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A fractional model of enzymatic competitive substrate inhibition with Ping-Pong mechanism

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ABSTRACT

Enzymes are biodegradable catalysts naturally present in living organisms. Enzymes can accelerate biochemical reactions by reducing the activation energy, and they are not consumed during reaction processes. Numerous applications of enzymes have been developed in biotechnology, industry, medicine, pharmaceuticals, food processing, biofuels, and so on. In this study, we develop a mathematical model describing enzymatic reactions with a Ping-Pong mechanism and competitive substrate inhibition. In order to obtain insights into the model behaviors, we use Python software to obtain numerical solutions for the model. Some discussions on the numerical results is provided. Finally, we briefly discuss a potential application of the model and some future work.

Keywords: *competitive inhibition; enzyme; fractional order derivative; mathematical model; peroxidase.*

1 Introduction

Enzymes are naturally present in living organisms and catalyze biochemical reactions in cellular metabolism to produce the metabolites necessary for the cells. Enzymes can accelerate biochemical reactions by reducing the activation energy, and they are not consumed during reaction processes. They are able to be degraded biologically, this feature suggests that enzymes may play an important role in environmental protection [3, 7, 9, 20].

Cells regulate the concentrations of metabolites at physiological levels by using many regulatory mechanisms. Among these, enzymatic inhibition processes are very common mechanisms. There exist three main inhibition processes of enzymes, and they are competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition processes. In the competitive inhibition process, the enzyme molecule has one binding site for the substrate and inhibitor. Inhibitor molecules and substrate molecules compete with each other for the binding sites of enzyme molecules. The binding of an inhibitor molecule to an enzyme molecule prevents the substrate molecules from binding to the enzyme molecule and form an enzyme-inhibitor complex, so this enzyme molecule is not able to catalyze reactions to form products. Competitive substrate

inhibition of an enzyme is a competitive inhibition process in which the substrate plays the role of inhibitor and enzymes often are bi-substrate enzymes [3, 7, 9, 10, 19, 20]. *Spanish broom* (*Cytisus multiflorus*) *peroxidase* is one of the enzymes that are inhibited by their substrates [10].

As mentioned above, enzymes offer numerous benefits. Nowadays, scientific and technological advances facilitate the study of enzymes and their applications [10, 14, 19]. New enzymes are being extracted and studied increasingly. A variety of applications of enzymes have been studied and developed in biotechnology, industry, and medicine. Some common applications of enzymes are in pharmaceuticals, food processing, biofuels, and so on [5, 8, 14, 18]. The mechanism of an enzyme must first be known before an application for it can be developed. Besides experimental studies, mathematical models are also helpful tools to help gain insights into the mechanism of action of an enzyme [**?**, 2, 12, 15, 16, 21].

In this study, we will develop a novel fractional model describing a system of enzymatic reactions with a Ping-Pong mechanism and competitive inhibition by a substrate. Numerical simulations will provide insights into how model outputs behave. This can be obtained by using Python software to numerically integrate the model. The model will be used to model the reactions of *Spanish broom peroxidase* under specific circumstances.

The rest of this paper is organized as follows. In Section 2, we describe the formulation of the mathematical model. Numerical solutions for the model are presented in Section 3, and Section 4 provides a potential application of the model to the reactions of *Spanish broom peroxidase* . Finally, we discuss some concluding remarks in Section 5.

2 The mathematical model

In this section, we develop a minimal fractional order model that describes the mechanism of competitive substrate inhibition of an enzyme, where the Caputo derivative is used. The fractional derivative of order α in the Caputo sense is defined as the operator $D_t^\alpha f(t)$ such that

$$D_t^\alpha f(t) = \frac{1}{\Gamma(m-\alpha)} \int_0^t \frac{f^{(m)}(t)}{(t-s)^{\alpha+1-m}} ds, \quad m-1 < \alpha < m,$$

where Γ is the Gamma function defined as follows

$$\Gamma(z) = \int_0^\infty u^{z-1} e^{-u} du, \Re(z) > 0.$$

For more details, the readers can find in [17]. In the current study, we use $\alpha \in (0, 1)$. Next, we provide a brief description of the kinetic mechanism that model is based on.

2.1 The kinetic mechanism

A Ping-Pong mechanism with competitive substrate inhibition of enzymatic reactions is depicted generally in Figure 1.

In this mechanism, an enzyme molecule has at least two binding sites for its substrates and produces two product molecules. First, a molecule of substrate A binds to an enzyme molecule E to form a substrate-enzyme complex EA. The enzyme molecule catalyzes the complex to form a product-enzyme complex $E'P$ and releases the product molecule P and an intermediate enzyme molecule E' then. When intermediate enzyme molecules E' occur, a substrate molecule of B is able to bind to an E' molecule to form a substrate-enzyme complex $E'B$. The E' enzyme

NOMENCLATURE

$[X]$ – the concentration of a species X; a function of time (mM)
E – a molecule of the enzyme
E' – a molecule of the intermediate enzyme
A – a molecule of the substrate A
B – a molecule of the substrate B
P – a molecule of the product P
Q – a molecule of the product Q
EA – an enzyme-substrate complex
$E'B$ – an enzyme-substrate complex
EB – an enzyme-substrate complex
$E'P$ – an enzyme-product complex
EQ – an enzyme-product complex
k_4 – catalytic rate for the intermediate enzyme acting on a substrate molecule B (s^{-1})
k_1 – adsorption rate of substrate molecules A to free enzyme molecules E $(mM^{-1}s^{-1})$
k_{-1} – desorption rate of substrate molecules from enzyme-substrate complexes EA (s^{-1})
k_2 – catalytic rate for the enzyme acting on a substrate molecule A (s^{-1})
k_{-2} – adsorption and catalytic rate for the intermediate enzyme to convert a product molecule P to substrate molecule $(mM^{-1}s^{-1})$
k_3 – adsorption rate of substrate molecules B to free intermediate enzyme molecules E' $(mM^{-1}s^{-1})$
k_{-3} – desorption rate of substrate molecules B from enzyme-substrate complexes $E'B$ (s^{-1})
k_5 – adsorption rate of substrate molecules B to free enzyme molecules E $(mM^{-1}s^{-1})$
k_{-5} – desorption rate of substrate molecules B from enzyme-substrate complexes EB (s^{-1})

molecule catalyzes the complex $E'B$ to form a product-enzyme complex EQ and releases the product molecule Q and enzyme molecule E. The competitive inhibition by the substrate is represented as follows: a substrate molecule B that is able to bind to an enzyme molecule E to form a substrate-enzyme complex EB. The binding of a molecule B to an enzyme molecule prevents A molecules from binding to the enzyme molecule E [4, 6, 10]. Figure 2

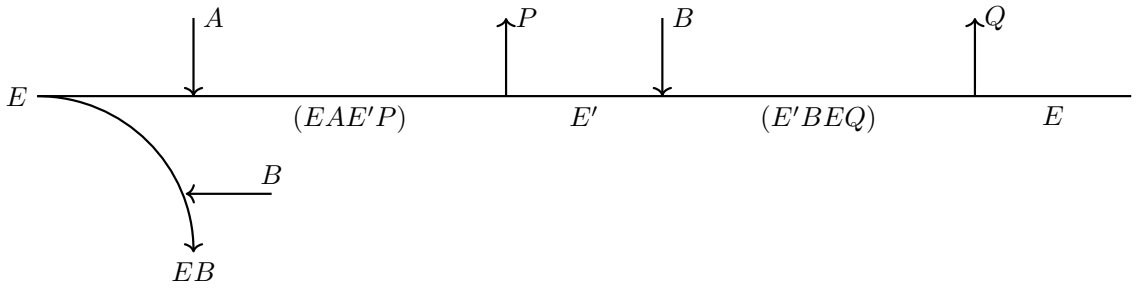

Figure 1: Diagram of Ping Pong mechanism with competitive inhibition by the substrate. Here E represents an enzyme molecule, A and B are the substrates molecules, P and Q are the product molecules. Further, E' corresponds to an intermediate enzyme molecule, EA, $E'B$, EB substrate-enzyme complexes, and EQ, $E'P$ product-enzyme complexes.

shows graphically the Ping-Pong part of the above mechanism.

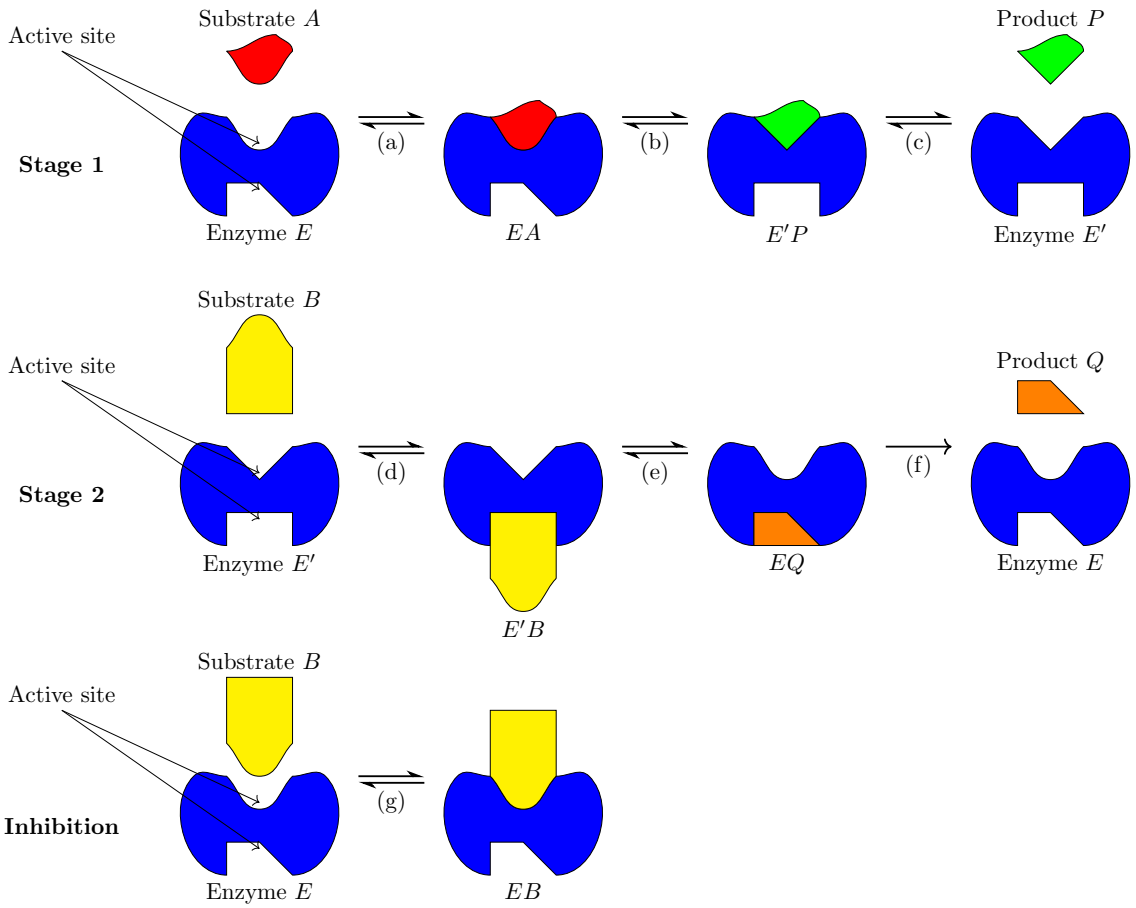

Figure 2: Illustration of a Ping-Pong mechanism.

To simplify the modeling, we reduce the above kinetic mechanism by a minimal set of chemical reactions as follows Assumptions are needed for developing a mathematical model. In the next section, we list some assumptions for the model.

$$
\begin{aligned}
E + A &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E' + P,\\
E' + B &\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E'B \xrightarrow{k_4} E + Q,\\
E + B &\underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} EB.
\end{aligned}
$$

Figure 3: A simplified scheme of the Ping-Pong mechanism. Notations used here are the same as those in Figure 1. Furthermore, the parameters k_i's are constant rates of chemical reactions. See the Nomenclature for more details.

2.2 Modeling assumptions

The model developed here is based on the law of mass action. Here are the necessary assumptions for the model.

- The mixture of substrate and enzyme is well-stirred throughout. This implies that diffusive effects in the process can be omitted and that the concentrations of the various species in the mixture can be described by functions of time only. This further implies that the evolution of the system can be modeled by a coupled system of nonlinear ordinary differential equations and that a partial differential equations model is not required [15, 22].

- We assume that mass action kinetics throughout; this implies that the rate of a reaction is taken to be proportional to the product of the concentrations of the reactants. We emphasize here that more complex formulas, such as the Michaelis–Menten formula for the rate of product production in an enzyme-catalysed reaction, are derivable from more fundamental mass action considerations under simplifying assumptions [15, 22].

2.3 The model equations

In order to be convenient for the modeling, we can rewrite the chemical reactions in Figure 3 as follows

$$
\begin{aligned}
&E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA,\\
&EA \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E' + P,\\
&E' + B \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E'B,\\
&E'B \xrightarrow{k_4} E + Q,\\
&E + B \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} EB.
\end{aligned}
$$

Under the above assumptions, using the law of mass action, the model equations that describe the concentrations of species in the mixture are given by

$$D_t^\alpha[E] = -k_1[E][A] + k_{-1}[EA] + k_4[E'B] - k_5[E][B] + k_{-5}[EB], \tag{2.1a}$$
$$D_t^\alpha[A] = -k_1[E][A] + k_{-1}[EA], \tag{2.1b}$$
$$D_t^\alpha[EA] = k_1[E][A] - (k_{-1} + k_2)[EA] + k_{-2}[E'][P], \tag{2.1c}$$
$$D_t^\alpha[E'] = -k_{-2}[E'][P] - k_3[E'][B] + k_2[EA] + k_{-3}[E'B], \tag{2.1d}$$
$$D_t^\alpha[P] = -k_{-2}[E'][P] + k_2[EA], \tag{2.1e}$$
$$D_t^\alpha[B] = -k_3[E'][B] + k_{-3}[E'B] - k_5[E][B] + k_{-5}[EB], \tag{2.1f}$$
$$D_t^\alpha[E'B] = -(k_{-3} + k_4)[E'B] + k_3[E'][B], \tag{2.1g}$$
$$D_t^\alpha[Q] = k_4[E'B], \tag{2.1h}$$
$$D_t^\alpha[EB] = k_5[E][B] - k_{-5}[EB], \tag{2.1i}$$

where $[X] = [X](t)$ denotes the concentration of species X at time t.

It is not necessary to provide discussions of these equations here. However, we do briefly discuss two of them to illustrate how the model equations are constructed. The chemical reactions for the model are displayed in Figure 3. We begin by considering the equation for E given by

$$D_t^\alpha[E] = \underbrace{-k_1[E][A]}_{①}\underbrace{+k_{-1}[EA]}_{②}\underbrace{+k_4[E'B]}_{③}\underbrace{-k_5[E][B]}_{④}\underbrace{+k_{-5}[EB]}_{⑤},$$

where

① this term accounts for the reduction in concentration of E due to substrate A binding.

② the increase in concentration of E due to substrate unbinding from the complex EA.

③ the increase in concentration of E due to enzyme catalyzing the complex $E'B$ and releasing the product then.

④ the reduction in concentration of E due to substrate B binding.

⑤ the increase in concentration of E due to substrate unbinding from the complex EB.

Next consider the equation (2.1c) for complex EA

$$D_t^\alpha[EA] = \underbrace{k_1[E][A]}_{ⓐ}\underbrace{-(k_{-1} + k_2)[EA]}_{ⓑ}\underbrace{+k_{-2}[E'][P]}_{ⓒ},$$

where

ⓐ this account for the increase in concentration of EA due to enzyme binding to the substrate A.

ⓑ the reduction in concentration of EA due to substrate unbinding from EA and enzyme catalyzing EA to form product P.

ⓒ the increase in concentration of EA due to enzyme E' binding to the substrate P and catalyzing the complex to form EA then.

The remaining equations (2.1b), (2.1d), (2.1e), (2.1f), (2.1g), (2.1h) and (2.1i) are interpreted similarly. These equations are to be solved subject to the initial conditions

$$\begin{array}{ll} [E](t=0)=E_0, & [E'](t=0)=0, \\ [A](t=0)=A_0, & [B](t=0)=B_0, \\ [EA](t=0)=0, & [E'B](t=0)=0, \\ [P](t=0)=0, & [Q](t=0)=0, \\ [EB](t=0)=0, & \end{array}$$

where E_0, A_0, and B_0 are positive constants corresponding to the initial concentrations of enzyme and substrates, respectively. Computing the sums of equations (2.1a)+(2.1c)+(2.1d)+(2.1g)+(2.1i), (2.1b)+(2.1c)+(2.1e) and (2.1f)+(2.1g)+(2.1h)+(2.1i), and integrating yields

$$[E]+[EA]+[EB]+[E']+[EB]+[E'B]=E_0, \tag{2.2a}$$
$$[A]+[EA]+[P]=A_0, \tag{2.2b}$$
$$[B]+[E'B]+[EB]+[Q]=B_0, \tag{2.2c}$$

which are the expressions of conservation of enzyme E, substrate A, and substrate B, respectively.

3 Numerical solutions

The system of differential equations was numerically integrated using the `fodeint` solver, the SciPy, and Numpy libraries [1,23,25]. SciPy [25] is an open source Python [24] library that contains numerical routines for applications in science and engineering. The initial conditions used here are given by

$$\begin{array}{ll} [E](t=0)=3.0\ mM, & [E'](t=0)=0.0\ mM, \\ [A](t=0)=5.0\ mM, & [B](t=0)=4.0\ mM, \\ [EA](t=0)=0.0\ mM, & [E'B](t=0)=0.0\ mM, \\ [P](t=0)=0.0\ mM, & [Q](t=0)=0.0\ mM, \\ [EB](t=0)=0.0\ mM, & \end{array}$$

and the values of model parameters are set as follows

$$\begin{array}{ll} k_1=3.0\ mM^{-1}s^{-1}, & k_{-1}=0.2\ s^{-1}, \\ k_2=2.0\ s^{-1}, & k_{-2}=0.1\ mM^{-1}s^{-1}, \\ k_3=3.0\ mM^{-1}s^{-1}, & k_{-3}=0.1\ s^{-1}, \\ k_4=2.0\ s^{-1}, & \\ k_5=3.0\ mM^{-1}s^{-1}, & k_{-5}=0.15\ s^{-1}. \end{array}$$

It should be noticed that the derivative order used here is $\alpha = 0.5$. In Figure 4, we plot the numerical solutions of the model corresponding to the initial conditions and the parameter values aforementioned. Each line corresponds to the concentration of one species in the mixture with respect to time t. Below, we give some discussions on these numerical results.

- The line ----- represents the concentration of enzyme during the process. In the first stage, the concentration of enzyme drops rapidly due to the binding of substrates to form

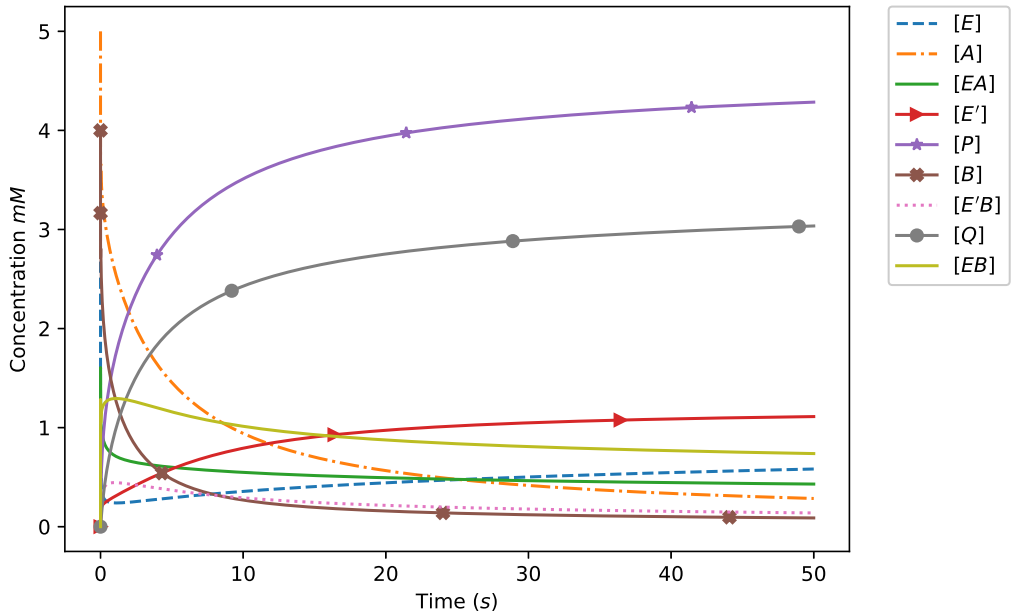

Figure 4: Numerical solutions of the model. Each line represents the concentration of one species in the mixture with respect to t. The values of model parameters are referred to the main text.

substrate-enzyme complexes EA and EB. As time goes on, the enzyme converts the substrates to the products. This reduces the concentrations of the substrates and makes the increase in the concentrations of products continuously. The concentration of the enzyme goes up and reaches a steady state at the end of the process. It should be noted that the steady concentration of the enzyme is lower than the initial concentration of it.

- The line describes the concentration of the substrate A. The concentration decreases rapidly and goes to a steady state at the end of the process. This happens because the intermediate enzyme E' can convert the product P to the substrate A.

- The line corresponds to the concentration of the substrate-enzyme complex EA. It can be seen that the concentration rises up rapidly in the early stage because of the binding of the substrate to the enzyme. Then, the concentration goes down quickly since the enzyme catalyzes the complex to form product P and the substrate unbinds from the complex. In the end, the concentration of the complex is completely converted to the product P and goes to a steady state. This is because the intermediate enzyme E' can convert the product P to the substrate A.

- The line describes the concentration of the intermediate enzyme E'. The concentration increases gradually and reaches a steady state at the end of the process. This agrees with the nature of the process. That is the intermediate enzyme is capable of converting the product P to the substrate A and the original enzyme, and the substrate B binds to the enzyme E' to form complexes $E'B$.

- The line corresponds to the concentration of the product P. The concentration goes up rapidly and reaches a steady state. It is clear that the concentration is lower than

the initial concentration of the substrate A. This is in line with the nature of the process since the enzyme is able to inter-convert between the substrate A and the product P.

- The line represents the concentration of the substrate B. The rapid decrease in the concentration is due to the binding of the substrate B to the intermediate enzyme E' to form the substrate-enzyme complex $E'B$. The concentration goes to zero at the end of the process since the substrate is completely converted to the product Q. This agrees with the fact that the enzyme is not able to convert the product Q to the substrate B at all.

- The line shows the concentration of the substrate-enzyme complex $E'B$. At the early stage, the rapid increase in the concentration is due to the binding of the substrate B to the intermediate enzyme E' to form the complex $E'B$. The concentration approaches zero at the end of the process since the complex is totally catalyzed to form the product Q and the substrate B is completely converted to the product Q. This is in line with the fact that the conversion of the complex to the product Q is an irreversible reaction.

- The line displays the concentration of the product Q with respect to time t. The concentration increases quickly at the early stage since the concentration of the complex $E'B$ increase quickly and the enzyme quickly catalyzes the complex and releases the product then. The concentration tends to the initial concentration of the substrate B as the substrate B is converted continuously.

- The line shows the concentration of the substrate-enzyme complex EB. At the early stage, the rapid increase in the concentration is due to the binding of the substrate B to the enzyme E to form the complex EB. It should be noted that this reaction is reversible. The concentration approaches zero at the end of the process since the substrate B is completely converted to the product Q.

In the next section, we describe how to use the model to model the enzymatic reactions catalyzed by *Spanish broom peroxidase*.

4 Model and H_2O_2-supported oxidation by *Spanish broom peroxidase*

Peroxidases are enzymes known as heme peroxidases [**?**]. Using H_2O_2 reduction, heme peroxidases catalyze the oxidation of a variety of substrates, including phenols, aromatic amines, thiosanisoles, halide and thiocyanate ions. Numerous application of peroxidase have been developed in clinical biochemistry, enzyme immunoassays [11], the treatment of waste water containing phenolic compounds, the synthesis of various aromatic chemicals, and the removal of peroxide from industrial wastes [13]. It is known that the kinetic mechanism of the *Spanish broom peroxidase* is a Ping-Pong mechanism with the presence of competitive inhibition by substrates [10]. Hence, our model may be used to model the oxidation catalyzed by the *Spanish broom peroxidase* with the support of H_2O_2 under specific conditions.

To successfully model the oxidation catalyzed by the enzyme, the mixture of species has to satisfy:

- the model assumptions;

- the concentration of H_2O_2 is not so high enough to make it able to inhibit the intermediate forms of enzyme;
- the concentration of other substrates are very much higher than that of the enzyme. See [10] for more details.

Then, the peroxidase enzyme plays the role of the native enzyme E in the model. The reactant H_2O_2 is the substrate A; AH_2 (*Pyrocatechol*, *o-Dianisidne*, an so on) corresponds to the substrate B; and EII is the intermediate enzyme E'. The products P and Q are H_2O and $AH^{\bullet}$, respectively [10].

5 Conclusions

Enzymes are biologically degradable catalysts with numerous applications in biotechnology, medicine, biofuels, food processing, pharmaceuticals, and so on. Cells use many regulatory mechanisms to regulate the concentrations of metabolites at physiological levels. Enzymatic competitive inhibition is one such mechanism, in particular, the competitive inhibition by the substrate. The kinetic mechanism of the reaction of an enzyme must be understood before the development of its applications. We know that the *Spanish broom peroxidase* is an enzyme that obeys a Ping-Pong mechanism with competitive inhibition by the substrates. Thus, from the point of view of applications, reliable mathematical models for enzymatic reactions with this mechanism are clearly desirable. In the current study, we developed a mathematical model for enzymatic reactions with this mechanism. The model is a system of fractional differential equations in the sense of Caputo. The model behaviors were investigated using Python software to numerically integrate the model. The system of interest was seen to model, under appropriate conditions, the H_2O_2-supported oxidation catalyzed by *Spanish broom peroxidase*. With high concentrations, however, the substrate H_2O_2 might competitively inhibit the *peroxidase* enzyme [10], this may provide an interesting research topic of mathematical modeling. Finally, the positivity of the model solutions remains to address. This might provide some expansions of the current study.

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