Schardinger Dextrin Interaction IV

Inhibition of Hydrolysis by Means of Molecular Complex Formation

By JOHN L. LACH and TING-FONG CHIN

Kinetic data presented substantiates the postulated 1:1 stoichiometric ratio for the benzocaine-cyclodextrin interaction. Data presented also show that the degree of retardation of benzocaine hydrolysis is considerably greater than that observed for the caffeine-type complexes indicating an interaction involving inclusion formation and other attractive forces.

THE APPLICATION of molecular complex formation as a means of drug solubilization and stabilization has received considerable attention in recent times. The complexing agents used in these studies include a variety of various types of molecules. Several reports have appeared in the literature concerning the application of such interaction in the stabilization of medicinal agents.

Recent studies (1–3) in these laboratories have indicated that the cyclodextrins[1] (homogeneous cyclic molecules containing 6, 7, or 8 D-glucopyranose units linked in a 1,4 position) also exhibit a marked tendency to solubilize various compounds. It was noted that the formation constants of these interactions were considerably greater than those reported for other interactions involving complex formation. The high values obtained for these formation constants indicate a higher degree of stability presumably due to an interaction involving inclusion formation and other attractive forces. It was of interest to study this interaction with respect to its ability to retard hydrolysis and to compare it to previously reported systems. The present study deals with the influence of beta-cyclodextrin on the base catalyzed degradation of benzocaine. Data from this kinetic study have been employed to verify the stoichiometric relationship of the cyclodextrin-benzocaine interaction and to compare the hydrolytic inhibition with other previously studied complexing agents.

EXPERIMENTAL

Reagents

Beta-cyclodextrin was prepared and purified by the modified method of French (4). $[\alpha]_D^{25}$ in water $= +162.5 \pm 0.5$. Benzocaine N.F. recrystallized; m.p. 87–88°.

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[1] The toxicity of the cyclodextrins has not been established.

Apparatus

A constant temperature water bath set at 30 ± 0.5° with rotating spindle; a "Tacan" thermostat water bath set at 30 ± 0.1, 35 ± 0.1, 40 ± 0.1, and 45 ± 0.1°; 20-ml. capacity vials with gum-rubber stoppers and aluminum caps; and a Beckman DU spectrophotometer, with 1-cm. silica cells were used.

Procedures

Benzocaine Interaction.—The solubility method of Higuchi and Lach (5) was used to study the interaction of benzocaine with cyclodextrin.

Benzocaine Hydrolysis.—An accurate amount of benzocaine was dissolved in hot distilled water. It was then allowed to cool down to a definite temperature in a constant temperature water bath. To this solution, a calculated amount of standard barium hydroxide solution was added and immediately brought up to a definite volume and separated into 20-ml. capacity vials (approximately 8 ml. was used for each vial). All of these procedures were conducted under nitrogen. Another series of samples was made under the same procedures and same conditions, containing various concentration of beta-cyclodextrin.

A 5-ml. sample solution was withdrawn from a vial at a designated time interval and immediately extracted with 3 × 10 ml. of chloroform. The chloroform extracts were combined and made up to 50 ml. with chloroform. The unhydrolyzed benzocaine was extracted by the chloroform and the hydrolyzed product, *para*-aminobenzoic acid, remained in the alkaline solution. The concentration of unhydrolyzed benzocaine was determinated spectrophotometrically at 285 mμ.

RESULTS AND DISCUSSION

The interaction of benzocaine with beta-cyclodextrin in aqueous solution is shown in Fig. 1. The straight-line relationship obtained indicates a first-order dependancy with respect to benzocaine concentration in the system. Since the concentration of benzocaine in this system studied is an invariant, a 1:1 stoichiometric relationship has been assigned to this interaction based on the slope obtained in Fig. 1.

The kinetic phase of this investigation was carried out at constant pH [0.04*N* $Ba(OH)_2$ solution] and at various temperatures. As has been reported previously benzocaine hydrolysis in aqueous solution follows a pseudo first-order reaction (6). The results of benzocaine hydrolysis in aqueous solution

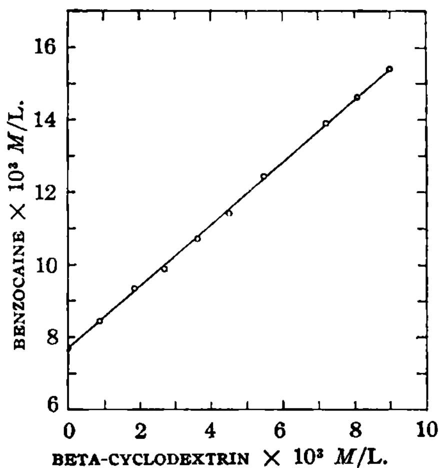


Fig. 1.—Interaction of benzocaine with beta-cyclodextrin at 30°.

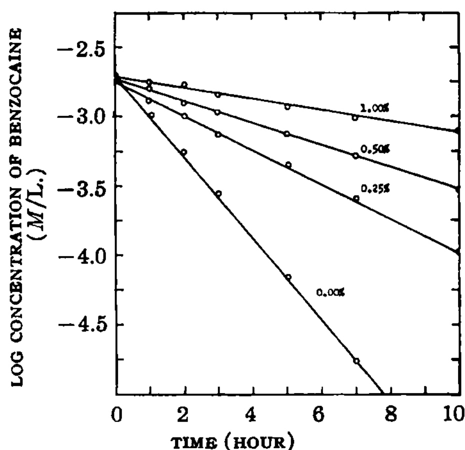


Fig. 2.—Influence of beta-cyclodextrin on benzocaine hydrolysis in 0.04 N $Ba(OH)_2$ at 30°.

with and without beta-cyclodextrin, shown in Fig. 2, demonstrate the marked ability of the cyclodextrin in inhibiting this hydrolytic reaction. It is interesting to point out here that this decrease in rate is proportional to the cyclodextrin concentration in the system. This is illustrated in Fig. 3 which shows the increase in half-life as a function of cyclodextrin in solution. The effect of beta-cyclodextrin on half-life and rate constant of benzocaine hydrolysis in aqueous solution is summarized in Table I.

From Table I it is clearly evident that addition of beta-cyclodextrin to this system produces a significant decrease in the hydrolytic rate of benzocaine. In a system containing 1% cyclodextrin a fivefold increase in the half-life of this reaction is achieved. This can be compared to the earlier work of Higuchi (6) in which he showed a 2.5-fold increase in the half-life with the addition of 1% caffeine. Pauli and Lach (7) reported smaller increases in the half-life of benzocaine with various complexing agents used in their study. This greater half-life observed in this cyclodextrin-benzocaine interaction resulting from inclusion formation is considerably stronger than those observed for the caffeine-type complex. It is reasonable to expect that inclusion formation would provide a better shield for the benzocaine ester linkage from hydroxyl ion attack. Rate constants and half-life of the hydrolysis of benzocaine with and without the cyclodextrin as a function of temperature are listed in Table II and Fig. 4.

This temperature dependancy for benzocaine degradation can also be illustrated by an Arrhenius plot as shown in Fig. 5. Plotted here are rate constants obtained at different temperatures for systems free of the cyclodextrin and those obtained at corresponding temperatures containing 1% beta-cyclodextrin. Interestingly the same slope is obtained in both cases, indicating that the degradation mechanism in systems containing the cyclodextrin is the same as that in systems without complexing agent, although the rate constants are different. The amount of free benzocaine existing in the two systems—the stoichiometric concentration or total benzocaine in each system being the same—causes the difference. In a benzocaine solution containing the cyclodextrin, the amount of free benzocaine is small and is governed by the dissociation constant of the complex. Since only one mechanism of degradation in the two systems studied

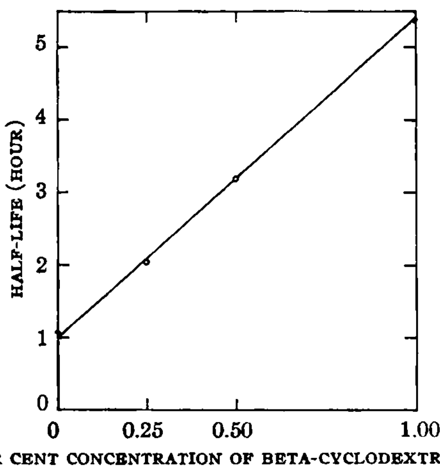


Fig. 3.—Beta-cyclodextrin influence on the half-life of benzocaine hydrolysis of 0.04 N $Ba(OH)_2$ at 30°.

TABLE I.—INFLUENCE OF BETA-CYCLODEXTRIN (β-CD) ON RATE CONSTANT AND HALF-LIFE OF BENZOCAINE HYDROLYSIS IN 0.04 N $Ba(OH)_2$ SOLUTION AT 30°

Per Cent of β-CD	Rate Constant (hr.$^{-1}$/M);	Half-life (hr.)
0.00	.666	1.04
0.25	.358	2.03
0.50	.229	3.19
1.00	.129	5.39

TABLE II.—INFLUENCE OF TEMPERATURE ON RATE CONSTANT AND HALF-LIFE OF BENZOCAINE HYDROLYSIS IN 0.04N $Ba(OH)_2$ IN A SYSTEM WITH AND WITHOUT BETA-CYCLODEXTRIN (β-CD)

	Rate Constant (hr.$^{-1}$/M)		Half-life (hr.)	
Temp.	No β-CD	1% β-CD	No β-CD	1% β-CD
30°	0.666	0.129	1.040	5.37
35°	0.849	0.170	0.828	4.14
40°	1.190	0.228	0.579	3.04
45°	1.576	0.339	0.439	2.05

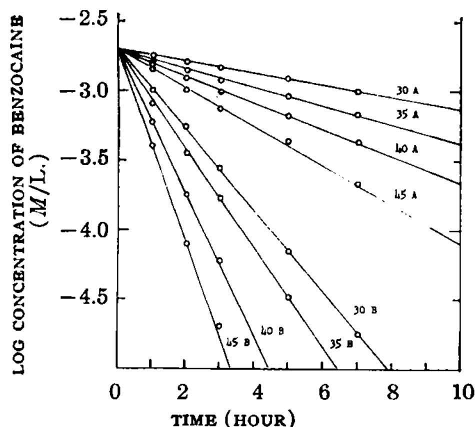
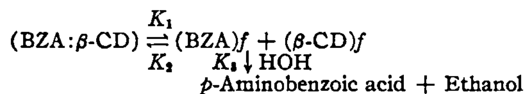


Fig. 4.—Influence of temperatures on benzocaine hydrolysis in 0.04 N $Ba(OH)_2$. Key: A, with beta-cyclodextrin; B, without beta-cyclodextrin.

is operative, one can conclude that this rate is dependent on the amount of free benzocaine in solution and not the total concentration present. The degradation rate of the complexed benzocaine species is negligible.

Based on the data obtained in this study and on the work of Guttman (8), the following degradation mechanism is proposed:



where K_1 is the dissociation constant, K_2 is the association constant, K_3 is the hydrolysis constant, $(BZA)f$ is free *benzocaine*, $(\beta\text{-CD})f$ is free beta-cyclodextrin, $(BZA)t$ is total benzocaine, and $(BZA{:}\beta\text{-CD})$ is the complexing compound.

Dissociation constant K_1 can be calculated from Eq. 1, if a 1:1 stoichiometric ratio is assumed for

$$K_1 = \frac{(BZA)f \cdot (\beta\text{-CD})f}{(BZA{:}\beta\text{-CD})} \qquad \text{(Eq. 1)}$$

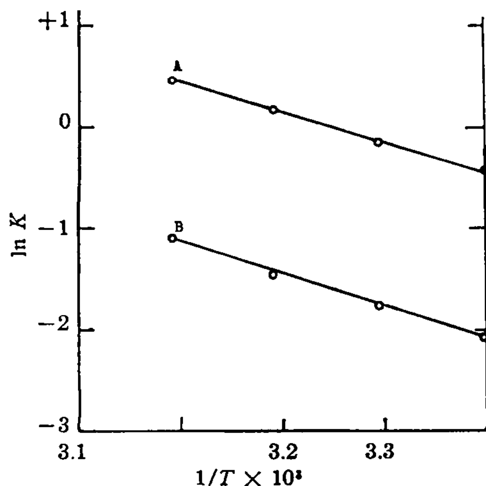


Fig. 5.—Arrhenius plot of hydrolysis of benzocaine in 0.04 N $Ba(OH)_2$ at different temperatures. Key: A, without beta-cyclodextrin; B, with beta-cyclodextrin.

this interaction. From experimental data, K_1 has been calculated to be 2.34×10^{-3}. The free benzocaine in the system containing beta-cyclodextrin can be calculated from Eq. 2. The molar

$$(BZA)f = \frac{k_1 \cdot (BZA{:}\beta\text{-CD})}{(\beta\text{-CD})f} \qquad \text{(Eq. 2)}$$

concentration of the (benzocaine:beta-cyclodextrin) complex is equal to the molar concentration of benzocaine present in the complexed form which in turn is equal to the difference of total benzocaine added to the system minus free benzocaine in solution. Since $(BZA{:}\beta\text{-CD}) = (BZA) = (BZA)t - (BZA)f$, therefore

$$(BZA)f = \frac{K_1[(BZA)t - (BZA)f]}{(\beta\text{-CD})f}$$

$$(BZA)f \cdot (\beta\text{-CD})f = K_1 \cdot [(BZA)t - (BZA)f] = K_1(BZA)t - K_1(BZA)f$$

$$(BZA)f \cdot (\beta\text{-CD})f + K_1 \cdot (BZA)f = K_1 \cdot (BZA)t$$

$$(BZA)f[K_1 + (\beta\text{-CD})f] = K_1(BZA)t$$

$$(BZA)f = \frac{K_1}{K_1 + (\beta\text{-CD})f}(BZA)t \qquad \text{(Eq. 3)}$$

Table III.—Comparison of Calculated and Experimental Apparent Rate Constants of Benzocaine Hydrolysis in a System Containing Beta-cyclodextrin (β-CD) in 0.04 N $Ba(OH)_2$ Solution at 30°

Per cent of β-CD	Experimental (hr.⁻¹/M)	Calcd. (hr.⁻¹/M)
0.00	0.666	
0.25	0.358	0.342
0.50	0.229	0.228
1.00	0.129	0.139

Equation 4 is rate equation for benzocaine hydrolysis in basic solution. Equation 5 is rate equation for

$$-d(BZA)f/dt = K_3(BZA)f \qquad \text{(Eq. 4)}$$

benzocaine hydrolysis in basic solution containing beta-cyclodextrin. The apparent rate constants,

$$-d(BZA)t/dt = K_3(BZA)f = K_3 \frac{K_1}{K_1 + (\beta\text{-CD})f}(BZA)t \qquad \text{(Eq. 5)}$$

evaluated for the reaction in a system containing beta-cyclodextrin are thus related to the true rate constant by Eqs. 6 and 7. From Eq. 6, the apparent

$$K_{app.} = K_3 \frac{K_1}{K_1 + (\beta\text{-CD})f} \qquad \text{(Eq. 6)}$$

$$K_3/K_{app.} = 1 + \frac{(\beta\text{-CD})f}{K_1} \qquad \text{(Eq. 7)}$$

rate constants can be calculated and can be compared with the experimental rate constant data obtained in a system containing beta-cyclodextrin. These comparison data are given in Table III.

The close agreement of the calculated and experimental apparent rate constant gives additional support to the assumption made of the 1:1 type of interaction between benzocaine and beta-cyclodextrin. If the interaction was not of this type, Eq. 1 would not be valid. Equation 7 shows a linear

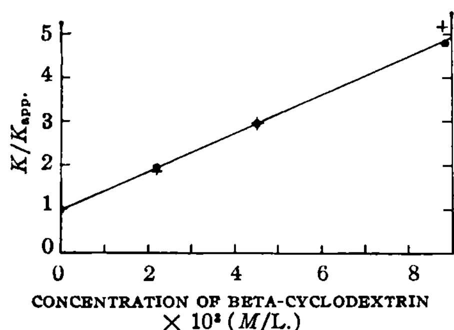


Fig. 6.—Effect of beta-cyclodextrin on the rate of benzocaine hydrolysis in 0.04 N $Ba(OH)_2$ at 30°. Key: +, experimental; ●, calculated.

relationship between the ratio of $K_3/K_{app.}$ and the concentration of free beta-cyclodextrin. This linear relationship is shown in Fig. 6, and further substantiates this 1:1 stoichiometry and the benzocaine hydrolysis in system containing the beta-cyclodextrin is dependant on the uncomplexed benzocaine in solution.

Studies involving inclusion formation and molecular complexation are of importance in the area of drug stabilization and solubilization. Since inclusion formation plays a role in the chemistry of living cells, studies of this type are also important in that data obtained may provide information with respect to the mechanism of drug absorption, transport, and metabolism in biological systems.

REFERENCES

(1) Cohen, J., and Lach, J. L., THIS JOURNAL, 52, 132 (1963).
(2) Lach, J. L., and Cohen, J., *ibid.*, 52, 137(1963).
(3) Lach, J. L., and Chin, T. F., *ibid.*, 53, 69(1964).
(4) French, D., Levine, M. L., Pazur, J. H., and Nordberg, E., *J. Am. Chem. Soc.*, 71, 353(1949).
(5) Higuchi, T., and Lach, J. L., THIS JOURNAL, 43, 394 (1954).
(6) Higuchi, T., and Lachman, L., *ibid.*, 44, 521(1955).
(7) Lach, J. L., and Pauli, W. A., DRUG STANDARDS, 27 104(1959).
(8) Guttman, D. E., THIS JOURNAL, 51, 1162(1962).

Behavior of Erythrocytes in Various Solvent Systems II

Effect of Temperature and Various Substances on Water–Glycerin and Water–Propylene Glycol Solutions

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Hemolytic behavior of rabbit and human erythrocytes in water–glycerin and water–propylene glycol solutions was studied at 25 and 37°, and the effect of various added substances to these systems was investigated. Human i values obtained for sodium chloride in aqueous glycerin or propylene glycol solutions at 37° were slightly greater than the corresponding i values at 25°. Increase in temperature from 25 to 37° decreased the concentrations of propylene glycol in 0.9 per cent saline solution needed to cause hemolysis of erythrocytes. Mono-monovalent salts, sugars and sugar alcohols, magnesium chloride, and sulfate and sodium succinate afforded essentially the same degree of protection as sodium chloride against hemolysis by propylene glycol. Isotonic concentrations of sodium or potassium sulfate, potassium sodium or disodium tartrate, or trisodium citrate afforded greater protection to erythrocytes than 0.9 per cent sodium chloride. The order in which the anions of the above salts appeared to protect human erythrocytes against propylene glycol hemolysis is citrate > tartrate > gluconate > sulfate; for rabbit erythrocytes the order is sulfate, tartrate > citrate. Much lower concentrations of propylene glycol were required to hemolyze erythrocytes in solutions containing isotonic concentrations of calcium chloride.

THE PREVIOUS PAPER in this series (1) discussed the behavior of erythrocytes in water–glycerin and water–propylene glycol systems in experiments carried out at 25°. Hemolytic i values were obtained for sodium chloride in the presence of various concentrations of glycerin and propylene glycol. It was observed that complete hemolysis took place in most glycerin solutions, but the addition of suitable amounts of sodium chloride prevented hemolysis of rabbit and human erythrocytes. Complete hemolysis occurred in all propylene glycol solutions, and in solutions containing 45–50% or more of propylene glycol the addition of iso- and hypertonic quantities of sodium chloride failed to prevent complete hemolysis of rabbit and human red blood cells.

The presently reported experiments are in three areas. (a) The behavior of rabbit and human red blood cells was compared at 37 and 25° in aqueous polyhydric alcohol systems,

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