Determination of Dissociation Constants of Selected Cyclodextrin–Benzaldehyde Inclusion Complexes Using Pulse Polarography[1]

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INTRODUCTION

Inclusion compounds of selected cyclodextrins (CD) have been studied extensively in recent years with regard to their structure, conformation, and use in enzyme modeling (1). Fundamental to such investigations is an accurate determination of the dissociation constant of these complexes. In the present work pulse polarography with its increased sensitivity is reported for the determination of dissociation constants of cyclodextrin complexes having guest molecules which cannot be determined by direct current polarography due to the lack of sensitivity of this method. The sensitivity of pulse polarography is reported to be about two orders of magnitude (10^{-7} *M*, compared to 10^{-5} *M*) greater than direct current polarography (2) and is comparable to the spectrophotometric method. The increased sensitivity is due to the advanced electronic design of the pulse polarograph and the current-sampling capability of the instrument when using the dropping mercury electrode. Together these improvements permit current sampling of the electroactive species and amplification of the resulting faradaic current after the capacitive current of the mercury drop has declined to a relatively small value. The pulse polarographic method should also be useful in the determination of dissociation constants of complexes in which the guest molecule lacks a chromophore for spectrophotometric measurement but which has a suitable electrophore. For comparative purposes in this study, dissociation constants determined by the spectrophotometric method are also reported.

MATERIALS AND METHODS

Instrumentation

Polarographic data were obtained with a PAR polarographic analyzer, Model 174A (Princeton Applied Research Corporation, Princeton, NJ), which is capable of performing four modes of polarography: direct current, current-sampled direct current, pulse, and differential pulse polarography. This instrument was equipped with a Model 174/70 drop timer, which permits the use of either natural or automated drop time. An automatic drop time of 1 sec was used throughout this study for all pulse polarographic data reported. The polarographic cell used was the PAR Model 9301 and 9300 cell top and bottom. The working electrode was the dropping mercury electrode, which consisted of a capillary connected to a mercury reservoir. The reference electrode was a standard saturated calomel electrode, Model 3-712 (Coleman Instrument, Oak Brook, IL), and the auxillary electrode was a platinum wire. An omnigraphic Model 2000 X-Y recorder (Houston Instrument, Bellaire, TX) was used to record all polarograms. Ultraviolet spectral measurements were made on a Beckman DU-7HS spectrophotometer controlled by a built-in high-speed microprocessor. Spectra were recorded on a Beckman video copier interfaced with the DU-7HS instrument.

Chemicals

α-Cyclodextrin (CDX) was obtained from Anspec, Ann Arbor, MI, and β-cyclodextrin was obtained from Chemical Dynamic Corp., South Plainfield, NJ. Permethyl-α-cyclodextrin (PM-α-CD) and permethyl-β-cyclodextrin (PM-β-CD) were prepared following a modification (dimethyl formamide was used instead of dimethyl sulfoxide) of the procedure reported by Szejtli *et al.* (3). PM-α-CD mp 203°C. [lit. (4) 203–205°C]; PM-β-CD mp 154°C [lit. (4) 156°C]. Benzaldehyde was Fisher Scientific Certified Grade.

Methods

Pulse Polarographic Determination of Dissociation Constants. In this research, solutions containing 1.0×10^{-4} *M* benzaldehyde and varying concentrations of selected cyclodextrins ranging from 1.0 to 6.0×10^{-4} *M* were prepared in 0.1 *M* KCl solution. Oxygen was removed by passing nitrogen gas through each solution for 7 min. The volume above the solution in the polarography cell was then flooded with nitrogen gas before running polarograms.

Spectrophotometric Determination of Dissociation Constants. The method reported by Cramer *et al.* (4) was used. Concentrations of benzaldehyde and cyclodextrin were the same as those used in the pulse polarographic method and were prepared in a 0.1 *M* KCl solution.

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Table I. Data Used to Determine Dissociation Constant of α-Cyclodextrin–Benzaldehyde Complex by Pulse Polarography

[Benzaldehyde]/[α-cyclodextrin][a]	Diffusion current i_d (μA)	Diffusion coefficient D_{obs} (cm^2 sec^{-1} × 10^5)[b]	$(D^\circ - D_{obs})^b/$ [CDX] × 10^2
1:1	2.35	1.80	4.00
1:2	2.12	1.47	3.65
1:4	1.95	1.24	2.40
1:8	1.49	0.72	1.85
1:10	1.36	0.60	1.60
1:16	1.24	0.50	1.06
1:20	1.17	0.45	0.88
1:0	2.60	$D^\circ = 2.20$	—

[a] [Benzaldehyde] = 1.00 × 10^{-4} *M*; [CDX] = [α-cyclodextrin].

[b] D° and $D_{obs} = [i_d (t^{1/2}\pi^{1/2}/nFAC)]^2 = [i_d (1.8 \times 10^{-3})]^2$.

RESULTS AND DISCUSSION

Osa *et al.* (5) reported that dissociation constants of cyclodextrin inclusion complexes may be determined using direct current polarographic half-wave potential values, diffusion currents, or diffusion coefficients. In those cases where the difference between the half-wave potential value of the guest molecule and the complex is not significant, the diffusion current or the diffusion coefficient may be used. Diffusion current is used if the dissociation–association rate of the complex is slow, and diffusion coefficients are used if the rate is rapid. Since the dissociation–association rate of the cyclodextrin complex is very rapid (4), diffusion coefficients were used in this research. These values were calculated using diffusion current obtained from pulse polarography (Table I) and the Cottrell equation (1).

$$D^{1/2} = i_d\, \pi^{1/2}\, t^{1/2}/nFAC \tag{1}$$

where

D = diffusion coefficient (cm^2 sec^{-2})
i_d = diffusion current (μA)
t = time current is measured after application of pulse (0.04 sec)
n = 2
F = 96,485 C/equiv
A = 0.01016 cm^2 (area of electrode surface was determined by rearranging the Cottrell equation and solving for A using dc and pulse polarographic data obtained for Cd^{2+})
C = concentration of benzaldehyde (m*M*)

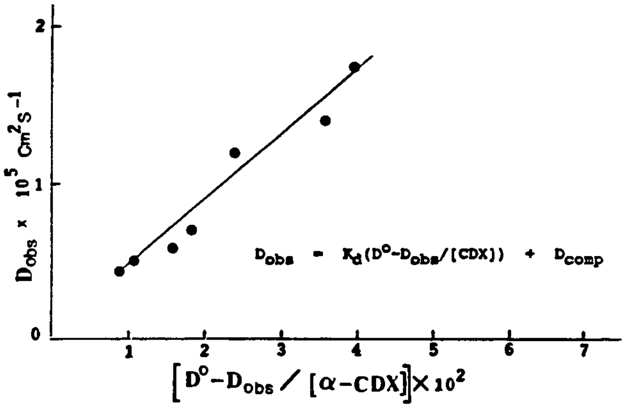

Fig. 1. A plot of pulse polarographic data from which the dissociation constant of benzaldehyde–α-cyclodextrin complex was calculated. All dissociation constants reported in Table II were obtained in a similar manner.

Equation (2) as reported by Osa *et al.* (5) was used to calculate dissociation constants.

$$D_{obs} = K_d(D^\circ - D_{obs})/[CD]) + D_{comp} \tag{2}$$

where D° and D_{comp} are the diffusion coefficients of benzaldehyde alone and of the benzaldehyde–cyclodextrin complex, respectively, and D_{obs} is the observed diffusion coefficient of benzaldehyde in solution with cyclodextrin. K_d is the dissociation constant of the complex, and [CD] is the molar concentration of the cyclodextrin being studied. K_d was obtained from the slope of a plot of the data as shown in Fig. 1.

Dissociation constants obtained spectrophotometrically were calculated using the method of Benesi and Hildebrand (6). As shown in Table II, the values of dissociation constants obtained by the pulse polarographic method are in good agreement with those obtained by the spectrophotometric method.

In conclusion, this research shows that dissociation constants of cyclodextrin and guest molecules with a suitable electrophore may readily be obtained using pulse polarography. This method should find increased application for similar compounds not amenable for analysis by other techniques.

Table II. Dissociation Constants Obtained by the Pulse Polarographic and Spectrophotometric Method

	Method	
	Polarographic	Spectrophotometric
α-Cyclodextrin–benzaldehyde	4.1 × 10^{-4} *M*	4.5 × 10^{-4} *M*
Permethyl-α-cyclodextrin–benzaldehyde	1.9 × 10^{-4} *M*	2.3 × 10^{-4} *M*
β-Cyclodextrin–benzaldehyde	5.6 × 10^{-4} *M*	6.1 × 10^{-4} *M*
Permethyl-β-cyclodextrin–benzaldehyde	3.1 × 10^{-4} *M*	3.9 × 10^{-4} *M*

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