Kinetics of Aspirin Hydrolysis and Stabilization in the Presence of 2-Hydroxypropyl-β-Cyclodextrin

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Received February 21, 1992; accepted June 3, 1992
KEY WORDS: aspirin; hydroxypropyl-β-cyclodextrin; complexation; stability; activation energies.

INTRODUCTION

Cyclodextrins (CDs) form noncovalent inclusion complexes with many substances (1). The commonly studied CD is βCD, which has a cavity whose inside diameter is ~7 Å, large enough to accommodate an aromatic ring. Inclusion complex formation may accelerate or decelerate the reactivity of the guest molecule, depending on the nature of the reaction and the orientation of the guest within the cyclodextrin cavity. βCD completely inhibits the alkaline hydrolysis of ethyl-*p*-aminobenzoate (2) but accelerates the hydrolysis of aspirin in alkaline solution (3). Deceleration of hydrolysis has been ascribed to the shielding of the functional ester group from the attacking nucleophile as the ester molecule undergoes total inclusion with the active center completely buried inside the void of the cyclodextrin cavity. Acceleration of the hydrolysis rate, on the other hand, stems from partial inclusion of the ester molecule into the cavity, leaving the active nucleophilic center sterically fixed in close proximity to the nucleophilic primary 6-hydroxyl group at the C_6 position of the cyclodextrin molecule. Chemically modified cyclodextrins may display greater complexing ability and improved aqueous solubility (4). 2-Hydroxypropyl-β-cyclodextrin (HPβCD) is obtained by replacing the C_6 primary hydroxyl group of βCD with the 2-hydroxypropyl group.

The focus of the present work was to study the effect of inclusion on the hydrolysis of aspirin at different pH levels (1.3–10) and temperature (40–60°C). Further, association constants were determined for complex formation using the model-predicted equation and to evaluate the effect of HPβCD on the activation energy (E_A) and frequency factor (A) associated with the hydrolysis of complexed aspirin.

MATERIALS AND METHODS

Materials

HPβCD was a gift from American Maize-Products Company, Hamond, IN. Aspirin and salicylic acid were obtained from Sigma Chemicals (St. Louis, MO). All other chemicals were of reagent grade and used as received.

Buffer System

All kinetic studies were performed in 0.05 *M* buffer solution at a fixed ionic strength of 0.1 *M*. The pH 1.3 buffer was prepared from HCl/KCl, pH 3.0 and 6.0 buffers from Na citrate/Na phosphate, pH 8.0 buffer from Na phosphate, and pH 10.0 buffer from glycine/NaOH.

Analytical Procedure

The amount of salicylic acid formed after hydrolysis was determined by a spectrophotometric method. The samples were analyzed by measuring the absorbance of iron (III)–salicylic acid complex (5) at 530 nm in a Perkin–Elmer spectrophotometer. In acidic pH, salicylic acid (3 molecules) combines with ferric ion (1 ion) to make a color complex with λ_{max} at 530 nm. Neither HPβCD nor aspirin interferes with this procedure. Ferric ion was supplied from a solution containing 4 g of $Fe(NO_3)_3$, 4 g of $HgCl_2$, and 12 ml of 1.0 *N* HCl per 100 ml of solution. This solution was filtered through 0.45-μm filter paper before use. An appropriate standard curve was prepared with salicylic acid (0.025–0.7 mg/ml). One milliliter of salicylic acid solution was mixed with 5 ml of ferric nitrate solution and kept for 15 min to develop the color. The absorbance was measured immediately after withdrawing the samples.

Degradation Kinetics of Aspirin in Aqueous Solution

Kinetic studies were carried out in specified-temperature and constant-pH buffered solution (I = 0.1 *M*) in a system with or without HPβCD. An accurate weight (10 mg) of powdered aspirin and different but accurate amount of HPβCD were taken in a 10-ml volumetric flask and brought to exactly 10 ml with buffer. The aspirin concentration was 5.55 m*M* and HPβCD concentrations were 0, 3.45, 6.9, 13.8, 27.6, and 41.4 m*M*. Hydrolysis was carried out at 40, 50, and 60°C in a Dubnoff constant-temperature water bath. Aliquots were withdrawn periodically from each flask and analyzed for salicylic acid concentration. Pseudo-first-order rate constants for the hydrolysis of aspirin were determined from the slope of the linear plot of the logarithm of residual aspirin against time.

Phase Solubility Study

This study was performed according to the method of Higuchi and Connors (6) at 25°C and pH 2.5, with various concentrations of HPβCD (0–40 m*M*). These temperature and pH conditions were used to minimize the degradation of aspirin during experimentation. An excess quantity of aspirin was suspended in constant buffer (pH 2.5) containing various concentrations of HPβCD in screw-cap vials. These vials were placed in a water bath at 25°C and shaken for 24 hr. An aliquot was filtered (0.45-μm, Millipore HV) and a portion was diluted and analyzed by UV spectrophotometry. Relative solubilities, S/S_o, where S_o is the solubility of aspi-

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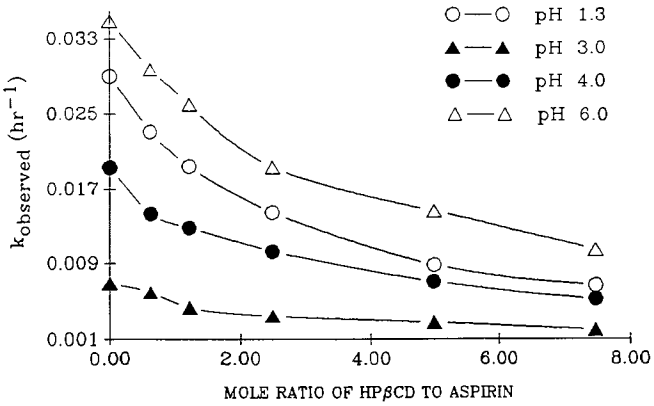


Fig. 1. Effect of HPβCD on the observed pseudo-first-order rate constant for the hydrolysis of aspirin at 40°C and different pH values.

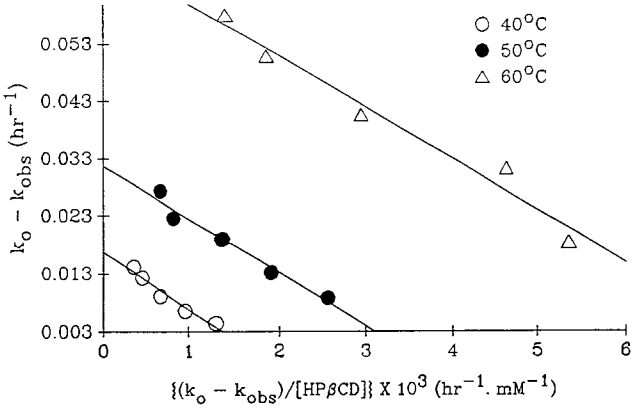


Fig. 2. Plots of rate data of aspirin hydrolysis according to the model predicted equation [Eq. (4)] at a constant pH 3.0 and different temperatures.

rin in the absence of cyclodextrins and S is the solubility at different HPβCD concentrations, were plotted as a function of total HPβCD concentration [HPβCD]. The complex formation rate constant was determined from the slope and intercept of the Eq. (1), where K_F is the equilibrium constant for aspirin–HPβCD complexation.

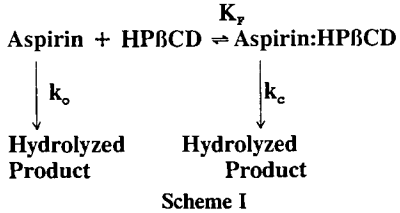
$$\frac{S}{S_o} = 1 + \frac{K_F[HP\beta CD]}{1 + K_F S_o} \quad (1)$$

RESULTS AND DISCUSSION

Aspirin is a substituted phenyl ester. As an aromatic ester, the most important reaction contributing to the instability of aspirin in aqueous solution is its hydrolysis, which yields salicylic and acetic acid. The hydrolysis of aspirin in aqueous buffered solution follows pseudo-first-order kinetics in the absence and presence of HPβCD.

Figure 1 illustrates the effect of HPβCD on the observed pseudo-first-order rate constant for the hydrolysis of aspirin. It is evident that k_{obs} is not a linear function of HPβCD concentration but, rather, asymptotically approaches a minimum value with increasing HPβCD concentrations. This saturation behavior is characteristic of reactions which proceed through complex formation occurring prior to the rate-determining step and may be accommodated by the reaction mechanism illustrated in Scheme I.

In Scheme I, k_o is the rate constant for hydrolysis for free aspirin, k_c is the rate constant for hydrolysis of complexed aspirin, and K_F is the apparent stability constant for complex formation. From this scheme the following rate expression can be derived:



Scheme I

$$\frac{-d[\text{Aspirin}]}{dt} = k_o[\text{aspirin}] + k_c[\text{aspirin:HP}\beta\text{CD}] \quad (2)$$

The observed rate of hydrolysis for aspirin in the presence of HPβCD is a weighted average of the rate of hydrolysis of free aspirin and rate of hydrolysis of aspirin complexed into HPβCD. The dependence of the observed rate constant (k_{obs}) of the hydrolytic reaction on the HPβCD concentration is quantitatively described by Eq. (3) (7):

$$k_{obs} = k_o + \frac{(k_c - k_o)[HP\beta CD]}{1/K_F + [HP\beta CD]} \quad (3)$$

Equation (3) can be rearranged to Eq. (4):

$$(k_o - k_{obs}) = \frac{-[1/K_F](k_o - k_{obs})}{[HP\beta CD]} + (k_o - k_c) \quad (4)$$

A plot of $(k_o - k_{obs})$ versus $(k_o - k_{obs})/[HP\beta CD]$ yields $-1/K_F$ as the slope and (k_o and k_c) as the intercept. In Fig. 2 the rate data at pH 3.0 and different temperatures have been plotted according to Eq. (4). The plots are linear, as expected, with a correlation coefficient >0.997. From the slope and intercept of the plots K_F and k_c are obtained as summarized in Table I. The agreement observed between the observed data and the linearity of the theoretic line according to Eq. (4) demonstrates that Scheme I adequately describes the degradation kinetics. The correlation also suggests that the interaction between aspirin and HPβCD results in the formation of a 1:1 stoichiometric complex.

The effect of temperature on the hydrolysis rate of aspirin has been investigated to gain further insight into the decomposition mechanism. Figure 3 depicts the Arrhenius

Table I. Stability and Rate Constants of Aspirin:HPβCD Complexes at pH 3.0 and Different Temperatures

Temperature	K_F (M^{-1})	k_c (hr^{-1})	k_o/k_{obs}
40°C	100.4	0.0027	7.19
50°C	108.2	0.0066	5.88
60°C	110.5	0.030	6.56

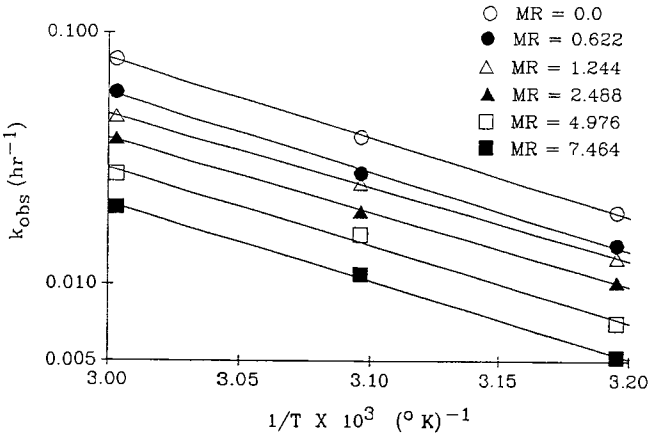


Fig. 3. Arrhenius plots for aspirin hydrolysis at pH 3.0 with various mole ratios (MR) of HPβCD to aspirin.

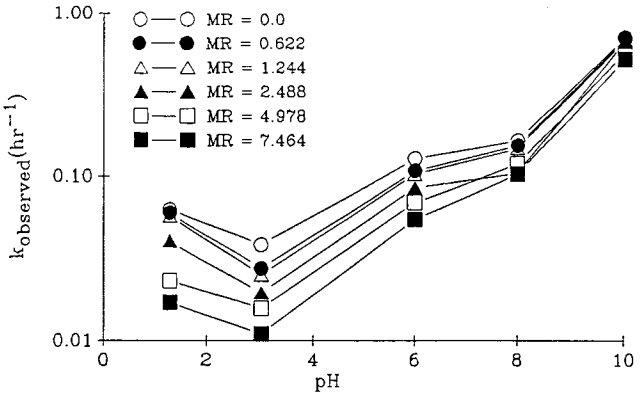


Fig. 4. The effect of pH on the hydrolysis of aspirin at different mole ratios (MR) of HPβCD to aspirin.

plots in the absence and presence of different concentrations of HPβCD over the temperature range of 40 to 60°C. The activation energy and the frequency factors have been calculated using the Arrhenius equation and are summarized in Table II. The activation energies (~14.5 kcal/mol) at all HPβCD concentrations are in good agreement with the value of 16.0 kcal/mol reported in literature (8). The consistent activation energies suggest that the mechanism of degradation probably remains unaltered. However, there is a significant decrease in frequency factor which asymptotically reaches a minimum. This is probably due to the inclusion of an aspirin molecule into the cavity of HPβCD, causing significant decrease in the hydrolysis of aspirin.

The effect of pH on the hydrolysis of aspirin at different concentrations of HPβCD is represented in Fig. 4. These pH rate profiles indicate that hydrolytic reaction is slowed down significantly at acidic pH's, but little or no effect was observed at higher pH's. Under alkaline conditions, an increased concentration of HPβCD did not stabilize aspirin in a proportionate manner. Chin *et al.* (3) observed an acceleration effect of βCD on the hydrolysis of aspirin. The conclusion was that aspirin was partially enclosed in the βCD cavity, exposing the reactive ester moiety to the catalytic effect of the ionized secondary hydroxyl group of βCD at higher pH's.

The stability constants of aspirin and HPβCD complex at various pH values (Fig. 5) indicate a dependency on pH. The stability constant tends to decrease with increasing pH of the medium. A possible explanation for this phenomenon is that greater solvation of the ionized carboxyl group at higher pH values may prohibit the aspirin molecule from penetrating as deeply into the cavity as the neutral carboxyl group. Ionization of aspirin with increased solvation may be another reason why the hydrolysis is not significantly inhibited at higher pH. In general, cyclodextrin cavity has a preferential affinity for the neutral form rather than the ionized form of a given substrate (9). The value for the stability constant of nonionized aspirin:HPβCD complex is 126 M^{-1} and that of ionized species is 31 M^{-1} (10).

In the phase solubility study for aspirin in an aqueous solution of HPβCD at pH 2.5 and 25°C, the solubility of aspirin increased linearly as a function of HPβCD concentration, and thus the phase solubility diagram can be classified as type A_L (6). The slope is less than one (~0.714), which is usually ascribed to the formation of a 1:1 complex. The stability constant, K_F, is determined from the slope and intercept of the straight line between aspirin and HPβCD concentration by Eq. (5).

$$K_F = \frac{\text{Slope}}{\text{Intercept}(1 - \text{Slope})} \tag{5}$$

The stability constant for complex formation at pH 2.5 (25°C) was found to be 98.9 M^{-1}, which is consistent with the constant derived from kinetic stability studies and also

Table II. Activation Energies and Frequency Factors of the Hydrolysis of Aspirin at 50°C and pH 3.0 with Various Mole Ratios of HPβCD to Aspirin

Mole ratio of HPβCD to aspirin	$A \times 10^3$ (sec^{-1})	E_A (kcal · mol^{-1})
0.0	83	14.4
0.612	71	12.8
1.224	49	13.5
2.448	11	13.5
4.976	10	14.0
7.464	8	14.0

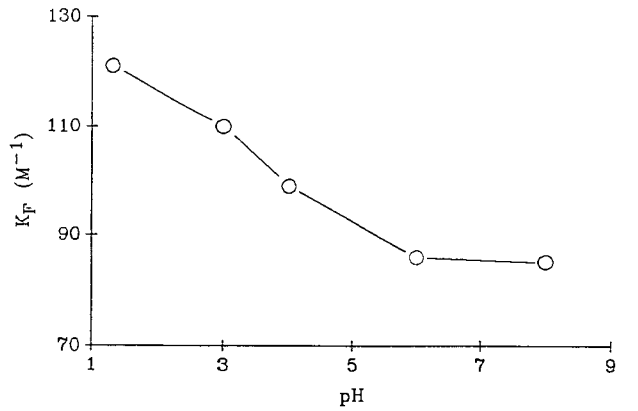


Fig. 5. The pH profile of stability constants of aspirin:HPβCD complex formation determined by the model-predicted equation.

reported in the literature (11). This agreement suggests that the model chosen (Scheme I) is appropriate.

In conclusion, complexation of aspirin with HPβCD has been shown to increase the solubility and stability of aspirin. These studies clearly demonstrate the feasibility of using HPβCD not only as solubilizers but also as stabilizers of highly unstable compounds.

ACKNOWLEDGMENT

The authors wish to thank Mr. Paul Howell, an undergraduate student at the School of Pharmacy, University of Wyoming, for his work in analyzing the aspirin samples by spectrophotometer.

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