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Alkaline hydrolysis of methyl, ethyl and *n*-propyl 4-hydroxybenzoate esters in the liquid and frozen states

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Summary

The alkaline hydrolysis of the methyl, ethyl and *n*-propyl esters of 4-hydroxybenzoic acid was studied in the liquid and frozen states in sodium hydroxide solutions. The temperature range was −26 to 60°C. Significant acceleration of the reaction rate was evident in the frozen state compared with rates found at liquid state temperatures. The maximum reaction rate in the frozen state occurred in the temperature range −12 to −10°C. Methyl 4-hydroxybenzoate showed more than a 20-fold increased rate constant from 7.17×10^{-6} s^{-1} at 30°C to 1.53×10^{-4} s^{-1} at −9°C in a 1.00×10^{-2} M solution of sodium hydroxide. Rate constants in the liquid and frozen states followed pseudo first-order kinetics over 2–4 half-lives of reaction. Data were fitted to a theoretical model describing the reaction rate in the frozen state as dependent upon the increased concentration of solutes in liquid vesicles in the frozen state and the predicted reduction in the reaction rate constant with temperature decrease. Although the data exhibited similar trends to that predicted by the model, there was frequently a 50% difference in the rates observed compared with those predicted. This study has clearly demonstrated that there is a significantly increased rate of hydrolysis of these esters in the frozen state. This is a further indication that it cannot be assumed that drugs stored in solution will necessarily be stabilized, or their stability enhanced, on freezing. Storage under refrigeration conditions (4–8°C) results in enhanced shelf-lives compared with deep-freeze storage at −20°C under the conditions of this study.

Introduction

Although a number of studies have examined the chemical stability of the methyl, ethyl and *n*-propyl esters of 4-hydroxybenzoic acid (parabens) (Aalto et al., 1953; Raval and Parrott, 1962; Kamada et al., 1973; Blaug and Grant, 1974; Sunderland and Watts, 1984, 1985) only Sunderland and Watts (1984, 1985) have reported detailed investigations in highly alkaline solutions. Under these conditions, the phenolic 4-OH group is dissociated which causes a diminution in rate compared with that predicted if dissociation did not occur.

Stability studies on these esters have thus far been reported only in the liquid state. There is an increased demand to store pharmaceuticals in the frozen state (for example, Dinel et al., 1977), with the objective of enhancing the shelf-life. Al-

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though improved stability frequently results, a number of studies on antibiotic solutions (Savello and Shangraw, 1971; Concannon et al., 1986; McDonald et al., 1989) have demonstrated significantly decreased drug stability in the frozen state when compared with liquid state storage conditions. The hydroxyl ion catalysed degradation of hexobarbital sodium has also shown enhanced reaction rates in the frozen state (Larsen and Jensen, 1969).

Freezing a solution initially results in partial crystallization of pure solvent. The remainder stays as a liquid phase containing the solutes. The concentration is dependent upon the frozen state temperatures and saturation solubilities (Pincock and Kiovsky, 1966) of the solutes present. This two-phase system will persist until the eutectic temperature is attained. In these circumstances it is possible for the reduced reaction rates expected from temperature lowering to be increased by the influence of concentration increase on second or higher order reactions occurring in the liquid regions of frozen solvent. The impact on pharmaceuticals is therefore dependent upon the initial concentration, solubility, reactivity, phase relationships and the presence of additives.

This study has investigated the influence of freezing on the alkaline hydrolysis of solutions of methyl, ethyl and *n*-propyl 4-hydroxybenzoate esters.

Materials and Methods

Methyl 4-hydroxybenzoate (British Drug Houses, LR), ethyl 4-hydroxybenzoate (British Drug Houses, LR) and *n*-propyl 4-hydroxybenzoate (Aldrich) were all recrystallised from ethanol-water admixtures. 4-Hydroxybenzoic acid (Hopkin and Williams, GPR) was recrystallised from water. Sodium hydroxide (Allwest, AR), potassium hydrogen phthalate (Ajax, AR) and sodium chloride (British Drug Houses, AR) were used as received. Water was de-ionised and passed through a Milli-Q (Millipore) apparatus prior to use and had a specific conductivity less than $5.5 \times 10^{-6}\ \Omega^{-1}\ \mathrm{cm}^{-1}$.

Kinetic studies in the liquid state involved mixing equal volumes of ester and sodium hydroxide solutions to give a final concentration of 2.5×10^{-5} M of ester and the required concentration of base. The reaction rates were investigated by placing approx. 2 ml in 1 cm quartz cells in a heated cell compartment located in a spectrophotometer, held at the temperature of the run (±0.1°C). The absorbances were measured, after temperature equilibration, at various timed intervals between 350 and 200 nm on a diode array UV-Vis spectrophotometer (Hewlett-Packard). The residual ester concentration was calculated from standardized extinction data normally at 300 nm, based on absorbance spectra obtained after 10 $t_{1/2}$ of reaction.

For the frozen state, solutions with ester concentrations of 2.5×10^{-4} M were mixed with a range of $[\mathrm{OH}^-]$. 2-ml aliquots were placed in 10-ml volumetric flasks and these were then frozen at −26°C in a freezer, for 30 min. They were then transferred to an environmental chamber set at the required temperature (±0.3°C) (Thermoline). After equilibration for 30 min, flasks were removed at zero and subsequent time intervals, thawed by the addition of water and made up to volume at room temperature. The absorbance values were measured immediately as before. Additionally, studies were carried out at −26.0°C in the freezer (±0.5°C). Reactions were normally followed for 10 $t_{1/2}$.

Effects of the initial ester concentration were examined using 1×10^{-4} and 1×10^{-5} M methyl 4-hydroxybenzoate in the liquid state and in the frozen state at 1×10^{-3} and 5×10^{-5} M. Similar methods of analysis were employed.

Hydroxide ion concentration was determined by titration with potassium hydrogen phthalate.

Analysis of results

All reactions were studied under pseudo first-order conditions. A plot of log $(A - A_\infty)$ vs time gives a linear relationship of slope $-k_{\mathrm{obs}}/2.303$, where A is the absorbance measured at any time t, and A_∞ denotes the measured absorbance after 10 $t_{1/2}$ (99,9%) of reaction.

Activation energy data were obtained by normal procedures of plotting log k vs $(1/T)$. All

TABLE 1

Observed pseudo first-order rate constants and calculated second-order rate constants for the alkaline hydrolysis of 4-hydroxybenzoate esters

Ester	Temperature (°C)	[NaOH] (M)	$10^5 k_{obs}$ (s^{-1})	$10^4 k_2$ ($M^{-1} s^{-1}$)
Methyl	30.0	0.010	0.7	7.2
		0.050	4.0	8.0
		0.100	8.7	8.7
	40.0	0.010	1.6	16.4
		0.050	7.9	15.9
		0.100	16.2	16.2
	50.0	0.010	3.7	37.2
		0.050	19.7	39.3
		0.100	39.0	39.0
	60.0	0.010	6.8	67.8
		0.050	36.7	73.4
		0.100	71.7	71.7
Ethyl	30.0	0.025	1.1	4.2
		0.050	2.2	4.4
		0.100	4.2	4.2
	40.0	0.010	0.9	9.3
		0.050	3.8	7.5
		0.100	8.0	8.0
	50.0	0.010	1.6	16.3
		0.050	8.3	16.6
		0.100	16.8	16.8
	60.0	0.010	3.4	34.0
		0.050	16.8	33.5
		0.100	34.7	34.7
n-Propyl	30.0	0.025	0.7	2.9
		0.050	1.4	2.8
		0.100	3.0	3.0
	40.0	0.025	1.7	6.8
		0.050	3.4	6.8
		0.100	6.8	6.8
	50.0	0.025	3.1	12.4
		0.050	6.2	12.4
		0.100	13.1	13.1
	60.0	0.025	5.7	22.7
		0.050	11.1	22.2
		0.100	24.5	24.5

data were fitted by a least-squares procedure and errors are stated for 95% confidence limits.

Results and Discussion

The observed pseudo first-order rate constants obtained in the liquid state are listed in Table 1 together with calculated second-order rate constants. In all cases, rate data showed excellent linearity over 4–5 $t_{1/2}$ of reaction. These rate data were reproducible within ±3%.

At high concentrations of hydroxide ion the dominant reaction involves hydroxide ion catalysis of ester anion hydrolysis (Sunderland and Watts, 1984):

$$E^- + OH^- \xrightarrow{k_2} \text{products} \qquad (1)$$

hence

$$-\frac{d[\text{total ester}]}{dt} = k_2[OH^-][E^-] \qquad (2)$$

Integration at constant $[OH^-]$ gives:

$$\log[E^-] = \log[E^-]_0 - k_{obs}t/2.303 \qquad (3)$$

where

$$k_{obs} = k_2[OH^-] \qquad (4)$$

and $[E^-]$ is the concentration of ester anion and $[E^-]_0$ corresponds to the initial concentration. k_2 is a second-order rate constant. As $[OH^-]$ was in considerable excess, the observed rate constant values, k_{obs}, were evaluated under pseudo first-order conditions.

There was a tendency for the values for second-order rate constants to increase slightly with increased ionic strength. This may indicate a minor salt effect. Although both species undergoing reaction are anions, the ester species at the reaction site is expected to be only partially negative by resonance. This may give rise to the small increase in rate that occurred with increased ionic strength. Ionic strength was not maintained constant, however, in these runs since the total concentration of all species present (reactants, products and other solutes) influences rates in the frozen state (see Eqns 6 and 7). Data at constant ionic strength have been previously reported (Sunderland and Watts, 1984).

When k_{obs} was plotted vs $[OH^-]$ second-order linear relationships were observed with intercept

TABLE 2

Activation energies for the alkaline hydrolysis of 4-hydroxybenzoate esters

Ester	Activation energies (kJ mol^{-1}) at hydroxide ion concentration (M)			
	0.010	0.025	0.050	0.100
Methyl	61.7±0.8	–	60.3±0.8	57.1±0.8
Ethyl	56.2±0.7	–	57.5±0.8	59.0±0.8
n-Propyl	–	56.7±0.8	57.8±0.8	59.0±0.5

values not statistically significantly different from zero.

Activation energy data

Data obtained using the Arrhenius equation at four sodium hydroxide concentrations are listed in Table 2. All data exhibited linear plots. The activation data were in acceptable agreement with those of Sunderland and Watts (1984). They are slightly lower than those of Blaug and Grant (1974). Their data were derived at lower pH and are mixed activation parameters for hydroxide ion catalysis of ester in the molecular and anionic forms.

Frozen state studies

Hydrolysis reactions were studied to 10 $t_{1/2}$ of reaction as in the liquid state. Pseudo first-order plots showed excellent linearity for 4 $t_{1/2}$ of the reaction. Some examples are shown in Fig. 1 and the observed rate constants found at various initial sodium hydroxide concentrations are recorded in Table 3 for all esters studied. Some liquid state runs at 0.01 M NaOH were very slow and hence were not evaluated, no data being presented for these. The times for attainment of equilibrium temperatures in the frozen state were checked by the measurement of temperatures with thermocouples immersed in the solutions. Once a set of conditions had been standardized, the actual runs were carried out without thermocouples in the solutions.

It is evident that some of the reactions studied in the frozen state are faster than in the liquid state at much higher temperatures. For example, methyl 4-hydroxybenzoate has a k_{obs} value of

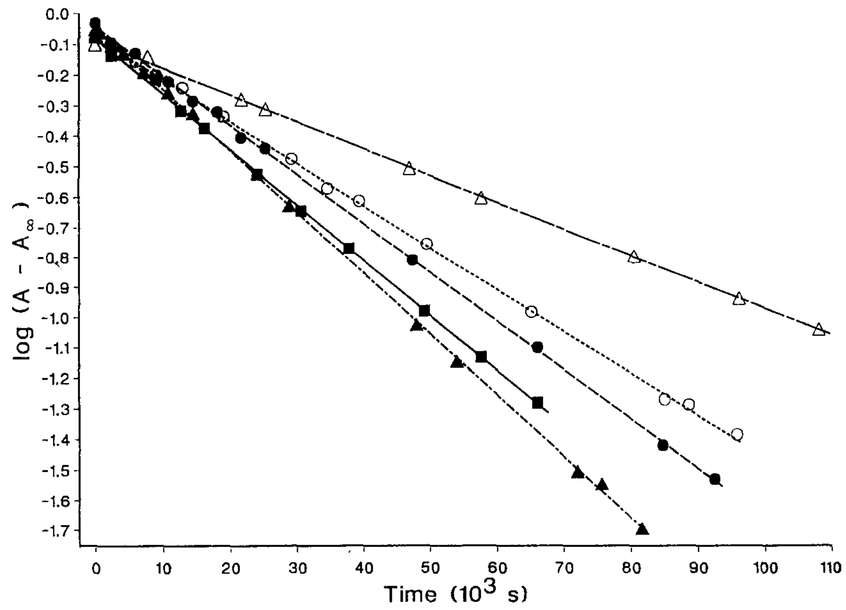

Fig. 1. Pseudo first-order plots for the hydrolysis of *n*-propyl 4-hydroxybenzoate in 0.05 M NaOH at −4.0 (●), −6.0 (▲), −13.8 (■), −19.0 (○) and −26°C (△).

TABLE 3

Rate constants for the hydrolysis of 4-hydroxybenzoate esters in frozen solutions

Ester	Temperature (°C)	NaOH liquid state concentration (M)	10^5k_{obs} (s^{-1})	$10^5k_2c_f$ [a] (s^{-1})
Methyl	−26.0	0.010	4.7	2.2
		0.050	6.3	2.5
		0.100	6.5	2.8
	−19.0	0.010	6.1	3.6
		0.050	7.8	4.1
		0.100	8.0	5.0
	−13.8	0.010	8.1	5.0
		0.050	11.1	5.5
		0.100	11.2	6.7
	−9.0	0.010	10.5	5.3
		0.050	11.7	5.9
		0.100	11.8	7.7
	−6.0	0.010	7.2	5.0
		0.050	8.6	5.7
		0.100	9.2	7.3
	−4.0	0.010	7.1	4.3
		0.050	7.6	4.7
		0.100	8.1	6.1
Ethyl	−26.0	0.010	1.6	1.6
		0.050	3.3	1.6
		0.100	3.5	1.0
	−19.0	0.010	2.0	2.7
		0.050	4.4	2.5
		0.100	2.9	1.9
	−13.8	0.010	5.2	3.6
		0.050	6.0	3.2
		0.100	6.2	2.6
	−9.0	0.010	5.3	4.1
		0.050	6.6	3.3
		0.100	6.7	3.0
	−6.0	0.010	5.0	3.8
		0.050	5.8	3.2
		0.100	5.9	2.9
	−4.0	0.010	4.3	3.1
		0.050	5.0	2.6
		0.100	5.2	2.4
n-Propyl	−26.0	0.010	0.3	– [b]
		0.050	2.0	1.3
		0.100	2.7	0.5
	−19.0	0.010	0.9	–
		0.050	3.2	2.0
		0.100	3.4	0.9
	−13.8	0.010	2.5	–
		0.050	4.1	2.6
		0.100	4.7	1.3
	−9.0	0.010	2.5	–
		0.050	5.1	2.6
		0.100	5.4	1.5
	−6.0	0.010	3.4	–
		0.050	4.6	2.5
		0.100	4.7	1.3
	−4.0	0.010	3.0	–
		0.050	3.7	2.1
		0.100	3.7	1.1

[a] Calculated from Eqn 10.

[b] Not evaluated in the liquid state.

7.17×10^{-6} s^{-1} at 30°C; at −6°C the value is 7.22×10^{-5} s^{-1} with an initial identical OH^- concentration of 1.00×10^{-2} M. This illustrates an approx. 10-fold increased degradation rate when storage occurs in the frozen state at −6°C compared with liquid state storage at 30°C. Some examples of this effect are illustrated in Fig. 2. Significant accelerations in rate in the frozen state compared with that of the liquid state have been reported previously (Grant et al., 1962; Larsen and Jensen, 1969; Concannon et al., 1986; McDonald et al., 1989).

This behaviour has been explained by Pincock and Kiovsky (1966) as being due to a concentration effect. Freezing of a dilute solution induces crystallization of pure solvent. In the frozen state a liquid phase containing solutes (in solution, dependent upon solubility) persists over a considerable range of temperatures below the initial freezing point. Hence, at any frozen state temperature there is an equilibrium between pure solid solvent and a liquid phase containing solutes in solution. At the eutectic temperature the system is completely solid. At or below this temperature only a solid-state reaction should occur.

Within the liquid phase of a frozen system soluble reactants become increasingly concentrated as the temperature is decreased. The effect is also influenced by the concentration of

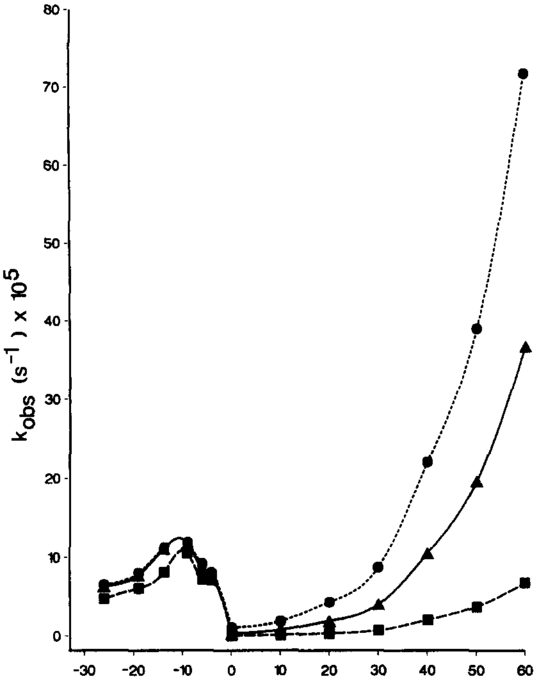

Fig. 2. Effect of temperature on the rate of degradation of methyl 4-hydroxybenzoate in (■) 0.01, (▲) 0.05 and 0.1 (●) M NaOH.

solutes present, which will include products and other solutes as well as reactants, and by the solubility-temperature relationships of these materials. Enhanced hydrolysis rates occurring in the frozen state as compared with the liquid state are mainly due to the crystallization of water molecules. This results in an increase in hydroxide ion concentration in the remaining liquid phase.

Within the liquid regions of the frozen system the rate expression for the reaction can be written as:

$$\frac{-d[E_f^-]}{dt} = k_2[OH_f^-][E_f^-] \tag{5}$$

where subscript f denotes concentrations in the liquid regions of the frozen systems. Normally rates are measured in thawed samples. Employing the concentration effect model derived by Pincock and Kiovsky (1966) this can be expressed as:

$$-\frac{d[E_s^-]}{dt} = k_2 c_f \cdot \frac{[OH_s^-][E_s^-]}{c_s} \tag{6}$$

where subscript s refers to concentrations measured in thawed solutions following reaction in the frozen state. The term c_f is the concentration in the liquid regions in the frozen state, c_s represents the concentration of all reactants, products and other solutes in the thawed system and k_2 is a second-order rate constant.

Under the conditions of this study, the concentration terms are dominated by $[OH^-]$. Hence, the rate of the frozen state reaction when measured in the thawed condition is:

$$-\frac{d[E_s^-]}{dt} = k_2 c_f \cdot \frac{[OH_s^-][E_s^-]}{[E_s^-] + [OH_s^-] + [P_s]} \tag{7}$$

where P relates to the concentrations of products formed. Since the bimolecular reaction should produce 2 moles of products the denominator term should be constant. Then integration yields:

$$\log[E_s^-] = \frac{k_2 c_f t}{2.303} \cdot \frac{[OH_s^-]_0}{[E_s^-]_0 + [OH_s^-]_0 + [P_s]_0} + \log[E_s^-]_0 \tag{8}$$

and under pseudo first-order conditions

$$k_{obs} = k_2 c_f \cdot \frac{[OH_s^-]_0}{[E_s^-]_0 + [OH_s^-]_0 + [P_s]_0} \tag{9}$$

the terms k_2 and c_f are dependent upon the temperature at which the frozen state reaction is studied. The subscript 0 refers to initial concentrations. Under the conditions of these studies, the last term in Eqn 9 is close to unity. Hence:

$$k_{obs} \cong k_2 c_f \tag{10}$$

The term k_2 is dependent upon the temperature at which the frozen state reaction is studied. From the Arrhenius equation:

$$k_2 = Ae^{-E_a/RT} \quad (11)$$

then from Eqn 10

$$\log k_{obs} = \log A - E_a/2.303RT + \log c_f \quad (12)$$

Application of this model is illustrated in Fig. 3 for the methyl ester in 0.1 M NaOH. The k_2 values at frozen state temperatures are estimated by extrapolation of the reaction rates obtained in the liquid state to these temperatures using the Arrhenius equation. Values of c_f are obtained from literature data of the phase diagram of aqueous solutions of sodium hydroxide at frozen temperatures (Pickering, 1893).

It is evident from Fig. 3 that the observed data correspond to consistently faster rates than predicted by the model over the whole of the frozen state temperature range. A similar difference (occasionally more marked) occurred at other hydroxide ion concentrations and with the other esters studied. One exception was noted for the ethyl ester in 0.01 M NaOH at low temperatures. It is possible to calculate c_f values from the relationship $c_f = (273.15 - T)/(2 \times 1.86)$ where 1.86 is the cryoscopic constant of water. This assumes ideal behaviour of sodium hydroxide in the frozen state, which at the high concentrations predicted by the model is unlikely.

Calculations of these values give similar c_f values to those measured by Pickering (1893) at and below −13.8°C. Hence, although there could be error in the c_f values, the calculated and experimental values are in close accord. Estimation of k_2 values involves a significant extrapolation of rate constants measured in the liquid state. This would induce a significant error in the calculated k_2 values. However, the consistency of the observed values, which are approx. 50% higher than those calculated, persists for all esters and all sodium hydroxide ion concentrations investigated with the exception previously mentioned. In this case, the E_a value was the lowest obtained, which would tend to predict higher k_2c_f values. The difference in the E_a values however was small.

According to the model, rates of hydrolysis in the frozen state should be independent of the initial hydroxide ion concentration. Higher [OH$^-$] should result in increased volumes of water (less

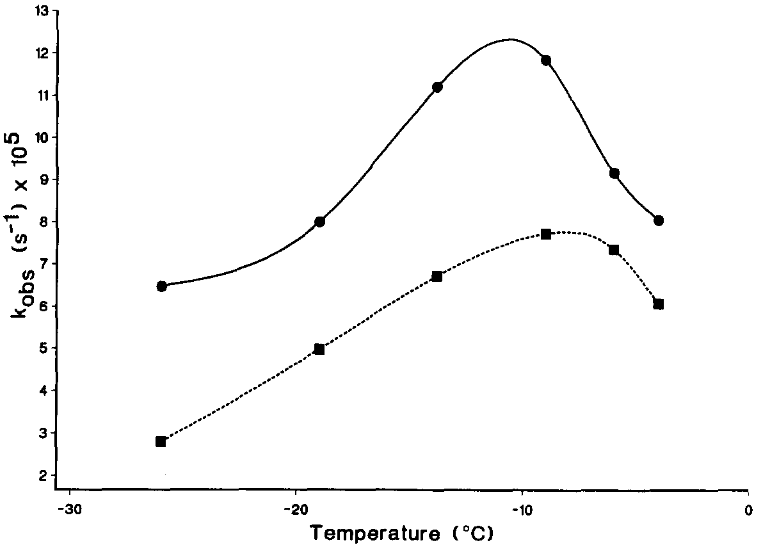

Fig. 3. The observed rate constants for the hydrolysis of methyl 4-hydroxybenzoate in 0.1 M NaOH (●) and that calculated from Eqn 10 (■).

ice), hence maintaining constant total species concentrations. This occurs to maintain the solid-liquid equilibrium in the frozen state which is dependent only on temperature. Degradation of the ester yields two species (acid and alcohol) which should be balanced by an equivalent reduction in [OH$^-$] and ester. The equilibrium between ice and water should be almost entirely controlled by the concentration of hydroxide ion. Although the rate constants showed little difference at higher concentrations of OH$^-$, a slower rate was consistently observed at 1.0×10^{-2} M hydroxide ion concentration. In this system the ester would undergo the greatest concentration enhancement. This would increase any interactions involving non-ideality (e.g. hydrogen bonding) that could influence the reaction rate. The other possibility could be a limitation of solubility of the esters. They are reported as highly soluble at this pH (1 in 1–2 of water at 20°C (Reynolds, 1989)). Precipitation of ester should result in zero-order kinetics. There was no evidence of this occurring even in the initial stage of a kinetic run.

To further investigate the trend, methyl 4-hydroxybenzoate ester was studied in 0.200 M NaOH. The rate constant of this run at −13.8°C was 1.13×10^{-4} s^{-1} compared with 1.12×10^{-4} s^{-1} in 0.100 M NaOH. This confirms that the rate is independent of the initial hydroxide ion concentrations at the higher concentrations.

TABLE 4

Rate constants for the hydrolysis of methyl 4-hydroxybenzoate at varying concentrations

Initial ester concentration (M)	$10^5\ k_{obs}$ (s^{-1}) in NaOH concentration (M)		
	0.010	0.050	0.100
−4.0°C			
5×10^{-5}	7.1	7.6	8.1
1×10^{-3}	–	7.4	7.8
40.0°C			
1×10^{-5}	1.6	7.7	16.2
1×10^{-4}	1.5	8.0	15.9

Effect of initial concentration of ester

Studies performed at −4°C using the methyl ester at initial concentrations of 1.00×10^{-5} and 1.00×10^{-3} M showed linearity over 4–5 $t_{1/2}$ of reaction. Data obtained from these studies at 5.00×10^{-2} and 0.10 M NaOH (Table 4) indicated the rate constants showed little variation with initial ester concentration. The higher concentration of ester was not studied at 1.00×10^{-2} M NaOH, since pseudo first-order conditions may not be maintained over the course of the reaction. This provides support for the pseudo first-order mechanism assigned to the hydrolysis reaction in the frozen state. The data for the liquid state temperature illustrate normal pseudo first-order behaviour (Blaug and Grant, 1974).

Conclusion

Significant alkaline hydrolysis of methyl, ethyl and *n*-propyl 4-hydroxybenzoates occurred in the liquid and frozen states. Marked acceleration of the hydrolytic degradation occurred in the frozen state. This occurs because of the enhanced concentration of OH$^-$ induced by freezing. These esters are therefore very unstable under highly alkaline conditions in the frozen state.

This study has shown that pseudo first-order conditions found in the liquid state continue in the frozen state. The rate of reaction under frozen state conditions showed very much less dependency on the initial hydroxide ion concentration. It is evident that freezing a drug solution does not always result in highly stable frozen systems. In this case, storage in a refrigerator (4–8°C) would result in a longer shelf-life than deep-freeze storage at −20°C.

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