Northeastern University

CHME 5137: Computational Modelling in Chemical Engineering

Computational Modelling of Drug-Protein interactions with Isothermal Titration Calorimetry.

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Contents

1 Background **3**

2 Theory **3**

3 Results and Discussion **4**

4 Conclusion **6**

5 References **6**

1 Background

Isothermal titration calorimetry is a biophysical technique used to probe the nature of interactions between a ligand and a macromolecule. In an ITC experiment, aliquots of a ligand (drug) in a syringe are periodically injected into a reaction cell containing a macromolecule (protein). The reaction is essentially a complexation reaction where the ligand is injected until the macromolecule reaches saturation. The saturation of the macromolecule indicates the end of the titration. A single ITC experiment provides data on thermodynamic properties such as Gibbs energy, entropy, binding sites, enthalpy, and dissociation constants. In our system the ligand is a drug, carvedilol and the protein is mucin. We probe the interaction between carvedilol and mucin because previous invitro research has established the essence of leveraging these interactions for drug development.

First, we generated a model from scratch using the principles of energy and mass conservation by writing energy and mass balance equations for our system. Then we tested our model with Ca-EDTA experimental data to validate the model and compared it with an elaborate theoretical model developed by (Dumas, 2019), here in referred to as the Dumas model. Fitting of our model to experimental data showed a step change between the saturated (lower section) and unsaturated region (upper section) of the binding isotherm. The fit returned a negative R squared value. This could be due to over approximation in our model. The Dumas Model yielded a better fit and an R squared value of 0.99.

We employed this model fit our carvedilol-mucin reaction.

2 Theory

The reaction between a ligand and a macromolecule is essentially described as

$$\mathrm{M} + \mathrm{L} \rightleftharpoons \mathrm{ML} \tag{1}$$

Where M,L,ML,$[M]$,$[L]$,$[ML]$,$[M]_T$,$[L]_T$ are macromolecule, ligand, complex, free macromolecule concentration, free ligand concentration, complex concentration, total macromolecule concentration and total ligand concentration, respectively.

According to the law of conservation of mass,

$$[\mathrm{M}]_\mathrm{T} \rightleftharpoons [\mathrm{M}] + [\mathrm{ML}][\mathrm{L}]_\mathrm{T} \rightleftharpoons [\mathrm{L}] + [\mathrm{ML}] \tag{2}$$

The association constant Ka is defined as

$$Ka = ([ML])/([M][L]) = 1/Kd \tag{3}$$

where Kd is the dissociation constant At each injection, the concentration of each species in the cell gets diluted. The concentrations of the macromolecule and the ligand in the cell are expressed as

$$[M]_T, i = [M]_0(1 - v/V0) \tag{4}$$

$$[L]_T, i = [L]_0(1 - (1 - v/V0)^i) \tag{5}$$

where $[L]_0$,$[M]_0$, v, and V_0 are initial concentrations of the ligand, macromolecule, injection volume and Initial volume of macromolecule respectively.

Substituting equations (1) and (2) into equation (3) yields

$$[ML] = ((K_a[M]_T + K_a[L]_T + 1) - \sqrt[2]{(K_a[M]_T + K_a[L]_T + 1)^2 - 4K_a^2[M]_T[L]_T})/2K_a \tag{6}$$

The equation of change of energy for multi-component systems can be written as

$$\frac{\mathrm{d}U}{\mathrm{d}t} = \sum_{k=1}^{K}(NH)_k + Q + W_s - P\frac{\mathrm{d}V}{\mathrm{d}N_k}N_k = \sum_{i=1}^{c}(N_i)_k(NH)_k = \sum_{i=1}^{c}(N_iH_i)_k \tag{7}$$

Where N,U,Q,W_s,P,V,t are Molar flow rate, Molar enthalpy, Partial molar enthalpy, internal energy, heat flow rate, work flow rate, pressure, volume and time, respectively.

Solving the the energy balance reduces the equation to

$$Q = \frac{\mathrm{d}U}{\mathrm{d}t} + P\frac{\mathrm{d}V}{\mathrm{d}t} - \sum^{K}(NH) \tag{8}$$

$$Q = V\Delta H\frac{\mathrm{d}C_i}{\mathrm{d}t} \tag{9}$$

Integrating equation (8) and substituting equation (6) yields

$$Q_i = V\Delta H \tag{10}$$

Where Q,I is the heat associated with each injection,ΔH is the molar enthalpy of the reaction.

Other thermodynamic parameters can be calculated by

$$\Delta G = -RT\ln K_a = RT\ln K_d \tag{11}$$

$$\Delta G = \Delta H - T\Delta S \tag{12}$$

Where G,R,T,S are Gibbs energy, molar gas constant, temperature and entropy respectively.

3 Results and Discussion

(a) Fit for CA-EDTA using our model

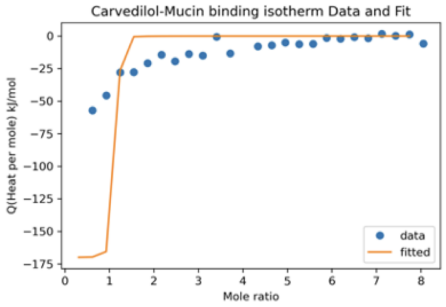
(b) Fit for CA-EDTA using Dumas model

Figure 1: CA-EDTA fit comparison

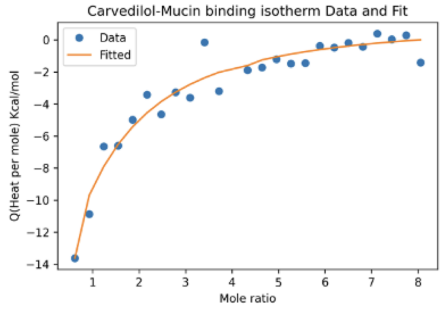
Dumas model yielded a better fit. For this reason, we analysed our carvedilol-mucin data with the Dumas model and found that it was able to fit the data correctly.

Model	$\Delta H, covariance(Kcal/mol)$	n,covariance	$K_d, covariance$	R^2
Our Model	$-17 + -1.69e^9$	nn	$2.5e^5 + -2.24e^7$	-14.44
Dumas Model	$-4.11 + -0.11$	$1.029 + -0.01$	$0.5 + -0.17$ uM	0.99

Table 1: Fit parameters estimation for CA-EDTA data



(a) Fit for Carvedilol-Mucin using our model



(b) Fit for Carvedilol-Mucin using Dumas model

Figure 2: Carvedilol-Mucin fit comparison

Experiment	$\Delta H, covariance(Kcal/mol)$	n,covariance	$K_d(uM), covariance$	$\Delta S, covariance(Kcal/mol/K)$
Carvedilol-Mucin Experiment	$-24.3 + -25.3$	$0.2 + -1.98$	$19.17 + -24$	$-0.0788 + -1.29$

Table 2: Fit parameters estimation for Mucin-Carvedilol data

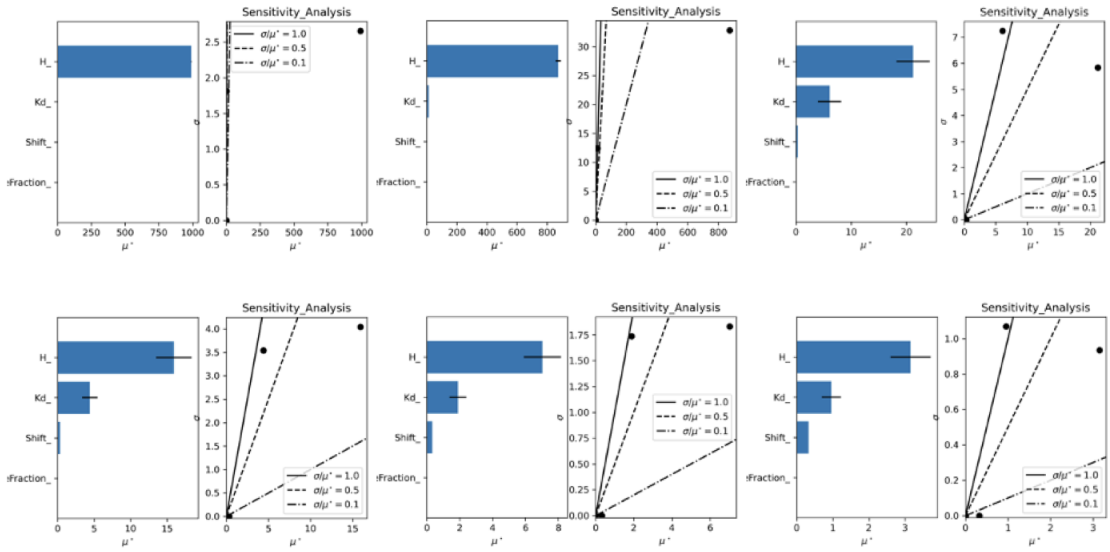


Figure 3: Sensitivity Analysis

We conducted a sensitivity analysis on the heat values in both unsaturated region (Qp_0 to Qp_13) and saturated region (Qp_{15} to Qp_{23}) of the binding isotherm and found that enthalpy was the dominant thermodynamic parameter in the reaction. This means that the reaction is enthalpy controlled and hints of electrostatic interactions or hydrogen bonding as the primary reaction mechanism of interaction.

4 Conclusion

We developed a model by writing mass and energy balances for our system of isothermal titration of carvedilol and mucin . Our model needs considerable revisions for proper fitting of experimental data. Comparison of our model with Dumas model shows that the dumas model fits the data more accurately. Sensitivity analysis shows that binding between carvedilol and mucin is enthalpy controlled and could be driven by hydrogen bonding. Also, 20% of the total binding site of mucin is available for carvedilol binding.

5 References

P. Dumas, Rigorous Equations for Isothermal Titration Calorimetry: Theoretical and Practical Consequences, (2019) https://doi.org/10.1101/512780.