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AUSTRALIAN CHEMISTRY OLYMPIAD

FINAL EXAMINATION — PART B

2000

Please note that this answer book will be photocopied when returned and then split so that answers are sent to the appropriate markers. For this reason it is extremely important that you observe instructions 6 to 8.

Instructions to candidate

(1) You are allowed **10 minutes** to read this paper, and **3 hours** to complete the questions.

(2) You are **not** permitted to refer to books, notes or periodic tables but you may use a non programmable electronic calculator.

(3) All questions to be attempted. A guide for time allocation is supplied at the beginning of each question.

(4) Data is supplied, where necessary, with each question.

(5) Answers **must** provide **clearly laid out working** and **sufficient explanation** to show how you reached your conclusions.

(6) Answers must be written in the blank space provided immediately below each question in the exam booklet. Rough working must be on the backs of pages. Only material presented in the answer boxes will be assessed.

(7) Ensure that your name is written in the appropriate place on **each page** of your examination booklet.

(8) Use **only black** or **blue pen** for your written answers, **pencil or other coloured pens are not acceptable.**

Question 1 (45 minutes)

In 1913, Leonor Michaelis and Maud Menten proposed a simple model to account for the kinetics of many enzyme-catalysed reactions. In their mechanism, an enzyme E binds with rate constant k_1 to its substrate S to form an enzyme-substrate complex. This complex can then either dissociate with rate constant k_{-1} to form E and S, or form the product P and the original enzyme E with rate constant k_2, as shown below:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

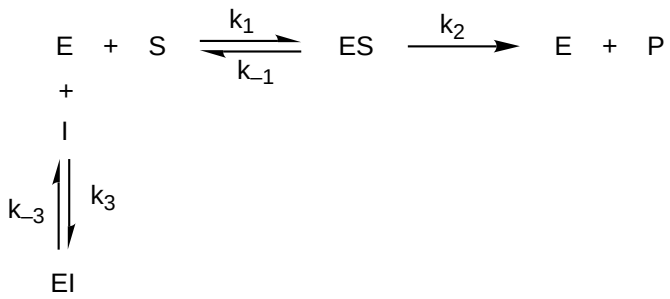
a) (i) Derive the rate law for this process by considering the velocity of the reaction, v, defined as the rate of production of the product, P. Use the Steady State Approximation in your answer.

(ii) Using the fact that the total enzyme concentration, $[E_T]$ is equal to the concentration of uncombined enzyme, [E], plus the concentration of enzyme-substrate complex, [ES], give your expression for [ES] in part (i) in terms of $[E_T]$ instead of [E]. This is more useful, as we usually know the initial amount of enzyme present, $[E_T]$.

(iii) Use the simplifications $v_{max} = k_2[E_T]$ and $K_m = (k_{-1} + k_2)/k_1$ to obtain v in terms of v_{max}, [S] and K_m. This is known as the Michaelis-Menten equation.

(iv) When $k_{-1} >> k_2$, what does K_m represent? What kinetics does this mechanism obey? (Hint: consider behaviour at both high and low substrate concentrations relative to K_m)

Competitive inhibition arises when an inhibitor, I, can bind with the enzyme E, forming an inactive enzyme-inhibitor complex. The reaction scheme is as follows:



(v) Take the same approach as in the case without inhibition (parts (i) to (iii)) to obtain a modified Michaelis-Menten equation. Use $K_i = k_{-3}/k_3$, and verify that this reduces to the Michaelis-Menten equation in the absence of inhibitor. Can v approach v_{max} even in the presence of this kind of inhibitor? If $K_m = K_i$, i.e. the enzyme has equal affinity for both substrate and inhibitor, what does this modified equation simplify to?

(vi) If a substrate concentration of 200 μM gives a rate of half v_{max} in the absence of a competitive inhibitor, calculate the concentration of inhibitor needed to reduce the rate of reaction to 5% of the uninhibited rate when the substrate concentration is 100 μM, assuming $K_m = K_i$.

b) In a method proposed to purify solid nickel, an impure sample of the metal was reacted with carbon monoxide gas at 298 K, where only the metal (and not the impurities) reacts according to the equilibrium reaction:

$$Ni\ (s) + 4CO\ (g) \rightleftharpoons Ni(CO)_4\ (g)$$

According to this proposal, at 298 K, the equilibrium should favour the product, which would be transported to another location where the temperature is 500 K. This is supposed to favour the reverse reaction, thereby producing pure Ni (s).

Data:

Substance	$\Delta_f H°_{298}$ (kJ mol^{-1})	$S°_{298}$ (J K^{-1} mol^{-1})
Ni (s)		29.9
CO (g)	–110.5	197.6
$Ni(CO_4)$ (g)	–602.9	410.6

$R = 8.314$ J K^{-1} mol^{-1}.

(i) Calculate the standard reaction Gibbs free energy at 298 K.

(ii) Calculate the equilibrium constant at 298 K.

(iii) Calculate the equilibrium constant at 500 K using the Van't Hoff equation:

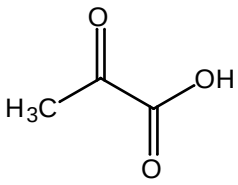
$$\ln\left(\frac{K_2}{K_1}\right) = -\left(\frac{\Delta_r H°}{R}\right)\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

(iv) Calculate the standard reaction Gibbs free energy at 500 K.

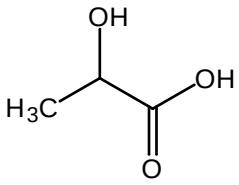
(v) Do your results support the above process? Explain your answer.

Question 2 (45 Minutes)

Pyruvic acid (**1**) and lactic acid (**2**) are biologically important acids.



(**1**)

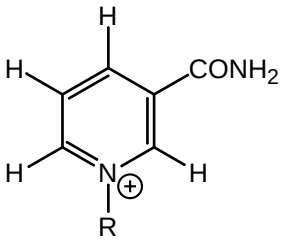


(**2**)

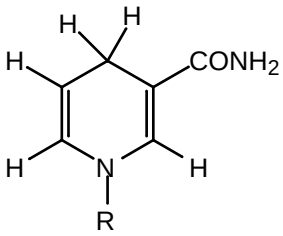
Under suitable conditions, the enzyme *lactate dehydrogenase* can interconvert pyruvate and lactate. It is often necessary to know the concentrations of both pyruvate and lactate in biological samples.

Data:

pK_a (pyruvic acid)	=	2.5
pK_a (lactic acid)	=	3.9
pK_a (ChH^{2+})	=	7.1
FW (ChCl)	=	138.5 g/mol



NAD^+



NADH

a) To analyse the amount of pyruvate and lactate in a suitably prepared solution of a cellular extract, the redox reactions between the following couples are used. (The redox potentials given are for reference biological conditions; ie, 298K and pH 7.0)

(**1**) pyruvate ⇌ lactate $E^{\circ\prime}$ = –0.19 V

(**2**) NAD^+ ⇌ NADH $E^{\circ\prime}$ = –0.32 V

Pyruvate and lactate are analysed independently; in each case, the method relies on the determination of the NADH concentration, obtained by measuring its absorption in UV light.

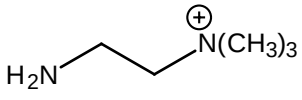
(i) Give balanced redox half-equations for reactions (**1**) and (**2**), indicating which species are being oxidised and which are being reduced (for the direction written).

(ii) Give the overall redox equation for the reaction between the two couples at biological reference conditions.

(iii) Calculate the values of $E^{\circ\prime}$ and K' (the biological standard cell potential and the corresponding equilibrium constant) for this reaction. Include in your answer the expression that gives K' in terms of the concentrations of the species present.

(iv) Give the expression for, and the value of, the true equilibrium constant, K.

b) The pyruvate present in a sample is analysed at pH 7.0 in a 0.05 M solution of cholamine (**3**). Cholamine is a base, and can accept a proton on the amine nitrogen. The acid form is often denoted ChH^{2+}, while the base form is given the notation Ch^+.



(**3**)

(i) Describe how you would prepare 500 mL of a 0.050 M pH 7.0 cholamine buffer using solid cholamine chloride (ChCl) and a 1.0 M HCl solution. (You can, of course, use water to dilute your buffer as necessary).

(ii) Explain why cholamine is a suitable choice for the preparation of a pH 7.0 buffer.

(iii) A large excess of NADH (relative to the pyruvate) is added to the buffered pyruvate sample, and then a catalytic amount of *lactate dehydrogenase*. When the mixture reaches equilibrium, the decrease in [NADH] is used to determine the initial concentration of pyruvate. Typical initial conditions for this analysis are:

$[\text{pyruvate}]_{init}$ ~ 2×10^{-5} M
$[\text{lactate}]_{init}$ ~ 3×10^{-4} M
$[\text{NADH}]_{init}$ ~ 2.5×10^{-4} M
$[\text{NAD}^+]_{init}$ = 0.0 M

Show that, under these conditions, and at pH 7.0, the decrease in the concentration of NADH by the time equilibrium is reached allows the initial concentration of pyruvate to be quantitatively measured (ie, to less than 1% error).

c) Finally, the lactate analysis is carried out in a pH 9.5 buffer, and with a large excess of NAD^+ (relative to the lactate). Again, a catalytic amount of *lactate dehydrogenase* is added. When equilibrium is reached, the concentration of NADH is measured and used to calculate the initial concentration of lactate.

(i) Write the balanced redox equation for the direction used in this lactate analysis.

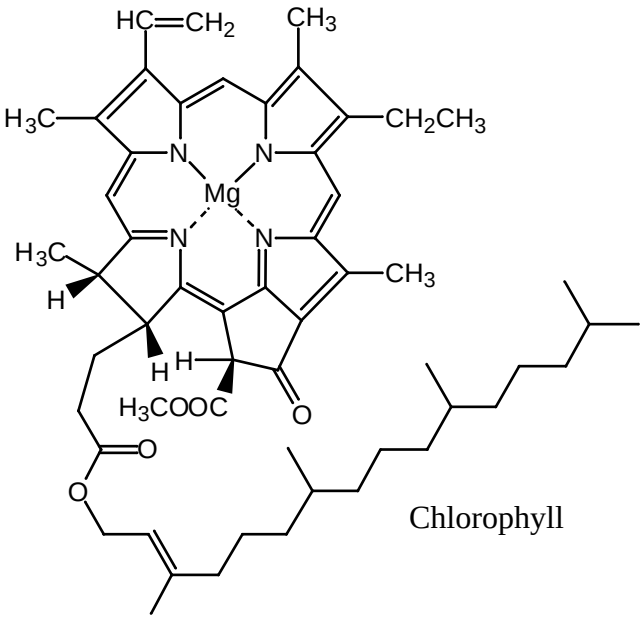
(ii) Calculate the equilibrium constants $K'_{9.5}$ and K'_7 at pH 9.5 and pH 7.0 respectively for this redox reaction.

(iii) Compare your values for $K'_{9.5}$ and K'_7 calculated in (ii) and explain why the lactate analysis is carried out at basic pH.

(iv) A student is given a sample to analyse that contains pyruvate at a concentration of around 2×10^{-5} M, and a lactate concentration of around 3×10^{-4} M (and no NADH). What approximate concentration of NAD^+ would they need in the solution so that the quantity of NADH produced was a quantitative indicator of the original lactate concentration? (Quantitative in this sense means the error is less than 1%).

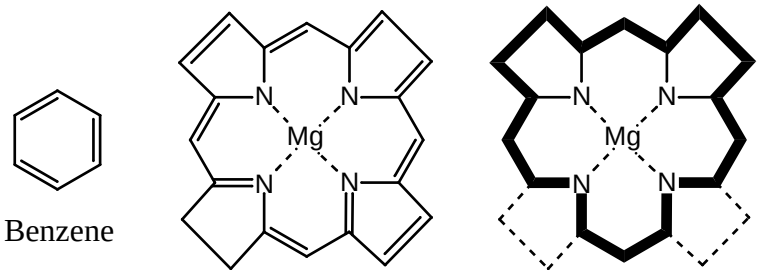
Question 3 (20 minutes)

Benzene is an aromatic ring system that approximates Hückel's rule of being a closed, cyclic, coplanar system with $(4n + 2; n = 0, 1, 2, 3\ldots)$ π electrons. Chlorophyll is also aromatic. There is some controversy over how to count the number of π electrons, however the total number is generally agreed to be either 18 or 22; either of which make the system aromatic.



Chlorophyll

Benzene and the core structure of chlorophyll are two planar species which approximate a circular ring structure: a hexagon (6) for benzene and a dodecagon (12) for chlorophyll. Each sp^2 hybridized carbon and two of the nitrogen atoms supply one π electron to the rings in these systems. Thus in benzene there are 6 π-electrons, whereas in chlorophyll there are 18 (or 22) π-electrons. For the purposes of this question, assume that there are 18 aromatic π electrons in chlorophyll which pass through the aza nitrogens but leave out the pyrrole-type nitrogens of the molecular core. Sigma (σ) electrons are in the plane of the molecule and π-electrons are perpendicular to the plane of the molecule.



Benzene

18 π system

Chlorophyll core

The energy of the molecular orbitals (MOs) of an electron confined to a ring of radius r is given by:

$$E_n = \frac{\mathrm{h}^2 l^2}{2mr^2} \qquad l = 0, \pm1, \pm2, \pm3\ldots$$

where $\mathrm{h} = \frac{h}{2\pi}$ (h = Planck's constant; 1 x 10^{-34} J sec), m is the mass of the electron, and l is the rotational quantum number of the electron (the equivalent of m_l in an atom). As an approximation, the C—C bond distance can be assumed to be 1.50 x 10^{-10} m.

a) Why does the magnesium not supply π-valence electrons to the chlorophyll ring?

b) What is the radius r_b of the benzene ring and r_c, that of the chlorophyll ring?

c) Find an expression for the energy of the highest occupied molecular orbital (HOMO) of each ring in terms of h, m, and r_b (or r_c). Similarly, find the expression for the energy of the lowest unoccupied molecular orbital (LUMO).

d) Find an expression in the same terms for the lowest (i.e. first) absorption band of each ring. You do NOT need to obtain numerical values for predicted absorptions. The experimental absorptions occur at 300 nm for benzene and at 600 nm for chlorophyll. Suggest an improvement which will bring the theoretical and experimental data (i.e. concerning relative absorptions) more into agreement. (**Hint**: Consider electron path).

e) Would you expect chlorophyll to be diamagnetic or paramagnetic? Explain in terms of the total spin of the π-system.

Question 4 (25 minutes)

Nitrogen forms a number of oxides. One of the important oxides of nitrogen is NO_2, a red-brown coloured reactive gas.

a) Draw the Lewis structure of NO_2 and predict its shape using valence shell electron pair repulsion (VSEPR) theory. Would the species NO_2^-, NO_2^+ and the related oxide of nitrogen N_2O be expected to have a similar geometry to that of NO_2? Explain.

b) An important commercial use of NO_2 is in the production of nitric acid *via* the Ostwald process. The process has three steps: the catalytic air oxidation of ammonia to nitric oxide and water; the air oxidation of nitric oxide to NO_2; and finally the reaction of NO_2 with water to give nitric acid and nitric oxide.

(i) Write a balanced equation for each of these three steps.

(ii) Why is a catalyst required in the first step of the process?

(iii) What type of reaction best describes the final step of the Ostwald process? Explain.

c) (i) NO_2 reacts with nitric oxide at low temperatures to produce an unstable oxide of nitrogen, N_2O_3. Sketch the molecular shape of N_2O_3.

(ii) When the same mixture of NO_2 and NO is passed through a solution of dilute aqueous potassium hydroxide, KNO_2 is produced. The latter further reacts with potassium bisulfite in water to give $K_3[N(SO_3)_3].2H_2O$. Write a balanced equation for the latter two reactions. [Note a second product is produced in each of these reactions.]

(iii) The nitrilosulfonate ion, $N(SO_3)_3^{3-}$, has a trigonal planar geometry rather than the more usual trigonal pyramidal geometry associated with, for example, NH_3. Give a rationale for this difference in molecular shape. Would you expect the phosphorus and arsenic analogues of the nitrilosulfonate ion to have a similar geometry?

(iv) The nitrilosulfonate ion, $N(SO_3)_3^{3-}$, readily hydrolyses in acidic or neutral solutions to give the imidosulfonate ion, $HN(SO_3)_2^{2-}$, and the pyrosulfate ion, $S_2O_7^{2-}$. The latter slowly hydrolyses further to the bisulfate ion. Write a balanced equation for these two reactions and sketch the geometries of the three products and the aforementioned bisulfite ion.

Question 5 (45 Minutes)

Ambucaine ($C_{17}H_{28}N_2O_3$) is a local anaesthetic derived from benzoic acid. A close relative of ambucaine, compound **H**, of synthetic interest itself, may be made from methyl benzoate. The synthesis starts with the reaction of methyl benzoate with a mixture of nitric and sulfuric acids, followed by saponification. After neutralisation of the sodium salt thus obtained, a compound **A** ($C_7H_5NO_4$) is isolated, which is submitted to reaction with hydrogen in the presence of palladium. The reduced product, **B**, is allowed to react with nitrous acid, followed by heating in water, to give **C** ($C_7H_6O_3$). Reaction of **C** with a nitric and sulfuric acid mixture affords compound **D**, 5-hydroxy-2-nitrobenzoic acid.

(i) Depict the reaction path from methyl benzoate to compound **D**.

(ii) Draw the structures of compounds **A** to **D**.

(iii) Give the other minor product(s) that may be formed during the final step, briefly explaining your choice.

Heating of **D** with ethanol in the presence of hydrochloric acid gives **E** ($C_9H_9NO_5$). **E** is treated with potassium hydroxide and *n*-butyl bromide and **F** ($C_{13}H_{17}NO_5$) is isolated. Subsequent saponification of **F** with KOH and reaction with thionyl chloride and finally with *N,N*-diethyl-2-aminoethanol affords **G** ($C_{17}H_{26}N_2O_5$). Compound **H** is obtained by catalytic hydrogenation of **G**.

(iv) Depict the reaction path from compound **D** to compound **H** ($C_{17}H_{28}N_2O_3$).

(v) Write the structures for compounds **E** to **H**.

(vi) Draw the product that would be obtained if *(R)*-2-bromobutane was used in place of *n*-butyl bromide in the reaction with compound **E**.

Thionyl chloride has molecular formula, $SOCl_2$.

(vii) Draw the Lewis stucture for thionyl chloride and indicate the electrophilic and nucleophilic sites on this molecule.

(viii) Suggest a mechanism for the above reaction with thionyl chloride, which results in the formation of an acid chloride.