CARBONIC ANHYDRASE: KINETICS OF REMOVAL OF Zn(II) By 2,6-PYRIDINECARBOXYLATE

Paul Nickerson*

Department of Chemistry, University of Florida, Gainesville, FL, US

E-mail: pvnick@ufl.edu

Abstract

In the past decade, supervised activity recognition methods have been studied by many researchers, however these methods still face many challenges in real world settings. Supervised activity recognition methods assume that we are provided with labeled training examples from a set of predefined activities. Annotating and hand labeling data is a very time consuming and laborious task. Also, the assumption of consistent pre-defined activities might not hold in reality. More importantly, these algorithms do not take into account the streaming nature of data, or the possibility that the patterns might change over time. In this chapter, we will provide an overview of the state of the art *unsupervised* methods for activity recognition. In particular, we will describe a scalable activity discovery and recognition method for complex large real world datasets, based on sequential data mining and stream data mining methods.

*To whom correspondence should be addressed

Introduction

Carbonic anhydrase (CA) catalyzes the interconversion of carbon dioxide and carbonic acid/bicarbonate as follows:

$$CO_2 + H_2O \overset{CA}{\rightleftharpoons} H_2CO_3 \tag{1}$$

In the active form, CA is bound to a Zn^{2+} cofactor (denoted as CA·Zn), which it relies upon for its catalytic activity. The zinc ion can be stripped from the enzyme using a Lewis base ligand, which donates electrons to the ion to form a covalent bond. The ligand being studied in this experiment is 2,6-pyridinecarboxylate, commonly called dipicolinate (or dipic). Figure 1 shows the structure of dipic. In this experiment, the rate of zinc removal by dipic will be measured.

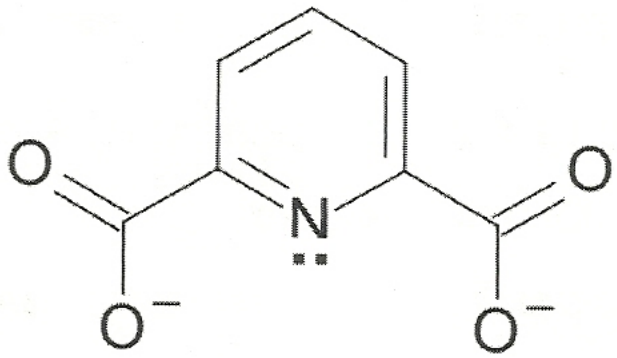

Figure 1: Structure of 2,6-pyridinecarboxylate (dipic)[1]

Mechanism

When $[dipic] >> [CA]$, that is, when $\frac{[dipic]}{[CA]} \geq 25$, then the removal of zinc is pseudo-first-order with respect to CA·Zn because the concentration of dipic, denoted as L, does not change appreciably. Thus the formation of the inactive enzyme, apoCA, can be modeled

using the following rate equation:

$$\frac{d[\text{apoCA}]}{dt} = k_{obs}[\text{CA·Zn}] \tag{2}$$

The pseudo-first-order rate constant, k_{obs}, increases as [L] increases, but levels off at sufficiently high concentrations of L. Biochemists will recognize behavior similar to Michaelis-Menten enzyme kinetics in which the enzyme, CA·Zn, and the substrate, L, reversibly form a CA·Zn·L complex with association constant K_{EML} (EML stands for Enzyme-Metal-Ligand):

$$\text{CA·Zn} + \text{L} \overset{\text{K}_{\text{EML}}}{\rightleftharpoons} \text{CA·Zn·L} \tag{3}$$

This can be modeled as follows:

$$K_{EML} = \frac{[\text{CA·Zn·L}]}{[\text{CA·Zn}][\text{L}]} \tag{4}$$

CA·Zn·L can either revert back to the original species or irreversibly convert to the inactive form of the enzyme, apoCA, and the covalently bound zinc-dipic molecule, ZnL:

$$\text{CA·Zn·L} \xrightarrow{\text{k}_\text{d}} \text{apoCA} + \text{ZnL} \tag{5}$$

This yields the following differential rate law:

$$\frac{d[\text{apoCA}]}{dt} = k_d[\text{CA·Zn·L}] \tag{6}$$

Recall that [L] >> [CA·Zn], so [L] can be assumed to stay constant at $[\text{L}]_0$, which is substituted into a rearranged form of equation (4),

$$[\text{CA·Zn·L}] = K_{EML}[\text{CA·Zn}][\text{L}]_0 \tag{7}$$

Carbonic anhydrase can exist in one of three forms: the metalloenzyme CA·Zn, the enzyme-metal-ligand complex CA·Zn·L, or the inactivated enzyme apoCA. Initially, all CA is tied up in the metalloenzyme, and none exists as CA·Zn·L or apoCA. As the activated form of the enzyme gets bound to L and then inactivated,

$$[\text{CA·Zn}] = [\text{CA·Zn}]_0 - [\text{apoCA}] - \text{CA·Zn·L}, \tag{8}$$

which can be combined with equation (7) to yield

$$[\text{CA·Zn}] = [\text{CA·Zn}]_0 - [\text{apoCA}] - K_{EML}[\text{CA·Zn}][\text{L}]_0 \tag{9}$$

and can be rearranged as follows:

$$[\text{CA·Zn}] = \frac{[\text{CA·Zn}]_0 - [\text{apoCA}]}{1 + K_{EML}[\text{L}]_0} \tag{10}$$

Equations (6) and (7) can be combined to give

$$\frac{d[\text{apoCA}]}{dt} = k_d K_{EML}[\text{CA·Zn}][\text{L}]_0 \tag{11}$$

Therefore, the rate of apoCA formation is first-order with respect to CA·Zn. Combining equations (11) and (10) yields

$$\frac{d[\text{apoCA}]}{dt} = k_d K_{EML}[\text{L}]_0 \frac{[\text{CA·Zn}]_0 - [\text{apoCA}]}{1 + K_{EML}[\text{L}]_0} \tag{12}$$

Rearranging and integrating,

$$\begin{aligned} \int_{[apoCA]_0}^{[apoCA]_t} \frac{d[\text{apoCA}]}{[\text{CA·Zn}]_0 - [\text{apoCA}]_t} &= \int_0^t \frac{k_d K_{EML}[\text{L}]_0}{1 + K_{EML}[\text{L}]_0} dt \\ &= \frac{k_d K_{EML}[\text{L}]_0}{1 + K_{EML}[\text{L}]_0} t \end{aligned} \tag{13}$$

The left side must be integrated using u-sub:

$$u = [\text{CA}\cdot\text{Zn}]_0 - [\text{apoCA}]_t$$

$$du = -d[\text{apoCA}]$$

To change the integral boundaries,

$u(t = 0) = [\text{CA}\cdot\text{Zn}]_0$, since no inactivated enzyme has been formed

$$u(t = t) = [\text{CA}\cdot\text{Zn}]_0 - [\text{apoCA}]_t$$

Therefore,

$$\begin{aligned}\int_{[apoCA]_0}^{[apoCA]_t} \frac{d[\text{apoCA}]}{[\text{CA}\cdot\text{Zn}]_0 - [\text{apoCA}]_t} &= -\int_{[\text{CA}\cdot\text{Zn}]_0}^{[\text{CA}\cdot\text{Zn}]_0-[\text{apoCA}]_t} \frac{du}{u} \\ &= -ln(u)\Big|_{[\text{CA}\cdot\text{Zn}]_0}^{[\text{CA}\cdot\text{Zn}]_0-[\text{apoCA}]_t} \\ &= -ln\left(\frac{[\text{CA}\cdot\text{Zn}]_0 - [\text{apoCA}]_t}{[\text{CA}\cdot\text{Zn}]_0}\right)\end{aligned} \tag{14}$$

Data and Results

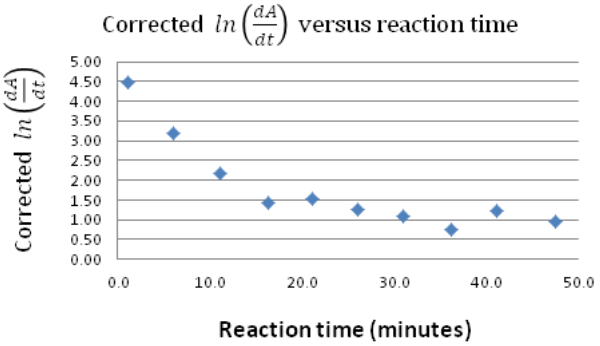

Figure 2: laksjdf askdfj asdkljfhaslkdjfhaklsdjfh asldkfjhasdlkjfhalksdjfhalksdjfh asldkfjhasdlkfjashdlfkajsdhflkajshdf asdfa sdiufhaisdu fas dfasd fasef asefasef asefas efasefkahsdkjfhaskldjfha sdfasldkfjhasdlfkjasdh falksdjfhalskdjfhasdlkfjah sdflkjashdlkfjah sdlkfjh aslkdjfh aslkdjfhalksdjfhalksjdh flkajsdhf alsdkjfash dflaskdjfah .

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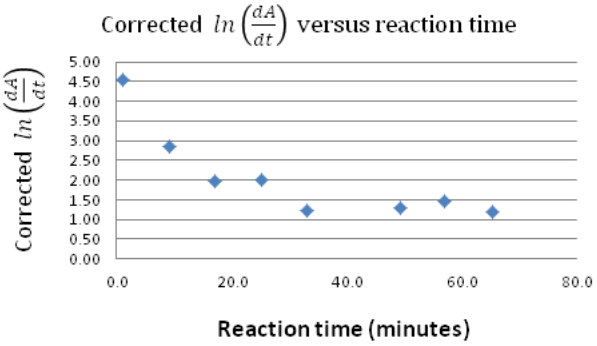

Figure 3: blahblah.

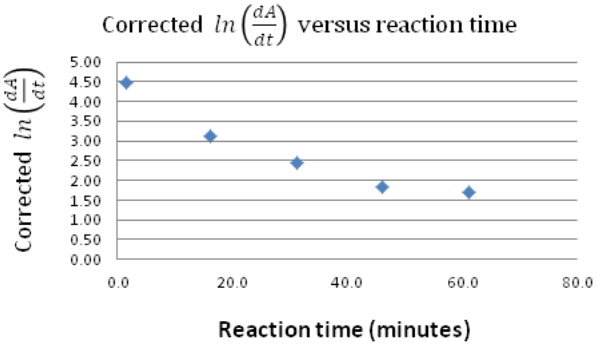

Figure 4: blahblah.

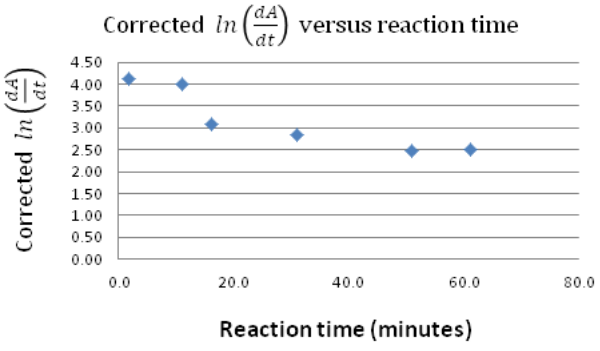

Figure 5: blahblah.

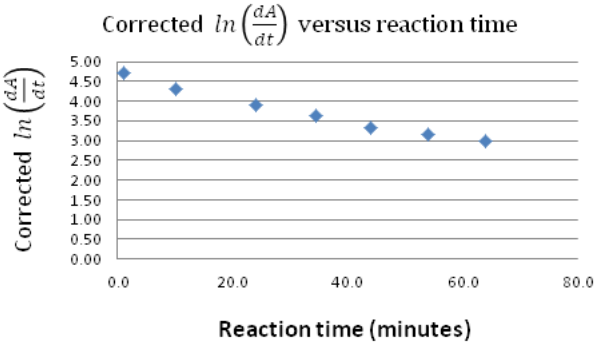

Figure 6: blahblah.

References

(1) Killian, B. J. *Experiments for Physical Chemistry Laboratory*, Summer 2014, Target Copy: Gainesville, **2014**. 45 - 50.