

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

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## 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.

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# Introduction

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



In the active form, CA is bound to a  $\text{Zn}^{2+}$  cofactor (denoted as  $\text{CA}\cdot\text{Zn}$ ), 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)<sup>1</sup>

## Mechanism

When  $[\text{dipic}] \gg [\text{CA}]$ , that is, when  $\frac{[\text{dipic}]}{[\text{CA}]} \geq 25$ , then the removal of zinc is pseudo-first-order with respect to  $\text{CA}\cdot\text{Zn}$  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:

$$\frac{d[\text{apoCA}]}{dt} = k_{obs}[\text{CA}\cdot\text{Zn}] \quad (2)$$

The pseudo-first-order rate constant,  $k_{obs}$ , increases as  $[\text{L}]$  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):



This can be modeled as follows:

$$K_{EML} = \frac{[\text{CA}\cdot\text{Zn}\cdot\text{L}]}{[\text{CA}\cdot\text{Zn}][\text{L}]} \quad (4)$$

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:



This yields the following differential rate law:

$$\frac{d[\text{apoCA}]}{dt} = k_d[\text{CA}\cdot\text{Zn}\cdot\text{L}] \quad (6)$$

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

$$[\text{CA}\cdot\text{Zn}\cdot\text{L}] = K_{EML}[\text{CA}\cdot\text{Zn}][\text{L}]_0 \quad (7)$$

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,

$$[\text{CA} \cdot \text{Zn}] = [\text{CA} \cdot \text{Zn}]_0 - [\text{apoCA}] - \text{CA} \cdot \text{Zn} \cdot \text{L}, \quad (8)$$

which can be combined with equation (7) to yield

$$[\text{CA} \cdot \text{Zn}] = [\text{CA} \cdot \text{Zn}]_0 - [\text{apoCA}] - K_{EML}[\text{CA} \cdot \text{Zn}][\text{L}]_0 \quad (9)$$

and can be rearranged as follows:

$$[\text{CA} \cdot \text{Zn}] = \frac{[\text{CA} \cdot \text{Zn}]_0 - [\text{apoCA}]}{1 + K_{EML}[\text{L}]_0} \quad (10)$$

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

$$\frac{d[\text{apoCA}]}{dt} = k_d K_{EML}[\text{CA} \cdot \text{Zn}][\text{L}]_0 \quad (11)$$

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

$$\frac{d[\text{apoCA}]}{dt} = k_d K_{EML}[\text{L}]_0 \frac{[\text{CA} \cdot \text{Zn}]_0 - [\text{apoCA}]}{1 + K_{EML}[\text{L}]_0} \quad (12)$$

Rearranging and integrating,

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

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,

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

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

Therefore,

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

Combining the evaluated integrals from equations (14) and (13) yields

$$\ln \left( \frac{[\text{CA}\cdot\text{Zn}]_0 - [\text{apoCA}]_t}{[\text{CA}\cdot\text{Zn}]_0} \right) = -\frac{k_d K_{EML} [\text{L}]_0}{1 + K_{EML} [\text{L}]_0} t \quad (15)$$

Assuming steady-state conditions for  $\text{CA}\cdot\text{Zn}\cdot\text{L}$ , that is,  $[\text{CA}\cdot\text{Zn}\cdot\text{L}]$  is always negligible,  $\frac{[\text{CA}\cdot\text{Zn}]_0 - [\text{apoCA}]_t}{[\text{CA}\cdot\text{Zn}]_0}$

is simply the fraction of  $\text{CA}\cdot\text{Zn}$  remaining after reaction time  $t$  and can be referred to as

$F_{\text{CA}\cdot\text{Zn}}$ :

$$\ln (F_{\text{CA}\cdot\text{Zn}}) = -\frac{k_d K_{EML} [\text{L}]_0}{1 + K_{EML} [\text{L}]_0} t \quad (16)$$

Since  $-\frac{k_d K_{EML} [\text{L}]_0}{1 + K_{EML} [\text{L}]_0}$  is a constant for a given concentration of dipic, equation (16) exhibits

linear behavior. Therefore, a linear least squares regression procedure can be performed for

measurements of  $\ln(F_{\text{CA}\cdot\text{Zn}})$  over time, and the slope, denoted  $-k_{obs}$ , will be

$$-k_{obs} = -\frac{k_d K_{EML}[\text{L}]_0}{1 + K_{EML}[\text{L}]_0} \quad (17)$$

Taking the reciprocal of equation (??) yields

$$\begin{aligned} \frac{1}{k_{obs}} &= \frac{1 + K_{EML}[\text{L}]_0}{k_d K_{EML}[\text{L}]_0} \\ &= \frac{1}{k_d K_{EML}[\text{L}]_0} + \frac{K_{EML}[\text{L}]_0}{k_d K_{EML}[\text{L}]_0} \\ &= \frac{1}{k_d K_{EML}} \times \frac{1}{[\text{L}]_0} + \frac{1}{k_d} \end{aligned} \quad (18)$$

Again, one can see a linear pattern; measuring  $k_{obs}$  at several different dipic concentrations enables performing a least squares regression procedure on  $\frac{1}{k_{obs}}$  versus  $\frac{1}{[\text{L}]_0}$  to determine the slope,  $m = \frac{1}{k_d K_{EML}}$ , and intercept,  $b = \frac{1}{k_d}$ .  $k_d$  and  $K_{EML}$  are thus calculated as follows:

$$k_d = \frac{1}{b} \quad (19)$$

$$K_{EML} = \frac{b}{m} \quad (20)$$

## Data and Results

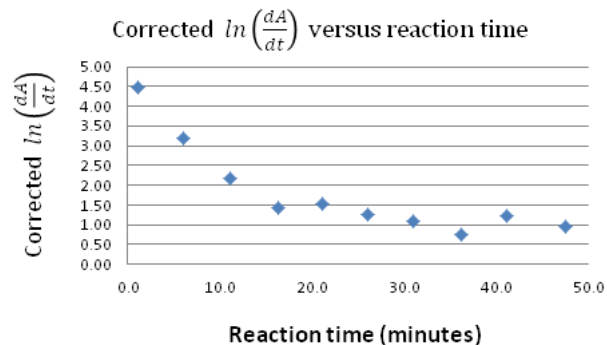


Figure 2: laksjdf askdfj asdkljfhaslkdjfhaklsdjfh asldkfjhasdlkjfhalksdjfhalksdjfh  
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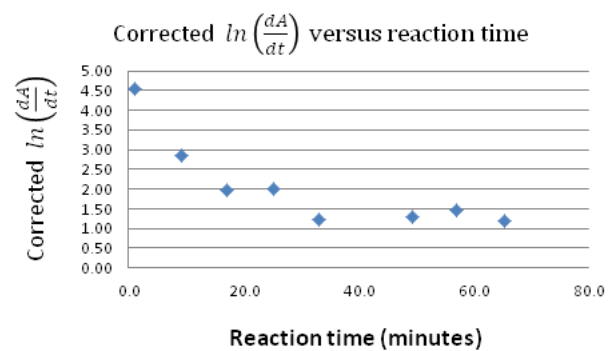


Figure 3: blahblah.

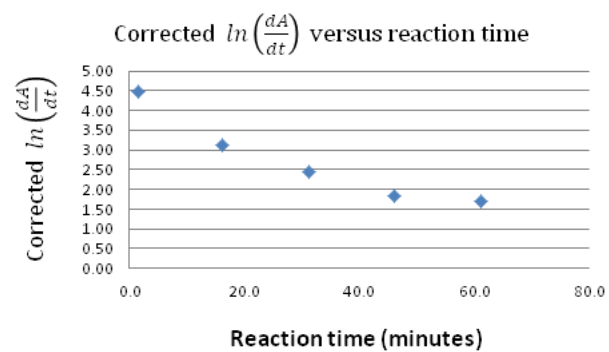


Figure 4: blahblah.

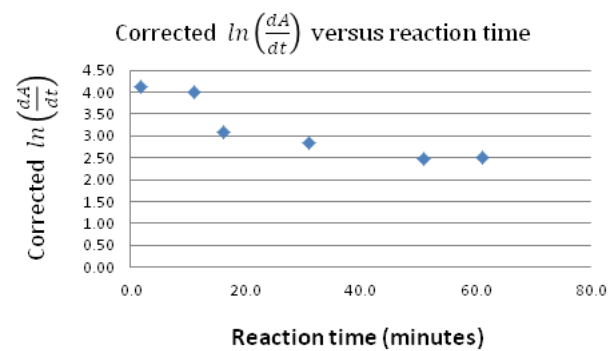


Figure 5: blahblah.



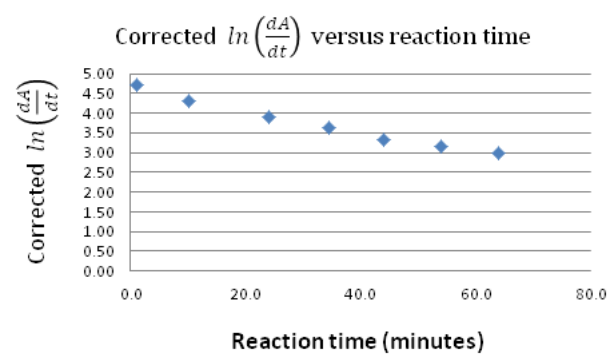


Figure 6: blahblah.

## References

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