Chemistry Units 3 & 4

Chapter 12 — Medicinal chemistry

Contents

Chapter 12 Medicinal chemistry — 12A Organic compounds as medicines

Theory

Most medicines are **organic molecules**. Some are made entirely in a laboratory, but many begin as compounds produced by living plants, from which a single **active ingredient** must be separated before it can be studied or used. Whether a molecule is extracted or synthesised, its behaviour in the body — how well it dissolves, how it is carried in the blood, and how tightly it binds to its biological target — is decided by two things: the **functional groups** it carries (the reactive groups met in Chapter 8) and its three-dimensional **shape**. This subtopic looks at how a plant compound is obtained and purified, how its functional groups are recognised and linked to its properties, and how the shape feature known as a **chiral centre** governs the way a drug interacts with the body.

Extraction of natural plant compounds — solvent extraction. A useful compound is usually present in plant tissue as part of a complex mixture. **Solvent extraction** separates it out by exploiting **differing solubility**: a solvent is chosen in which the target compound is much more soluble than the unwanted plant material. The guiding rule is *like dissolves like* — a **polar** target (one carrying groups such as –OH or –COOH that can hydrogen-bond) dissolves best in a **polar** solvent such as water or ethanol, whereas a **non-polar** target (a large hydrocarbon or aromatic molecule) dissolves best in a **non-polar** solvent. The crushed plant material is stirred with the chosen solvent so that the target dissolves; the solid residue is then filtered off, and evaporating the solvent leaves the compound behind. Choosing the right solvent is therefore a matter of matching its polarity to that of the compound being sought.

Purification by distillation. Distillation separates and purifies **volatile** components by differences in boiling point.

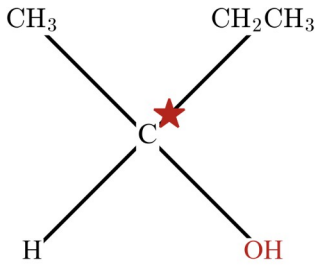
- In **fractional distillation**, a mixture of liquids is heated and passed up a fractionating column; the component with the **lower boiling point** vaporises and distils over first, and is collected separately from the higher-boiling components. This is used to separate two or more volatile liquids from each other.
- In **steam distillation**, steam is passed through the plant material. A volatile compound that does not mix with water co-distils **with** the steam and boils off at a temperature **below its own normal boiling point**. The vapours are condensed, giving two immiscible layers — water and the compound — which are then separated. Because the compound never has to be heated to its own (often high) boiling point, steam distillation is well suited to **heat-sensitive** plant oils that would decompose if distilled directly.

Identifying functional groups in a medicine. Recognising a drug's functional groups is the same skill practised in Chapter 8, now applied to larger molecules: locate each reactive group and name its family. Common groups in medicines include the **hydroxyl** group –OH (alcohols and phenols), the **carboxyl** group –COOH (carboxylic acids), the **amine** group –NH_2, the **carbonyl** group $C=O$ (aldehydes and ketones), the **ester** linkage –COO–, the **amide** linkage –CONH– (–$CONH_2$ in a primary amide), the **benzene ring**, and carbon–halogen bonds.

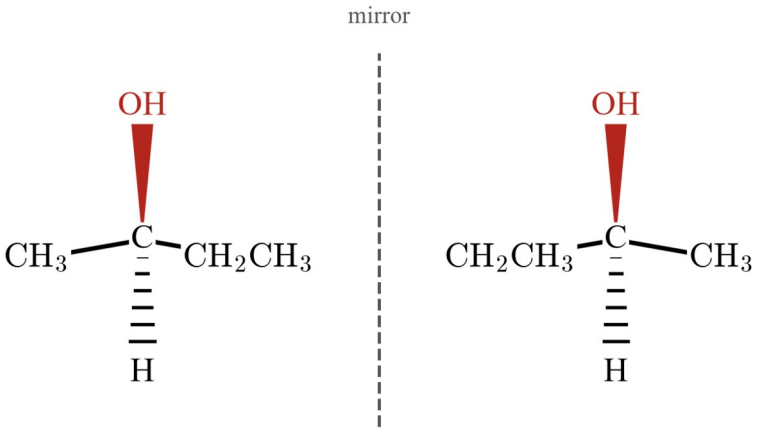
The groups present control the molecule's **polarity** and therefore its **solubility**. Polar groups able to **hydrogen-bond** with water — –OH, –COOH, –NH_2 and $C=O$ — raise a molecule's **water solubility**, and groups such as –COOH (acidic) or –NH_2 (basic) can also **ionise** at body pH, raising it further. In contrast, large **non-polar** regions such as a benzene ring or a long hydrocarbon chain cannot hydrogen-bond with water and instead lower water solubility while raising solubility in non-polar (lipid) surroundings. A drug's overall solubility reflects the balance between its polar and non-polar parts, and this balance affects how the drug is absorbed and transported.

Isomers and the effectiveness of medicines. Isomers are molecules with the **same molecular formula** but a different arrangement of atoms. Because a molecule's biological effect depends on its precise shape and on which groups are exposed, isomers of one another can behave very differently in the body — a small structural change can strengthen, weaken or abolish the effect. **Structural isomers** differ in the way the atoms are connected; **stereoisomers** have the same connections but a different arrangement in space. The stereoisomers most important in medicinal chemistry arise at a **chiral centre**.

Chiral centres. A **chiral centre** is a carbon atom bonded to **four different groups**. Such a carbon has no plane of symmetry, and as a result the molecule can exist in two forms that are **non-superimposable mirror images** of one another, called **enantiomers** — related to each other as a left hand is to a right hand.



a chiral centre: one carbon bonded to four different groups



enantiomer 1 enantiomer 2

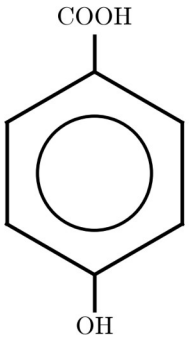
non-superimposable mirror images

To decide whether a particular carbon is a chiral centre, list the four groups attached to it and check that **all four are different**. A carbon carrying two or more identical groups — for example a $-CH_2-$ group (two hydrogens) or a carbon bearing two $-CH_3$ groups — is **not** a chiral centre; nor is a carbon that is part of a $C=O$ or $C=C$ double bond, since it is not bonded to four separate groups. Counting the chiral centres in a structure is done one carbon at a time.

Why chirality matters in the body. The targets a drug acts on — enzymes and receptors — are built from amino acids and are themselves **chiral**. A chiral target can tell two enantiomers apart in the same way that a right hand fits only a right-handed glove. Consequently the two enantiomers of a chiral drug can have **different biological (physiological) effects**: commonly one enantiomer is **active** (it produces the intended therapeutic effect) while the other is **inactive**, or is less active, or in some cases produces an unwanted or harmful effect. This is why the chirality of a medicine, and the number of chiral centres it contains, are important features to identify.

Worked examples

Worked Example 1 — The molecule shown below is a candidate medicine isolated from a plant: a benzene ring carrying a –COOH group and an –OH group. (i) Identify its functional groups. (ii) Give its molecular formula. (iii) State one feature that raises, and one that lowers, its solubility in water.



Working	Comment
The six-membered aromatic ring is a **benzene ring**.	A recognisable Chapter-8 group.
One substituent is –COOH (a carbonyl and a hydroxyl on the same carbon): a **carboxyl** group.	The molecule is a carboxylic acid.
The other substituent is –OH bonded directly to the ring: a **hydroxyl** group (a phenol).	A second polar group.
Counting atoms (ring C_6H_4, plus –OH and –COOH): $C_7H_6O_3$.	Molecular formula.
The polar –OH and –COOH groups **hydrogen-bond** with water → **raises** water solubility (and –COOH can ionise).	Polar, H-bonding groups increase solubility.
The **non-polar benzene ring** cannot hydrogen-bond with water → **lowers** water solubility.	The overall solubility is a balance of the two.

Worked Example 2 — For butan-2-ol, $CH_3CH(OH)CH_2CH_3$: (i) identify any chiral centre; (ii) explain how you decided; (iii) state how many enantiomers the molecule has.

Working	Comment
Number the chain C1–C4 and examine the four groups on each carbon.	A chiral centre carries four **different** groups.
C1 and C4 are $-CH_3$ groups (three H's each); C3 is $-CH_2-$ (two H's).	A repeated group rules these out.
C2 is bonded to $-H$, $-OH$, $-CH_3$ and $-CH_2CH_3$ — **four different groups**.	C2 is the **chiral centre** — the starred carbon in the chiral-centre figure in the Theory section.
All four groups differ, so C2 has no plane of symmetry.	The test is passed only at C2.
One chiral centre ⇒ the molecule exists as **two enantiomers**.	Non-superimposable mirror images.

Worked Example 3 — Crushed plant leaves contain both a fragrant, water-insoluble volatile oil and a bitter, water-soluble compound. Describe, with the principle each relies on, how (i) steam distillation and (ii) solvent extraction could each be used to isolate one of these substances.

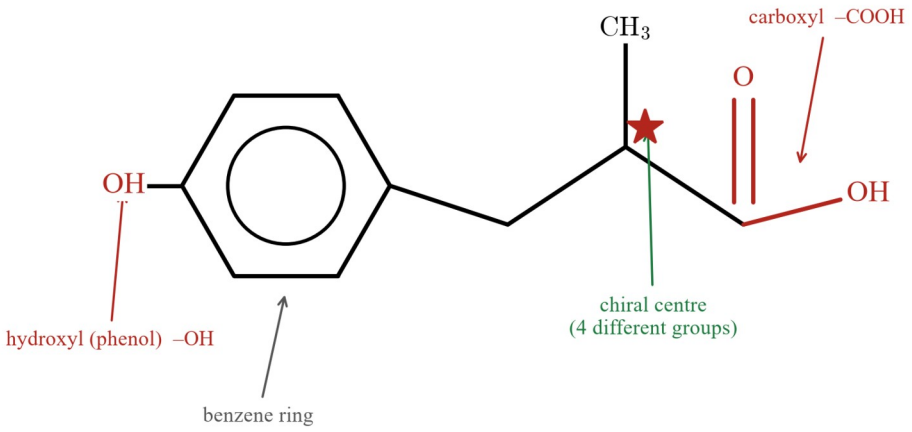
(i) The fragrant oil is **volatile** and does **not mix with water**, so it is suited to **steam distillation**. Steam is passed through the leaves; the oil co-distils with the steam and boils off at a temperature **below its own normal boiling point**. The mixed vapours are condensed to give two immiscible layers — water and the oil — which are separated. The principle is that a volatile, water-immiscible compound distils together with steam at a reduced temperature, which also **protects a heat-sensitive oil** from decomposing.

(ii) The bitter compound is **water-soluble**, so it is suited to **solvent extraction**. The leaves are stirred with a solvent (such as water or ethanol) in which this compound is far more soluble than the rest of the plant

material; the compound dissolves, the solid residue is filtered off, and the solvent is evaporated to recover the compound. The principle is **differing solubility** — *like dissolves like* — so the solvent's polarity is matched to that of the target.

∴ steam distillation isolates the volatile water-immiscible oil by co-distillation with steam, while solvent extraction isolates the water-soluble compound by exploiting its greater solubility in an appropriately chosen solvent.

Worked Example 4 — The structure below is a candidate anti-inflammatory drug extracted from the bark of a shrub, $HOC_6H_4CH_2CH(CH_3)COOH$. (i) Identify its functional groups. (ii) Predict its solubility in water, justifying your answer from the groups present. (iii) Identify and count its chiral centre(s). (iv) Explain why its two enantiomers might have different effects in the body.



(i) The molecule contains a **benzene ring**, a **hydroxyl** group –OH bonded to the ring (a phenol), and a terminal **carboxyl** group –COOH (a carboxylic acid). Counting the atoms gives the molecular formula $C_{10}H_{12}O_3$.

(ii) The –OH and –COOH groups are polar and can **hydrogen-bond** with water (and the –COOH can ionise), which promotes water solubility; but the large **non-polar** benzene ring and hydrocarbon chain oppose it. The molecule is therefore expected to be only **sparingly soluble** in water, and more soluble in non-polar solvents — the outcome of the balance between its polar and non-polar regions.

(iii) Examine each carbon. The $-CH_2-$ carbon carries two hydrogens, so it is not a chiral centre. The carbon bearing the $-CH_3$ branch (starred) is bonded to **four different groups**: $-H$, $-CH_3$, $-COOH$ and the $-CH_2C_6H_4OH$ group. This carbon is a **chiral centre**, and it is the only one — the molecule has **one chiral centre** and so exists as **two enantiomers**.

(iv) The enzymes and receptors the drug acts on are themselves chiral, so they can distinguish the two non-superimposable mirror images. Typically only one enantiomer has the shape that fits the target and produces the therapeutic effect; the other may be inactive or may cause an unwanted effect.

∴ the compound is $C_{10}H_{12}O_3$ with benzene-ring, hydroxyl and carboxyl groups, is only sparingly water-soluble, and — having one chiral centre — exists as two enantiomers whose biological effects may differ.

Practice questions

Question 1 — A medicine consists of a benzene ring bearing an –OH group and a $-CH_2CONH_2$ group, giving the semi-structural formula $HOC_6H_4CH_2CONH_2$.

a. Identify the functional groups present.
b. State which of these groups are able to hydrogen-bond with water.
c. Predict whether the molecule is more soluble in water or in a non-polar solvent, and give a reason.

Question 2 — Consider 2-chlorobutane, $CH_3CHClCH_2CH_3$.

a. State what is meant by a chiral centre.
b. Identify the chiral centre in 2-chlorobutane by giving the four groups bonded to it.
c. State the number of enantiomers this molecule has.

Question 3 — Two techniques for obtaining plant compounds are solvent extraction and distillation.

a. State the property of the target compound that solvent extraction relies on.
b. Explain why steam distillation is suitable for isolating a heat-sensitive volatile oil from a plant.
c. Explain how fractional distillation could separate two volatile liquids that have different boiling points.

Question 4 — Deciding whether a carbon is a chiral centre.

a. Is the central carbon (C2) of propan-2-ol, $CH_3CH(OH)CH_3$, a chiral centre? Justify your answer.
b. Is C2 of butan-2-ol, $CH_3CH(OH)CH_2CH_3$, a chiral centre? Justify your answer.
c. State the general rule used to decide whether a carbon is a chiral centre.

Question 5 — Chirality and biological activity.

a. Define the term *enantiomers*.
b. Explain why a chiral biological receptor can distinguish between two enantiomers of a drug.
c. State the consequence for the body when a chiral drug is a molecule with one active and one harmful enantiomer.

Question 6 — Consider 2-hydroxypropanoic acid, $CH_3CH(OH)COOH$.

a. Give its molecular formula.
b. Identify its chiral centre by listing the four groups bonded to it.
c. Explain why the carboxyl carbon of this molecule is not a chiral centre.

Question 7 — Identify the functional groups present in the medicine methyl 2-hydroxypropanoate, $CH_3CH(OH)COOCH_3$.

Question 8 — State, with a reason, whether pentan-3-ol, $CH_3CH_2CH(OH)CH_2CH_3$, contains a chiral centre.

Question 9 — Determine the number of chiral centres in 3-chlorobutan-2-ol, $CH_3CH(OH)CHClCH_3$, identifying each one.

Question 10 — A non-polar compound is to be extracted from plant material by solvent extraction. Explain how a suitable solvent is chosen, referring to the principle *like dissolves like*.

Question 11 — A plant compound is a non-volatile crystalline solid that dissolves readily in ethanol. State whether steam distillation is a suitable technique for obtaining it, justifying your answer from the requirements of that technique, and name the technique that should be used instead.

Question 12 — A candidate drug contains a single carboxyl group attached to a large benzene-ring system. Explain, in terms of the groups present, why the molecule is only sparingly soluble in water.

Question 13 — Explain why the two enantiomers of a chiral drug can produce different physiological effects when taken into the body. Your answer should form a clear reasoning chain.

Question 14 — Consider 2-aminopropanoic acid, $CH_3CH(NH_2)COOH$.

a. Identify its functional groups.
b. Identify its chiral centre by listing the four groups bonded to it.
c. Give its molecular formula.

Question 15 — State, with a reason, whether 2-methylbutan-2-ol, $(CH_3)_2C(OH)CH_2CH_3$, contains a chiral centre.

Question 16 — Identify the chiral centre in 3-methylhexane, $CH_3CH_2CH(CH_3)CH_2CH_2CH_3$, and explain why that carbon is a chiral centre even though the molecule contains no functional group.

Question 17 — Distinguish between solvent extraction and distillation as techniques for obtaining a plant compound, and state one situation in which each is the better choice.

Question 18 — A new painkiller is isolated from tree bark; its structure is $HOC_6H_4CH_2CH(CH_3)COOH$ (the molecule of Worked Example 4).

a. Suggest a technique for obtaining the compound from the crushed bark, and state the property it exploits.
b. Identify the functional groups present and predict whether the compound is likely to be very soluble in water.
c. Identify and count the chiral centre(s), and explain why only one enantiomer may be pharmacologically active.

Answers

1 a benzene ring, hydroxyl (–OH, a phenol) and primary amide (–$CONH_2$); b the –OH and the –$CONH_2$ (both can hydrogen-bond with water); c more soluble in water — it carries two polar, hydrogen-bonding groups, though the benzene ring reduces solubility somewhat. **2** a a carbon atom bonded to four different groups; b C2, bonded to $-H$, $-Cl$, $-CH_3$ and $-CH_2CH_3$; c two enantiomers. **3** a differing solubility (the target is much more soluble in the chosen solvent than the rest of the material); b it co-distils with steam below its own boiling point, so the heat-sensitive oil is not decomposed; c the liquid with the lower boiling point vaporises and distils over first, separating it from the higher-boiling liquid. **4** a no — C2 carries two identical $-CH_3$ groups, so it is not bonded to four different groups; b yes — C2 is bonded to four different groups ($-H$, $-OH$, $-CH_3$, $-CH_2CH_3$); c a carbon is a chiral centre if it is bonded to four different groups. **5** a two non-superimposable mirror-image forms of a molecule containing a chiral centre; b the receptor is itself chiral, so only one mirror image fits it (like a hand and a glove); c one enantiomer gives the intended effect while the other causes harm, so the mixture can be both therapeutic and toxic. **6** a $C_3H_6O_3$; b C2, bonded to $-H$, $-OH$, $-CH_3$ and $-COOH$; c it is part of a $C=O$ double bond, so it is attached to only three groups (the doubly bonded O, the –OH and C2) rather than to four different groups. **7** hydroxyl (–OH) and ester (–COO–). **8** no — the carbon bearing the –OH (C3) is bonded to two identical $-CH_2CH_3$ (ethyl) groups, so it is not bonded to four different groups. **9** two chiral centres — C2 ($-H$, $-OH$, $-CH_3$, $-CHClCH_3$) and C3 ($-H$, $-Cl$, $-CH_3$, $-CH(OH)CH_3$). **10** choose a non-polar solvent, because the non-polar target dissolves best in a solvent of similar (non-polar) character (*like dissolves like*), while polar plant material stays behind. **11** no — steam distillation requires the target to be **volatile** (and immiscible with water) so that it can co-distil with the steam; a non-volatile solid will not vaporise with the steam, so nothing would come over. Solvent extraction, using ethanol, should be used instead. **12** the single –COOH group can hydrogen-bond with water, but the large non-polar benzene-ring region dominates and cannot hydrogen-bond, so overall the molecule is only sparingly soluble in water. **13** the drug's biological targets are chiral, so they bind the two mirror-image enantiomers differently; usually only one fits and is active, so the two enantiomers can differ in effect. **14** a amine (–NH_2) and carboxyl (–COOH); b C2, bonded to $-H$, $-NH_2$, $-CH_3$ and $-COOH$; c $C_3H_7NO_2$. **15** no — the carbon bearing the –OH (C2) is bonded to two identical $-CH_3$ groups, so it is not bonded to four different groups (a chiral centre does not require a hydrogen, but it does require all four groups to differ). **16** C3, bonded to $-H$, $-CH_3$ (methyl), $-CH_2CH_3$ (ethyl) and $-CH_2CH_2CH_3$ (propyl) — four different groups; chirality depends only on the four groups being different, not on any functional group being present. **17** solvent extraction dissolves the target in a solvent of matching polarity and leaves other material behind (best when the compound is not volatile, or has a distinctive solubility); distillation separates by boiling point and suits volatile components (steam distillation for heat-sensitive water-immiscible oils, fractional distillation for separating volatile liquids). **18** a solvent extraction — exploiting the compound's much greater solubility in a chosen solvent than that of the rest of the bark (distillation is ruled out, as this hydrogen-bonded solid is not volatile); b benzene ring, hydroxyl (phenol) and carboxyl; not very soluble in water — the polar –OH and –COOH promote solubility but the large non-polar ring/chain limits it; c one chiral centre (the carbon bearing the $-CH_3$ branch); only the enantiomer whose shape fits the chiral biological target is active.

Fully-worked solutions

Question 1 a. The six-membered aromatic ring is a **benzene ring**. The –OH bonded to the ring is a **hydroxyl** group (a phenol). The $-CH_2CONH_2$ group ends in a carbonyl bonded to $-NH_2$, which is a primary **amide** (–$CONH_2$). b. Groups able to hydrogen-bond with water are the polar ones with O–H or N–H bonds: the **–OH** and the **–$CONH_2$**. (The benzene ring cannot hydrogen-bond.) c. The molecule is more soluble in **water**: it carries two polar groups (–OH and –$CONH_2$) that hydrogen-bond with water, and although the benzene ring is a non-polar region that lowers solubility, the two hydrogen-bonding groups make the molecule overall able to dissolve appreciably in water.

Question 2 a. A **chiral centre** is a carbon atom bonded to four different groups. b. Numbering $CH_3CHClCH_2CH_3$, the second carbon (C2) is bonded to $-H$, $-Cl$, $-CH_3$ and $-CH_2CH_3$. All four groups are different, so **C2 is the chiral centre**. c. A molecule with one chiral centre has **two enantiomers** (non-superimposable mirror images).

Question 3 a. Solvent extraction relies on **differing solubility**: the compound is much more soluble in the chosen solvent than the rest of the plant material. b. In steam distillation the volatile oil co-distils with the steam and boils off at a temperature **below its own normal boiling point**. It is therefore never heated to the high temperature at which it

would decompose, so a heat-sensitive oil is recovered undamaged. c. When the mixture is heated, the liquid with the **lower boiling point** vaporises more readily, passes up the fractionating column and distils over first, so it is collected separately from the liquid with the higher boiling point.

Question 4 a. **No.** In propan-2-ol the central carbon (C2) is bonded to $-\mathrm{H}$, $-\mathrm{OH}$ and **two** $-\mathrm{CH_3}$ groups. Because two of the groups are identical, the carbon is not bonded to four different groups and so is not a chiral centre. b. **Yes.** In butan-2-ol, C2 is bonded to $-\mathrm{H}$, $-\mathrm{OH}$, $-\mathrm{CH_3}$ and $-\mathrm{CH_2CH_3}$. All four groups are different, so C2 is a chiral centre. c. A carbon is a chiral centre if, and only if, it is bonded to **four different groups**.

Question 5 a. **Enantiomers** are the two non-superimposable mirror-image forms in which a molecule containing a chiral centre can exist. b. A biological receptor is built from chiral building blocks and so is itself **chiral** (it has a definite handedness). Only one of the two mirror-image enantiomers has the matching shape to fit into the receptor — just as only a right hand fits a right-handed glove — so the receptor binds the two enantiomers differently. c. Because the receptor distinguishes them, one enantiomer produces the intended therapeutic effect while the other produces a harmful effect. Administering the molecule as a mixture of both therefore delivers the harmful enantiomer alongside the active one, which is why the enantiomers must be distinguished.

Question 6 a. Counting the atoms of $\mathrm{CH_3CH(OH)COOH}$: C 3, H 6, O 3 $\rightarrow \mathrm{C_3H_6O_3}$. b. The middle carbon (C2) is bonded to $-\mathrm{H}$, $-\mathrm{OH}$, $-\mathrm{CH_3}$ and $-\mathrm{COOH}$ — four different groups — so **C2 is the chiral centre**. c. The carboxyl carbon of $\mathrm{CH_3CH(OH)COOH}$ is **doubly bonded** to one oxygen and singly bonded to the –OH group and to C2. Because it is part of a $\mathrm{C=O}$ double bond it is attached to only **three** separate groups, not four, so it cannot be a chiral centre. A chiral centre must be a carbon forming **four single bonds** to **four different** groups.

Question 7 The carbon bearing the –OH is a **hydroxyl** group (alcohol). The –COO– linking the two carbon groups is an **ester** linkage. So methyl 2-hydroxypropanoate contains a **hydroxyl** group and an **ester** group.

Question 8 No. Number the five-carbon chain C1–C5; the –OH sits on C3. C3 is bonded to $-\mathrm{H}$, $-\mathrm{OH}$ and a $-\mathrm{CH_2CH_3}$ (ethyl) group **on each side**. The two ethyl groups are identical, so C3 is not bonded to four different groups. (C1 and C5 are $-\mathrm{CH_3}$ groups and C2 and C4 are $-\mathrm{CH_2}-$ groups, each carrying repeated hydrogens, so none of them qualifies either.) Pentan-3-ol therefore has **no chiral centre** — the identical groups need not be as small as methyl groups for the test to fail.

Question 9 Examine each carbon of $\mathrm{CH_3CH(OH)CHClCH_3}$. C1 and C4 are $-\mathrm{CH_3}$ groups (three identical H's), so neither is chiral. C2 is bonded to $-\mathrm{H}$, $-\mathrm{OH}$, $-\mathrm{CH_3}$ and $-\mathrm{CHClCH_3}$ — four different groups → a chiral centre. C3 is bonded to $-\mathrm{H}$, $-\mathrm{Cl}$, $-\mathrm{CH_3}$ and $-\mathrm{CH(OH)CH_3}$ — four different groups → a chiral centre. There are **two chiral centres**, at C2 and C3.

Question 10 Because *like dissolves like*, a **non-polar** compound dissolves best in a **non-polar** solvent, whose molecules interact with it through the same kind of (dispersion) forces. A non-polar solvent is therefore chosen: the target compound dissolves in it, while the more polar plant material remains largely undissolved and can be separated off.

Question 11 No — steam distillation is not suitable here. The technique works only because the target compound is **volatile**: steam passed through the plant material carries the compound's vapour along with it, and the two co-distil below the compound's own boiling point. It further requires the compound to be **immiscible with water**, so that the condensed distillate separates into two layers. A **non-volatile crystalline solid** fails the first requirement — it does not enter the vapour phase alongside the steam — so nothing of it would distil over and the technique would recover none of the compound. The stated high solubility in ethanol points instead to **solvent extraction**: the crushed plant material is stirred with ethanol, in which the compound is far more soluble than the rest of the material; the solid residue is filtered off and the ethanol evaporated to leave the compound behind.

Question 12 The molecule has one polar group, –COOH, which can hydrogen-bond with water and would by itself favour dissolving. However, it is attached to a **large non-polar benzene-ring system**, which cannot hydrogen-bond with water and instead disrupts the hydrogen bonding between water molecules. Because the non-polar region is large relative to the single polar group, the non-polar character dominates, and the molecule is only **sparingly soluble** in water.

Question 13 A chiral drug exists as two enantiomers — non-superimposable mirror images that arise from its chiral centre. The drug acts by binding to a biological target (an enzyme or receptor) that is built from chiral units and is therefore itself chiral, with a definite handedness. Only the enantiomer whose three-dimensional shape **matches** that of the target can bind effectively, while the mirror-image enantiomer fits poorly or not at all. Because binding is what produces the biological response, the two enantiomers therefore produce **different physiological effects** — commonly one is active and the other is inactive or harmful.

Question 14 a. The $-NH_2$ group is an **amine**; the terminal –COOH is a **carboxyl** group (carboxylic acid). b. The central carbon (C2) is bonded to $-H$, $-NH_2$, $-CH_3$ and $-COOH$ — four different groups — so **C2 is the chiral centre**. c. Counting the atoms of $CH_3CH(NH_2)COOH$: C 3, H 7, N 1, O 2 → $C_3H_7NO_2$.

Question 15 No. The carbon bearing the –OH (C2) is bonded to $-OH$, $-CH_2CH_3$ and **two** $-CH_3$ groups. A chiral centre does **not** need to carry a hydrogen atom, but it does need all four attached groups to be **different**. Here two of the groups are identical $-CH_3$ groups, so C2 is not bonded to four different groups and the molecule has no chiral centre.

Question 16 Number the six-carbon chain of $CH_3CH_2CH(CH_3)CH_2CH_2CH_3$; the branch is on C3. C3 is bonded to $-H$, a $-CH_3$ (methyl) branch, a $-CH_2CH_3$ (ethyl) group on one side, and a $-CH_2CH_2CH_3$ (propyl) group on the other side. These four groups are all different, so **C3 is a chiral centre**. Chirality is decided solely by whether the four groups attached to a carbon are different; it does not require any functional group, so a purely hydrocarbon molecule can still have a chiral centre.

Question 17 Solvent extraction dissolves the target compound in a solvent chosen to match its polarity (*like dissolves like*), leaving other plant material behind; it is the better choice when the compound is **not volatile**, or when it has a distinctive solubility that a suitable solvent can exploit. **Distillation** separates components by **boiling point** and so suits **volatile** substances: steam distillation is the better choice for a **heat-sensitive, water-immiscible** oil (it distils below its boiling point), while fractional distillation is better for **separating two or more volatile liquids** of different boiling points.

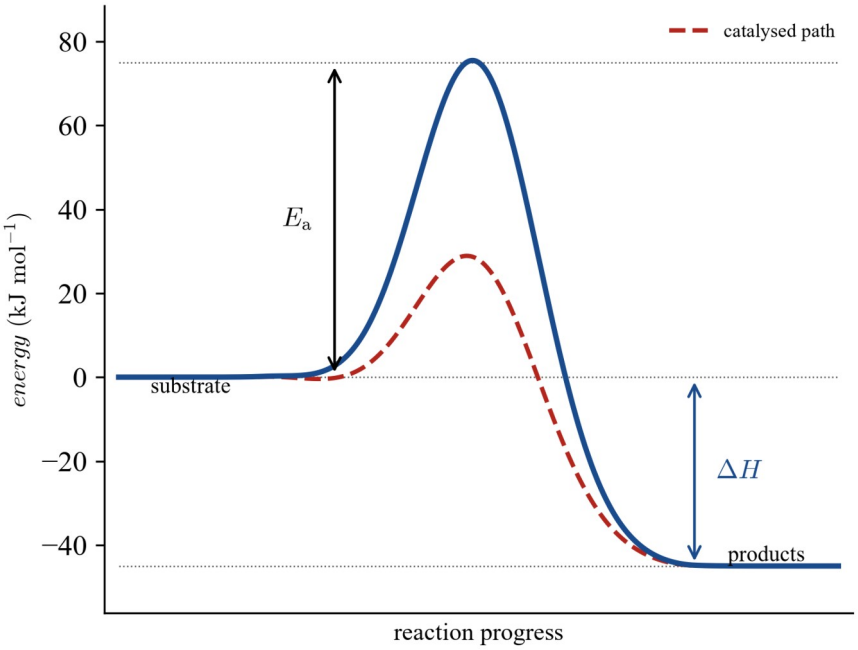
Question 18 a. **Solvent extraction** should be used: the crushed bark is stirred with a solvent in which the compound is much more soluble than the rest of the bark, exploiting **differing solubility** (*like dissolves like*); the residue is then filtered off and the solvent evaporated. Distillation is not an option for this compound — carrying both an –OH and a –COOH group, it is an extensively hydrogen-bonded solid and is **not volatile**, whereas steam distillation requires a volatile, water-immiscible target. b. The molecule contains a **benzene ring**, a **hydroxyl** group (phenol, –OH) and a **carboxyl** group (–COOH). The –OH and –COOH can hydrogen-bond with water and favour dissolving, but the large non-polar benzene ring and hydrocarbon chain oppose it, so the compound is **not very soluble** in water (only sparingly soluble). c. Checking each carbon, the only one bonded to four different groups is the carbon carrying the $-CH_3$ branch, which is bonded to $-H$, $-CH_3$, $-COOH$ and $-CH_2C_6H_4OH$. There is **one chiral centre**, so the molecule has two enantiomers. Only the enantiomer whose shape matches the chiral biological target can bind to it and produce the pharmacological effect, so only that one enantiomer is active.

Chapter 12 Medicinal chemistry — 12B Enzymes

Theory

Living cells carry out thousands of reactions at body temperature and near-neutral pH — conditions far too mild for most of those reactions to proceed at a useful rate on their own. They are made fast enough by **enzymes**: large protein molecules that act as **biological catalysts**. Like any catalyst, an enzyme speeds a reaction up without being used up, by providing an **alternative reaction pathway of lower activation energy** (Chapter 5). It does not change the enthalpy change ΔH of the reaction, nor the position of any equilibrium — it only lets the existing reaction reach equilibrium sooner.

Enzymes lower the activation energy. Only those collisions in which the reacting particles have at least the activation energy E_a can react. By binding the reactants and holding them in a favourable orientation, an enzyme opens a new pathway with a much smaller E_a, so a far greater fraction of collisions is successful and the rate rises steeply. The energy profile below compares the uncatalysed pathway with the enzyme-catalysed one: the peak (the barrier) is lower for the catalysed path, but the substrate and product energy levels — and therefore ΔH — are identical.

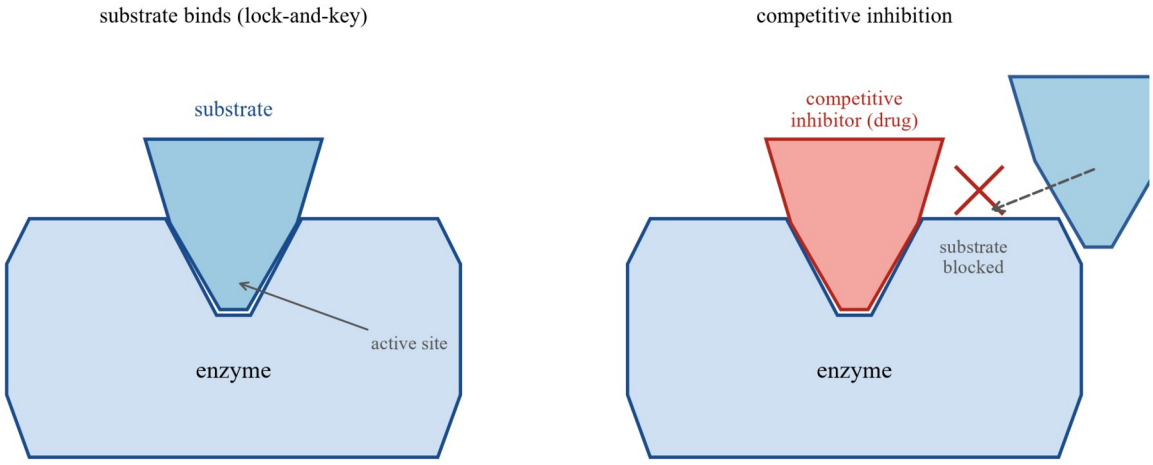


Specificity and the active site. Each enzyme catalyses only one reaction, or one small group of closely related reactions. This **specificity** arises because the reactant — called the **substrate** — must bind to a particular region of the enzyme, the **active site**, whose three-dimensional shape and pattern of charges are complementary to the substrate. Only a molecule of the right shape fits, so the enzyme "recognises" its substrate and ignores others. Because the active site is built from the folded protein chain, its shape depends entirely on how the protein is folded.

The four levels of protein structure. A protein's shape is described at four levels, each held together by particular bonding.

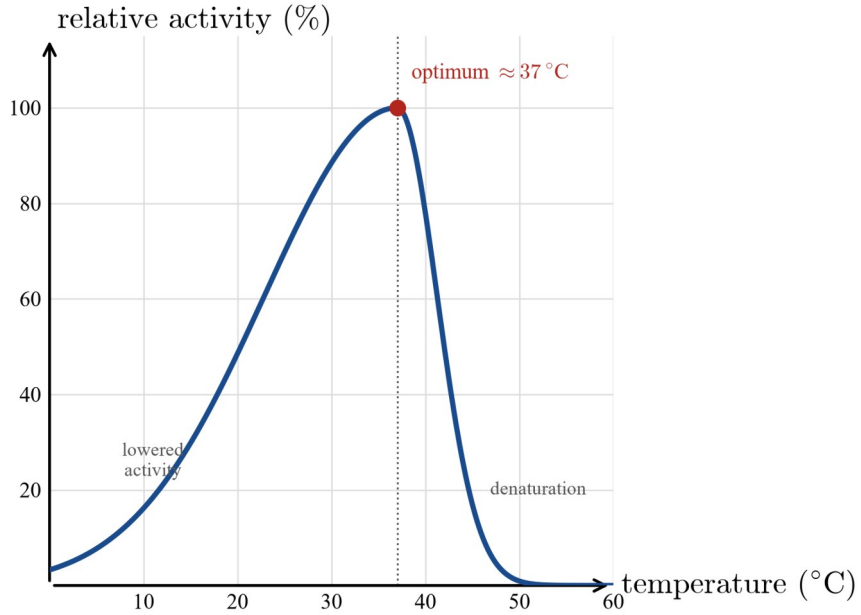
- **Primary structure** — the sequence of amino acids joined in a chain. Neighbouring amino acids are linked by **peptide bonds** (covalent amide bonds formed by condensation). The primary structure is fixed by strong covalent bonds and sets everything that follows.
- **Secondary structure** — regular local folding of the chain into an **α-helix** or a **β-pleated sheet**, held in place by **hydrogen bonds** between the backbone C=O and N–H groups.
- **Tertiary structure** — the overall three-dimensional folding of the whole chain, held by interactions between the amino-acid **R groups**: hydrogen bonds, ionic bonds (between oppositely charged R groups), dispersion forces, and covalent disulfide bridges. The tertiary structure creates the precise shape of the active site.
- **Quaternary structure** — the assembly of two or more folded polypeptide **subunits** into one functional protein, held together by the same kinds of R-group interactions acting between the subunits.

Because the active-site shape is produced by this folding, anything that breaks the bonds holding the fold together will change the active site and alter — or destroy — the enzyme's function.



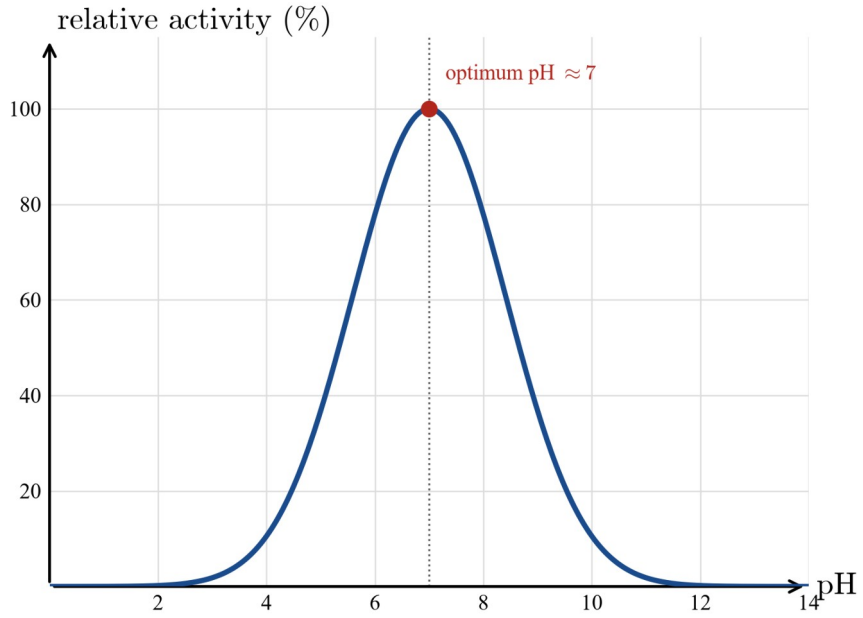
The lock-and-key model. The complementary fit between substrate and active site is often pictured as a key (the substrate) fitting a lock (the active site): only the correctly shaped key fits, and once bound the enzyme–substrate complex reacts and releases the products, leaving the enzyme unchanged (left panel above).

Temperature and enzyme activity. As temperature rises from low values, particles move faster and collide more often and more energetically, so the rate of the catalysed reaction increases — the curve climbs to an **optimum temperature** (near 37 °C for many human enzymes). At **low temperatures** the enzyme is undamaged but sluggish: molecules move slowly and few collisions succeed, so activity is low. This **lowered activity** is fully reversible — warming the enzyme back toward the optimum restores it. Above the optimum, however, the extra thermal energy shakes apart the weak hydrogen bonds and other R-group interactions that hold the tertiary fold, so the protein unravels. This is **denaturation**: the active-site shape is lost, the substrate can no longer bind, and activity falls sharply. Because the fold is destroyed rather than merely slowed, denaturation by heat is essentially **irreversible** — cooling does not restore the original shape.



pH and enzyme activity. Each enzyme also has an **optimum pH** at which its activity is greatest; activity falls away on either side, giving a bell-shaped curve. Many amino-acid R groups (and the chain ends) carry acidic –COOH or basic –NH_2 groups. At a suitable pH an amino acid exists as a **zwitterion** — a single species carrying both a positive (–NH_3^+) and a negative (–COO^-) charge. When the pH is changed, these groups gain or lose H^+, so their charges change: added H^+ at low pH protonates –COO^- groups, while added OH^- at high pH removes H^+ from –NH_3^+ groups.

Altering these charges disrupts the ionic bonds and hydrogen bonds that maintain the fold, so the active site loses its shape — the enzyme is **denatured**. This is why activity is highest only over a narrow pH range around the optimum.



Medicines as competitive enzyme inhibitors. Many medicines work by slowing down a target enzyme. A **competitive inhibitor** is an organic molecule — often a drug — whose shape is similar enough to the substrate that it binds to the enzyme's **active site** by the same **lock-and-key mechanism**. While the inhibitor occupies the active site, the real substrate cannot bind, so fewer enzyme–substrate complexes form and the reaction slows (right panel of the lock-and-key figure). This links directly to 12A: designing a drug that fits a chosen enzyme's active site is one of the main ways a medicine produces its effect.

Worked examples

Worked Example 1 — An uncatalysed biochemical reaction has a forward activation energy of $75\ \text{kJ mol}^{-1}$ and an enthalpy change of $\Delta H = -45\ \text{kJ mol}^{-1}$. In the presence of its enzyme, the same reaction proceeds by a pathway whose forward activation energy is only $28\ \text{kJ mol}^{-1}$. (a) By how much does the enzyme lower the activation energy? (b) State ΔH for the enzyme-catalysed reaction. (c) Determine the activation energy of the reverse reaction for both the uncatalysed and the enzyme-catalysed pathway.

Working	Comment
lowering $= 75 - 28 = 47\ \text{kJ mol}^{-1}$	The enzyme provides an alternative pathway; the forward barrier is lowered by this amount.
$\Delta H = -45\ \text{kJ mol}^{-1}$ (unchanged)	A catalyst lowers E_a only; it does not alter the energy of the substrate or of the products, so ΔH is unchanged.
reverse (uncatalysed): $E_a - \Delta H = 75 - (-45) = 120\ \text{kJ mol}^{-1}$	On the energy profile the reverse barrier is measured from the product level up to the same peak.
reverse (enzyme): $28 - (-45) = 73\ \text{kJ mol}^{-1}$	The enzyme lowers the barrier in *both* directions, so the reverse reaction is also faster.

Worked Example 2 — The graph *enzyme activity vs temperature* above is for an enzyme taken from human cells; its activity at 45 °C is only about 17% of the maximum. (a) State the enzyme's optimum temperature. (b) Explain, in terms of bonding and the active site, why the activity is so low at 45 °C. (c) Explain why the activity is also low at 5 °C, and state how this situation differs from that at 45 °C.

Working	Comment
(a) optimum ≈ 37 °C	Read from the peak of the curve — the temperature at which activity is greatest.
(b) Above the optimum the extra thermal energy breaks	High-temperature loss of activity is caused by

Working	Comment
the hydrogen bonds and other R-group interactions holding the tertiary fold; the protein unravels (**denaturation**) and the active site loses its shape, so the substrate can no longer bind.	denaturation — bonds break and the active-site shape is lost.
At 45 °C this has largely happened, so activity is only ~17% of maximum and the loss is **irreversible**.	Cooling does not refold the protein.
(c) At 5 °C the enzyme is undamaged but the particles move slowly, so collisions are infrequent and low-energy and few substrate molecules bind per second — **lowered activity**.	Low temperature slows the enzyme without breaking bonds.
This is **reversible**: warming back toward 37 °C restores full activity, unlike the irreversible denaturation at 45 °C.	The key contrast: reversible (cold) vs irreversible (heat).

Worked Example 3 — Explain how the four levels of protein structure give rise to the active site of an enzyme, naming the main type of bonding responsible for each level.

The **primary structure** is the sequence of amino acids joined by covalent **peptide bonds**; this sequence fixes all the higher levels. Local stretches of the chain then coil or fold into **secondary structure** — α-helices and β-pleated sheets — held by **hydrogen bonds** between backbone C=O and N–H groups. The whole chain then folds into its **tertiary structure**, the overall three-dimensional shape, held by interactions between the amino-acid **R groups**: hydrogen bonds, ionic bonds, dispersion forces and disulfide bridges. Where the protein is built from more than one polypeptide, these subunits assemble into the **quaternary structure**. It is the tertiary (and where relevant quaternary) folding that brings particular R groups together to form a pocket of a precise shape and charge distribution — the active site. ∴ the active site is a direct consequence of how the primary sequence folds, so the shape needed to bind the substrate exists only while all of these bonds hold the fold in place.

Worked Example 4 — A medicine is described as a competitive enzyme inhibitor. Explain, using the lock-and-key idea, how such a molecule reduces the rate of the reaction catalysed by its target enzyme.

A competitive inhibitor is an organic molecule whose shape is complementary to the enzyme's **active site**, so it binds there by the same **lock-and-key** fit as the substrate. While the inhibitor occupies the active site, the actual **substrate cannot bind**, so the enzyme cannot form the enzyme–substrate complex needed to catalyse the reaction. With a fraction of the enzyme's active sites blocked at any moment, fewer product molecules form per second. ∴ the medicine lowers the enzyme's catalytic rate not by damaging the enzyme, but by competing with the substrate for its active site.

Practice questions

Question 1 — A functional enzyme is built from two folded polypeptide chains that fit together as a single unit. Within each chain, the fold is cross-linked by covalent disulfide bridges between R groups.

a. Name the level of protein structure represented by the assembly of the two chains.
b. A reagent is added that breaks the disulfide bridges but leaves every peptide bond intact. Explain, in terms of bonding and the active site, why the enzyme loses its catalytic activity.
c. State whether the enzyme's *primary* structure has been changed by this treatment, and justify your answer.

Question 2 — An enzyme isolated from a hot spring was assayed at several temperatures. Its activity, as a percentage of the greatest value measured, is:

temperature (°C)	30	45	60	70	80	90	95
relative activity (%)	10.1	32.5	69.3	91.2	100	21.0	3.0

a. State the optimum temperature of this enzyme.
b. Explain, in terms of bonding and the active site, why the activity has fallen to 3.0% at 95 °C.
c. A human enzyme has an optimum temperature of 37 °C. Explain why that enzyme would show almost no activity at 80 °C, even though 80 °C is the temperature at which the hot-spring enzyme works best.

Question 3 — An uncatalysed reaction has a forward activation energy of $60\,\text{kJ mol}^{-1}$ and $\Delta H = -30\,\text{kJ mol}^{-1}$. With its enzyme, the forward activation energy is $22\,\text{kJ mol}^{-1}$.

a. Calculate how much the enzyme lowers the activation energy.
b. State ΔH for the enzyme-catalysed reaction, and justify your answer.
c. Describe how the energy profile of the catalysed reaction differs from that of the uncatalysed reaction.

Question 4 — Enzymes have an optimum pH.

a. State what is meant by the *optimum pH* of an enzyme.
b. Explain, referring to zwitterions and charged R groups, why activity falls when the pH is moved far from the optimum.
c. Explain why a graph of activity against pH is bell-shaped rather than a straight line.

Question 5 — Some medicines act as competitive enzyme inhibitors.

a. Define a *competitive enzyme inhibitor*.
b. Explain why such an inhibitor reduces the enzyme's activity even though it breaks none of the bonds that hold the enzyme's folded shape.
c. Explain why a molecule whose shape is very different from the substrate would *not* act as a competitive inhibitor of that enzyme.

Question 6 — A solution of an enzyme is heated to 70 °C for several minutes and then cooled back to 37 °C. Its catalytic activity does not return.

a. Name the process that has occurred at 70 °C.
b. Explain, in terms of bonding and the active site, why the enzyme has lost its activity.
c. Explain why cooling the solution back to 37 °C does not restore the activity.

Question 7 — Explain the role of an enzyme as a catalyst, referring to activation energy and the reaction pathway, and state the effect (if any) of the enzyme on ΔH.

Question 8 — Explain what is meant by the *specificity* of an enzyme, referring to the active site and the shape of the substrate.

Question 9 — An uncatalysed reaction has a forward activation energy of $80\,\text{kJ mol}^{-1}$ and $\Delta H = +22\,\text{kJ mol}^{-1}$. Its enzyme provides a pathway with a forward activation energy of $34\,\text{kJ mol}^{-1}$. Calculate (i) how much the enzyme lowers the forward activation energy, (ii) ΔH for the catalysed reaction, and (iii) the activation energy of the reverse reaction on the enzyme-catalysed pathway.

Question 10 — Name the four levels of protein structure and, for each, state the main type of bonding or interaction that maintains it.

Question 11 — Explain why the primary structure of a protein ultimately determines the shape of an enzyme's active site.

Question 12 — An industrial process uses an enzyme whose optimum temperature is 35 °C. An operator proposes running the process at 60 °C instead, arguing that raising the temperature always speeds a reaction up. Explain why this change would in fact slow the process almost to a stop.

Question 13 — Samples of an enzyme whose optimum temperature is 37 °C are stored in a refrigerator at 4 °C, rather than at 37 °C or at 60 °C. Explain, in terms of enzyme structure and activity, why 4 °C is a suitable storage temperature, and state what must be done to a sample before it is used.

Question 14 — One enzyme has an optimum pH of 2, while a second enzyme is denatured within minutes at pH 2. Explain how both statements can be true. Refer to the R groups of the two proteins, the charges they carry, the bonding that holds each fold, and the active site.

Question 15 — For a certain enzyme, the relative activity (as a percentage of its maximum) measured at whole-number pH values is:

pH	4	5	6	7	8	9	10
relative activity (%)	10.5	36.8	77.9	100	77.9	36.8	10.5

a. State the optimum pH.

b. Estimate the pH range over which the activity is at least half its maximum value.

c. The activity at pH 5 and at pH 9 is the same, yet one solution is acidic and the other basic. Explain, referring to the H^+ and OH^- present, what happens to the R groups in each case.

Question 16 — A chiral medicine exists as two enantiomers, but only one of them acts as a competitive inhibitor of the target enzyme. Explain, referring to the active site and to the lock-and-key mechanism, why the other enantiomer has no inhibitory effect.

Question 17 — A patient is given a larger dose of a medicine that acts as a competitive inhibitor, so its concentration at the target enzyme is higher. Explain why the enzyme's overall activity falls further as the concentration of the drug is raised.

Question 18 — An enzyme from the small intestine works best at about 37 °C and pH 8. A sample of it is placed in a solution held at 60 °C and pH 2, and it loses essentially all of its catalytic activity. Explain, referring to structure, bonding and the active site, how *each* of the two changed conditions causes the enzyme to stop working.

Answers

1 a quaternary structure; b the disulfide bridges are among the R-group interactions holding the tertiary fold, so breaking them unravels the fold and the active site loses its specific shape, and the substrate can no longer bind; c no — the primary structure is the amino-acid sequence joined by peptide bonds, and every peptide bond is intact. **2** a 80 °C; b above the optimum the thermal energy breaks the hydrogen bonds and other R-group interactions holding the tertiary fold, so the protein unravels (denaturation) and the active site loses its shape; c 80 °C is far above the human enzyme's own optimum, so the interactions holding *its* fold are broken and it denatures, while the different R-group interactions of the hot-spring enzyme still hold its fold at 80 °C — the optimum is a property of the individual protein. **3** a $38\,\text{kJ mol}^{-1}$; b $\Delta H = -30\,\text{kJ mol}^{-1}$ (a catalyst does not change ΔH); c same substrate and product energy levels, but a lower peak (lower E_a). **4** a the pH at which the enzyme's activity is greatest; b changing pH alters the charges on the acidic/basic R groups (zwitterion equilibria shift), disrupting ionic and hydrogen bonds so the active site loses shape (denaturation); c activity is high only near the optimum and falls on either side, giving a bell shape. **5** a an organic molecule that binds an enzyme's active site by a lock-and-key fit, preventing the substrate from binding; b it physically occupies the active site, so no enzyme–substrate complex can form — the fold and active-site shape are undamaged, so activity is blocked rather than destroyed (unlike denaturation); c a very different shape is not complementary to the active site, so it cannot bind there and cannot block the substrate. **6** a denaturation; b the bonds holding the tertiary fold break, so the active-site shape is lost and the substrate can no longer bind; c the change is irreversible — cooling does not refold the protein to its original active shape. **7** an enzyme is a catalyst: it provides an alternative pathway of lower activation energy, so more collisions succeed and the rate rises; the enzyme is not consumed and ΔH is unchanged. **8** each active site has a specific shape/charge pattern complementary to one substrate, so only that substrate binds (lock-and-key). **9** (i) $46\,\text{kJ mol}^{-1}$; (ii) $\Delta H = +22\,\text{kJ mol}^{-1}$; (iii) reverse $E_a = 34 - 22 = 12\,\text{kJ mol}^{-1}$. **10** primary — peptide (covalent amide) bonds; secondary — hydrogen bonds; tertiary — R-group interactions (H-bonds, ionic bonds, dispersion forces, disulfide bridges); quaternary — the same R-group interactions between subunits. **11** the amino-acid sequence determines how the chain folds, and the fold builds the active site; so the primary structure fixes the active-site shape. **12** 60 °C is far above this enzyme's optimum, so it denatures — the bonds holding the fold break, the active site loses its shape and the substrate can no longer bind, so the low-E_a catalysed pathway is lost; the faster collisions cannot make up for having almost no working enzyme, and the damage is irreversible. **13** at 4 °C the enzyme is undamaged (no bonds broken) but molecules move slowly, so activity is very low — lowered activity, which is reversible; 60 °C would denature it irreversibly and 37 °C would leave it fully active; warm the sample back toward 37 °C before use. **14** each protein has its own R groups, so its own charge pattern and its own optimum: the first enzyme's ionic and hydrogen bonds hold its fold *at* pH 2, while pH 2 is far from the second enzyme's optimum, so the excess H^+ protonates its $–COO^–$ groups, the charges change, those bonds are disrupted and its active site is lost. **15** a pH 7; b about pH 5.3 to 8.7 (activity crosses 50% between pH 5 and 6 on the low side and between pH 8 and 9 on the high side); c at pH 5 the added H^+ protonates $–COO^–$ groups (removing negative charges); at pH 9 the $OH^–$ removes H^+ from $–NH_3^+$ groups (removing positive charges) — a comparable change in charge either way, so a comparable loss of active-site shape. **16** the two enantiomers are non-superimposable mirror images, so only one has the shape complementary to the active site and can bind by the lock-and-key fit; the other cannot fit the site, so it cannot occupy it or block the substrate. **17** with more inhibitor present, a greater proportion of the active sites is occupied at any instant, so fewer enzyme–substrate complexes form per second and less product is made. **18** at 60 °C heat denatures the enzyme (bonds break, active site lost, irreversible); at pH 2 the excess H^+ changes R-group charges (zwitterion shift), breaking ionic/hydrogen bonds and denaturing the enzyme — both destroy the active-site shape.

Fully-worked solutions

Question 1 a. The two folded chains are **subunits**, and their assembly into one functional protein is the **quaternary** structure. (The three-dimensional fold of each chain on its own is its tertiary structure.) b. Disulfide bridges are covalent links between R groups, and they are one of the **R-group interactions** — along with hydrogen bonds, ionic bonds and dispersion forces — that hold the **tertiary** fold in place. Breaking them removes those cross-links, so the chain can no longer hold its folded shape. The **active site** is a pocket created by bringing particular R groups together in space, so as the fold is lost the active site loses its specific shape and charge pattern. The substrate is no longer complementary to it and cannot bind, no enzyme–substrate complex forms, and the enzyme cannot catalyse its reaction. c. **No.** The primary structure is the *sequence* of amino acids joined by covalent **peptide bonds**. The reagent breaks only disulfide bridges, so every peptide bond — and therefore the entire sequence — is left unchanged. The

enzyme is inactive even though its primary structure is intact, because catalytic activity depends on the higher levels of folding that build the active site.

Question 2 a. Activity is greatest at **80 °C** (100%), so this is the optimum temperature. b. 95 °C is well above the optimum, so the molecules have enough thermal energy to break the hydrogen bonds and other weak R-group interactions that hold the tertiary fold together. The protein unravels — **denaturation** — and the active site loses its specific shape, so the substrate can no longer bind. Only the small fraction of enzyme molecules still correctly folded can work, which is why the activity has fallen to 3.0%. c. An optimum temperature is a property of the individual protein: each has its own amino-acid sequence and therefore its own set of R-group interactions holding its fold. In the human enzyme those interactions are broken well below 80 °C, so at 80 °C it is denatured — its active site has lost its shape and the substrate cannot bind. The hot-spring enzyme's fold is held by interactions that still survive at 80 °C, so its active site keeps the correct shape and it works fastest there. The same temperature is therefore denaturing for one enzyme and optimal for the other.

Question 3 a. Lowering $= 60 - 22 = 38\ \text{kJ mol}^{-1}$. b. $\Delta H = -30\ \text{kJ mol}^{-1}$, the same as the uncatalysed reaction. A catalyst provides an alternative pathway of lower activation energy; it changes neither the energy of the substrate nor that of the products, so ΔH is unchanged. c. The catalysed energy profile has the **same** substrate and product energy levels (so the same ΔH), but a **lower peak** — a smaller activation energy — than the uncatalysed profile.

Question 4 a. The optimum pH is the pH at which the enzyme's catalytic activity is at its maximum. b. Many R groups (and the chain ends) are acidic (–COOH) or basic ($–NH_2$) and are normally charged, existing as zwitterions with both $–NH_3^+$ and $–COO^–$ groups. Changing the pH adds H^+ (low pH) or $OH^–$ (high pH), which alters these charges. The ionic bonds and hydrogen bonds that depend on those charges are disrupted, so the tertiary fold — and with it the active-site shape — is lost (denaturation), and the substrate can no longer bind. c. Activity is greatest only within a narrow pH range around the optimum; moving the pH either lower or higher progressively disrupts the bonding and reduces activity. Because activity is high in the middle and falls away on both sides, the graph is bell-shaped rather than linear.

Question 5 a. A competitive enzyme inhibitor is an organic molecule (often a medicine) that binds to an enzyme's active site by a lock-and-key fit, preventing the actual substrate from binding. b. The inhibitor does not attack the bonding that holds the protein's fold: the tertiary structure, and with it the shape of the active site, is left intact. Instead the inhibitor **physically occupies** the active site, so while it is bound the substrate cannot enter and no enzyme–substrate complex can form. Fewer product molecules are made per second, so the measured activity falls. The loss of activity is therefore caused by *blocking* the active site, not by destroying it as denaturation does. c. A molecule with a very different shape is not complementary to the active site, so it cannot fit into and bind the site. Because it cannot occupy the active site, it cannot prevent the substrate from binding, and so it does not act as a competitive inhibitor.

Question 6 a. Denaturation. b. At 70 °C the thermal energy breaks the hydrogen bonds and other weak R-group interactions that maintain the tertiary structure, so the protein unravels and the active site loses its specific shape. The substrate can no longer bind, so the enzyme cannot catalyse the reaction. c. Denaturation is irreversible: once the fold has been destroyed, cooling does not cause the chain to re-fold into its original, correctly shaped active site, so the activity does not return.

Question 7 An enzyme is a biological catalyst: it provides an **alternative reaction pathway with a lower activation energy**, so a greater fraction of collisions has enough energy to react and the reaction rate increases. The enzyme is not consumed in the reaction, and — like any catalyst — it does **not** change ΔH (nor the position of equilibrium); it only lets the reaction reach equilibrium faster.

Question 8 Specificity means an enzyme catalyses only one reaction (or a few closely related ones). The active site has a definite three-dimensional shape and charge pattern that is complementary to just one substrate. Only a substrate of the matching shape can fit and bind (lock-and-key), so other molecules are not catalysed — the enzyme is specific to its substrate.

Question 9 (i) Lowering $= 80 - 34 = 46\ \text{kJ mol}^{-1}$. (ii) $\Delta H = +22\ \text{kJ mol}^{-1}$, unchanged — a catalyst does not alter the enthalpy change. (iii) On an energy profile the reverse activation energy is the barrier measured from the product level: $E_a(\text{reverse}) = E_a(\text{forward}) - \Delta H = 34 - 22 = 12\ \text{kJ mol}^{-1}$. (On the uncatalysed pathway it would be $80 - 22 = 58\ \text{kJ mol}^{-1}$.)

Question 10 - **Primary** — the amino-acid sequence, held by covalent **peptide (amide) bonds**. - **Secondary** — α-helix / β-pleated sheet, held by **hydrogen bonds** between backbone C=O and N–H groups. - **Tertiary** — the overall 3-D fold, held by **R-group interactions**: hydrogen bonds, ionic bonds, dispersion forces and disulfide bridges. - **Quaternary** — the assembly of two or more subunits, held by the **same R-group interactions** acting between the subunits.

Question 11 The primary structure is the order of amino acids in the chain. It is this sequence — which particular R groups appear, and where — that dictates how the chain folds into its secondary and tertiary structures, because the folding is driven by the interactions between those specific R groups. The tertiary fold builds the active site by bringing the right R groups together in the right positions. Since the fold, and therefore the active-site shape, is set entirely by the sequence, the primary structure ultimately determines the shape of the active site.

Question 12 The operator's argument is only half the story. Raising the temperature does make the enzyme and substrate molecules move faster, so they collide more often and more energetically — and up to the optimum this does increase the rate. Above the optimum, however, the extra thermal energy breaks the hydrogen bonds and other weak R-group interactions that hold the tertiary fold together. At 60 °C — far above this enzyme's 35 °C optimum — the protein unravels (**denaturation**) and the active site loses its specific shape, so the substrate can no longer bind and the enzyme–substrate complex cannot form. The low-activation-energy pathway the enzyme provided is therefore lost, and the reaction is left to proceed by the slow uncatalysed pathway. The increase in collision energy cannot compensate for having almost no working enzyme, so the overall rate falls sharply. ∴ the process would slow almost to a stop, and because denaturation is irreversible, cooling the vessel back to 35 °C would not recover the enzyme.

Question 13 Storage at 4 °C keeps the enzyme **undamaged but sluggish**. At this temperature no bonds are broken: the hydrogen bonds, ionic bonds, dispersion forces and disulfide bridges holding the tertiary fold are all intact, so the active site retains its shape. What is reduced is molecular motion — enzyme and substrate molecules move slowly and collide infrequently and with little energy — so very few reactions occur per second and the sample shows only **lowered activity**. Because this is purely a kinetic effect, it is fully **reversible**.

Storing at 60 °C would be far above the optimum and would **denature** the enzyme, destroying the active site irreversibly. Storing at 37 °C would hold the enzyme at its optimum, where it is at its most active, so any substrate present in the sample would be catalysed away during storage. Refrigeration at 4 °C is the choice that suppresses the enzyme's activity without damaging the protein.

∴ before use, the sample must be **warmed back toward 37 °C**, which restores full activity because the fold and active site were never lost.

Question 14 Both statements are true because an optimum pH is a property of the *individual* protein, not a universal value. Each enzyme has its own amino-acid sequence, so its own particular set of acidic (–COOH) and basic ($–NH_2$) R groups, each carrying a charge that depends on the pH; the zwitterion equilibria of those groups set the pattern of charges the protein carries.

For the first enzyme, that pattern of charges at **pH 2** is the one that forms the ionic bonds and hydrogen bonds holding its tertiary fold in the correct shape, so at pH 2 its active site is complementary to the substrate and the enzyme is at its most active. Moving *this* enzyme to a higher pH would be what disrupts its fold.

For the second enzyme, pH 2 lies far from its own optimum. The large excess of H^+ protonates its $–COO^–$ groups (shifting the zwitterion equilibria), so the charges that its ionic and hydrogen bonds depend on are removed. Those bonds break, the tertiary fold unravels (**denaturation**), the active site loses its shape and the substrate can no longer bind.

∴ denaturation by pH depends on how far the pH has been moved from that particular enzyme's optimum, not on the value of the pH by itself.

Question 15 a. The optimum pH is 7, where the relative activity is 100%. b. The activity is 100% at pH 7 and falls symmetrically on either side. It is above 50% at pH 6, 7 and 8 (77.9%, 100%, 77.9%) but below 50% at pH 5 and 9 (36.8%). So the activity crosses half-maximum between pH 5 and 6 on the low side and between pH 8 and 9 on the high side — a half-maximum range of roughly **pH 5.3 to 8.7**. c. The two sides involve chemically different changes that have a comparable effect. At **pH 5** the solution is acidic, so the added H^+ **protonates the** $–COO^–$ **groups** of acidic R groups ($–COO^– \rightarrow$ –COOH), removing negative charges. At **pH 9** the solution is basic, so $OH^–$ **removes** H^+ **from**

the $-NH_3^+$ groups of basic R groups ($-NH_3^+ \rightarrow -NH_2$), removing positive charges. In each case the zwitterion equilibria shift and the pattern of charges on the protein is altered — by a comparable amount, since pH 5 and pH 9 are each two units from the optimum. The ionic bonds and hydrogen bonds that depended on those charges are disrupted to a similar extent, so the active site is distorted to a similar extent and the two solutions give the same relative activity of 36.8%.

Question 16 A chiral molecule exists as two **enantiomers** — non-superimposable mirror images of each other (12A). The enzyme's active site is itself a chiral environment: it is a pocket of a definite three-dimensional shape and charge distribution, built by the folding of the protein chain. For a molecule to act as a competitive inhibitor it must be **complementary** to that pocket, so that it binds there by the **lock-and-key** mechanism the substrate would use.

Only one of the two mirror images has that matching handedness — just as only one hand fits a given glove. That enantiomer fits into the active site, occupies it, and so prevents the substrate from binding, which lowers the rate. Its mirror image presents the same groups in the opposite spatial arrangement, so it is *not* complementary to the site and cannot fit into it.

∴ because the second enantiomer cannot bind the active site, it cannot block the substrate, and the enzyme's activity is unaffected by it.

Question 17 A competitive inhibitor and the substrate compete for the **same active sites**, and any one active site can hold only one of them at a time. Raising the concentration of the drug increases the number of inhibitor molecules available to collide with, and bind to, those sites, so at any instant a **greater proportion of the enzyme's active sites is occupied by the drug**.

Every occupied site is one at which the substrate cannot bind, so fewer enzyme–substrate complexes are formed per second and less product is made. ∴ as the drug concentration rises, a larger fraction of the enzyme is blocked at any moment and the enzyme's measured activity falls further — which is why the effect of such a medicine depends on the dose given.

Question 18 Both changes destroy the active site, but by breaking bonds in different ways. - **60 °C (temperature):** heating well above the 37 °C optimum supplies enough thermal energy to break the hydrogen bonds and other weak R-group interactions holding the tertiary fold. The protein unravels (**denaturation**), the active site loses its shape, and the substrate can no longer bind. This is irreversible. - **pH 2 (pH):** the large excess of H^+ changes the charges on the acidic and basic R groups (the zwitterion equilibria shift as $-COO^-$ groups are protonated). The ionic bonds and hydrogen bonds that depended on those charges are broken, so the fold and active-site shape are again lost (**denaturation**).

In both cases the specific three-dimensional shape of the active site is destroyed, so the substrate cannot bind and the enzyme stops catalysing the reaction.

Topic 4 Test A — Organic analysis and medicines (multiple choice)

Time allowed: 30 minutes · 25 marks · One scientific calculator permitted · The Chemistry Data Sheet (back of the Practice Examinations booklet) may be used.

Choose the response that is correct or that best answers the question. A correct answer scores 1 mark; an incorrect answer scores 0. Marks are not deducted for incorrect answers. No marks are given if more than one answer is completed for any question. Relative atomic masses and the characteristic data (infrared absorption ranges, NMR chemical-shift ranges) are those of the Chemistry Data Sheet.

Question 1

A few drops of orange bromine water are added to a sample of hept-1-ene in the dark. Which one of the following describes the observation and what it shows?

A. The bromine water is decolourised (orange to colourless), showing that a carbon–carbon double bond is present.

B. The bromine water turns from purple to colourless, showing that a carbon–carbon double bond is present.

C. The bromine water turns from orange to green, showing that an oxidisable group is present.

D. The bromine water remains orange, showing that a carbon–carbon double bond is present.

Question 2

Which one of the following observations identifies a compound as a carboxylic acid rather than an alcohol?

A. It decolourises orange bromine water.

B. It turns acidified potassium dichromate from orange to green.

C. It produces effervescence of carbon dioxide with aqueous sodium hydrogencarbonate.

D. It gives no reaction with aqueous sodium carbonate.

Question 3

Which one of the following compounds is **not** oxidised by warm acidified potassium permanganate (the purple colour is unchanged)?

A. propan-1-ol

B. butan-2-ol

C. propanal

D. butan-2-one

Question 4

A white crystalline solid melts over the range $108 - 115\,°C$. The pure compound is known to melt sharply at $121\,°C$. The sample is best described as

A. pure, because part of it melts below $121\,°C$.

B. impure, because it melts over a broad range at temperatures below the literature value.

C. a different pure compound, because its melting range is narrow.

D. pure, because its melting range includes a temperature close to $121\,°C$.

Question 5

Two volatile liquids have boiling points of $64\,°C$ and $70\,°C$. The most suitable technique for separating them is

A. simple distillation.

B. solvent extraction.

C. fractional distillation.

D. filtration.

Question 6

A $20.00\,mL$ aliquot of a solution of 2-methylpropanoic acid, $(CH_3)_2CHCOOH$, is titrated against $0.200\,mol\,L^{-1}$ sodium hydroxide, requiring an average titre of $15.00\,mL$. The concentration of the 2-methylpropanoic acid is closest to

A. $0.150\,mol\,L^{-1}$

B. $0.300\,mol\,L^{-1}$

C. $0.267\,mol\,L^{-1}$

D. $0.0750\,mol\,L^{-1}$

Question 7

A $20.00\,mL$ portion of $0.100\,mol\,L^{-1}$ iron(II) sulfate solution is acidified, and $20.00\,mL$ of $0.0250\,mol\,L^{-1}$ potassium permanganate is then added in a single portion. Given the mole ratio $n(MnO_4^-):n(Fe^{2+})=1:5$, the reactant left in excess, and the amount of it remaining unreacted, are

A. Fe^{2+}, $1.90\times10^{-3}\,mol$

B. MnO_4^-, $1.00\times10^{-4}\,mol$

C. Fe^{2+}, $1.50\times10^{-3}\,mol$

D. MnO_4^-, $4.00\times10^{-4}\,mol$

Question 8

A $4.00\,g$ sample of an oil reacts by addition with $3.20\,g$ of iodine, I_2, saturating all of its carbon–carbon double bonds. The iodine number of the oil is

A. 80.0

B. 125

C. 40.0

D. 0.800

Question 9

Which one of the following is suitable as a primary standard for standardising a solution of sodium hydroxide?

A. sodium hydroxide

B. sodium chloride

C. hydrochloric acid

D. ethanedioic (oxalic) acid

Question 10

In a mass spectrum, the molecular ion appears at $m/z\,88$ and a fragment ion appears at $m/z\,57$. The neutral fragment lost from the molecular ion to give the peak at $m/z\,57$ is

A. CH_3 (mass 15)

B. C_2H_5 (mass 29)

C. OCH_3 (mass 31)

D. OH (mass 17)

Question 11

Near the molecular ion of a compound, two peaks of almost equal height appear two mass units apart. This indicates that the molecule contains

A. one chlorine atom.

B. one bromine atom.

C. two oxygen atoms.

D. one nitrogen atom.

Question 12

In a mass spectrum, the relative molecular mass of the compound is given by the m/z value of

A. the molecular ion peak (the highest-m/z peak of significant abundance).

B. the base peak.

C. the $M+2$ isotope peak.

D. the peak at $m/z\,43$.

Question 13

An infrared spectrum shows a very broad absorption spread across $2500-3300\,\text{cm}^{-1}$ together with a strong band at $1710\,\text{cm}^{-1}$. The compound is most likely

A. an alcohol.

B. a ketone.

C. a carboxylic acid.

D. an amine.

Question 14

In which one of the following wavenumber ranges does the broad O–H absorption of an alcohol appear?

A. $1000 - 1300\,\text{cm}^{-1}$

B. $1670 - 1750\,\text{cm}^{-1}$

C. $2850 - 3090\,\text{cm}^{-1}$

D. $3200 - 3550\,\text{cm}^{-1}$

Question 15

How many peaks appear in the low-resolution ^{13}C NMR spectrum of 1,2-dichloroethane, $\text{ClCH}_2\text{CH}_2\text{Cl}$?

A. 1

B. 2

C. 3

D. 4

Question 16

In the high-resolution ^{1}H NMR spectrum of chloroethane, $\text{CH}_3\text{CH}_2\text{Cl}$, the two $-\text{CH}_2\text{Cl}$ protons appear as a

A. singlet.

B. doublet.

C. triplet.

D. quartet.

Question 17

Which one of the following protons appears at the highest chemical shift (is the most deshielded)?

A. the protons of an alkyl R–CH_3 group

B. the protons of an R–O–CH_2- group in an ester

C. the proton of an R–CHO (aldehyde) group

D. the protons of a benzene ring

Question 18

In HPLC, a component in a sample is identified by comparing its _____ with that of a pure standard run under identical conditions.

A. peak area

B. retention time

C. molecular ion m / z

D. chemical shift

Question 19

An HPLC calibration line through the origin is $A = 16.0\,c$, where A is the peak area and c is the concentration in mg L^{-1}. A sample gives a peak area of 208. The concentration of the compound in the sample is

A. $13.0\,\text{mg L}^{-1}$

B. $192\,\text{mg L}^{-1}$

C. $3330\,\text{mg L}^{-1}$

D. $0.0769\,\text{mg L}^{-1}$

Question 20

A chiral centre in an organic molecule is a carbon atom that is bonded to

A. two carbon–carbon double bonds.

B. four different groups.

C. four identical groups.

D. two identical groups and two other, different groups.

Question 21

How many chiral centres are present in 3-chloropentane, $\text{CH}_3\text{CH}_2\text{CHClCH}_2\text{CH}_3$?

A. 3

B. 2

C. 1

D. 0

Question 22

Which one of the following describes the functional groups present in the candidate anaesthetic methyl 4-aminobenzoate, $\text{H}_2\text{NC}_6\text{H}_4\text{COOCH}_3$?

A. an amine group, an ester group and a benzene ring

B. an amide group, a carboxyl group and a benzene ring

C. an amine group, a carboxyl group and a benzene ring

D. an amine group, a ketone group and a benzene ring

Question 23

The order in which the amino acid residues are joined by peptide links along an enzyme's polypeptide chain is the enzyme's

A. secondary structure.

B. primary structure.

C. tertiary structure.

D. quaternary structure.

Question 24

One sample of an enzyme solution is cooled to well below the enzyme's optimum temperature; a second sample of the same enzyme is heated to well above it. Which one of the following best describes the two samples?

A. Both samples are denatured, so both lose their activity permanently.

B. The cooled sample is denatured, while the heated sample is unaffected.

C. The cooled sample has a lower activity because collisions are fewer and less energetic, while the heated sample is denatured because its tertiary structure is disrupted.

D. The cooled sample loses its activity permanently, while the heated sample regains full activity once it is cooled again.

Question 25

The pH of a solution of an enzyme is lowered to well below the enzyme's optimum pH, and the rate of the reaction it catalyses falls almost to zero. This is best explained by the added H^+ ions

A. lowering the activation energy of the catalysed reaction.

B. converting the substrate molecules into zwitterions.

C. hydrolysing the peptide links, breaking the polypeptide chain into individual amino acids.

D. protonating the $-COO^-$ groups of the R groups, so that the ionic interactions holding the tertiary structure are lost and the active site changes shape.

End of Topic 4 Test A

Topic 4 Test A — solutions

Question	Answer	Question	Answer
1	A	14	D
2	C	15	A
3	D	16	D
4	B	17	C
5	C	18	B
6	A	19	A
7	B	20	B
8	A	21	D
9	D	22	A
10	C	23	B
11	B	24	C
12	A	25	D
13	C		

Question 1 — A. Bromine water is the specific test for a C=C double bond: the alkene adds Br_2 across the double bond, consuming the bromine, so the orange colour is decolourised. **B** quotes the colour of permanganate (purple), the wrong reagent; **C** quotes the orange-to-green change of dichromate (an oxidation test); **D** describes the negative (saturated) result while wrongly claiming a C=C is present.

Question 2 — C. Only a carboxylic acid is acidic enough to react with hydrogencarbonate, giving effervescence of CO_2; an alcohol is neutral and gives no bubbles. **A** is the alkene test; **B** is the *positive* test for a primary or secondary alcohol (and for an aldehyde) — a carboxylic acid is not oxidised by dichromate and gives no colour change at all, so this observation points to the alcohol, the opposite of what is asked; **D** is the result an alcohol would give, not a positive identification of the acid.

Question 3 — D. A ketone's carbonyl carbon carries no hydrogen, so it cannot be oxidised and the permanganate stays purple. **A** (primary alcohol), **B** (secondary alcohol) and **C** (aldehyde) all have a removable hydrogen on the carbon bearing the oxygen, so all three are oxidised and decolourise the permanganate.

Question 4 — B. The range is broad ($108-115\,°C$, a spread of $7\,°C$) and lies below the literature value of $121\,°C$ — both hallmarks of impurity, since an impurity lowers and broadens the melting range. **A** and **D** misread a depressed, broad range as "pure"; **C** wrongly calls the range narrow.

Question 5 — C. The boiling points differ by only $6\,°C$, too close for a single vaporisation, so fractional distillation (many vaporisation–condensation cycles) is needed. **A** simple distillation suits a large ($>25-30\,°C$) gap; **B** and **D** do not separate two miscible volatile liquids.

Question 6 — A. $(CH_3)_2CHCOOH + NaOH \rightarrow (CH_3)_2CHCOONa + H_2O$ (1 : 1, the acid is monoprotic).
$n(\text{NaOH}) = 0.200 \times 0.01500 = 3.00 \times 10^{-3}\,\text{mol} = n(\text{acid})$; $c = \dfrac{3.00 \times 10^{-3}}{0.02000} = 0.150\,\text{mol L}^{-1}$. **B** — 0.300 — inverts the ratio, taking $n(\text{acid}) = 2n(\text{NaOH})$; **C** — 0.267 — swaps the aliquot and titre volumes ($0.200 \times 20.00 / 15.00$); **D** — 0.0750 — treats the acid as diprotic, taking $n(\text{acid}) = 1/2\,n(\text{NaOH})$.

Question 7 — B. $n(Fe^{2+}) = 0.100 \times 0.02000 = 2.00 \times 10^{-3}\,\text{mol}$ and
$n(MnO_4^-) = 0.0250 \times 0.02000 = 5.00 \times 10^{-4}\,\text{mol}$. Oxidising all of the Fe^{2+} needs
$1/5 \times 2.00 \times 10^{-3} = 4.00 \times 10^{-4}\,\text{mol}$ of MnO_4^-; more than this is available, so Fe^{2+} is the limiting reactant and
$5.00 \times 10^{-4} - 4.00 \times 10^{-4} = 1.00 \times 10^{-4}\,\text{mol}$ of MnO_4^- is left over (the mixture stays purple). **A** inverts the ratio, requiring only $1/5 \times 5.00 \times 10^{-4} = 1.00 \times 10^{-4}\,\text{mol}$ of Fe^{2+}; **C** ignores the mole ratio and subtracts the amounts directly ($2.00 \times 10^{-3} - 5.00 \times 10^{-4}$); **D** quotes the amount of MnO_4^- that *reacted* rather than the amount remaining.

Question 8 — A. Iodine number $= \dfrac{m(\mathrm{I_2})}{m(\text{oil})} \times 100 = \dfrac{3.20}{4.00} \times 100 = 80.0$. **B** — 125 — inverts the ratio ($4.00 / 3.20 \times 100$); **C** — 40.0 — halves the correct value; **D** — 0.800 — omits the $\times 100$.

Question 9 — D. Ethanedioic (oxalic) acid is pure, stable and of known composition, so it can be weighed directly to make a standard solution. **A** NaOH absorbs water and $\mathrm{CO_2}$, so it is not a primary standard; **B** sodium chloride is not an acid–base standard for this titration; **C** hydrochloric acid is a volatile solution of uncertain concentration, not a weighable primary standard.

Question 10 — C. The neutral lost equals the difference in m/z: $88 - 57 = 31$, which is a methoxy group, $\mathrm{OCH_3}$ (the characteristic loss from a methyl ester). **A** (15), **B** (29) and **D** (17) are the masses of other common neutral losses but do not match the difference of 31.

Question 11 — B. Two peaks of almost equal height ($\approx 1:1$), two mass units apart, are the Br-79 : Br-81 signature of one bromine atom. **A** is wrong because one chlorine gives a $3:1$ ($M / M+2$) pattern, not $1:1$; **C** and **D** do not produce an $M+2$ doublet of this kind.

Question 12 — A. The molecular ion M^+ has essentially the mass of the whole molecule and appears at the highest m/z of significant abundance, so its m/z equals M_r. **B** the base peak is only the tallest peak (usually a fragment); **C** the $M+2$ peak is an isotope peak; **D** a fixed fragment m/z says nothing about M_r.

Question 13 — C. A very broad O–H over $2500 - 3300\ \mathrm{cm^{-1}}$ **together with** a strong C=O at $1710\ \mathrm{cm^{-1}}$ is the signature of a carboxylic acid. **A** an alcohol shows a broad O–H but **no** C=O; **B** a ketone shows the C=O but no O–H; **D** an amine shows N–H ($3300 - 3500$), not this pattern.

Question 14 — D. The broad, hydrogen-bonded O–H of an alcohol lies at $3200 - 3550\ \mathrm{cm^{-1}}$. **A** is the C–O range, **B** the C=O range and **C** the C–H range — none is the alcohol O–H.

Question 15 — A. In $\mathrm{ClCH_2CH_2Cl}$ the molecule's symmetry makes the two $\mathrm{CH_2Cl}$ carbons chemically equivalent, so there is one carbon environment and **1** peak. **B**, **C** and **D** ignore the symmetry that relates the two identical carbons.

Question 16 — D. The $\mathrm{CH_2Cl}$ protons have three equivalent hydrogen neighbours (the $\mathrm{CH_3}$ group), so $n = 3$ and $(n+1) = 4$: a quartet. **C** (triplet) uses $n = 2$; **B** (doublet) uses $n = 1$; **A** (singlet) uses $n = 0$ — all miscount the neighbouring protons.

Question 17 — C. The aldehyde proton, R–CHO, is the most deshielded of these: it is bonded directly to a carbon that is itself doubly bonded to the strongly electronegative oxygen, which draws electron density away from the proton and leaves it poorly shielded, so the Data Sheet places it at $\delta\ 9.4 - 10.0$. **D** aromatic ring H ($6.5 - 8.5$) is the next highest but is still below it; **B** R–O–$\mathrm{CH_2}$– ($3.3 - 4.5$) and **A** alkyl R–$\mathrm{CH_3}$ ($0.8 - 1.0$) are far upfield.

Question 18 — B. Under fixed conditions each compound has a characteristic retention time, so a component is identified by matching its retention time to that of a pure standard. **A** peak area gives the amount, not the identity; **C** and **D** belong to mass spectrometry and NMR, not HPLC.

Question 19 — A. $c = \dfrac{A}{k} = \dfrac{208}{16.0} = 13.0\ \mathrm{mg\ L^{-1}}$. **B** subtracts ($208 - 16$); **C** multiplies ($208 \times 16.0$); **D** inverts the calculation ($16.0 / 208$).

Question 20 — B. A chiral centre is a carbon bonded to four **different** groups; it then has no plane of symmetry and gives rise to two non-superimposable mirror images. **A** a doubly-bonded carbon is not bonded to four separate groups; **C** four identical groups is fully symmetric; **D** has only three kinds of group, because two of the four are the same — that carbon lies on a plane of symmetry, so it is not a chiral centre.

Question 21 — D. The only candidate is C3, but it is bonded to –Cl, –H and **two identical ethyl groups** (–$\mathrm{CH_2CH_3}$). Because two of its groups are the same, C3 is not bonded to four different groups, so there are **0** chiral centres. **A**, **B** and **C** overlook the two identical ethyl groups.

Question 22 — A. Methyl 4-aminobenzoate, $H_2NC_6H_4COOCH_3$, has an amine group ($-NH_2$) on the ring, an ester linkage ($-COO-$) and a benzene ring. **B** misreads the amine as an amide and the ester as a carboxyl; **C** confuses the ester ($-COO-$) with a carboxyl ($-COOH$); **D** misreads the ester carbonyl as a ketone.

Question 23 — B. The **primary** structure is the sequence of amino acid residues along the chain, joined by covalent peptide links. **A** the secondary structure is the local folding of the backbone into α-helices and β-pleated sheets, held by hydrogen bonds between the backbone C=O and N–H groups; **C** the tertiary structure is the overall three-dimensional fold of one chain, held by interactions between R groups (hydrogen bonds, ionic attractions, dispersion forces and disulfide bridges); **D** the quaternary structure is the assembly of two or more folded chains into one functional protein.

Question 24 — C. Cooling and heating act by different mechanisms. Below the optimum the enzyme is intact but the particles move more slowly, so collisions between enzyme and substrate are **fewer and less energetic** and the activity falls — reversibly. Well above the optimum the extra kinetic energy disrupts the hydrogen bonds, ionic attractions and dispersion forces holding the **tertiary** structure, so the chain unfolds, the active site loses its shape and the enzyme is **denatured**. **A** wrongly denatures the cooled sample; **B** reverses the two effects; **D** reverses which change is permanent — it is denaturation, not chilling, that is irreversible.

Question 25 — D. At its optimum pH the enzyme's acidic and basic R groups are ionised as **zwitterions** ($-NH_3^+$ and $-COO^-$), and the ionic attractions between them help hold the tertiary structure. Added H^+ protonates the $-COO^-$ groups to $-COOH$, destroying those attractions, so the chain unfolds, the active site changes shape and the enzyme is denatured. **A** reverses the direction — lowering E_a would *raise* the rate; **B** misplaces the zwitterion: it is the enzyme's own R groups, not the substrate, whose charges matter (and strong acid protonates a zwitterion rather than forming one); **C** peptide links are covalent and are not broken by denaturation — the primary structure survives.

Topic 4 Test B — Organic analysis and medicines (short and extended response)

Time allowed: 50 minutes · 40 marks · One scientific calculator permitted · The Chemistry Data Sheet (back of the Practice Examinations booklet) may be used.

Answer all questions in the spaces provided. Give simplified answers to all numerical questions, with an appropriate number of significant figures. Show all working; no marks are given for an incorrect answer unless accompanied by working. Ensure chemical equations are balanced and that formulas include states, e.g. $\mathrm{H_2(g)}$, $\mathrm{NaCl(s)}$. *Where required, use relative atomic masses* $\mathrm{H}\,1.0$, $\mathrm{C}\,12.0$, $\mathrm{O}\,16.0$.

Question 1 (6 marks)

Octan-1-ol, octan-2-ol and 2-methylhexan-2-ol are colourless liquids. A separate sample of each is warmed with an excess of acidified potassium dichromate.

a. State the colour change observed with octan-2-ol, name the organic product, and write a balanced ionic equation, including states, for the reaction. 2 marks

b. Octan-1-ol is oxidised in two successive steps. Name the two organic products in the order in which they are formed, and write a balanced equation, including states, for the reaction of the **final** product with aqueous sodium hydrogencarbonate. 2 marks

c. State what is observed when 2-methylhexan-2-ol is warmed with acidified potassium dichromate, and explain the result by referring to the carbon atom that carries the $-\mathrm{OH}$ group. 2 marks

Question 2 (7 marks)

An antiseptic solution is analysed for hydrogen peroxide, $\mathrm{H_2O_2}$, by redox titration. A $25.0\,\mathrm{mL}$ sample of the antiseptic is diluted with water to $250.0\,\mathrm{mL}$ in a volumetric flask. A $25.00\,\mathrm{mL}$ aliquot of the diluted solution is acidified with excess sulfuric acid and titrated against $0.0250\,\mathrm{mol\,L^{-1}}$ potassium permanganate, which oxidises the hydrogen peroxide to oxygen gas. After a rough trial titre of $16.7\,\mathrm{mL}$, three careful titres are recorded: 15.95, 16.00 and $16.05\,\mathrm{mL}$.

a. From the recorded titres, determine the average titre that should be used in the calculation, and explain which titres you include. 1 mark

b. Write a balanced ionic equation, including states, for the reaction between permanganate ions and hydrogen peroxide in acidic solution.

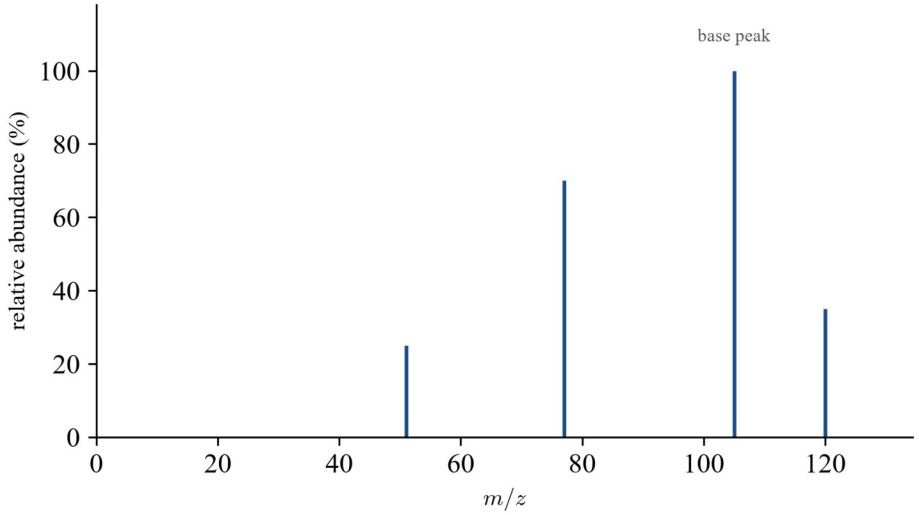
1 mark

c. Determine the concentration of hydrogen peroxide in the undiluted antiseptic, in mol $\mathrm{L^{-1}}$, and hence in g $\mathrm{L^{-1}}$. 3 marks

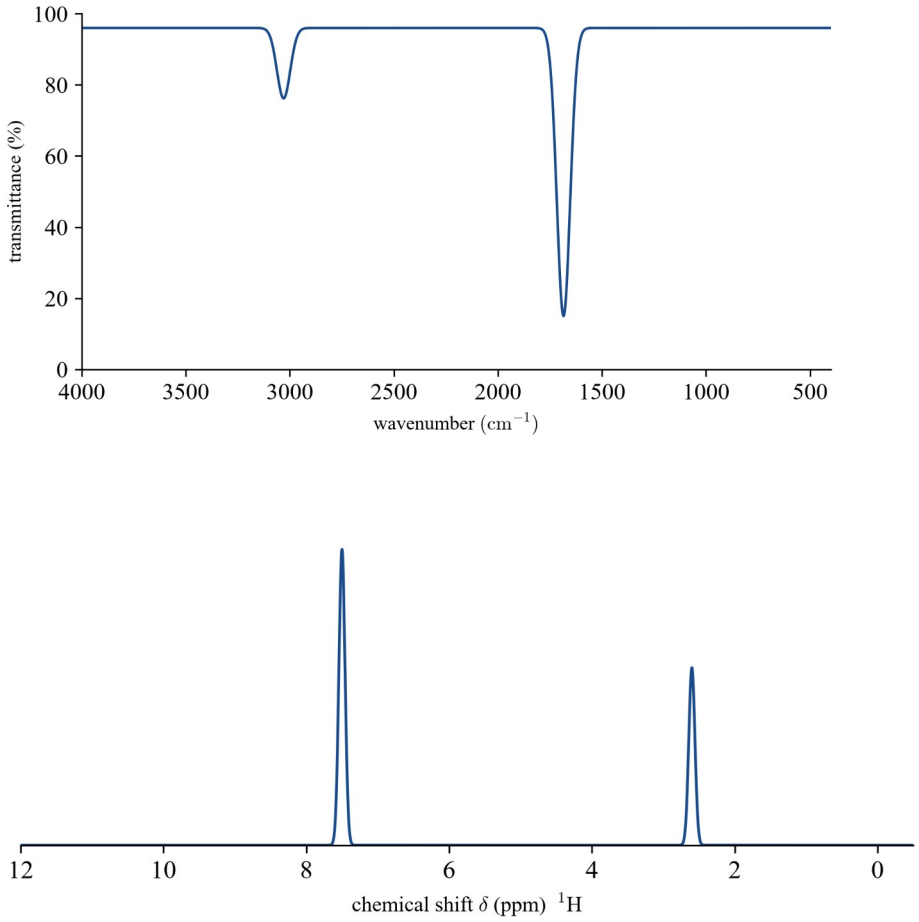
d. The burette is graduated at intervals of $0.1\,\mathrm{mL}$, and each titre is the difference between two burette readings. State the uncertainty in a titre, and explain what the three concordant titres show about the results — and what would additionally be needed to judge the result *accurate*. 2 marks

Question 3 (6 marks)

An organic liquid **Q** contains only carbon, hydrogen and oxygen. It is analysed by mass spectrometry, infrared spectroscopy and ^{1}H NMR. The mass spectrum shows its highest significant peak at $m/z\,120$ (with no $M+2$ peak), a base peak at $m/z\,105$, and a further peak at $m/z\,77$.



The infrared spectrum shows a sharp band near $3030\,\text{cm}^{-1}$ and a strong band at $1685\,\text{cm}^{-1}$, but no broad absorption in either O–H region (nothing over $3200-3550\,\text{cm}^{-1}$ and no very broad band across $2500-3300\,\text{cm}^{-1}$). The ^{1}H NMR spectrum shows a singlet at $\delta = 2.6$ ppm (3 H) and a multiplet at $\delta = 7.4-8.0$ ppm (5 H).



a. State the relative molecular mass of **Q**, and state what the absence of an $M+2$ peak tells you about the molecule. 1 mark

b. Identify the bond responsible for the strong band at $1685\,\text{cm}^{-1}$, and explain how the infrared spectrum rules out both a carboxylic acid and an alcohol. 2 marks

c. State what each of the two ^{1}H NMR signals indicates about the structure of **Q**. 1 mark

d. Using the molecular ion and the fragments at m/z 105 and 77 together with the data above, deduce the structure of **Q**, draw its structure and give its name. 2 marks

Question 4 (5 marks)

A candidate medicine extracted from a plant has the semi-structural formula $(CH_3)_2CHCH(OH)COOH$ (2-hydroxy-3-methylbutanoic acid).

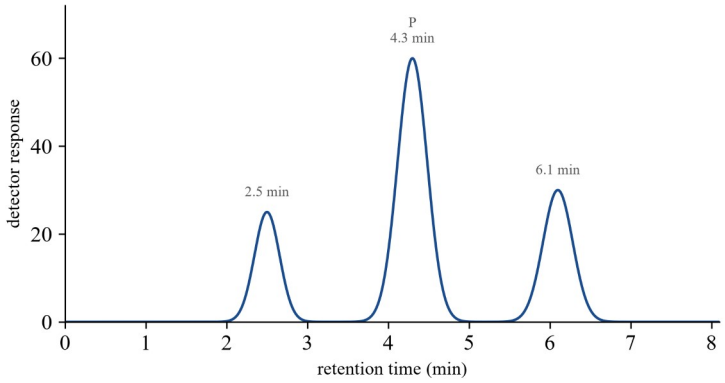
a. Identify the two functional groups present in the molecule. 1 mark

b. Identify the chiral centre by listing the four groups bonded to it, and state the total number of chiral centres in the molecule. Explain why the $-CH(CH_3)_2$ carbon is **not** a chiral centre. 2 marks

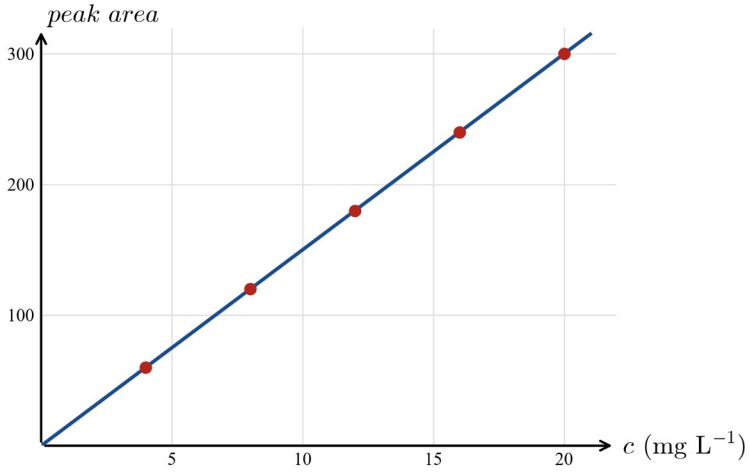
c. The molecule exists as two enantiomers. Explain, as a clear reasoning chain, why the two enantiomers may have different physiological effects in the body. 2 marks

Question 5 (6 marks)

A soft drink is analysed by HPLC for a preservative, compound **P**, whose peak appears at a retention time of $4.3\,\text{min}$. Standard solutions of **P** give the calibration data below.



Concentration of **P** (mg L^{-1})	4.0	8.0	12.0	16.0	20.0
Peak area (arbitrary units)	60	120	180	240	300



a. Explain why the calibration line is drawn through the origin. 1 mark

b. Determine the gradient of the calibration line, including its units. 2 marks

c. The drink is diluted 5.0-fold before injection; the diluted sample gives a peak area of 210. Determine the concentration of **P** in the original drink (in mg L^{-1}) and the mass of **P** in a $250\,\text{mL}$ serve (in mg). 2 marks

d. A second, stronger drink gives a peak area of 360 for the *diluted* sample. Explain why its concentration should **not** be found by extending the calibration line, and state what should be done instead. 1 mark

Question 6 (5 marks)

An enzyme catalyses a biochemical reaction. In the absence of the enzyme the forward reaction has an activation energy of $64\ \text{kJ mol}^{-1}$ and $\Delta H = -26\ \text{kJ mol}^{-1}$; in the presence of the enzyme the forward activation energy is $27\ \text{kJ mol}^{-1}$.

a. State how an enzyme increases the rate of the reaction, and state the effect (if any) of the enzyme on ΔH. 1 mark

b. Calculate (i) the amount by which the enzyme lowers the forward activation energy, and (ii) the activation energy of the reverse reaction on the enzyme-catalysed pathway. Give each answer in kJ mol⁻¹. 2 marks

c. A medicine acts as a competitive inhibitor of this enzyme. Explain, using the lock-and-key model, how it reduces the rate of the reaction. 2 marks

Question 7 (5 marks)

A pharmaceutical company obtains active ingredients for medicines from plant material.

a. A volatile, heat-sensitive oil that does not mix with water is recovered from citrus peel by **steam distillation** rather than by simple distillation. Explain why steam distillation protects the oil. 1 mark

b. A second active ingredient in the peel is a **non-polar** solid. Explain, referring to the principle *like dissolves like*, what kind of solvent should be chosen to extract it and why, and state how the solvent should be removed so that it can be re-used. 2 marks

c. Evaluate the sustainability of producing the medicine, referring to **two** green chemistry principles (for example, using renewable feedstocks and designing safer chemicals), and give a concluding statement.

2 marks

End of Topic 4 Test B

Topic 4 Test B — solutions

Question 1

(a) Octan-2-ol is a **secondary** alcohol, so it is oxidised to the ketone **octan-2-one**, and the dichromate changes from **orange to green** as $Cr_2O_7^{2-}$ is reduced to Cr^{3+}.

$$3\,CH_3CH(OH)(CH_2)_5CH_3(l) + Cr_2O_7^{2-}(aq) + 8\,H^+(aq) \rightarrow 3\,CH_3CO(CH_2)_5CH_3(l) + 2\,Cr^{3+}(aq) + 7\,H_2O(l)$$

Atoms balance and the charge is $+6$ on each side. **(1 mark** orange-to-green and product named as octan-2-one; **1 mark** balanced ionic equation with states.)

(b) Octan-1-ol is a **primary** alcohol, so with an excess of the oxidant it is oxidised first to the aldehyde **octanal** and then further to the carboxylic acid **octanoic acid**. Only the acid reacts with hydrogencarbonate, giving brisk effervescence of carbon dioxide:

$$CH_3(CH_2)_6COOH(l) + NaHCO_3(aq) \rightarrow CH_3(CH_2)_6COONa(aq) + H_2O(l) + CO_2(g)$$

(1 mark octanal then octanoic acid, in that order; **1 mark** balanced equation with states.)

(c) **No colour change** — the dichromate stays **orange**, because 2-methylhexan-2-ol is not oxidised. It is a **tertiary** alcohol: the carbon carrying the –OH group is bonded to three other carbon atoms and so carries **no hydrogen atom**. Oxidation by dichromate requires the removal of that hydrogen along with the hydrogen of the –OH, so with no C–H bond on that carbon there is no reaction and the $Cr_2O_7^{2-}$ is not reduced. **(1 mark** no colour change, the solution stays orange; **1 mark** tertiary alcohol — the carbon bearing the –OH has no hydrogen to be removed, so it cannot be oxidised.)

Question 2

(a) The three careful titres 15.95, 16.00 and 16.05 mL agree within 0.10 mL, so they are **concordant** and are averaged; the rough trial (16.7 mL) is discarded. Average titre $= \dfrac{15.95 + 16.00 + 16.05}{3} = 16.00$ mL. **(1 mark: average titre** 16.00 mL **from the three concordant titres only.)**

(b) The permanganate is reduced to Mn^{2+} and the hydrogen peroxide is oxidised to oxygen gas:

$$2\,MnO_4^-(aq) + 5\,H_2O_2(aq) + 6\,H^+(aq) \rightarrow 2\,Mn^{2+}(aq) + 5\,O_2(g) + 8\,H_2O(l)$$

Atoms balance and the charge is $+4$ on each side; ten electrons are transferred. **(1 mark: balanced ionic equation, atoms and charge, with states.)**

(c) $n(MnO_4^-) = cV = 0.0250 \times 0.01600 = 4.00 \times 10^{-4}$ mol. From the equation $n(MnO_4^-) : n(H_2O_2) = 2:5$, so

$$n(H_2O_2)\text{ in the aliquot} = 5/2 \times 4.00 \times 10^{-4} = 1.00 \times 10^{-3}\text{ mol},$$

$$c(\text{diluted}) = \frac{1.00 \times 10^{-3}}{0.02500} = 0.0400\text{ mol L}^{-1}.$$

The 25.0 mL sample was diluted to 250.0 mL, a 10-fold dilution, so $c(\text{antiseptic}) = 0.0400 \times 10 = 0.400$ mol L^{-1}. With $M(H_2O_2) = 2(1.0) + 2(16.0) = 34.0$ g mol^{-1},

$$c(\text{antiseptic}) = 0.400 \times 34.0 = 13.6\text{ g L}^{-1}.$$

$\therefore$ the antiseptic contains 0.400 mol L^{-1}, or 13.6 g L^{-1}, of hydrogen peroxide. **(1 mark** $n(H_2O_2) = 1.00 \times 10^{-3}$ mol via the 2 : 5 ratio; **1 mark** $c(\text{antiseptic}) = 0.400$ mol L^{-1} with the dilution factor applied; **1 mark** 13.6 g L^{-1}.)

(d) A single reading carries an uncertainty of ± 0.05 mL (half the smallest graduation). A titre is the difference between two such readings, and the two uncertainties add, so the uncertainty in a titre is ± 0.1 mL — about

0.6 % of the 16.00 mL titre. The three concordant titres show the results are **precise** (they agree within 0.10 mL, so random error is small); to judge the result **accurate**, it would have to be compared with a true or accepted value — for example by titrating a standard of known hydrogen peroxide concentration, or by standardising the permanganate against a primary standard — since precise results can still be systematically wrong. **(1 mark** ± 0.05 mL per reading, doubled to ± 0.1 mL for the titre because it is a difference of two readings; **1 mark** concordant = precise, and accuracy requires comparison with a true/accepted value to detect systematic error.)

Question 3

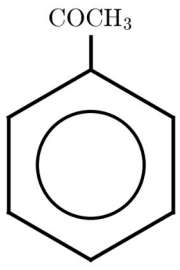
(a) The molecular ion is at m/z 120, so the relative molecular mass is 120. The absence of an $M+2$ peak shows the molecule contains **no chlorine or bromine** (only C, H and O, as stated). **(1 mark:** $M_r = 120$ **and no Cl/Br.)**

(b) The strong band at $1685\ \text{cm}^{-1}$ lies in the C=O range ($1670 - 1750\ \text{cm}^{-1}$), so the bond responsible is a **carbonyl, C=O**. There is **no** very broad absorption across $2500 - 3300\ \text{cm}^{-1}$, which is where the hydrogen-bonded O–H of a carboxylic acid appears, so **Q** is not a carboxylic acid; and there is no broad band over $3200 - 3550\ \text{cm}^{-1}$, the alcohol O–H range, so **Q** is not an alcohol either. (The sharp band near $3030\ \text{cm}^{-1}$ lies in the C–H range, $2850 - 3090\ \text{cm}^{-1}$; it is an aromatic C–H, not an O–H.) **(1 mark** 1685 assigned to C=O from the $1670 - 1750\ \text{cm}^{-1}$ range; **1 mark** neither O–H region shows a broad band, ruling out both the acid and the alcohol.)

(c) The 3 H **singlet** at $\delta = 2.6$ is a CH_3 group bonded **directly to the carbonyl carbon** (R–CO–CH_3, δ 2.0 – 2.9 ; a CH_3 bonded to oxygen would lie further downfield at δ 3.3 – 4.5), and it is a singlet because the adjacent carbon carries no hydrogen. The 5 H **multiplet** at $\delta = 7.4 - 8.0$ is the signal of a **monosubstituted benzene ring** (five aromatic hydrogens). **(1 mark:** δ 2.6 **singlet** = CH_3 **next to C=O;** δ 7.4 – 8.0**, 5 H** = **monosubstituted benzene ring.)**

(d) The 5 H aromatic signal gives a C_6H_5- group and the 3 H singlet a CH_3-, so **Q** contains 8 hydrogens; with $M_r = 120$ this fixes the molecular formula as C_8H_8O (96.0 + 8.0 + 16.0 = 120.0). Only **one** oxygen is present, so **Q** cannot be an ester or an acid (both need two) — the carbonyl must be a **ketone**. The degree of unsaturation is $1/2(2 \times 8 + 2 - 8) = 5$: four for the benzene ring and one for the C=O. The base peak at m/z 105 is a loss of $120 - 105 = 15$ (CH_3), leaving the benzoyl acylium ion $C_6H_5CO^+$; the peak at m/z 77 is a further loss of $105 - 77 = 28$ (CO), giving the phenyl cation $C_6H_5^+$ — the benzene ring. Joining C_6H_5-, –CO– and –CH_3 gives $C_6H_5COCH_3$.

1-phenylethanone



∴ **Q** is **1-phenylethanone**, $C_6H_5COCH_3$ ($M_r = 120$; also known as acetophenone). **(1 mark** fragments assigned — $105 = C_6H_5CO^+$ (loss of CH_3), $77 = C_6H_5^+$ (loss of CO); **1 mark** correct structure drawn and named as 1-phenylethanone — accept phenylethanone, acetophenone or methyl phenyl ketone.)

Question 4

(a) The carbon bearing –OH is a **hydroxyl** group (alcohol); the terminal –COOH is a **carboxyl** group (carboxylic acid). **(1 mark: hydroxyl and carboxyl.)**

(b) The chiral centre is the carbon bearing the –OH (C2). It is bonded to four different groups: –H, –OH, –COOH and $-CH(CH_3)_2$ (an isopropyl group). There is **one** chiral centre. The $-CH(CH_3)_2$ carbon is bonded to –H, –CH(OH)COOH and **two identical** $-CH_3$ **groups**; because two of its groups are the same, it is not bonded to four different groups and so is not a chiral centre. **(1 mark** C2 bonded to –H, –OH, –COOH, $-CH(CH_3)_2$ = one chiral centre; **1 mark** the isopropyl carbon has two identical CH_3 groups, so it is not chiral.)

(c) The molecule has one chiral centre, so it exists as two **enantiomers** — non-superimposable mirror images. The biological targets it acts on (enzymes and receptors) are built from chiral amino acids and are themselves chiral, with a definite handedness. Only the enantiomer whose three-dimensional shape **matches** the target can bind to it (like a hand fitting only the correctly handed glove), while the mirror-image enantiomer fits poorly or not at all. Because binding is what produces the biological response, the two enantiomers therefore produce **different physiological effects** — commonly one is active and the other is inactive or harmful. **(1 mark** the chiral target distinguishes the two mirror images (only one fits); **1 mark** so the enantiomers differ in effect — one active, the other inactive/harmful.)

Question 5

(a) A solution containing **none** of compound **P** gives **no peak** — zero peak area at zero concentration — so the point $(0, 0)$ lies on the line and the calibration line passes through the origin. **(1 mark: zero concentration gives zero peak area, so the line passes through the origin.)**

(b) Peak area is proportional to concentration, so the line runs through the origin. Using the top point,

$$k = \frac{\Delta A}{\Delta c} = \frac{300 - 0}{20.0 - 0} = 15.0 \text{ area units per mg L}^{-1}.$$

(1 mark gradient calculated from the origin and a data point; **1 mark** $k = 15.0$ with units area per mg L^{-1}.)

(c) Concentration in the diluted, injected sample: $c_{\text{diluted}} = \frac{A}{k} = \frac{210}{15.0} = 14.0 \text{ mg L}^{-1}$ (interpolation, within the standards). Multiplying by the 5.0-fold dilution factor:

$$c_{\text{drink}} = 14.0 \times 5.0 = 70.0 \text{ mg L}^{-1}.$$

Mass in a 250 mL serve: $m = c \times V = 70.0 \text{ mg L}^{-1} \times 0.250 \text{ L} = 17.5 \text{ mg}$. **(1 mark** $c_{\text{drink}} = 70.0 \text{ mg L}^{-1}$ (dilution factor applied); **1 mark** $m = 17.5 \text{ mg}$.)

(d) A peak area of 360 lies **above** the most concentrated standard (area 300 at 20.0 mg L^{-1}). The detector response is only known to be linear over the calibrated range, and it may curve or saturate beyond it, so extending (extrapolating) the line could give a wrong value. Instead, the sample should be **diluted by a known factor** and re-run so that its peak area falls **within** the calibrated range, then the measured concentration multiplied back by that factor. **(1 mark: response may be non-linear beyond the top standard, so dilute by a known factor and re-run within the calibrated range.)**

Question 6

(a) An enzyme increases the rate by providing an **alternative reaction pathway of lower activation energy**, so a greater fraction of collisions has enough energy to react. It does **not** change ΔH (ΔH is unchanged at -26 kJ mol^{-1}). **(1 mark: alternative lower-E_a pathway AND ΔH unchanged.)**

(b)

(i) The enzyme lowers the forward activation energy by $64 - 27 = 37 \text{ kJ mol}^{-1}$.

(ii) On an energy profile the reverse activation energy is measured from the product level: $E_a(\text{reverse}) = E_a(\text{forward}) - \Delta H = 27 - (-26) = 53\ \text{kJ mol}^{-1}$. **(1 mark** lowering $= 37\ \text{kJ mol}^{-1}$; **1 mark** reverse $E_a = 53\ \text{kJ mol}^{-1}$.)

(iii) A competitive inhibitor is a molecule whose shape is complementary to the enzyme's **active site**, so it binds there by the same **lock-and-key** fit as the substrate. While the inhibitor occupies the active site, the real substrate **cannot bind**, so fewer enzyme–substrate complexes form. With a fraction of the active sites blocked at any instant, fewer product molecules form per second and the reaction rate falls. **(1 mark** the inhibitor binds the active site by a complementary lock-and-key fit; **1 mark** the substrate is blocked, so fewer enzyme–substrate complexes form and the rate falls.)

Question 7

(a) Because the oil does not mix with water, the boiling oil and the boiling water each exert their own vapour pressure, so the mixture boils when the **sum** of the two reaches atmospheric pressure — that is, at a temperature **below** $100\,^{\circ}\text{C}$ **and well below the oil's own boiling point**. Simple distillation would have to heat the oil to its own (much higher) boiling point, at which a heat-sensitive oil would decompose. **(1 mark: the immiscible oil co-distils with the steam, so the mixture boils below the oil's own boiling point, avoiding the temperature at which simple distillation would decompose it.)**

(b) Because *like dissolves like*, a **non-polar** organic solvent (for example hexane or dichloromethane) should be chosen: a non-polar solute is held in place by dispersion forces, and only a non-polar solvent can form comparable dispersion forces with it, so the target dissolves while the polar and ionic components of the peel — and any water present — do not. The extract is then separated from the solid, and the solvent is removed by **distillation** rather than simply being evaporated to the air, so that the condensed solvent is collected and re-used and the active ingredient is left behind. **(1 mark** a non-polar solvent chosen via *like dissolves like*, justified by matching intermolecular forces; **1 mark** the solvent is distilled off and condensed so that it can be recovered and re-used, leaving the compound.)

(c) *Model answer (rubric).* **Renewable feedstocks:** the active ingredient is obtained from a plant, a feedstock that can be regrown, rather than from a finite fossil resource, which supports sustainable long-term supply. **Designing safer chemicals / safer solvents:** choosing a low-toxicity, less hazardous extraction solvent (and, where possible, recovering and reusing it) reduces harm to workers and the environment and cuts waste. **Conclusion:** obtaining the medicine from a renewable plant source using a safer solvent is the more sustainable option, provided the plant is harvested sustainably and the solvent is recovered rather than discarded. **(1 mark** two green chemistry principles applied with evidence (renewable feedstocks; designing safer chemicals/solvents); **1 mark** a concluding statement that weighs the evidence.)