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# Effect of 2-hydroxypropyl-β-cyclodextrin on the stability of prostaglandin $E_2$ in solution

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**Abstract**

The potential use of 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) in the stabilization of prostaglandin $E_2$ ($PGE_2$) was investigated. Inclusion complexation of $PGE_2$ with HP-β-CyD was estimated by circular dichroism spectroscopy. The effect of HP-β-CyD on the dehydration rate of $PGE_2$ and the isomerization rate of $PGA_2$ was examined by comparing the rate constant of $PGE_2$ alone with that of the inclusion complex of $PGE_2$ with HP-β-CyD in each buffered solution. The dehydration rate of $PGE_2$ and the isomerization rate of $PGA_2$ under acidic conditions were decreased by HP-β-CyD, while the rates under basic conditions were increased, probably due to positive catalytic effects on the reactions. The retardation of the dehydration and isomerization rates at low pH might be the effect of inclusion complexation with HP-β-CyD.

*Key words:* Prostaglandin $E_2$; Prostaglandin $A_2$; Prostaglandin $B_2$; Inclusion complex; 2-Hydroxypropyl-β-cyclodextrin; Hydrolysis; Stability

## 1. Introduction

Prostaglandin is a biologically active compound with a wide spectrum of pharmacological response such as labor induction, contraception, control of ulcers, asthma, and blood pressure regulation. The β-hydroxyketo group in the five-membered ring of prostaglandin $E_2$ ($PGE_2$) is dehydrated readily in aqueous solution to yield prostaglandin $A_2$ ($PGA_2$) which is consecutively isomerized to prostaglandin $B_2$ ($PGB_2$) under basic conditions (Connors et al., 1986). This chemical instability of $PGE_2$ makes aqueous formulation unfavorable.

In the currently available PGE formulations, inclusion complex formulations with natural cyclodextrin (CyD) such as α-, β- and γ-CyDs have been utilized for the solubilization and stabilization of PGEs (Uekama et al., 1981, 1984; Wiese et al., 1991). Recently, extensive efforts have been directed toward the development of CyD derivatives to improve the low aqueous solubility or to decrease the toxicity in parenteral applications of natural CyDs. An example of these derivatives is 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) which is highly water soluble because of its amor-

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phous structure and also these derivatives are useful in intravenous and other parenteral preparations because of their low hemolytic activity and irritation (Yoshida et al., 1988; Duchêne and Wouessidjewe, 1990).

Kinetic information on the solution stability of $PGE_2$ in terms of these dehydration and isomerization reactions has been limited due to the lack of a practical separation and stability-indicating method for $PGE_2$, $PGA_2$ and $PGB_2$ simultaneously under acidic or basic conditions. $PGA_2$ and $PGB_2$ are structurally very similar and have similar polarities. $PGE_2$, on the other hand, is more polar than $PGA_2$ and $PGB_2$. Hence, isocratic high-performance liquid chromatography (HPLC) has not provided adequate kinetic data for $PGE_2$ due to poor resolution and long retention time. Recently, a simple and simultaneous separation and assay method for $PGE_1$, $PGA_1$ and $PGB_1$ (Lee and DeLuca, 1991) as well as $PGE_2$, $PGA_2$ and $PGB_2$ (Lee et al., 1994) by reversed-phase HPLC was developed.

In the present study, this method was applied to investigate the effect of HP-$\beta$-CyD on the dehydration rate of $PGE_2$ and the isomerization rate of $PGA_2$ in aqueous solutions by comparing the rate constant of $PGE_2$ alone with that of the inclusion complex of $PGE_2$ with HP-$\beta$-CyD. Furthermore, the stabilizing effect of the inclusion complex as a function of HP-$\beta$-CyD concentration in each buffered solution was also examined.

## 2. Materials and methods

### 2.1. Chemicals

$PGE_2$, $PGA_2$ and $PGB_2$ were purchased from Sigma (St. Louis, MO, U.S.A.) and HP-$\beta$-CyD was from Aldrich (St. Louis, MO, U.S.A.). Acetonitrile, methanol and absolute ethanol were HPLC grade from Fisher (Pittsburgh, PA, U.S.A.). All other compounds were reagent grade obtained from commercial sources and were used as received without purification. Aqueous solutions were prepared using deionized and double-distilled water filtered with a 0.22 $\mu$m membrane of a Milli-Q system (Millipore, Bedford, MA, U.S.A.).

### 2.2. HPLC method

The concentration of $PGE_2$, $PGA_2$ and $PGB_2$ was measured by HPLC using a modular system which consisted of a pump (Model 501, Waters, U.S.A.), an injector (U6K, Waters, U.S.A.), a turnable UV detector (Model 484, Waters, U.S.A.) and a computing integrator (C-R6A, Shimadzu, Japan). The HPLC analyses were performed at ambient temperature using an LC-18 reversed-phase column (3 $\mu$m, 15 cm $\times$ 4.6 mm i.d.; Supelco, Bellefonte, PA, U.S.A.) with a mobile phase of 35% acetonitrile in 0.002 M phosphate buffer at pH 3.5. The HPLC chromatograph of $PGE_2$, $PGA_2$ and $PGB_2$ was operated at a flow rate of 1.5 ml/min using an injection volume of 5 $\mu$l. The eluent was monitored spectrophotometrically at 192 nm and peak areas were measured to determine the concentration.

### 2.3. Detection of inclusion complex by circular dichroism

The circular dichroism (CD) spectra were taken using a spectropolarimeter (I-500C, Jasco, Japan) at a detection wavelength of 230–340 nm. The measuring values of CD were expressed in terms of molecular ellipticity $[\theta]_\lambda$.

### 2.4. Kinetic methods

Stock solutions of 2 mg/ml of $PGE_2$, $PGA_2$ and $PGB_2$ were prepared in absolute ethanol and stored in vials at 4°C. The working solutions of $PGE_2$, $PGA_2$ and $PGB_2$ were prepared by dilution of the respective stock solution with absolute ethanol. The $PGE_2$ solution at a concentration of 20 $\mu$g/ml was prepared at pH 2.0, 7.2 and 10.0 by placing 0.1 ml of 200 $\mu$g/ml diluted stock solution of $PGE_2$ and 0.9 ml of the appropriate buffer solution into screw-cap vials. Buffers used were 0.05 M hydrochloric acid-0.05 M potassium chloride (pH 2.0), 0.002 M sodium dihydrogen phosphate-0.002 M sodium hydroxide (pH 7.2) and 0.002 M sodium bicarbonate-0.002 M sodium hydroxide (pH 10.0). The vials of working solutions were placed into a water bath maintained at 60°C. At appropriate intervals, each vial was removed from the water bath, equilibrated to room

$$\begin{array}{ccc} & \overset{K}{\Leftrightarrow} & \\ PGE_2 + HP\text{-}\beta\text{-}CyD & & PGE_2\text{-}(HP\text{-}\beta\text{-}CyD) \\ \downarrow k_0 & & \downarrow k_c \\ \text{Degradation products} & & \text{Degradation products} \\ & & + HP\text{-}\beta\text{-}CyD \end{array}$$

Scheme 1.

temperature and thoroughly shaken. The solutions were taken from the vial using a syringe and directly injected into the HPLC system without any modification. The disappearance of $PGE_2$ and the appearance of $PGA_2$ and $PGB_2$ were simultaneously determined by HPLC according to the method described above.

### *2.5. Stability kinetics of $PGE_2$ and its inclusion complex*

Stability tests for $PGE_2$ and its HP-β-CyD complex in buffered solutions were conducted at 60°C as a function of HP-β-CyD concentration. The inclusion complex solutions of $PGE_2$ with HP-β-CyD were obtained by mixing 0.1 ml of 200 mg/ml diluted stock solution of $PGE_2$ and 0.9 μl of each buffer solution of HP-β-CyD. The concentration of HP-β-CyD in the buffer solution was in the range of $10^{-4}$–$10^{-3}$ M.

The apparent first-order rate constant, $k_{obs}$ for the dehydration of $PGE_2$ in aqueous buffer solution was determined from the slope of a linear plot of the logarithm of residual $PGE_2$ concentration vs time. The dehydration rate constant of $PGE_2$ in aqueous buffered solution was compared with that of the inclusion complex of $PGE_2$ with HP-β-CyD in aqueous buffered solution. The dependency of $k_{obs}$ on the HP-β-CyD concentration was quantitatively treated according to Eq. 1 to obtain the stability constant of the complex, $K$ and pseudo first-order rate constant of the complex, $k_c$, on the following 1:1 complexation as shown in Scheme 1, where $k_0$ and $[HP\text{-}\beta\text{-}CyD]_t$ represent the rate constant in the absence of HP-β-CyD and the total concentration of HP-β-CyD, respectively (Brewster et al., 1992).

$$\frac{[HP\text{-}\beta\text{-}CyD]_t}{k_0 - k_{obs}} = \frac{1}{k_0 - k_c}[HP\text{-}\beta\text{-}CyD]_t + \frac{1}{K(k_0 - k_c)} \quad (1)$$

Lineweaver-Burk plots are obtained by plotting $[HP\text{-}\beta\text{-}CyD]_t/(k_0 - k_{obs})$ vs the HP-β-CyD concentration.

## 3. Results and discussion

The CD spectra of $PGE_2$ in the absence and presence of HP-β-CyD are shown in Fig. 1. Yamamoto et al. (1992) reported that $PGE_1$ exhibited a negative CD band around 292 nm due to the $n \rightarrow \pi^*$ transition of the C-9 carbonyl chromophore. Fig. 1 shows that the minimum molecular ellipticity of $PGE_2$ in 0.05 M HCl-0.05 M KCl buffer solution (pH 2.0) was observed at a wavelength of 277 nm. On addition of HP-β-CyD, the optical activity of $PGE_2$ was slightly increased and the negative peak was shifted to a shorter wavelength. Therefore, this result suggests that the intrinsic CD of $PGE_2$ may be more induced by complexing with HP-β-CyD.

The dehydration reaction of $PGE_2$ in aqueous solution of various composition of HP-β-CyD was examined. Typical effects of HP-β-CyD on the degradation of $PGE_2$ in aqueous buffered solu-

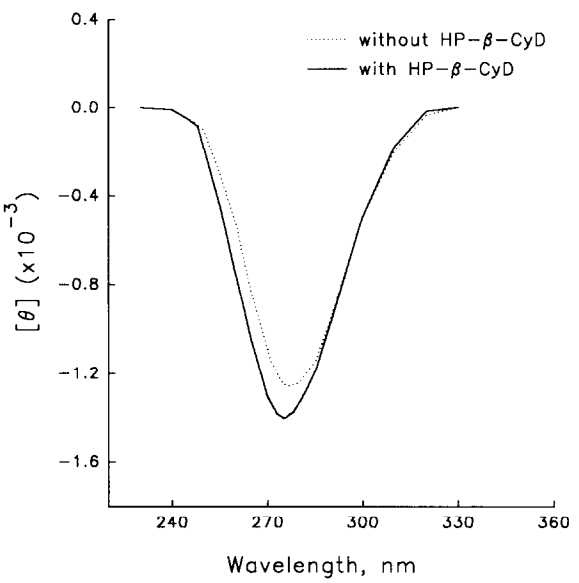


Fig. 1. CD spectra of $PGE_2$ ($5.674 \times 10^{-5}$ M) in the absence and presence of HP-β-CyD. (Dotted line) Without HP-β-CyD, (continuous line) with HP-β-CyD ($5.674 \times 10^{-4}$ M).

tions are shown in Fig. 2. The smooth line in Fig. 2 was calculated from the first-order rate equation. The $PGE_2$ concentration decayed exponentially with time in aqueous buffered solutions of pH 2.0, 7.2 and 10.0. For all solutions studied, the disappearance of $PGE_2$ showed first-order kinetic behavior. The degradation of $PGE_2$ under acidic pH conditions was suppressed by complexation with HP-β-CyD. On the other hand, HP-β-CyD accelerated degradation of $PGE_2$ with increasing HP-β-CyD concentration in the medium and under basic pH conditions.

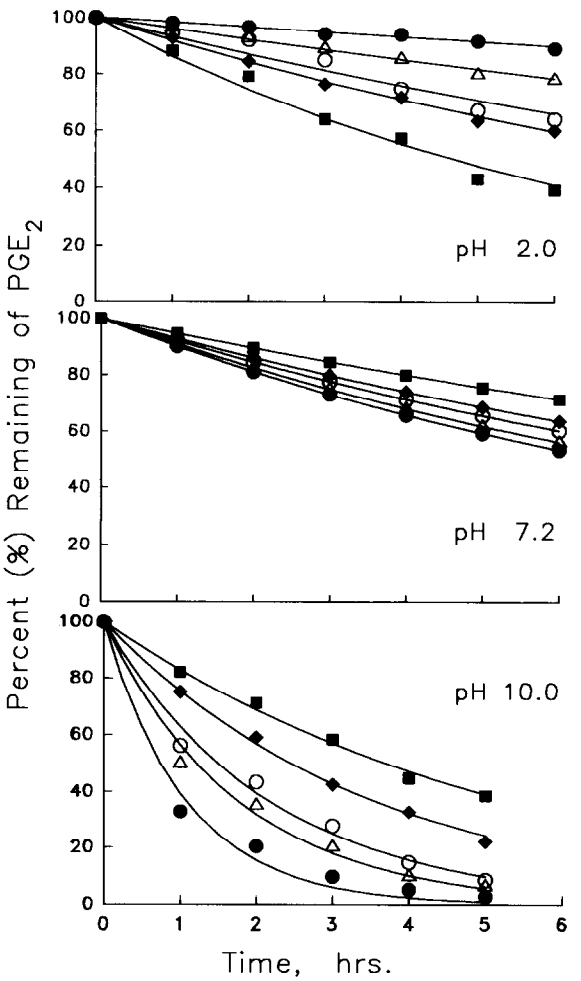


Fig. 2. Effect of HP-β-CyD on the dehydration reaction of $PGE_2$ at 60°C. (■) $PGE_2$ alone, (♦) $1.13\times10^{-3}$ M, (○) $2.27\times10^{-3}$ M, (△) $3.40\times10^{-3}$ M, (●) $5.67\times10^{-3}$ M.

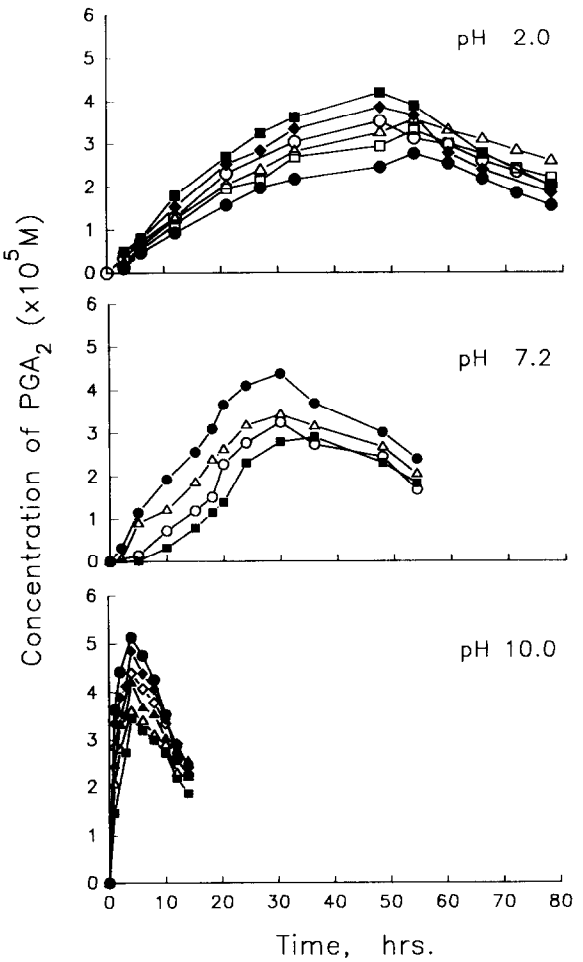


Fig. 3. Concentration profiles of $PGA_2$ depending on the concentration of HP-β-CyD at 60°C. (■) $PGE_2$ alone, (♦) $1.13\times10^{-3}$ M, (○) $2.27\times10^{-3}$ M, (△) $3.40\times10^{-3}$ M, (□) $4.54\times10^{-3}$ M, (●) $5.67\times10^{-3}$ M.

The concentration profiles of $PGA_2$ and $PGB_2$ with time as a function of HP-β-CyD concentration and pH are shown in Figs. 3 and 4, respectively. Under acidic conditions, isomerization of $PGA_2$ was also retarded by HP-β-CyD and therefore the rate of reaction decreased with increasing HP-β-CyD concentration. However, under basic conditions, HP-β-CyD accelerated the isomerization of $PGA_2$.

Fig. 5 shows the effect of HP-β-CyD concentration on the observed reaction rate constant, $k_{obs}$ of $PGE_2$ in aqueous buffered solutions of

pH 2.0, 7.2 and 10.0 at 60°C. Under acidic conditions, $k_{obs}$ decreased with increasing concentration of HP-β-CyD. Under basic conditions, $k_{obs}$ increased with increasing HP-β-CyD concentration. The apparent stability constant, $K$, and the rate constant of the complexes, $k_c$, were calculated based on the relation of $k_{obs}$ with HP-β-CyD concentration. The plots of the kinetic data derived from Eq. 1 are illustrated graphically in Fig. 6 as a plot of the ratios of $[\text{HP-}\beta\text{-CyD}]_t/(k_0 - k_{obs})$ vs HP-β-CyD concentration, $[\text{HP-}\beta\text{-CyD}]_t$, at pH 2.0, 7.2 and 10.0. A linear relationship was obtained for the inclusion complex of $PGE_2$ with HP-β-CyD, confirming 1:1 complexation. From the slope and intercept of those plots, values of $K$ and $k_c$ were calculated. The kinetic parameters calculated such as $k_0$, $k_c$, $k_c/k_0$ and $K$ are given in Table 1. The $K$ values for the HP-β-CyD

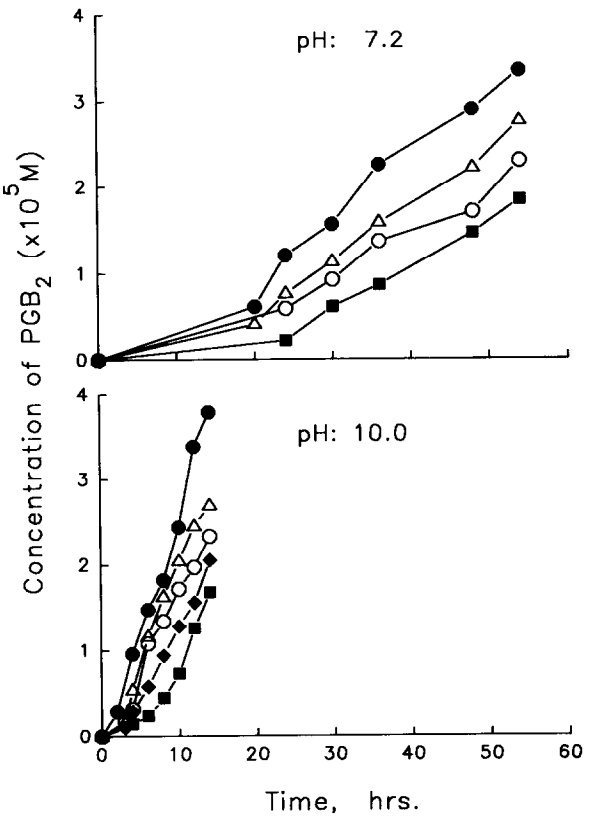


Fig. 4. Concentration profiles of $PGB_2$ depending on the concentration of HP-β-CyD at 60°C. (■) $PGE_2$ alone, (♦) $1.13\times10^{-3}$ M, (○) $2.27\times10^{-3}$ M, (△) $3.40\times10^{-3}$ M, (●) $5.67\times10^{-3}$ M.

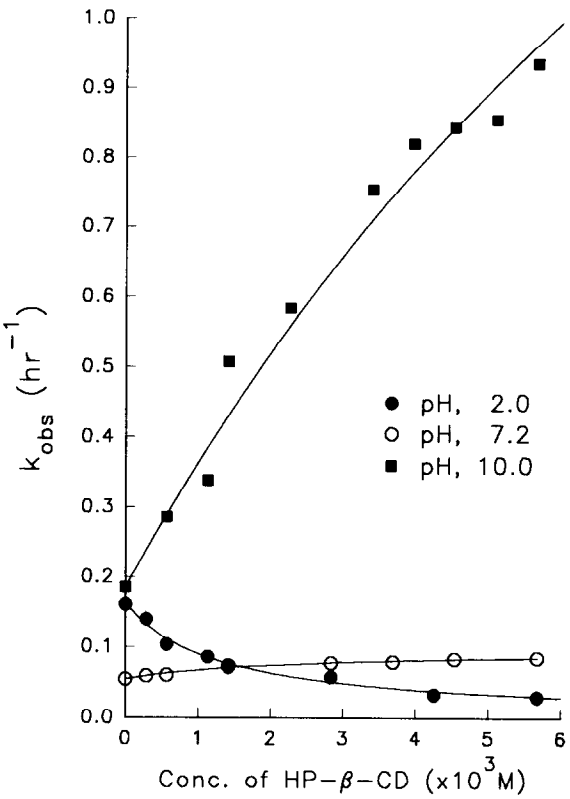


Fig. 5. Observed rate constants for the dehydration of $PGE_2$ as a function of HP-β-CyD concentration at various pH.

complex were dependent on the pH. Since the dissociation constant, $pK_a$, of $PGE_2$ is approx. 4.9–5.0 (Stehle, 1982) and the unionized form of $PGE_2$ can be bound more easily into the cavity of HP-β-CyD than the ionized form, the stability constant of the complex at high pH may be smaller than that at low pH. On comparison with the $k_c/k_0$ values, the dehydration of $PGE_2$ under acidic conditions was significantly suppressed by HP-β-CyD, probably due to the inclusion of the β-hydroxyketo moiety of $PGE_2$. In the presence of HP-β-CyD, the degradation rate of $PGE_2$ decreased more than 30-fold as compared with that of $PGE_2$ alone. However, under basic conditions, $PGE_2$ was destabilized, since the degradation rate of the inclusion complex of $PGE_2$ with HP-β-CyD increased more than 15-fold as compared with that of $PGE_2$ alone. This acceleration effect of HP-β-CyD on the degradation of $PGE_2$ under basic conditions was probably due to positive

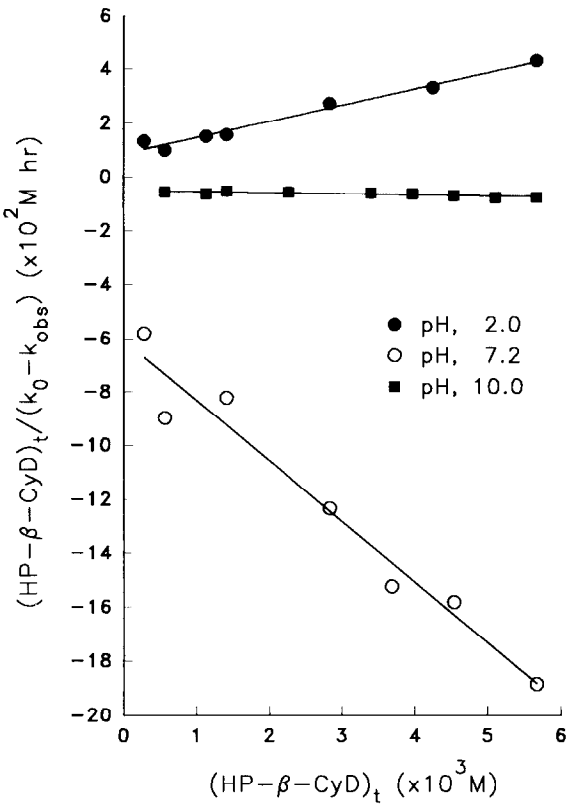


Fig. 6. Determination of $K$ and $k_c$ for $PGE_2$-(HP-β-CyD) inclusion complex by plotting kinetic data (Fig. 5) according to Eq. 1.

catalytic effects on the intramolecular general base catalysis of enol-enolate formation (Hirayama et al., 1984, 1986).

In conclusion, the effect of HP-β-CyD on the stability of $PGE_2$ in solutions was examined and it was confirmed that the dehydration rate of $PGE_2$ and the isomerization rate of $PGA_2$ under acidic conditions were decreased by HP-β-CyD, while the rates under basic conditions were increased, probably due to positive catalytic effects on the reactions. The retardation of the dehydration and isomerization rates at low pH might be the effect of inclusion complexation with HP-β-CyD.

Table 1
Apparent first-order rate constant, $k_c$, and stability constant, $K$, for the inclusion complex of $PGE_2$ with HP-β-CyD at various pH values and 60°C

| System | $k_0$ | $k_c$ | $k_c/k_0$ | $K$ |
|---|---|---|---|---|
| pH 2.0 | 0.1612 | 0.0052 | 0.03 | 695 |
| pH 7.2 | 0.0543 | 0.0992 | 1.83 | 363 |
| pH 10.0 | 0.1855 | 2.950 | 15.90 | 69 |

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