※ 注意：請於試卷上依序作答，並應註明作答之部份及其題號。

一、 填空(45%)：請使用下列答案庫填答，限用英文、每題3分、文法錯誤扣1分

答案庫(含單數名詞、原型動詞、形容詞與副詞)：activation; active; catalyst; catalyze; competitive; enzyme; first order; higher; intersect; kinetic; logarithmic; lower; Michaelis constant; non-competitive; reciprocal; selective; second order; slope; specificity; steady; substrate; thermal; transient; uncompetitive.

Biological reactions __(1)__ by enzymes are generally very __(2)__ to certain chemicals known as the __(3)__ of the enzymes; the chemicals bind to the __(4)__ site of the enzyme to become a __(5)__ complex (ES complex) with a much lower __(6)__ energy.

$$E + S \xleftrightarrow{K_1/K_{-1}} ES \xrightarrow{K_2} E + P$$

The reaction therefore can occur under mild conditions (e.g. temperature and pressure) with sufficient efficiency. Based on Michaelis-Menten model, the reaction rate is related to the concentration of ES complex as the following __(7)__ kinetics.

$$V = -\frac{d[S]}{dt} = \frac{d[P]}{dt} = K_2[ES]$$

In the __(8)__ state, the concentration of ES complex can be solved as follows.

$$\frac{d[ES]}{dt} = 0$$

$$K_1[E][S] = (K_{-1} + K_2)[ES]$$

$$\frac{[ES]}{[E]} = \frac{K_1[S]}{K_{-1} + K_2} = \frac{[S]}{K_M}$$

$$\frac{[ES]}{[E]+[ES]} = \frac{[ES]}{[E]_t} = \frac{[S]}{K_M + [S]}$$

$$[ES] = \frac{[S]}{K_M + [S]}[E]_t$$

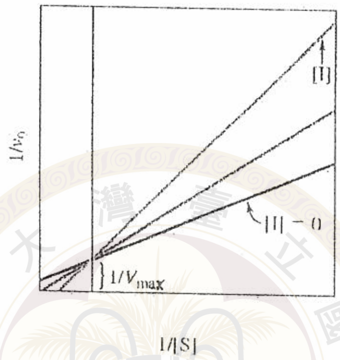
An enzymatic reaction rate therefore increases non-linearly with the substrate concentration and reaches a maximum as the substrate concentration being much higher than __(9)__.

$$V = K_2[ES] = K_2[E]_t \frac{[S]}{K_M + [S]} = V_{\max} \frac{[S]}{K_M + [S]}$$

Since the kinetics is not linear, it will be more convenient to analyze the parameters by plotting the ___(10)___ values of the reaction rate against those of the substrate concentration. The ___(11)___ of the plot will be proportional to the Michaelis constant.

$$\frac{1}{V}=\frac{1}{V_{\max}}\left(\frac{K_M+[S]}{[S]}\right)=\frac{1}{V_{\max}}+\left(\frac{K_M}{V_{\max}}\right)\frac{1}{[S]}$$

The following plots show the existence of ___(12)___ inhibitors which will alter the apparent K_M of the enzyme kinetics. The higher the inhibitor concentration, the ___(13)___ the apparent K_M.



According to Arrehenius expression, the rate constant (k_2) of an enzyme will be affected by the temperature (T) and the activation energy (Ea). The reaction for the substrate possesses an Ea___(14)___ than other chemicals, which is the origin of the substrate ___(15)___ of the enzyme.

$$k_2 = Ae^{-Ea/RT}$$

二、 **簡答(20%)：每題 10 分，請列出算式，中文可**

For lysine oxidase, chemicals with similar structures such as ornithine will also be oxidized but with much slower speed.

$$\text{lysine} + O_2 + H_2O \xrightarrow{\text{lysine oxidase}} H_2O_2 + NH_3 + \text{an aldehyde}$$
$$k_2 = Ae^{-Ea/RT}$$
$$\text{ornithine} + O_2 + H_2O \xrightarrow{\text{lysine oxidase}} H_2O_2 + NH_3 + \text{an aldehyde}$$
$$k_2' = Ae^{-Ea'/RT}$$

At 27°C, the rate constant for oxidizing ornithine (k_2') is 10% of that for lysine (k_2).

1. Calculate the difference in activation energy ($\Delta Ea = Ea' - Ea$) for the two reactions (R= 8.3 JK^{-1}, $0^{o}K=-273^{o}C$, ln 0.1 = -2.3).
2. Suggest a method to reduce the side reaction of ornithine to 8% of lysine.

三、簡答(35%)：每題 7 分，請列出算式，中文可

Assuming Avogadro number to be 10^{24}……

1. Calculate the average distance (in Å) between two adjacent enzyme molecules in a 1μM enzyme solution.

Based on Einstein's diffusion theory, the mean traveling distance of a particles in Brownian motion is a function of the square root of time.

$$\overline{\Delta x} = \sqrt{2Dt}$$

If the diffusion constant of the substrate molecules is approximately 10^{-6} cm^2/S, ……

2. Calculate the average time required for a substrate to reach an enzyme molecule in a 1μM enzyme solution.

Since enzyme is expensive and will not be consumed in the reaction, immobilized enzyme strategies are frequently adopted for bio-industrial applications. The enzymes are to be immobilized onto/within insoluble particles with diameter larger than 100μm.

3. Describe three possible manners for the immobilization procedures.
4. Calculate the average time required for a substrate to reach an enzyme molecule embedded in the center of the insoluble particle of 100μm.
5. Describe possible influence of the diffusion constrain on enzyme kinetics.