THE APPLICATION OF EQUILIBRIUM DIALYSIS TO THE DETERMINATION OF DRUG-CYCLODEXTRIN STABILITY CONSTANTS

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ABSTRACT

The equilibrium dialysis method was applied to the determination of drug-cyclodextrin stability constants using diflunisal and 2-hydroxypropyl-β-cyclodextrin (HPBCD) as a model system. Analysis of data showed the existence of a linear Scatchard plot, which is indicative of the formation of a 1:1 diflunisal:HPBCD complex. The stoichiometry of the complex was verified using the appropriate mass action law equation. The complexation constant (K_c) was 3801 ± 541 M^{-1}. The K_c obtained using the equilibrium dialysis method was comparable to that obtained using a potentiometric method (5564 ± 56 M^{-1}).

1. INTRODUCTION

The equilibrium dialysis method has been employed in several studies investigating binding of drugs to proteins and other macromolecules (1). However, this method has not been widely applied to the characterization of drug binding to cyclodextrins. Equilibrium dialysis has several advantages over the commonly used phase-solubility method. Saturated drug solutions are not required for this method, therefore, only relatively small amounts of drug are needed. The control of pH and ionic strength of solutions is relatively easy compared to the saturated solutions used in phase-solubility studies. It is also possible to study the effects on complexation of varying either the drug or ligand concentration. This method also avoids some of the problems associated with the accurate determination of S_o (the solubility of the drug in the absence of ligand) reported elsewhere (2). Furthermore, it is possible to measure free drug, bound drug, and ligand concentrations. Since the concentrations of free drug, bound drug and ligand are known, the stability constant can be calculated directly without resorting to data transformation.

In the present study, the equilibrium dialysis method was applied to the determination of the stability constant of HPBCD:diflunisal complexes since the binding parameters for this

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complex were recently evaluated using the potentiometric method and an ion-selective electrode (3), and thus, could be compared to parameters obtained in this study. The results obtained by the equilibrium dialysis and potentiometric titration methods were then compared.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in this study were as follows: potassium phosphate, monobasic crystals, and potassium phosphate, dibasic crystals (J.T. Baker, Phillipsburg, NJ), diflunisal (5[2,4-Difluorophenyl] salicylic acid), Sigma Chemical Co., St. Louis, MO), Encapsin HPB (2-Hydroxypropyl-ß-cyclodextrin; HPBCD) (D.S. = 4.1, MW = 1,372.8, American Maize Products Company, Hammond, IN), sodium hydroxide (MCB Manufacturing Chemists, Inc., Cincinnati, OH), methanol, and acetonitrile (Burdick & Jackson, Muskegon, MI), potassium chloride (J.T. Baker, Phillipsburg, NJ), nylon membrane, 0.6-mil, acrylic plastic dialysis cells (Model 289, Bel-Art Products, Pequannock, NJ), filters, 0.45-µm (Millipore Corp., Bedford, MA). All solutions were prepared using deionized, distilled water.

2.2 HPLC Assay

The HPLC system consisted of an Altex (Altex Scientific Inc., Berkeley, CA) Model 110A pump, a Rheodyne (Cotati, CA), Model 7125 injector with a 50 µL loop and a Hitachi/Spectra-Physics (Fremont, CA) Model 100-30 variable-wavelength UV detector set at 262 nm. The analytical column was a Phenomenex (Torrance, CA) C-18 column (10 µm, 300 X 3.9 mm i.d.) fitted with a Whatman (Clifton, NJ) C_{18} (30 µm) guard column (10 X 4.6 mm). The mobile phase consisted of 58% v/v of 0.01 M phosphate buffer, pH 7.0: 26.3% v/v acetonitrile: 15.7% v/v methanol. A flow rate of 1.0 mL per min was utilized. Duplicate 50-µL injections were made for each sample.

2.3 Equilibrium dialysis studies

The nylon membranes were washed for 1 hr in deionized, distilled water to remove any contaminants. The dialysis cells were assembled with the membrane acting as a semipermeable barrier between the two compartments. Then, 0.8 mL of buffer solution was added to one cell compartment (the aqueous compartment) and 0.8 mL of solutions consisting of varying volume ratios of diflunisal (0.01 M) in HPBCD (0.009 M) and HPBCD (0.009 M) was added to the other cell compartment (binder compartment). The cells were placed on a water bath shaker (GCA/Precision Scientific, Chicago, IL) and agitated at 100 oscillations/min for 21 hours at room temperature (23-25°C) until equilibrium was achieved. Samples were removed from both cell compartments and analyzed for diflunisal using the above HPLC method.

The impermeability of the nylon membrane to HPBCD was verified by placing 0.8 mL of buffer solution in one cell compartment and 0.8 mL of 0.009 M HPBCD solution in the other compartment. After equilibration (21 hr), both compartments were assayed for HPBCD using a previously reported method (4). Possible changes in the volume of the two compartments, due to an osmotic pressure gradient (5), were insignificant during the period of the study. Binding of diflunisal to the nylon membrane was also determined to be insignificant (< 0.5%).

2.4 Data Analysis

In the equilibrium dialysis studies, the concentration of diflunisal measured on the buffer side of the cell compartment represents the free (unbound) concentration, and the difference in diflunisal concentration on the buffer and binder sides of the cell compartments represents the bound diflunisal concentration. The binding of diflunisal to HPBCD and the calculation of the associated binding parameters were analyzed by the methods of Scatchard (6) and Plumbridge et al. (7).

Assuming that only one class of sites exists, and that there are n independent and equivalent binding sites per molecule of cyclodextrin, each having a complexation constant (Kc), the following equation may be written:

$$r/D_f = K_c(n-r) \qquad \text{(Eq. 1)},$$

where r is the average number of moles of diflunisal bound per mole of HPBCD; D_f is the concentration of free (unbound) diflunisal, n is the number of binding sites per molecule of HPBCD and K_c is the complexation constant for the binding of diflunisal to HPBCD. In accordance with the recommendation of Plumbridge et al. (7) the binding parameters (n, K_c) were obtained by regressing D_f (the free or unbound drug concentration) on D_t (the total drug concentration) according to the following equation:

$$D_f = D_t(1 + K_cD_f)/(1 + K_cD_f + nK_cC_{Lt}) \qquad \text{(Eq. 2)},$$

where C_{Lt} is equal to the total ligand (HPBCD) concentration.

3. RESULTS AND DISCUSSION

The data obtained from the equilibrium dialysis experiments (N=4) were subjected to a Scatchard analysis. The regression of r/D_f vs. r produced highly significant linear correlations ($p < 0.0005$). The observed linearity of the data plots is indicative of the presence of one class of binding sites and the adherence of the binding data to Eq. 1. The mean values for K_c and n, which were calculated from the regression of D_f on D_t are 3801 $\pm$ 541 M^{-1} and 0.906 $\pm$ 0.059, respectively. The %CV for Kc and n were 14.20 and 6.58, respectively.

The stability constant for the diflunisal-HPBCD interaction determined by the equilibrium dialysis method was comparable to the Kc determined by the potentiometric method (3),

reported to be 5564 ± 56 M^{-1}. This indicates that the equilibrium dialysis method described here can be used reliably for studying drug binding to cyclodextrins. The potentiometric method is also a relatively simple method; however, it requires the use of ion-selective electrodes which are not always easy to construct. As previously discussed, there are several advantages of equilibrium dialysis over phase solubility methods which can often make it the preferred method.

4. CONCLUSIONS

The major factor limiting the widespread use of the equilibrium dialysis method is the availability of a dialysis membrane with an appropriate selectivity for the cyclodextrin and drug molecules to be studied. Ideally, the membrane should totally restrict the passage of the cyclodextrin but allow free movement (equilibration) of drug molecules. At the present time, there is a limited commercial availability of dialysis membranes with a low molecular weight cutoff (≤ 500 Daltons). The molecular weight cutoff of the nylon membrane is not known, however this membrane proved to be selectively permeable to HPBCD and diflunisal in this study. The molecular weight of diflunisal (MW = 250) is in the same MW range as many other typical drug molecules, therefore the nylon membrane should be applicable to the study of binding of many other drugs to cyclodextrins.

REFERENCES

1. M.C. Meyer and D.E. Guttman. The binding of drugs by plasma proteins. J. Pharm. Sci., 57:895-918 (1968).
2. K.A. Connors. Binding Constants: The Measurement of Molecular Complex Stability, John Wiley & Sons, New York, NY, 1987, p. 266.
3. E.E. Sideris, M.A. Koupparis and P.E. Macheras. Effect of cyclodextrins on protein binding of drugs: the diflunisal/hydroxypropyl-β-cyclodextrin model case. Pharm. Res. 11:90-95 (1994).
4. M. Vikmon. Rapid and simple spectrophotometric method for determination of micro-amounts of cyclodextrins. 1st Int. Sympos. Cyclodextrins, 1981, Budapest, Hungary, pp. 69-74.
5. G.F. Lockwood and J.G. Wagner. Plasma volume changes as a result of equilibrium dialysis. J. Pharm. Pharmacol. 35:387-399 (1983).
6. G. Scatchard. The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci., 51:660-672 (1949).
7. T.W. Plumbridge, L.J. Aarons and J.R. Brown. Problems associated with analysis and interpretation of small molecule/macromolecule binding data. J. Pharm. Pharmacol. 30:69-74 (1977).