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A KINETIC APPROACH FOR THE DETERMINATION OF HOST-GUEST BINDING CONSTANTS

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The kinetics of the alkaline hydrolysis of methyl acetate (E) was studied in the presence of β-cyclodextrin (CD), employing a conductance method. In the presence of CD, the rate of the $E + OH^-$ reaction decreases because a host-guest complex is formed between E and CD and the ester bound to the CD cavity is not accessible to the OH^- attack. The experiment consists of two steps: (1) the rate constant that describes the alkaline hydrolysis of methyl acetate is determined, and (2) the kinetics of the reaction is measured once again in the presence of CD. An algorithm is presented in the Mathematica platform to estimate the equilibrium constant for the methyl acetate/CD host-guest complex using the kinetic data.

Методом кондуктометрии изучена кинетика щелочного гидролиза метилацетата (E) в присутствии β-циклодекстрина (ЦД). В присутствии ЦД скорость $E+OH^-$ реакции снижается из-за образования "хозяин-гость" комплекса между E и ЦД, а эфирная связь в полости ЦД становится недоступной к атаке OH^- групп. Эксперимент состоит из двух этапов: (1) определение константа скорости, что представляет щелочной гидролиз метилацетата, и (2) измерение еще раз кинетики реакции в присутствии ЦД. С использованием кинетичаских данных представлен математический алгоритм для оценки константа равновесия комплекса метил ацетат/ЦД "хозяин-гость".

Կոնդուկտոմետրիայի մեթոդով ուսումնասիրվել է մեթիլացետատի (E) հիմնային հիդրոլիզը β-ցիկլոդեքստրինի (ՑԴ) ներկայությամբ: ՑԴ-ի ներկայությամբ $E+OH^-$ ռեակցիայի արագությունը դանդաղում է E-ի և ՑԴ-ի միջև «տեր-հյուր» համալիրագոյացման պատճառով և եթերային կապը ՑԴ-ի խոռոչում դառնում է անմատչելի OH^- խմբերի հարձակման համար: Գիտափորձը տարվել է երկու էտապով. (1) արագության հաստատունի որոշում, որը ներկայացնում է մեթիլացետատի հիմնային հիդրոլիզը, և (2) ռեակցիայի կինետիկայի չափում մեկ անգամ էլ ՑԴ ներկայությամբ: Օգտագործելով կինետիկական տվյալները, ներկայացվել է մաթեմատիկական ալգորիթմ, գնահատելու հավասարության հաստատունը մեթիլացետատ/ՑԴ «տեր-հյուր» համալիրի համար:

Introduction

Cyclodextrins (CDs) are water soluble molecules that posses a hydrophobic cavity which enables them to form host-guest complexes with molecules ranging widely in their physical properties and size. In particular, non-polar substrates that fit tightly inside the cavity tend to form host-guest complexes with large binding constants [1-4]. The formation of a host-guest complex may alter properties of the guest such as the fluorescence yield, the absorption extinction coefficient, and the position of NMR signals [5]. Because CDs are chiral, a substrate may exhibit the induced circular dichroism phenomenon upon interacting with the CD cavity. Monitoring changes in these properties it has been possible to study a large number of host-guest equilibria and an understanding of the physical factors underlying the formation of these complexes is emerging. More related to the present work is the fact

that CDs can reduce the accessibility of a guest with respect to a reactant or a quencher. This provides a means to control the rate of chemical reactions and to measure host-guest equilibrium constants (K_{eq}) [5-8].

Physical methods that require high concentrations of the guest may not be adequate for measuring equilibrium constants because the guest is prone to aggregate and the stoichiometry may deviate from 1:1, the host-guest complexes may precipitate out of the solution, or form homodimers [7, 8]. Clearly, if the assumption for the stoichiometry is correct, then different physical methods should yield similar values of K_{eq}. Hennrich and Cramer [9] determined the dissociation constants for different substrates by kinetic, spectroscopic and competitive inhibition methods, obtaining good agreement among the different methods. While under certain experimental conditions the use of approximations may introduce errors, to some extend this problem can be reduced employing non-linear techniques and regressions [6].

This paper describes a procedure to measure the equilibrium constant for the formation of a host-guest complex between β-cyclodextrin (CD) and methyl acetate (E) employing a conductimetric method:

$$CD + E \Leftrightarrow CD{\bullet}E \qquad (1)$$

where CD•E represents the host-guest complex [10-14]. E binds to the CD cavity through van der Waals forces and hydrophobic interactions. The formation of host-guest complexes is enthalpy driven. As implied in equation 1, in an aqueous solution containing CD the ester exists as a free species (E) as well as bound to the CD cavity (CD•E). The experiment involves various measurements. First, the rate constant that describes the alkaline hydrolysis of methyl acetate, k_f, is determined following the method presented by Crockford et al. [15]

$$CH_3COO\,CH_3 + OH^- \xrightarrow{k_f} CH_3OH + CH_3COO^- \qquad (2)$$

Second, the kinetics of reaction 2 is monitored again in the presence of a specified concentration of CD. Qualitatively, it is observed that the rate of reaction 2 decreases upon adding CD. The ester is consumed in reaction 2 and its total amount at a given reaction time is given by, $[E]_T = [E] + [CD{\bullet}E]$. In contrast, the OH^- ion exists exclusively as a free species. The rate of reaction 2 decreases upon adding CD to the reaction mixture because the ester molecules associated to the cavity of CD are inaccessible to the OH^- attack. Therefore, in the presence of CD the rate of reaction 2 also depends on the concentration of free ester, which is dictated by the following expression of K_{eq}:

$$K_{eq} = \frac{[CD \bullet E]}{[CD][E]} \qquad (3)$$

Substituting $[E]_T - [E]$ for $[CD{\bullet}E]$ in equation 3, an expression for the free ester in terms of $[E]_T$, $[CD]_o$ and K_{eq} is obtained,

$$[E] = X_E[E]_T = \frac{1}{K_{eq}[CD]_o + 1}\,[E]_T \qquad (4)$$

where $[CD]_0$ is the analytical concentration of CD. In obtaining equation 4 it was assumed, $[CD]_o >> [CD{\bullet}E]$, in order to substitute $[CD]_o$ for $[CD]$. It is important to emphasize that the

reaction rate decreases upon adding CD because [E] is reduced and not because the magnitude of k_f for reaction 2 has decreased. The magnitude of k_f only depends on the temperature of the system, which is kept constant. An interesting aspect of this method is that measuring the kinetics of reaction 2 using a simple conductimetric method one obtains thermodynamic data (*i.e.*, $\Delta G^{\circ} = -RT \ln K_{eq}$) about equilibrium 1.

Experimental Method

Methyl acetate (99+%, Aldrich) and β-cyclodextrin (Aldrich) were used as received. Deionized water was used to prepare all solutions. The experiments reported were performed at ambient temperature, although the reaction tube was placed in a water bath to minimize temperature variations. Conductance measurements were made using a YSI Model 35 conductance meter coupled with a YSI 3401 conductivity cell.

Results and Discussion

Alkaline Hydrolysis of Methyl Acetate. A task of this experiment is to measure the rate constant of reaction 2. However, strictly speaking the hydrolysis of methyl ester must be written as an equilibrium:

$$CH_3COOCH_3 + OH^- \Leftrightarrow CH_3OH + CH_3COO^- \qquad (5)$$

As stated by Crockford et al. [10], because the ionic conductivity of OH^- is larger than that of CH_3COO^- at a given temperature, it is possible to monitor the kinetics of the reaction by measuring the conductance decrease of the solution as a function of time (see Figure 1). The limiting ionic conductivities in water of Na^+, CH_3COO^- and OH^- are 50.11, 40.9 and 197.6 siemens•cm^2•mol^{-1}, respectively. Although the sodium ion contributes to the conductivity of the solution, its concentration remains constant. The kinetic equation that describes reaction 5 is,

$$-\frac{d[E]}{dt} = k_f[E][OH^-] - k_b[EtOH][Ac^-] \qquad (6)$$

where [E], [OH^-], [MeOH] and [Ac^-] represent the ester, hydroxide, methanol, and acetate ion concentrations, respectively, and k_f and k_b the corresponding forward and backward rate constants. Equation (6) can be written in terms of the reaction progress variable as follows,

$$\frac{dx}{dt} = k_f(a-x)(b-x) - k_b x^2 \qquad (7)$$

where a and b denote the ester and hydroxide initial concentrations, which we chose to be equal. In writing equation 7 it is assumed that no products are present at the beginning of the reaction. Furthermore, for short reaction times, equation 7 may be written in the following approximate form,

$$\frac{dx}{dt} = k(a-x)^2 \qquad (8)$$

Integrating equation 8 from zero to infinity one obtains,

$$kt = \frac{x}{a(a - x)} \tag{9}$$

Since a and x are proportional to $(L_0 - L_r)$ and $(L_0 - L)$, respectively, the following relations between a, x, and $a - x$ can be derived in terms of L, L_0 and L_r, which denote the conductances at time = t, 0, and infinity, respectively.

$$\frac{x}{a} = \frac{L_0 - L}{L_0 - L_\infty} \tag{10.a}$$

$$\frac{a - x}{a} = \frac{L - L_\infty}{L_o - L_\infty} \tag{10.b}$$

Inserting equations 10.a and 10.b into equation 9 leads to

$$akt = \frac{L_o - L}{L_o - L_\infty} \tag{11}$$

Thus, k can be calculated plotting $(L_0 - L)/(L - L_r)$ against time (taking into account that a is one half the concentration of the stock solutions). The inset of Figure 1 shows such a plot, as well as the linear fit from which one obtains, $k = (0.2279 \pm 0.0026)\ M^{-1}\ s^{-1}$.

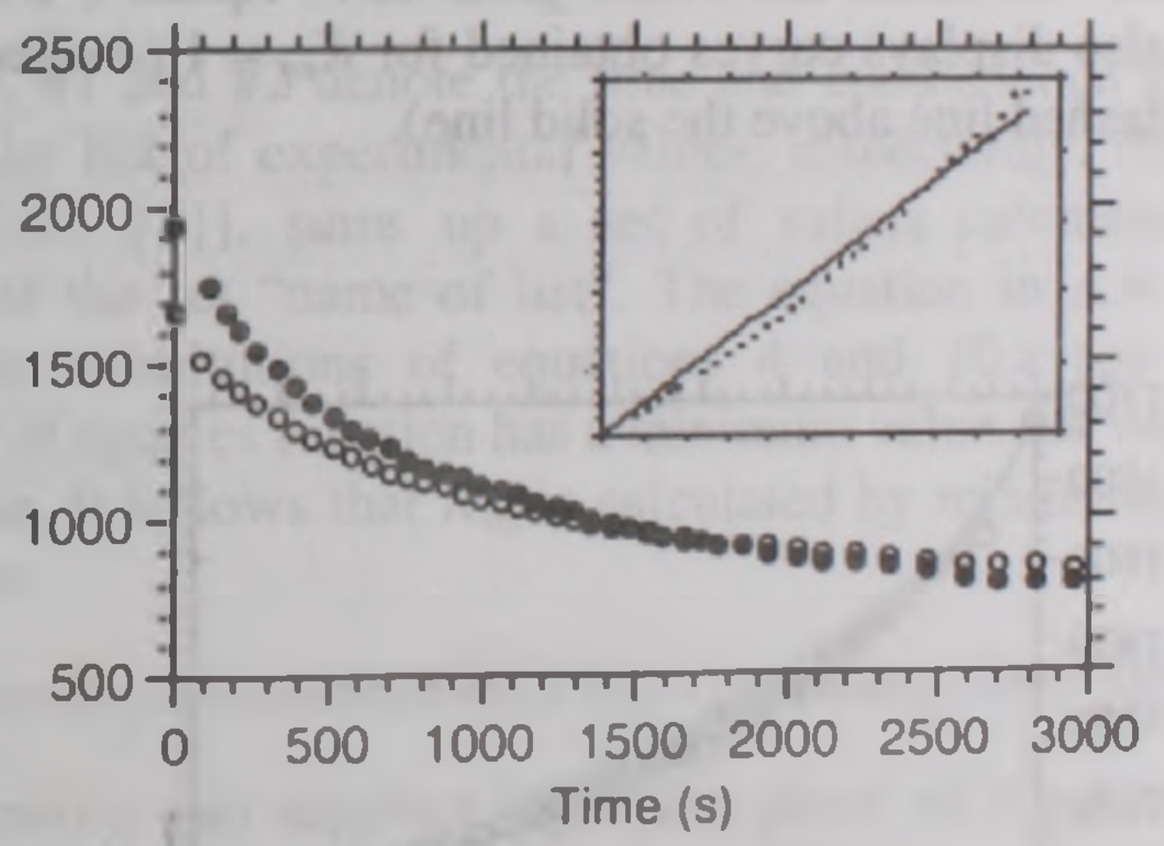

Figure 1. Conductance decays for the alkaline hydrolysis of methyl acetate with (open circles) in the presence of CD and without adding CD to the reaction medium. Not all the experimental points are shown for clarity. The following concentrations were used: 0.01M methyl acetate, 0.01M sodium hydroxide, and 0.005M CD. The inset shows conductance data for the experiment without CD expressed in linear form, along with the corresponding fit. The rate constant is estimated from the slope of the linear fit using equation 11.

Determination of the Binding Constant. As mentioned in the Introduction, the concentration of free ester available to react decreases when CD is added to the solution, as it is apparent from Figure 1. Therefore, to describe the kinetics of reaction 2 in the presence of

CD, equation 7 needs to be modified considering that a fraction of the ester molecules are forming a host-guest complex

$$\frac{dx}{dt} = k(a X_t \pm x)(b \pm x) \tag{12}$$

Letting $aX_E = a'$, and integrating the above equation using the boundary conditions employed to solve equation 8, the following standard result is obtained,

$$kt = \frac{1}{a' - b} \ln\left(\frac{b(a' - x)}{a'(b - x)}\right) \tag{13}$$

The above equation can be re-written as

$$kt = \frac{1}{E_0 X_E - E_0} \ln\left(\frac{(E_0 X_E - x)}{X_E(E_0 - x)}\right) \tag{14}$$

To calculate K_{eq}, X_E and x are substituted in equation 14, as defined in equations 4 and 10.a (or 10.b), respectively. Note that X_E introduces K_{eq} (see equation 4), which is the only parameter to be adjusted to the kinetic data. The final equation and its application into a least squares routine is explained in the Appendix. We analyzed data points up to 1000 s, which is the approximate time when the backward reaction begins to interfere significantly. Fitting the data in Figure 2 (solid curve) to our model for reaction times up to 1000 s, one obtains K_{eq} to be (11.8±1.2) M^{-1} at 295.3 K, which gives ΔG° equals (–6.05±0.60) kJ/mol. For comparison, Figure 2 also displays curves obtained for $K_{eq} = 1$ (dashed line below the solid line) or $K_{eq} = 25\ M^{-1}$ (dashed line above the solid line).

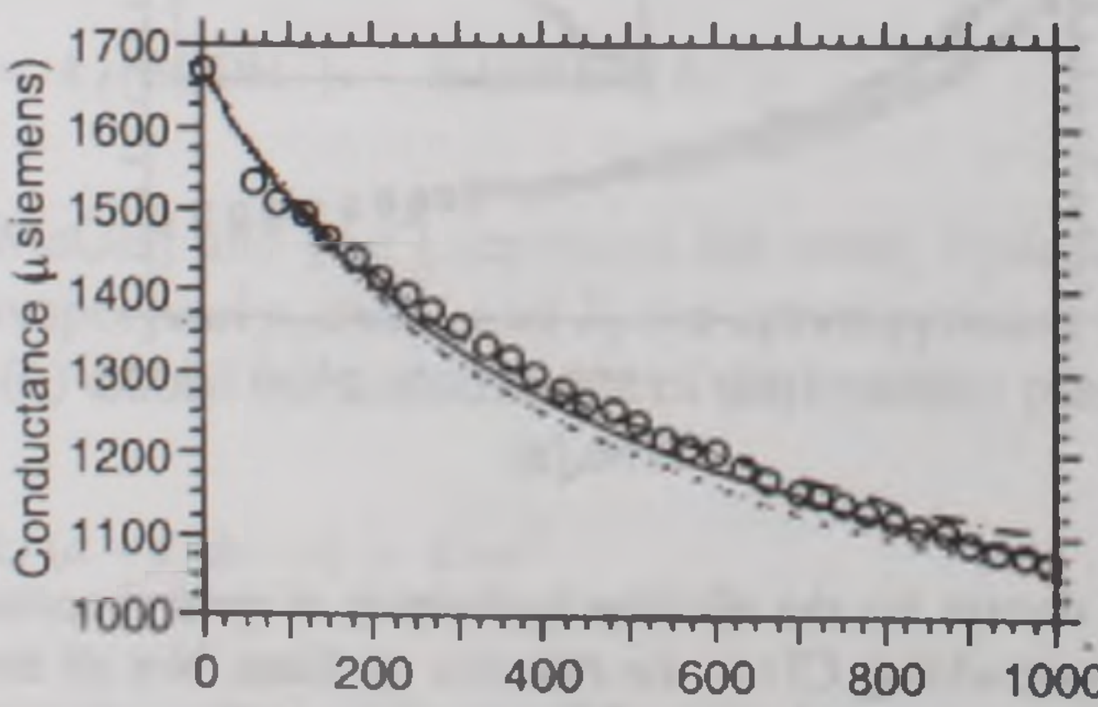

Figure 2. Conductance decay for the alkaline hydrolysis of methyl acetate in the presence of CD. The solid line represents the best fit, calculated using the estimated CD/ester binding constant. The dashed line represent the curves obtained if one puts $K_{eq} = 1$ (curve below the solid line) or $K_{eq} = 25\ M^{-1}$ (dashed line above the solid line).

Appendix

This appendix describes the algorithm developed in the Mathematica platform [16] to calculate K_{eq} using a regression. Lines in quotes mean that the respective values are chosen or determined by the user. First, an array of experimental data (the kinetic data set) is entered into the program. In the present case, times longer than 1000 seconds are not considered:

"name of list"={{t_1,L_1},{t_2,L_2},...} (A.1)

Next, a set of constants are assigned values that were previously determined in the experiment. These are the ester initial concentration (E0), the rate constant for the reaction in free water without CD (k), the CD analytical concentration (CD0), the conductance at time zero (L0), and the conductance at infinity (Li):

{E0,k,CD0,L0,Li}={"E0","k","CD0","L0","Li"} (A.2)

For instance, the input line using the values obtained in the present work would be:

{E0,k,CD0,L0,Li}={0.01,0.2279,0.005,1670,780.64} (A.3)

In order to get a regression, a function is defined for the sum of squares. It depends only K_{eq}, and is minimized to find the best estimate of K_{eq}:

```
sumofsquares[Keq_] =
Plus @@ Apply[((Log[E0]-Log[E0-(E0-(#2-Li)/(L0-Li)*E0)]-Log[E0*1/(CD0*Keq+1)]
+ Log[-(E0-(#2-Li)/(L0-Li)*E0)+E0*1/(CD0*Keq+1)])/
((E0*k)+E0*k*1/(CD0*Keq+1))-#1)^2 &,"name of list", {1}]
```
(A.4)

In equation A.4, #1 and #2 denote the time and conductance elements of each time-conductance pair in the list of experimental values, respectively. Plus @@ Apply[(t(#2)-#1)^2 &,"name of list", {1}], sums up a set of values calculated from each pair of coordinates member of the set "name of list". The equation in A.4, in this case t(#2), is equation 14 after the substitutions of equations 4 and 10.a (or 10.b) for X_E and x, respectively. The sum of squares function has a minimum value, for which the corresponding K_{eq} is the best estimate. It follows that K_{eq} is calculated by minimizing the sum of squares. This is done as follows:

FindMinimum[sumofsquares[Keq],{Keq,5},MaxIterations->100] (A.5)

where {Keq,5} represents a user supplied "educated guess" of K_{eq} for the function to initiate the iteration process. A.5 returns the best estimate for K_{eq}.

The following routine generates a set of theoretical values (fit) of conductance (L) against time (t). In this case, the routine supplies a sequence of conductance values starting at "initial L" and ending at "final L", and returns a list of time values:

```
Do[
L=i;
x=(E0-(L-Li)/(L0-Li)*E0);
t=(Log[E0]-Log[E0-x]-Log[E0*1/(CD0*Keq+1)]
+ Log[-x+E0*1/(CD0*Keq+1)])/(-(E0*k)+E0*k*1/(CD0*Keq+1));
Print[N[t]," / ",L],
{i,"initial L","final L","increase"}]
```
(A.6)

These latter values can be entered into a new list the same way as in A.1. In order to graphically compare both lists, and visually evaluate the adequacy of the fit, type the following lines:

```
g1=ListPlot["name of experimental data list"]
g2=ListPlot["name of theoretical data list"]
Show[g1, g2]                                                    (A.7)
```

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