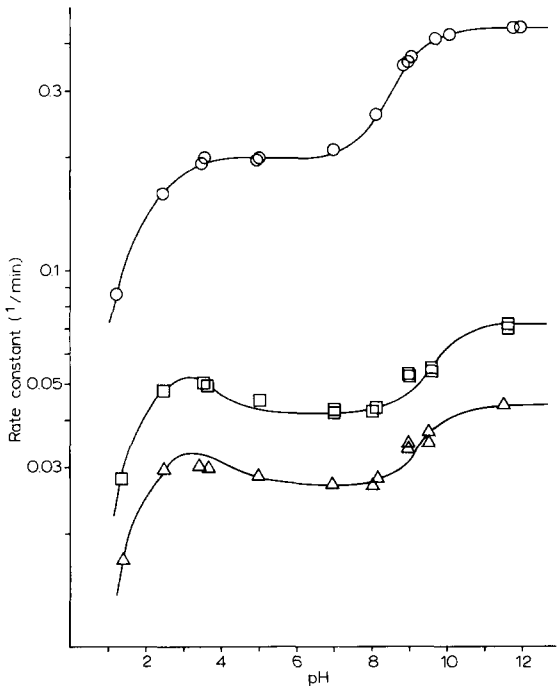


Fig. 3. The pH-rate profiles for the observed first-order degradation of melphalan in aqueous 2-HPCD buffer solutions at 60 °C: ○, 0% (w/v); □, 5% (w/v) and △, 10% (w/v) 2-HPCD. The curves are calculated using Eqn. 4 and the constants in Table 2.

solutions, compare well with the pKa values of 1.42, 2.75 and 9.17 obtained from a pH-rate profile at 0.4 M ionic strength and 37 °C (Stout and Riley, 1985). Also in this case the observed pKa values in aqueous 2-HPCD solutions were different from those obtained when no 2-HPCD was present, an artifact which at least partly can be explained by the different values of the stability constant for the four ionic species. Addition of 5% (w/v) 2-HPCD results in 5–6-fold increase in stability and addition at 10% (w/v) 2-HPCD results in 7–10-fold increase in stability of melphalan in aqueous buffer solutions.

TABLE 2

Apparent microscopic rate constants and acidity constants for the degradation of melphalan in aqueous 2-HPCD buffer solutions at 60.0 °C

Parameter	Value		
	0% w/v 2-HPCD	5% w/v 2-HPCD	10% w/v 2-HPCD
$k_{H2M^{\oplus}}$	0.13 min^{-1}	0.055 min^{-1}	0.035 min^{-1}
$k_{HM^{\oplus\oplus}}$	0.20 min^{-1}	0.042 min^{-1}	0.027 min^{-1}
$k_{M^{\oplus}}$	0.44 min^{-1}	0.072 min^{-1}	0.044 min^{-1}
Ka_1	1.2	1.5	1.5
Ka_2	2.7	4.0	4.0
Ka_3	8.7	9.7	9.5

Influence of 2-HPCD concentration

Increasing the 2-HPCD concentration in the reaction medium decreases the rate of degradation of both chlorambucil and melphalan and a non-linear relationship is obtained between the pseudo-first-order rate constants calculated from the slopes and the 2-HPCD concentration (Fig. 4). The rate decreases fast when the 2-HPCD concentration is increased from 0 to about 1×10^{-2} M (from 0 to about 2%, w/v) but then levels off at higher 2-HPCD concentration. These results are consistent with a kinetic system where a drug degrades at a higher rate outside than inside the complex

$$\text{Drug} + \text{2-HPCD} \underset{}{\overset{K_c}{\rightleftharpoons}} \text{Drug} \cdot \text{2-HPCD}$$

$$\text{Drug} \xrightarrow{k_o} \text{Degradation products} \qquad \text{Drug} \cdot \text{2-HPCD} \xrightarrow{k_c} \text{Degradation products}$$

Where k_o represents the pseudo-first-order rate constant for the degradation of the free drug, k_c the pseudo-first-order rate constant for the degradation of the drug in the complex and K_c the stability constant of the inclusion complex assuming 1:1 complexation (Lin and Yang, 1984; Hirayama and Uekama, 1987):

$$K_c = \frac{[\text{Drug} \cdot \text{2-HPCD}]}{[\text{Drug}]([\text{2-HPCD}] - [\text{Drug} \cdot \text{2-HPCD}])} \approx \frac{[\text{Drug} \cdot \text{2-HPCD}]}{[\text{Drug}][\text{2-HPCD}]} \quad (5)$$

The general rate equation for the change of the total drug concentration ($[\text{Drug}]_T$) is

$$-\frac{d[\text{Drug}]_T}{dt} = k_o[\text{Drug}] + k_c[\text{Drug} \cdot \text{2-HPCD}] \quad (6)$$

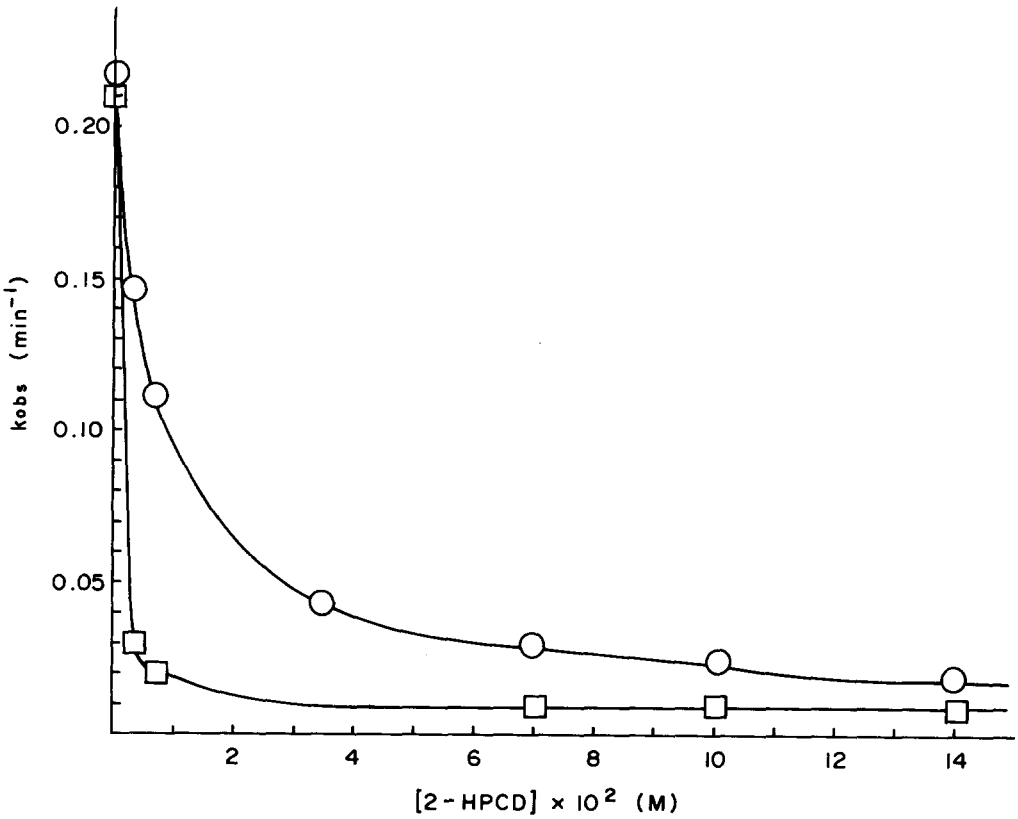


Fig. 4. Influence of 2-HPCD on the observed rate constants for degradation of chlorambucil at pH 7.25 and 50 °C and initial concentration of 1.1×10^{-4} M (□), and melphalan at pH 7.00 and 60 °C and initial concentration of 3.3×10^{-5} M (○).

Rearrangement of Eqn. 5 and substitution into Eqn. 6 gives Eqn. 7:

$$-\frac{d[Drug]_T}{dt} = \left[k_o \frac{1}{1 + K_c[2\text{-HPCD}]} + k_c \frac{K_c[2\text{-HPCD}]}{1 + K_c[2\text{-HPCD}]}\right][Drug]_T = k_{obs}[Drug]_T \quad (7)$$

where k_{obs} is the pseudo-first-order rate constant for the drug degradation:

$$k_{obs} = \frac{k_o + k_c K_c[2\text{-HPCD}]}{1 + K_c[2\text{-HPCD}]} \quad (8)$$

Rearrangement of Eqn. 8 yields Eqn. 9:

$$\frac{k_o}{k_o - k_{obs}} = \frac{k_o}{K_c(k_o - k_c)[2\text{-HPCD}]} + \frac{k_o}{(k_o - k_c)} \quad (9)$$

Knowing k_o, k_c and K_c can be calculated after construction of Lineweaver–Burk plots using Eqn. 9 (Fig. 5). The value of k_c is obtained from the ordinate intercept and k_o, and K_c is obtained by dividing the slope into the ordinate intercept. The values of k_c were both much smaller and much less sensitive to changes in pH than k_o (Tables 3 and 4). The stability constants were also affected by pH, being significantly larger at high than low pH. Thus, the anionic forms of the drugs appear

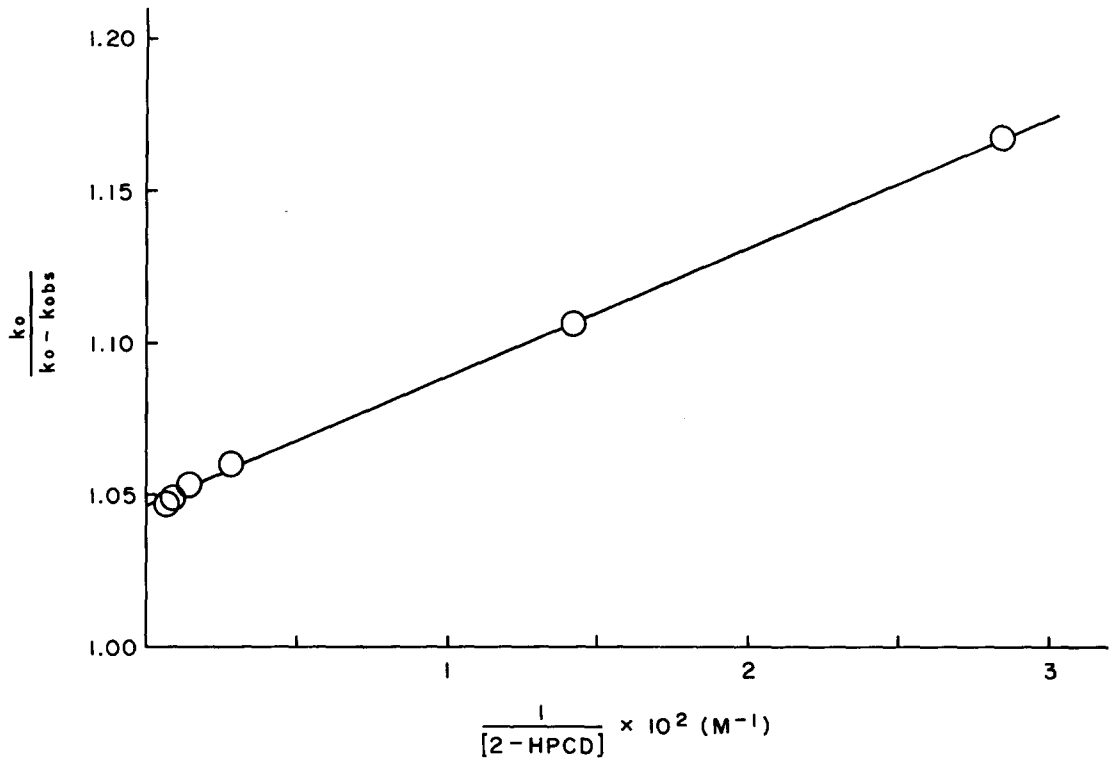


Fig. 5. Representative Lineweaver–Burk plot for degradation of chlorambucil in pH 7.25 aqueous buffer solutions at 50.0 °C. The initial chlorambucil concentration was 1.1×10^{-4} M.

TABLE 3

The first-order rate constants and the stability constants for the degradation of chlorambucil in aqueous 2-HPCD buffer solutions at 50.0 °C

pH ± S.D.	$k_o \times 10^2$ (min^{-1})	$k_c \times 10^3$ (min^{-1})	K_c (M^{-1})
1.34 ± 0.04	1.95	9.98	391.8
7.25 ± 0.07	21.0	9.53	2518

TABLE 4

The first-order rate constants and the stability constants for the degradation of melphalan in aqueous 2-HPCD buffer solutions at 60.0 °C

pH ± S.D.	$k_o \times 10^2$ (min^{-1})	$k_c \times 10^3$ (min^{-1})	K_c (M^{-1})
1.30 ± 0.08	8.61	5.03	77.7
2.46 ± 0.07	16.0	6.95	80.8
7.00 ± 0.05	22.0	7.51	121
11.65 ± 0.24	43.4	10.8	175

to form more stable complexes with 2-HPCD than the cationic forms.

Influence of temperature

Linear plots of $\ln(k/T)$, where k is k_c, k_o or

TABLE 5

The first-order rate constants, the stability constants and activation parameters for the degradation of chlorambucil in aqueous 2-HPCD pH 7.25 ± 0.07 McIlvaine Buffer solutions

°C	$k_o \times 10^2$ (min^{-1})	$k_c \times 10^3$ (min^{-1})	K_c (M^{-1})
40.0	6.14	2.62	3244
45.0	12.2	4.80	2808
50.0	21.0	9.53	2518
60.0	51.3	26.7	1664
$\Delta H^{\ddagger}$ (kJ/mol)	87.7	98.4	−31.5
$\Delta S^{\ddagger}$ (kJ/mol/deg)	−21.7	−14.4	−278

TABLE 6

The first-order rate constants, the stability constants and the activation parameters for the degradation of melphalan in aqueous 2-HPCD pH 7.00 ± 0.05 McIlvaine buffer solutions

°C	$k_o \times 10^2$ (min^{-1})	$k_c \times 10^3$ (min^{-1})	K_c (M^{-1})
50.0	7.64	2.85	171
55.0	13.2	4.70	145
60.0	22.0	7.51	121
70.0	51.3	18.0	89.5
$\Delta H^{\ddagger}$ (kJ/mol)	85.1	82.2	−32.6
$\Delta S^{\ddagger}$ (kJ/mol/deg)	−37.4	−73.9	−303

K_c, versus 1/T, based on the Eyring equation:

$$\ln(k/T) = \ln(k_B/h) + (\Delta S^{\ddagger}/R) - (\Delta H^{\ddagger}/R)1/T \quad (10)$$

where k_B is the Boltzmann constant, T is the absolute temperature and R is the gas cónstant, were used to determine the enthalpy of activation ($\Delta H^{\ddagger}$) and the entropy of activation ($\Delta S^{\ddagger}$) at the plateau regions of the pH-rate profiles in Figs. 2 and 3 (Tables 5 and 6). The values of the enthalpies and entropies for the degradation of the free drug are conforming with what would be expected for a reaction with ordered cyclic transition state. Both the enthalpy and the entropy of activation for the stability constants have negative values. The enthalpy obtained for the stability constant of an inclusion complex formation is always negative and the complex dissociates when the temperature is increased (Saenger, 1980), and the entropy has a large negative value associated with formation of highly ordered state.

Conclusion

Our data shows that the aqueous solubility of chlorambucil and melphalan can be significantly improved by addition of 2-HPCD. About 18-fold increase in aqueous solubility of chlorambucil and about 6-fold increase in the aqueous solubility of melphalan was observed at 30 °C when 20% (w/v) 2-HPCD was added as solution medium.

The aqueous stability of chlorambucil and melphalan can be increased by forming an inclusion complex with 2-HPCD since both drugs degrade at much slower rate within the 2-HPCD cavity than outside in the aqueous solution. About 19-fold increase in the aqueous stability of chlorambucil and about 5-fold increase in that of melphalan were obtained at neutral pH and 40 and 60 °C, respectively, when 5% (w/v) was added to the reaction medium.

The results also indicate that the stabilizing and solubilizing effects of 2-HPCD increase with decreasing temperature.

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