Question 2

0.1

Using the law of mass action, we could get the equations of the rate of changes as follows. Here we will use the same symbol E refers to the enzyme, S refers to the substrate, ES and P refer to the intermediate and final product.

At first, the rate of change equation of product P is:

$$\frac{dP}{dt} = k_3 ES \tag{1}$$

Then we could obtain the equations of ES and S as follows:

$$\frac{dES}{dt} = k_1 E \cdot S - k_2 ES - k_3 ES \tag{2}$$

$$\frac{dS}{dt} = -k_1 E \cdot S + k_2 ES \tag{3}$$

Notice that, the total enzyme concentration E_0 in the system equal to

$$E_0 = E(t) + ES(t) \tag{4}$$

Hence we could get:

$$\frac{dE}{dt} + \frac{dES}{dt} = 0 \tag{5}$$

Substituting Eq.(5) into (2), we could write the rate of change equation of E as:

$$\frac{dE}{dt} = -k_1 E \cdot S + k_2 ES + k_3 ES \tag{6}$$

Thus, we obtain the rate of change equations of four species:

$$\begin{cases} \frac{dES}{dt} = k_1 E \cdot S - k_2 ES - k_3 ES, \\ \frac{dE}{dt} = -k_1 E \cdot S + k_2 ES + k_3 ES, \\ \frac{dS}{dt} = -k_1 E \cdot S + k_2 ES, \\ \frac{dP}{dt} = k_3 ES, \end{cases} \tag{7}$$

where k_1, k_2, and k_3 are the rate constants.

0.2

For a given initial value problem:

$$\begin{cases} y' = f(t,y), \ \ a \le t \le b \\ y(a) = y_a. \end{cases} \tag{8}$$

The fourth-order Runge-Kutta method is as follows:

$$\begin{cases} k_1 = f(t_i, y_i), \\ k_2 = f(t_i + \frac{h}{2}, \ y_i + \frac{h}{2}k_1), \\ k_3 = f(t_i + \frac{h}{2}, \ y_i + \frac{h}{2}k_2), \\ k_4 = f(t_i + h, y_i + hk_3), \\ y_{i+1} = y_i + \frac{h}{6}(k_1 + 2k_2 + 2k_3 + k_4), \end{cases} \tag{9}$$

where $a = t_0 \le t_1 \le ... \le t_N = b, h = \frac{b-a}{N} = t_{i+1} - t_i$ and $i = 0, 1, ..., N-1$.

In question 2.2, we initialized $E(0) = 1\mu M$, $S(0) = 10\mu M$, $ES(0) = 0$, and $P(0) = 0$. The rate constants are: $k_1 = 100/\mu M/min$, $k_2 = 600/\mu M/min$, $k_3 = 150/\mu M/min$. We could through MATLAB to construct the ordinary differential equation (ODE) and numerically solve these four equations using the fourth-order Runge-Kutta method.

At first, we combined the Eq. (7) into *fun_set.m* with default rate of changes and input the initial values corresponding to E, S, ES, and P.

```
function f = fun_set(t, y)
    % this is the ODE
    % y = [E, S, ES, P]
    % k = [k1, k2, k3]

    k = [100 600 150];

    f(1) = -k(1)*y(1)*y(2) + k(2)*y(3) + k(3)*y(3);    %dE
    f(2) = -k(1)*y(1)*y(2) + k(2)*y(3);                %dS
    f(3) = k(1)*y(1)*y(2) - k(2)*y(3) - k(3)*y(3);     %dES
```

```
    f(4) = k(3)*y(3);                                    %dP

    f = f(:);
```

We also constructed a function which called *Runge.m* to calculate the numerical solution through Eq. (9).

```
function [t, y] = Runge(func, y0, h, t0, tn)
    % func: the function of ODE
    % y0: the initial value
    % h: step wise
    % [t0, tn]: interval

    t = t0:h:tn;
    n = length(t);
    y = zeros(length(y0), n);
    y(:,1) = y0(:);

    for i = 1:n-1
        if y(2,i)>0
           K1 = feval(func, t(i), y(:,i));
           K2 = feval(func, t(i) + h/2, y(:,i) + h/2*K1);
           K3 = feval(func, t(i) + h/2, y(:,i) + h/2*K2);
           K4 = feval(func, t(i) + h, y(:,i) + h*K3);
           y(:,i+1) = y(:,i) + (h/6)*(K1 + 2*K2 + 2*K3 + K4);
        end
    end
end
```

Need to mention that, in practice, once the reactants are depleted, the entire chemical reaction stops. Therefore we have introduced this qualifying factor into the procedure.

At the end, with the initial value, we applied $h = 0.001$, $[t_0, t_n] = [0, 1]$ to solve these four equations.

The final answers are: $E = 1$, $S = 0$, $ES = 0$ and $P = 10$.

When the enzyme is $1\mu M$, and the substrate is $10\mu M$ at the beginning, with the rates of changes k_1, k_2, and k_3 equal to 100, 600, and $150/\mu M/min$, correspondingly. The total reaction was completed within one minute.

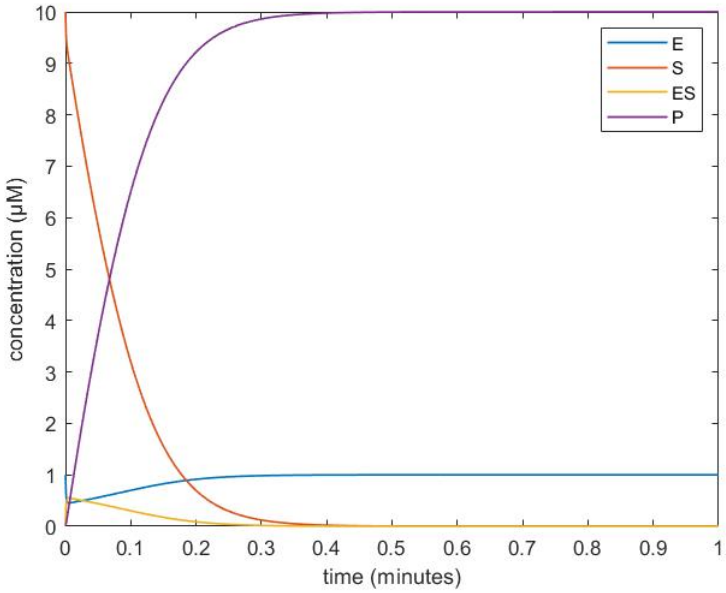

Figure 1: Visualization of concentration

Besides, we plot the concentrations of four species belonging to time to apply a better visualization in Fig. (1).

0.3

Following the definition of velocity,

$$V = \frac{dP}{dt} = k_3 ES \tag{10}$$

Since ES is an intermediate whose concentration is unknown, we could use the *steady-state* approximation to express V in terms of known quantities.

The rates of ES are formed and falls apart equal to:

$$rate\ of\ formed\ of\ ES = k_1 E * S \tag{11}$$

$$rate\ of\ falls\ apart\ of\ ES = (k_2 + k_3) ES \tag{12}$$

That is, under the steady-state approximation, the concentration of ES stays a constant when its rate of formed and falls apart equal to each other:

$$k_1 E * S = (k_2 + k_3) ES \tag{13}$$

Rearranging it,

$$ES = \frac{k_1 E * S}{k_2 + k_3} \tag{14}$$

According to the definition of Michaelis constant K_M:

$$K_M = \frac{k_2 + k_3}{k_1} \tag{15}$$

Substituting Eq. (15) into (13)

$$ES = \frac{E * S}{K_M} \tag{16}$$

Since the total concentration of $E_0 = E + ES$. We could substitute it into Eq. (16) then get:

$$ES = \frac{(E_0 - ES)S}{K_M} \tag{17}$$

Solving it for ES,

$$ES = E_0 \frac{S}{S + K_M} \tag{18}$$

Then substitute Eq. (18) into (10) we could obtain the velocity V as a function of the concentration of the substrate S,

$$V = k_3 E_0 \frac{S}{S + K_M} \tag{19}$$

In the end, following the initial value in the above question, we plot the relationship between V and S in Fig. (2).

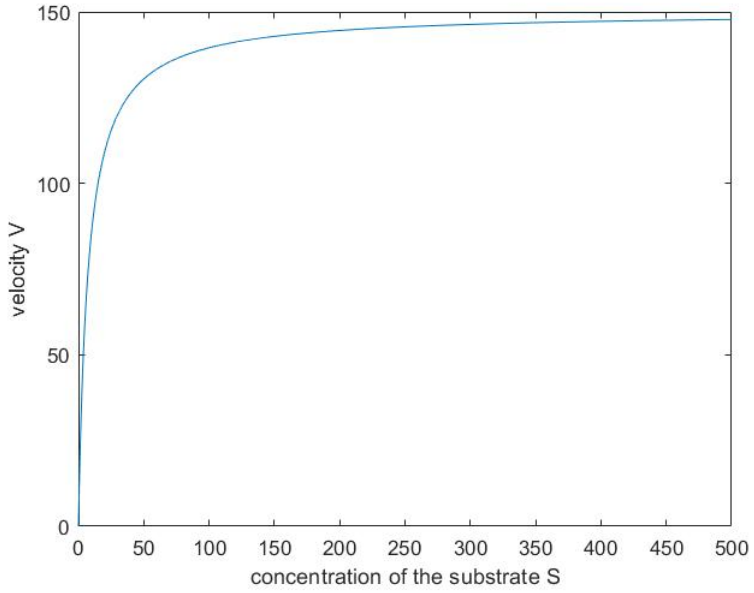

Figure 2: The relationship between S and V

According to the figure above, we verify that when the concentrations of S are small, the velocity V shows an approximate liner proportion with S. When the concentrations of S are very large, the velocity V saturates its maximum value. In this simulation, the V_m is equal to 150.