LAB-Biochemistry

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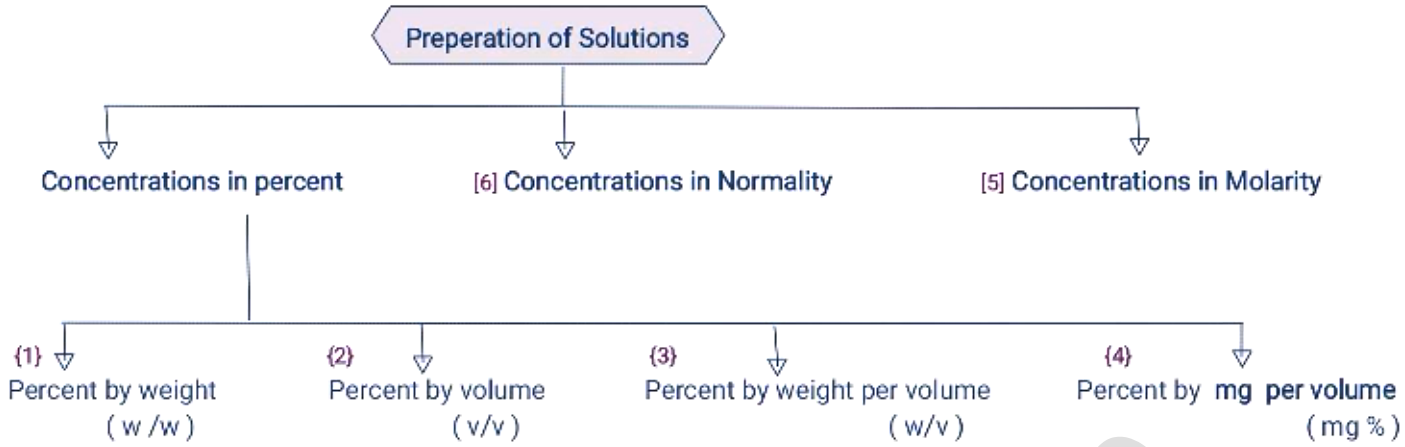
[Exp.1] Calculation in Biochemistry

(مرفق مع الماتيوال)



{1} شرح :

(X g of solute / 100 g of solvent) × 100 = X %

10 % (wt / wt) solution

10 g of solute & 90 g of solvent .

E.X :- 30 gm Hydrocortisone (من ال solution)
1 % Hydrocortisone

بالنسبة 1 % (wt / wt) --> لذالك التعامل مع الأوزان(wt)

1 g → 100 g
X g → 30 g

X * 100 = 1 * 30

X = 1 * 30 / 100 = 0.3 gm of hydrocortisone

{2} شرح :

(X mL / 100 mL of total solution) × 100 = X %

10 % (v/v) solution

يتم إضافة 10mL من ال (stock solution = concentrated solution)
إلى 90mL من ال dilutent المخفف .

E.X :-

بدنا نحضر 200mL من ال topical product
ضاف 6mL من ال phenol

percentage (v/v) of phenol in the topical product ??

يعني آخر إنه...

6 mL phenol in 200 mL topical product
how much phenol in 100 mL of this solution = ?? mL

6 mL phenol ⟶ 200 mL of topical solution

"z" ?? mL phenol ⟶ 100 mL of topical solution

$$\frac{6 * 100}{200} = \frac{z * 200}{200}$$

z = 3 mL of phenol in 100 mL solution . (topical solution)

or 3 %

{3} شرح :

(X g of solute / 100 mL total volume) × 100 = X %

E.X (1) :- 30 % NaCL solution

يحضر من خلال إضافة 30g of salt على وعاء يحتوي على 50mL water .

" Quantity Sufficent (QS) to measure 100 mL "

لا تضيف ال 30gm salt إلى 100mL water خطأ

لماذا ؟! لأنه رح يطلع المحلول الناتج , مخففا أكثر من ما هو متوقع

the resulting solution would be more dilute than planned .

E.X (2) :- m_{NaCL} = ?? gram in 500 mL of 0.9 % NaCL solution .

0.9 g of NaCL ⟶ 100 mL solution

"y" ?? g of NaCL ⟶ 500 mL solution

$$\frac{0.9 * 500}{100} = \frac{y * 100}{100}$$

y = 4.5 gm of NaCL

E.X (3) :- $m_{detrose}$ = ?? grame

3000mL مطلوب تحضير of 10 % solution

10 g of destrose ⟶ 100 mL solution

"z" ?? g of destrose ⟶ 3000 mL solution

$$\frac{z * 100}{100} = \frac{10 * 3000}{100}$$

z = 300 g of de trose

300 grum of detrose is need of to prepare 3000 mL of a 10% solution

{4} شرح :

mg per volume | mg % | ← تستخدم في المختبرات السريرية (clinical Laboratories)

(mg / mL)

mg of solution / 100 mL of total volume .

E.X :- 225 mg of glucose per 100mL of blood serum .

[5] شرح :

$$\text{Molarity (M)} = \frac{n}{V_{solution(L)}} = \frac{\frac{mass}{M.mass}}{\frac{V}{1}} = \frac{mass}{M.mass \times V_{(L)}}$$

E.X :- n_{NaCl} = ?? mole in 150 mL of 1.5 M solution

$$M = \frac{n\ ??}{V_{(L)}} \qquad n = M \times V_{(L)} = \frac{1.5\ mol}{1\ L} \times 150\ mL \times \frac{1\ L}{1000\ mL} = 0.225\ mole\ NaCL .$$

ولأنه إحنا بيفيدنا بالشغل العملي بالاب الكتله لل NaCL أكثر من عدد مولاتها . لذلك حسب كمية ال NaCL بال gram التي تستخدم معنا .

$M.m_{NaCL} = 58.5\ g / moL$

$$0.225\ moL\ NaCL \times \frac{58.5\ g\ NaCL}{1\ moL\ NaCL} = 13.1625\ g\ of\ NaCL$$

m	milli	10^{-3}	n	nano	10^{-9}
μ	Micro	10^{-6}	p	pico	10^{-12}

- $1\ m\ \mu \xrightarrow{\text{تضرب}} 1 * 10^{-3}\ \mu$

  $1\ m\ mol/L \xrightarrow{\text{تضرب}} 1 * 10^{-3}\ mol/L$ أو نستطيع التحويل عنها بطريقة أخرى

  وهي .....

$$\frac{1 \times 10^{-3}\ mol}{1\ L} * \frac{1\ L}{1000\ mL} = 1 * 10^{-6}\ mol/mL = 1\ \mu\ mol/mL$$

- $1\ \mu M = 1 * 10^{-6}\ (M) = \frac{1\ M\ mol}{L}$

$$\frac{1\ \mu\ mol}{L} = \frac{1 \times 10^{-6}\ mol}{1\ L} * \frac{1\ L}{1000\ mL} = 1 \times 10^{-9}\ \frac{mol}{L} = 1\ n\ mol/L$$

وكذلك التحويل عنها أيضاً من طريق الـ .....

- $1\ nM = 1 * 10^{-9}\ M = \frac{1 * 10^{-9}\ mol}{\cancel{1\ L}} * \frac{\cancel{1\ L}}{1000\ mL} = 1\ p\ mol/L$

[6] شرح :

E.X :- n = ?? moles M = ?? 0.5 N solution H_2SO_4 ($2H^+$) V = 500 mL .

$$N = n \times M ?? \longrightarrow M = \frac{N}{n} = \frac{0.5}{2} = 0.25\ M$$

$$M = n ?? \longrightarrow n = M \times V = \frac{0.25\ moL}{1\ \cancel{L}} \times 500\ \cancel{mL} \times \frac{1\ \cancel{L}}{1000\ \cancel{mL}} = 0.125\ moL$$

Example .(1) :- 50 mL of 2.0 N solution

نخفف من 5.0 N of stock solution

$$M_1 \times V_1 ?? = M_2 \times V_2$$

$$\frac{1\ \cancel{moL} \times \cancel{5\ N} \times V_1\ ??}{\cancel{5}} = \frac{\cancel{1\ moL} \times 2\ \cancel{N} \times 50\ mL}{5}$$

V_1 = 20 mL of stock solution add 30mL of diluent

(عشان يصير حجم الكلي 50ml)

Example . (2) :- V_1 = 7 mL V_2 = ?? mL

M_1 = 5 % (w/v) solution M_2 = 3% (w/v) solution

$$M_1 \times V_1 = M_2 \times V_2 ?? \longrightarrow \frac{5\ \% \times 7\ mL}{3\ \%} = \frac{\cancel{3\ \%} \times V_2}{\cancel{3\ \%}}$$

$$V_2 = 11.66 \sim 11.7\ mL$$

to 7 mL of initial solution add 4.7 mL of diluent.

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تفريغ منيح ومفيد :-

التجربة الأولى من لاب البيوكم ، بسيطة وكلها حسابات وأغلبها أشياء أخذناها من قبل .
شغلنا بهاذ الاب على المحاليل ، فيعني لازم تستخدم طرق لحتى تعبر عن تركيز هاي المحاليل
يعني النسبة المئوية للمحلول ونعبر عنها perent solution بالطرق التالية :-

Concentrations in percent

يعني ان جبت مقدار من أي مادة سائلة وذوبتها بمقدار مُعين من مادة ثانية سائلة ، على سبيل المثال جبت 10mL كحول و 90mL ماء
وذوبتهم ببعض فصار عندي محلول .
ستكون النسبة 10% ، من وين اجت ؟!
كحول 100mL / من التوتل 100 (ماء + كحول).

2 - Percent by weight (w/w)

نفس الحكي إلّي فوق ونفس القانون بس هون بدنا نحط كتلة مادة مُعينة على كتلة من مادة ثانية
أو ممكن نفس المادة المهم يكون عندنا مذيب ومذاب .

3 - Percent by weight per volume (w/ v)

برضو نفس الإشي بس الفرق ضيف مادة على حجم مادة .
حكالك مثلاً ذوب سكر في ماء بنسبة 45% بتجيب 45g من السكر وبتذوبهم بمقدار مُعين من المي ، بعدين بتكمل الحجم ل 100mL .

4 - Percent by mg per volume (mg %)

نفس فرع ثلاثة بس وحدة المذيب mg وليس g .

Concentration in molarity

هي أكثر الطرق تعبيراً عن التركيز في البيوكم .
يعني جيب مادة وحطها بمادة ثانية نفس الكلام ، لأنه كله يعبر عن التركيز بس هون القانون أنه بنحسب عدد المولات المادة سواء مركب أو أيون ، بس ما تكون حمض أو قاعدة .

من خلال الكتلة على الكتلة المولية وبتقسمها على الحجم ، بس أنتبه --> وحدة الحجم باللتر مو بالملي لتر
مثل نسبة التركيز اللي بنقطة 1,3,4

Concentration in Normality

نفس المولارية بس الفرق هون عدد مولات هم عدد مولات أيون الهيدروكسيد OH- إذا كان قاعدة
أو عدد مولات البروتون H+ إذا كان حمض .

القانون اللي بالمانيوال فلسفه ، فإنه هوه نفس قانون المولارية بس الفرق هون إنه عدد مولات OH- or H+ ، وبنضربهم بمعاملاتهم .
مثلاً عندنا H2SO4 لما تتفاعل مع المي بيعطينا 2mole of +H ، فإنه بتضرب ب 2 يعني المولارية مضروبه ب 2 .

Dilutions

بنجي على التخفيف ، هوه إنه يكون عندك محلول مُركز
stock solutions (concentrated solutions)
وبدك تخفف تركيزه عن طريق إضافة حجم نفس المادة عليه
طيب ليش نفس المادة ؟!
لأنه إحنا بدنا نخفف تركيز فقط بدون ما نغير خصائصه ، ونفاعل مواد مع بعض .

بما إنه نفس المادة حتضل عدد المولات ثابتة قبل التخفيف وبعده
فالقانون حيكون إنه عدد المولات قبل يساوي عدد المولات بعد

$$Moles_1 = Moles_2$$
$$M_1 \times V_1 = M_2 \times V_2$$

M_1 : concentration of initial solution تركيز المادة المركزة
V_1 : volume of initial solution حجم المادة المركزة

M_2 : concentration of desired (final , diluted) solution تركيز المادة المخففه
V_2 : volume of desired (final , diluted) حجم المادة المخففة

The " X " Factor

بيحكيلك هون إنه stock solution الموجود عندك ممكن إنه يحتوي على أكثر من مركب
يعني أكثر من مادة كيميائية ، وهاذ طبيعي لأنه المحلول هو عبارة عن مزيج المواد .

وطبعاً بدك تكون على علم إنه أكيد كل مادة إلها تركيز يختلف عن المادة الأخرى في نفس المحلول . على سبيل المثال عنا مركب فيه أسيتون بتركيز 2M
وكحول بتركيز 1M وبرضو حمض بتركيز 0.5M وبكميات مختلفة .

وبالمنطق هاذ صح لأنه مثلاً حمض ما رح يكون تركيزه مثل الأسيتون
ولا مثل الحمض ما رح يكون تركيزه مقل الأسيتون ولا الكحول لأنه مادة حارقة فالإختلاف التركيز والكميات
يهدف إلى إنتاج المحلول إلي لازمني بالشغل .
فال X هي عبارة عن تركيز المادة الكيميائية في المركب والقانون يكون بهاذ الشكل :-

Volume of stock solution (A) needed = Volume of 1X needed / concentrated factor

حجم المحلول المركز ، الذي بدنا نخففه — لحتى ننتج الحجم المطلوب ، بضيف

diluted soln. الحجم المخفف

إنه حجم المحلول المركز إلي معنا وإلي بدك تخففه لحتى تنتج الحجم المطلوب ، يعني الحجم المخفف
بتقدر تطبق على هذا القانون أو قانون المولات قبل التخفيف يساوي عدد المولات بعد التخفيف .

$M_1 \times V_1 = M_2 \times V_2$

المثال إلي بالمانيوال :-

V_2

التركيز الذي بدنا الوصول إليه ، الحجم الذي بدنا نحضره بحاجته

Let's say you need 50 mL of 1X running buffer to perform an experiment
& the stock concentration is 50X .

المركز ، الأول

بدنا نحضر الحجم الذي أخذناه V_1 من المحلول المركز .

50 mL ⟶ 50X
"Z" ?? mL ⟶ 1X

"Z" = $\frac{50\ mL}{50X}$ = 1mL is required From A + 49 mL diluent

(diluent المخفف هو 49mL اللي استخدمنا)

or you can use :-

$M_1 * V_1 = M_2 * V_2$

$\frac{50X * V_1}{50X} = \frac{1X * 50mL}{50X}$ ⟶ V_1 = 1 mL is required From A + 49 mL diluent.

بيحكيلك إنه إحنا بنحتاج 50 مل بتركيز 1X وكان عندنا تركيز المحلول المركز 50X من خلال القانون ، تطبيق مباشر فبينتج عندك حجم المحلول المركز ،
أو على الأقل قانون ثاني تاع المولات إلي حكيناه فوق .

ووضحلك إنه طلع حجم المحلول المركز 1mL ، وأنا بدي 50mL مخفف يعني بضيف على 1 + 49mL
عشان بيتخفف المحلول ، وبالمنطق المحلول رح يقل تركيزه .
أو طريقة سريعة ثانية --> التركيز كان 50 وصار 1 ، يعني خفف بمقدار 49 مرة
فزاد الحجم بمقدار 49 مرة .

Dilutions

There are two main methods for interpreting dilutions (تفسر التخفيف) 1 to 10 :-

[A] means that there is one part of concentrate in 10 parts of final solution .

(1 mL of concentrate & 9 mL solvent with a final volume of 10 mL)

[B] means that there is one part of concentrate to 10 parts of solvent .
Or (1 in 11)

مثلاً : (1mL of concentrate & 10 mL vehicle with a final volume of 11 mL)

--> على ورقة المانيوال

مثلاً عندك كاسة شاي بتسع 100mL وبدهم بيخففوها لمقدار 5 : 2 يعني 2to5

$\frac{100}{5}$ = 20 mL وهكذا بنكون طبقنا على قانون volume of each part .

وبما إنه لمقدار 5 : 2 يعني حيكون جزيئين من الشاي المركز - وثلاث اجزاء من المي لحتى يكملو الخمس اجزاء كاملين وبيكون الحجم كامل مكمل .

وبما إنه الجزاء الواحد يساوي 20mL ، إذا رح يكون الشاي المركز بمقدار 40mL والماء المغلي بمقدار 60mL .

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Serial dilution

برضو طريقة التخفيف وتختص بالكميات الصغيرة للأبحاث أو أكتشاف حلول أو أسباب لأمراض معينه ، أو لرسم منحنيات يعني أي شيء يتطلب أحجام وتراكيز قليلة .

يعني بيكون عندنا محلول مُركز وبدنا نخففه ، بس مش شرط نزيد حجمه لأنه ممكن نقلله أو نجعله ثابت مع تقليل التركيز وهاذ هو الفرق بينه وبين التخفيف الطبيعي .

إذا أجينا بدنا نعرفه --> بنحكي إنه تخفيف متتالي يعني أكثر من تخفيف لكن في العملية نفسها .

طيب شو خطوات هذه العملية ؟!

أولًا بنجيب عدد من الأنابيب أحكي مثلًا خمسة أنابيب ، وبحط بكل واحد مقدار مُعين من المادة ، أحكي مثلًا 100mL (وكلهم نفس التركيز)

نأتي على أول أنبوب ... وبضيف عليه 100mL بتركيز أقل لحتى يتخفف ، فصار في الأنبوب الأول 200mL مُخفف ... فبروح بوخذ منه 100mL مخفف وبضيفهم على الأنبوب الثاني .

وبتالي الأنبوب الثاني ... تخفف أكثر من الأنبوب الأول ، وصار حجمه 200mL .

--> فبوخذ منه برضو 100mL مخفف ، وبحطهم على الأنبوب الثالث وهكذا حتى نصل إلى الأنبوب الخامس والأخير ، بعدما صار 200mL بروح بوخذ منه 100mL وبحطهم على جنب هذول ما بدي إياهم .
فصار عندي محلول مخفف بالتركيز المطلوب وبقدر أتحكم بحجمة مثل ما بدي .

طيب فهمنا بس لازم نحل رياضيات ، عشان نفهم أكثر بيحكيلك في قانون **dilution factor = C_1 / C_2** يعني تركيز الأول على التركيز الثاني .

نجي على المثال الذي شرح سابقًا ، الأنبوبة الأولى خففت إلى النص يعني 100/200 = 0.5
والثانية إلى النص برضو والثالثة والرابعة والخامسة ، أو بقدر بنحكي نسبة التخفيف الأنبوبة الأولى كان نص ، والثانية ربع ، طيب كيف ربع ؟!

أنا بحكيلك ؛ لأنها نسبة الأنبوبة الثانية صح هي نص ولكن برضو خففت بنص تخفيف الأنبوبة الأولى ، يعني النص ربع .

والثالثة ثُمن لأنه بالأصل هي نص وأجاها ربع نص الربع يساوي ثُمن وهكذا ...
لو بدنا نحسب التخفيف كامل كتوتل بنحكي ...

$$0.5 \times 0.5 \times 0.5 \times 0.5 \times 0.5 = 0.03125$$

ضرب وليس جمع 🙂

فإحنا بنستخدم هاذ التوتل لحتى نحسب التركيز من المحلول المركز الأصلي stock solution يعني نطلع التركيز النهائي إلّي بدنا إياه ، بنحكي تركيز المحلول المركز وضليت اخفف وألعب فيه لحد ما وصلت للي بدي إياه .

وهاذ المثال دليل على كلام إلّي مفرغ ... فوق إنه الحجم ما زاد عنا مع إنه التركيز قل وهاذ بسبب التخفيف المستمر والمتكرر وأهمية هاي الطريقة ببساطة لو بدي أقلل تركيز بنسبة كثير كثير قليلة ، أكيد ما رح أقعد أجيب كميات كثير كثير كبيرة لحتى أخففه.

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إذا كنا نرغب بتحديد أحجام كل جزء — يعني إنه نخفف المحلول المركز إلى 1X من التركيز الحالي

If you wish determine the volumes of each part. A dilution of 1:X means your concentrated solution should be diluted to 1Xth of its current concentration.

$$\text{Volume of each part.} = \frac{\textit{total volume}}{\textit{total parts "X"}}$$

Add the concentrated solution to (X-1) volumes of diluent. ***Always add a small amount to a larger amount.***

Example: a dilution of 1:5, means that the concentrated solution should be diluted to 5^{th} of its current concentration

I have 100 ml solution of concentration 10%,
Volume of each part= 100/5= 20 ml for each part
Add 20 ml from concentrated stock to X-1 parts
i.e. 5-1 = 4 parts
4 parts* volume of each part
= 4* 20 ml = 80 ml diluent (final solution concentration is 2%)

1 : 5 —يعني→ 1 to 5 — معناته بدي أحصل على 5ml بالزيادة

| 100 ml solution ~ concentration 10 % | standard — هاد الكلام بدي ياه |

هلأ بطبق القانون مباشرة — Volume of each part = $\frac{\text{total volume}}{\text{total part}} = \frac{100 \text{ mL}}{5 \text{ mL}} = 20$ ml

1 part * 20 mL = 20 mL of concentration
4 part * 20 mL = 80 mL of diluent
100 mL of final solution

لو احتجت تحققي نسبة تركيزه ، بتطلع هيك:

100 mL → 10 %
20 mL → ?? %

= 2 %

Serial dilution 📌

التخفيف على مراحل
يتم إستخدامها عندما تحتاج إلى حجم أو كمية صغيرة جدًا لا يمكن قياسها

Serial dilution is used when you need a volume or amount that is too small to measure
(example , prepare 0.00007 mg/ml)

options in this case is :

1- Prepare larger volumes than what you need.

2- Make a small volume of a higher concentration and then perform multiple dilution steps (serial dilution) to reach the required concentration.

أعمل حجم صغير بتركيز عالي ثم قم بتنفيذ خطوات التخفيف المتعددة للوصول إلى التركيز المطلوب

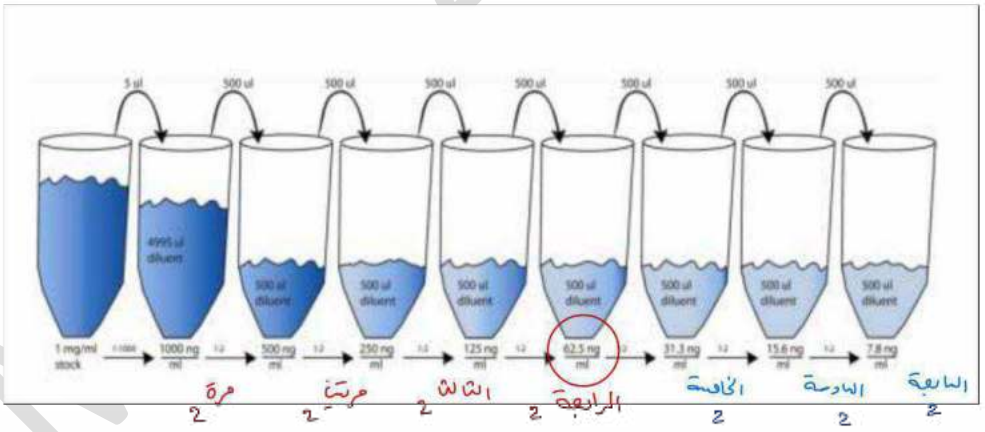
A serial dilution is a stepwise series of dilutions which starts with a small amount of starting material and amplifies the dilution factor serially by using diluted material as a source for subsequent dilutions.

Serial dilution advantages include :

1- Saving reagents/spaces
2- Used in experiments which require standard curve.

✓ Serial two-fold & ten-fold dilutions are commonly used to prepare diluted analytes.
Serial dilutions are also commonly used to avoid having to pipette very small volumes

تستخدم التخفيفات التسلسلية ذات الشقين والعشرة أضعاف لتحضير التحليلات المخففه
تستخدم التخفيفات المتسلسلة أيضًا بشكل شائع لتجنب الإحتياج لإستخدام ماصة كميات صغيرة جدًا



☆ لفهم الطبخه هون بالرسمه لمعرفة عدد مرات التخفيف الي لازم اعملها بالتجربه عندي بالاب ....

عندي قوانين مهمه جداً لازم اعرفها منيح

☆ **DF total = DF (dilution factor) × Z** (هاي الرمز اخترعتو من عندي 😉)
= concentration of stock solution / concentration of final solutio
(التركيز المخفف الي بدي احصل عليه بالآخر)

☆ $Z = 2^{n}$ طيب أنا الآن شو بدي استفيد من هذول القانونيين 🤔

هسى بنجي على الرسمه أو بدونها أحسن - بس تتبع أفكار الحل على الرسمه لتكتمل الأمور أحسن وأحسن 😉

◇ عندي سؤال مبلش بتركز 1mg /ml من ال stock solution ← حسب وحدة التركيز عندي للتخفيف النهائي
الي هيه مُعطاه بال ng /ml عشان هيك بدي احول (ال mg لل ng) وحدة الكتله
ولاحظ إنه ما بلشت بوحدة الحجم ضلت ml

$$1\,mg = \frac{1 * 10^{-3}\,g}{10^{-9}} = 1 * 10^{6}\,ng = 1000\,000\,ng$$

وبدي أصل لتركيز نهائي concentration of final solution مثلاً مقداره 62.5ng/ml

هسى تركيز أول تخفيفه primary concentration ، بيكون معطيني كم بده وهون بالسؤال 1000ng/ml

بدي أعرف اولاً ال DF ، طيب كيف !؟ من القانون تاعو

DF (dilution factor) = concentration 1 ÷ concentration 2
= concentration of stock solution. ÷ concentration of primary
= 1000000 ÷ 1000 = 1000

DF total = DF × z = concentration of stock solution / concentration of final solution
1000 × z = 1000000 / 62.5

Z = 16

$(2)^4 = 16 = Z = 2^n$
$n = 4$ مزاااح

◇ عندي مثال آخر لنتمكن من الفكره منيح ، سؤال مبلش بتركيز لل stock solution = 1000000ng/ml

وبدي أصل لتركيز نهائي concentration of final solution مثلاً مقداره 7.8ng/ml
وعندي ال DF = 1000ng/ml (هاي من الفرع الي فوق نفسها)

DF total = DF × z = concentration of stock solution ÷ concentration of final solution
1000 × z = 1000000 ÷ 7.8

Z = 128.205 = 128

$(2)^7 = 128 = Z = 2^n$
$n = 7$ صحتك
مزاااح

حاولت قد ما أقدر افهم الموضوع وبصيغه بطريقة سهله ويسيره لتناسب الجميع للمثالين الي بالمانيوال موجودين على شكل حكي عن الرسمه طبعاً الشغل العملي سهل وحل الأسله مباشره ومش صعبه ، وبالتوفيق للجميع ✨

Lab 1 Report sheet

Calculations

1- What is the % (w/v) of 1.0 L solution that contains 5.0 g of NaCl?

5 g NaCl → 1000 mL
"z" ?? g → 100 mL

$?\ "z" = \frac{5 * 100}{1000} = 0.5$ g of NaCl in 100 mL or 0.5 % (w/v)

2- Find the percent volume (v/v%) of ethanol in a solution prepared by diluting 30.0 mL of ethanol to 250 mL.

30 mL ethanol → 250 mL solution
"x" ?? mL ethanol → 100 mL solution

$x = \frac{100 * 30}{250} = 12$ mL of ethanol in 100 mL solution

or 12 % (v/v)

3- Carry out the following calculations:

a) How many moles of NaCl (MW = 58.5) are required to prepare 100 mL of a 1.6 M solution?

$M = \frac{n\ ??}{v} \longrightarrow n = M * v = \frac{1.6\ mol}{1\ L} * 100\ mL * \frac{1\ L}{1000\ mL} = 0.16$ moles

b) How many grams ?! of NaCl are required, MW NaCl= 58.5 g/mole?

0.16 moles of NaCl * $\frac{58.5\ g\ of\ NaCl}{1\ moles\ NaCl}$ = 9.36 gram of NaCl

4. How many moles ?? of HCl are present in 50 mL of 3.0 M HCl solution?

$n_{HCl} = M * v = \frac{3\ mol}{1\ L} * 50\ mL * \frac{1\ L}{1000\ mL} = 0.15$ mole of HCl

5. What are the normalities N of **(a)** 0.213 M HCl, and **(b)** 0.010 M $Ca(OH)_2$?

a) HCl: $N = n * M = 1 * 0.213$ $M = 0.213$

b) $Ca(OH)_2$: $N = n * M = 2 * 0.010$ $M = 0.020$

6. How would you prepare 240 mL of a 1.0% (w/v) solution of glucose from a 6.0% (w/v) solution? How would you prepare 2.5 L of a 2% (w/v) glucose solution from 5% (w/v) aqueous glucose?

① $C_1 * V_1$?? $= C_2 * V_2$

$\frac{6\ \%}{6} * V_1 = \frac{1\ \%}{6} * 240\ mL$

V_1 = 40 mL of solution of glucose

(add 200 mL of diluent)

② $C_1 * V_1$?? $= C_2 * V_2$

$\frac{5\ \%}{5\ \%} * V_1 = \frac{2\ \%}{5\ \%} * 2.5\ L$

V_1 = 1 L of glucose solution (or 1000 mL)

(add 1.5 mL of diluent)

تجربة 2
تطبيق حسابات
على قوانين وحسابات
تجربة 1 سبق

Experiment 2

Experimental applications on solution preparation and dilution.

In this laboratory you will be preparing and diluting solution as per given in the lab. In all steps distilled water will be used as a diluent

Using the following materials, prepare the solution below

Materials

- NaCl
- KCl
- Red buffer
- 10% bromophenol blue stock (3mls)
- H_2SO_4
- Volumetric flasks (50 mL)
- Digital balance
- Graduated pipettes
- Pipette filler.
- Distilled water
- Test tubes
- Erlenmeyer flask
- Beaker

اسألة التركيز
التخفيف ممنوع ، نحلها نسبة
وتناسب ... الحل الصح على

$M_1 \times V_1 = M_2 \times V_2$

1- Prepare 4 mls of 0.078% of bromophenol blue, given that the stock concentration is 10% bromophenol blue. Write down the procedure you followed in your report sheet.

2- Prepare 50 ml of 20% w/v NaCl?

3- Prepare 50 ml of 136.8 mM NaCl using the stock solution (20 % w/v) you prepared in the last step, knowing that the molecular weight of NaCl is 58.44 g/mole.

4- You have a 10X red buffer, prepare 10 ml of 1X buffer.

5- Prepare 50 ml of 150 mM KCl knowing that the molecular weight of KCl is 74.5513?

6- Prepare 50 ml of 0.2 N H_2SO_4, from a stock of 2 M H_2SO_4 found in the fume hood?

NaCl
20.04849

Lab 2 Report sheet

Experimental applications on solution preparation and dilution

Student name:

Student ID: **section:**

A. Write down the calculation that you used to prepare the solutions (mention volumes, weight, glassware used and any other steps involved (you can draw schemes)?

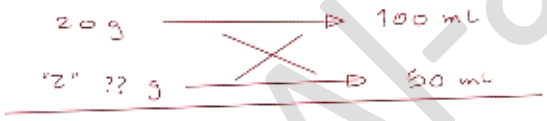
1- Solution 1

$C_1 * V_1 ?? = C_2 * V_2$

$\frac{10\% * V_1}{10\%} = \frac{0.078\ \% * 9\ mL}{10\ \%}$

$V_1 = 0.0312\ mL$

2- Solution 2



$Z = \frac{20 * 50}{100} = 10\ g$ of NaCl.

3- Solution 3 $136.8\ mM\ NaCl = 136.8 * 10^{-3}\ M\ NaCl$

molarity

$\frac{136.8 * 10^{-3}\ mol}{1000\ mL} * \frac{58.44\ g}{1\ mol} = 0.00799 \approx 0.008\ g/mL$ → concentration C_2: $0.008 * 100 = 0.8\%$ (w/v)

$V_2 = 50\ mL$
$C_1 = 20\ \%$
$V_1 = ??$

$C_1 * V_1 ?? = C_2 * V_2$

$\frac{20\% * V_1}{20\%} = \frac{0.8\% * 50\ mL}{20\%}$ → $V_1 = 2\ mL$ (take 2 mL From the 20% stock & dilute to 50 mL)

4- Solution 4

$C_1 * V_1 ?? = C_2 * V_2$

$\frac{10\% * V_1}{10\%} = \frac{1\% * 10\ mL}{10\%}$

$V_1 = 1\ mL$ is required From red buffer

add 9 mL diluent.

5- Solution 5

$m_{KCl} = M_{KCl} * U_{soln.} * M.m_{KCl} = 150 * 10^{-3} * 50 * 10^{-3} * 74.5513$

$= 0.55913$ g of KCl

6- Solution 6

$N_{H_2SO_4} = n * M_{H_2SO_4} \rightarrow \frac{0.2}{2} = \frac{2 * M}{2} \rightarrow M_{H_2SO_4} = 0.1$ M (C_2)

$C_1 * V_1 ?? = C_2 * V_2$

$\frac{2 * V_1}{2} = \frac{0.1 * 50 \text{ mL}}{2} \rightarrow V_1 = 2.5$ mL

C. A solution contains 15 g of $CaCl_2$ in a total volume of 200 mL. Express the concentration of this solution in terms of **(a)** g per L, **(b)** % (w/v), **(c)** M? (MW of $CaCl_2$ = 110.986 g/mole)?

(a) g/L ? $= M * M.m = \frac{\frac{15}{110.986}}{\frac{0.2}{1}} * 110.986 = 75$ g/L

(b) % (w/v) ??

15 g → 200 mL soln.

x ? g → 100 mL soln.

$x = \frac{100 * 15}{200} = 7.5$ g of $CaCl_2$

% (w/v) = 7.5 %

(c) $M = \frac{n}{V} = \frac{15 / 110.986}{0.2} = 0.0676$ M

[Exp.3] Use of Micropipettes

(فرفق مع المانيوال ، رح أدمج ملاحظاتي مع المانيوال وبغير الترتيب حبتين - بس رح يكون كافي وافي ان شاء الله✨)

☆ Micropipettes :- are the standard laboratory equipment used to measure and transfer small volumes of liquids.

هي المعدات المخبرية القياسية المستخدمة لقياس ونقل كميات صغيرة من السوائل

☆ you will use them throughout this semester & in advanced courses that you take in the future .

☆ it is essential that you master their use if you are to be successful in your experiments .

من الضروري أن تتقن إستخدامها

☆ these devices are expensive and somewhat delicate .

هذه الأجهزة باهضة الثمن وحساسة إلى حد ما

☆ To obtain accurate and precise data , correct operation of the micropipettes is essential .

☆ for this reason , we are going to start the course with an exercise of farmiliarize everyone with the micropipettes.🧐

Precise : هي تقارب القيم الي رح نحسبهم وندرسهم

accurate : الدقة ، قرب القيم للقيمة الحقيقية أي القيمة المحددة والمدروسة

(التعرف على أجزاء الجهاز ال Micropipettes)

- Accuracy and precision are fundamental in the field of biochemistry because reproducibility is a must and measured volumes are extremely small.

الدقة والتقارب القيم من بعضها البعض أمران أساسيان في مجال الكيمياء الحيوية ، لأن ال reproducibility أمر لابد منه والكميات المقاسة صغيرة جدا

- You are already familiar with how to read accuracy of simple laboratory glassware (i.e., graduated cylinder) and the more complicated burette.

أنت بالفعل على علم بكيفية قراءة دقة الأواني الزجاجية المختبرية البسيطة

مثل ال graduated cylinder وال burette الاكثر تعقيدا قليلًا

- You are now going to begin use with a new measuring device known as an adjustable micropipette.

Objective : 📌

to learn how to use adjustable micropipettes and maintain two things : accuracy and precision.

لتعلم كيفية إستخدام الماصات الدقيقة القابلة لتعديل والحفاظ على شيئين : الدقة ، وتقارب القيم من بعضها البعض

Parts of a micropipette

The volume indicator consists of three number dials and is read from bottom to top.

يحتوي على ٣ أقراص رقمية ، ويتم قرائتها من الأسفل إلى الأعلى

The digits on dial display indicate the volume selected.

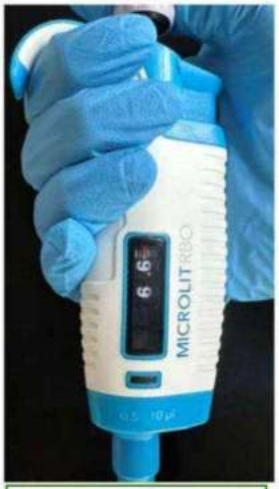
تشير الأرقام الموجودة على الجهاز إلى الحجم المحدد الفراد إستخدامه

Different sizes of micropipettes

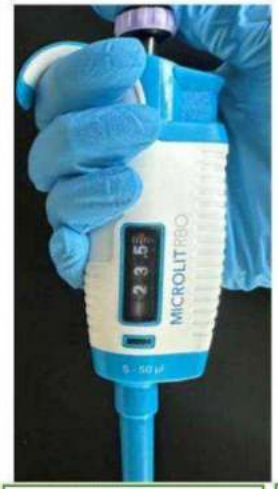
The micropipettes in this laboratory come in three different sizes each of which measures a different range of volumes.
تأتي بمذلاة أحجام مختلفة يقيس كُل منها نطاقًا مختلفًا من الاحجام

The three sizes are P10, P50 and P1000
Micropipette are named based on the upper range volume. يتم تسميتها بناءا على حجم النطاق الأعلى

Micropipette Size	Lower – upper volume range
P-10	0.5-10µl
P-50	5-50µl
P-1000	100-1000µl



P-10, range (0.5-10 µL)
Display: 6.6 µL



P-50, range (5-50 µL)
Display: 23.5 µL

P-1000, range (100-1000 µL)
Display: 530 µL

Volume is read from bottom to top.

فعظم الماصات الصغيرة قابلة للتعديل ويمكن أن تقدم أحجامًا متغيرة
بناءً على الحجم الذي يحددة المستخدم .

- Most micropipettes are adjustable and can deliver variable volumes (e.g., P-1000 can deliver 500, 735, 945µl) depending on the volume set by the user.

الماصات الدقيقة ذات الحجم المتغير تأتي مع نطاقات مختلفة وحدود علوية وسفلية للقياس

- Variable volume micropipette comes with different ranges and upper & lower limits of measurement. In such cases, error percentage may vary as per the measured liquid.

في مثل هذه الحالات قد تختلف نسبة الخطأ حسب السائل المقاس

- Trying to dispense less than the lower value of the range will result in inaccurate liquid measurements, whereas
trying to dispense over the upper range will completely fill the tip and allow the liquid to enter into the pipette body.

ستؤدي محاولة الإستغناء عن القيمة الأقل من النطاق إلى القياسات الغير دقيقة للسائل
بينما محاولة الإستغناء عن النطاق العلوي ستملاء ال Tip تمامًا وتسمح للسائل بالدخول إلى جسم الماصة .

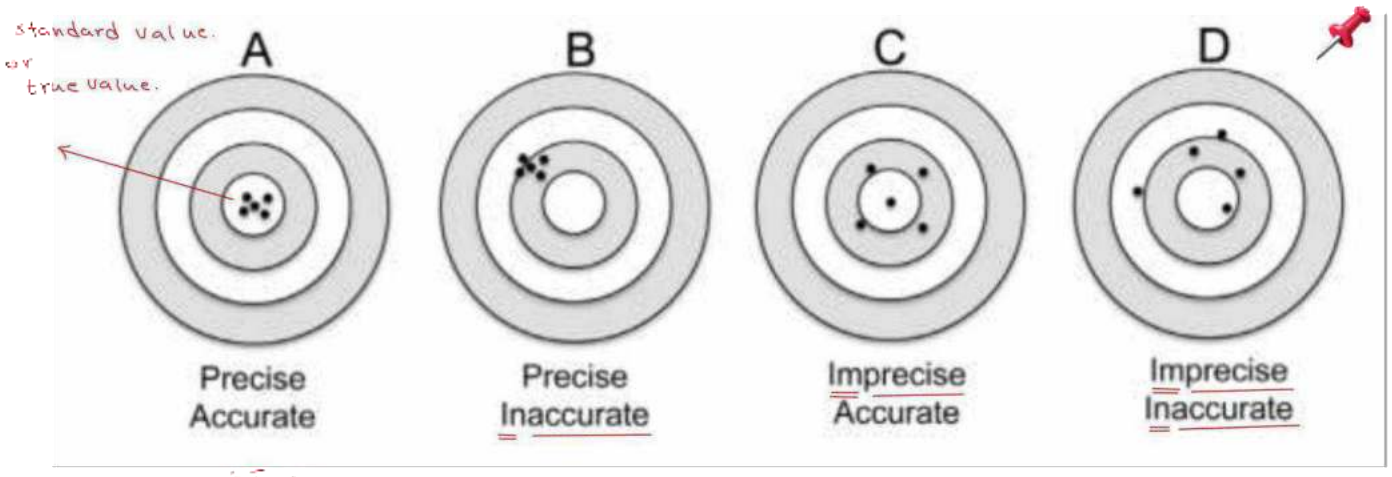
C] Accuracy and precision

لا توخذو بالكم من الترجمة كتير 👀 وسيلة مساعدة لا اكتر 😅👍

Accuracy depends on the micropipette delivering the correct volume.
Precise results are reproducible.

Manufacturers determine the accuracy and precision of micropipettes by using them to transfer defined volumes of distilled water that is then weighed on an analytical balance.

يحدد المصنعون دقة الماصات الدقيقة ودقتها باستخدامها لنقل أحجام محددة من الماء المقطر
الذي يتم وزنه بعد ذلك على ال analytical balance 😅



وش آخر

Hight accuracy | low accuracy | Hight accuracy | low accuracy

Hight precision | Hight precision | low precision | low precision

This is called **the gravimetric method** which determines the delivered mass of distilled water at a reference temperature (25˚C), given that the density of water is 1.0 gram per mL at 25˚C. This means that every (µl) should weigh exactly 0.001g.

{ مثال بسيط بالمانيوال }

1 mL ⟶ weight exactly 0.001 g

density of water = 1 g/mL

$D = \frac{m}{v} \longrightarrow m = d \times v = \frac{1\ g}{1\ cm^3} \times 1 \times 10^{-6}\ cm^3 \times \frac{1000\ cm^3}{1\ L}$

$= 1 \times 10^{-3}\ g = 0.001\ g$

500 mL ⟶ weight exactly = ? g

density of water = 1 g/mL

$m = d \times v = \frac{1\ g}{1\ cm^3} \times 500 \times 10^{-6}\ cm^3 \times \frac{1000\ cm^3}{1\ L}$

$= 500 \times 10^{-3}\ g = 0.5\ g$

The process is repeated several times during the calibration process, and the data is used to calculate the accuracy and precision of a micropipette.

تكرر العملية عدة مرات اثناء عملية المعايرة
ويتم إستخدام البيانات لحساب دقة الماصة الدقيقة .

Accuracy : is the closeness of **the dispensed volume** (الحجم الذي يتم إستخدامه بالماب) to the true (standard/nominal) volume as set on the pipette.

Accuracy is expressed as **mean error or % error** (متوسط الخطاء) , the percent by which the mean value of a large number of replicate measurements of the same volume will deviate from the expected or "true" volume.

هو نسبة المئوية التي تنحرف بها القيمة المتوسطة لعدد كبير من القياسات المكررة
لنفس الحجم عن الحجم المتوقع أو الحجم الحقيقي

عامل المعايرة

The accuracy of these micropipettes is determined by the factory calibration as shown in Table 1 and checked gravimetrically using distilled water and an analytical balance.

» القوانين المستخدمة في ال accuracy :

[1] Average (mean المتوسط) = $\bar{x}$ = sum of all values / total number of values

[2] % Error (% accuracy) = | Experimental value - Standard value | / Standard value × 100

[3] % accuracy = mean volume ($\bar{x}$) - nonimal volume / nominal volume × 100

(ال nonimal volume تعني ال true volume فعطى بالسؤال)

Precision - Precision provides information about **reproducibility** of your measurements and refers to as the "scatter" of individual measurements around the mean of replicate measurements, without any reference to a standard.

% Relative Standard Deviation (%precision) is usually used to express how precise is your data. Sometimes it is called coefficient of variation (cv%).

» القوانين المستخدمة بال Precision :

[1] Standard deviation (SD) = $\sigma = \sqrt{\frac{\sum_i (x_i - \bar{x})}{n-1}}$

($\bar{x}$: average)

[2] % RSD (% precision) = sample standard deviation (SD) / average measurment ($\bar{x}$) × 100

where **Xi** is one of n **individual values in the data set** ,
and **X** is **the data set's mean (average) value.**

The % precision is used to calculate the accuracy and precision of a micropipette, which is determined by the factory calibration as shown in Table 1.

{ مثال بسيط بالمانيوال - على الجدوال إلّي تحت }

volume 80 mL ⟶ accuracy % (0.8 - 1.6) % between

precision (0.3 - 0.6) % between

Calibration guidelines of micropipettes

Table 1: Calibration guidelines for the listed pipettes from the International Organization for Standardization's (ISO) - EN ISO 8655.

Range	Volume (µl)	Accuracy (±%)	Precision (±%)
0.2-2µl	2	4.00	2.00
	1	8.00	4.00
	0.2	40.00	20.00
2-20µl	20	1.00	0.50
	10	2.00	1.00
	2	10.00	5.00
10-100µl	100	0.80	0.30
	50	1.60	0.60
	10	8.00	3.00
200-1000 µl	1000	0.80	0.30
	* 500	1.60	0.60
	200	4.00	1.50
1-5ml	5000	0.8	0.30
	2500	1.60	0.60
	1000	4.00	1.50
1-10ml	10000	0.6	0.30
	5000	1.20	0.60
	1000	6.00	3.00

☆ هسى ال 0.08 طلعت أقل من ال 1.60 لذلك ستعتبر accuracy.

☆ طيب لو طلعت تساويها معناته كمان accuracy ، عادي

☆ بس لو كانت أعلى القيمة إلي طلعت معي من ال 1.60 فهون خلص بنحكي not accuracy

طيب لو أجيت على ال precision

أنا عندي بالسؤال طلعت القيمة 8.06 وهاي القيمة أعلى من ال 0.60

لذلك خلص بنحكي not precision

{ Example -1 } Calculate the SD of the following data set (84 , 84 , 89 , 91 , 110 , 114 , and 116) ??

$$\bar{X} = \text{average} = \frac{84 + 84 + 89 + 91 + 110 + 114 + 116}{7} = 98.28 = 98.3$$

$$SD = \sqrt{\frac{(84-98.3)^2 + (84-98.3)^2 + (89-98.3)^2 + (91-98.3)^2 + (110-98.3)^2 + (114-98.3)^2 + (116-98.3)^2}{7-1}} = 14.4$$

(على الآلة الحاسبة أسرع وأسهل ودقيق)

Standard deviations are very sensitive to extreme values (outliers) in the data .
For example, if the highest value in the dataset had been 150
instead (بدلاً من) of 116, the SD would have gone up from 14.4 to 23.9

بنحكي بالسؤال Standard Volume or Nominal Volume.

{ Example - 2 } Calculate mean , SD , % accuracy & % precision for result of 1000 microliter pipette set to 900 microliter I got :

(910 microliter , 887 microliter , 882 microliter , 902 microliter , 921 microliter)

الحل :-

① $\bar{x}$ = average or mean = 900.4 μL

② SD = 16.10 ≈ 16

③ $$\% \text{ Accuracy} = \frac{(\text{mean volume} - \text{nominal volume})}{\text{nominal volume}} * 100$$

$$= \frac{(900.4 - 900)}{900} * 100 = 0.04$$

وزنه نسبة 0.04 %

الجدول عندي القيم التالية
0.80 %
طيب القيمة عندي إلي طلعة أقل منها ، معناته accuracy

Accuracy : Comparing this to **Table 1** this is well within calibration limits.

④ $$\% \text{ precision} = \% \text{ RSD} = \frac{\text{sample standard deviation (SD)}}{\text{mean volume } (\bar{x})} * 100 = \frac{16}{900.4 \ \mu L} * 100 = 1.776$$

$= 1.8$

وزنه نسبة 1.8 %

Precision : This is above the calibration limits shown in **Table 1** so this pipette should not be used as the results are very variable between pipetting. However,

it could also be poor pipetting technique **! So** it may be worth checking with someone else 🙂

0.30 % طيب القيمة إلي طلعت معي بالحساب أعلى منها ، وبتالي
Imprecise (not precision)

◇◇

📌 How Pipetting Technique Contributes to Error :

(1) Tip Wiping --> Unnecessary tip wiping can lead to material loss يعني لا تمسك ال Tip وتمسحها بإيدك مثلاً ، لأنه رح تسحب من السوائل وبقلل من المواد

(2) Choosing the Wrong Pipetting Mode

(3) Working Too Quickly

الصح الصح أن تستخدم وبتسحب بشكل عامودي مش بزاوية

(4) Pipetting at an Angle

إستخدام طرف الماصة الخطأ

(5) Using the Wrong Pipette Tips

(6) If at any time a pipette is dropped or for any reason you suspect the pipette is not functioning properly 😒 INFORM YOUR INSTRUCTOR IMMEDIATELY .

Pre - Lab Questions

1) Fill in the blanks 25000 µL = 250 mL

1 mL → 1000 µL
250 mL → ? µL

= 1000 * 250 / 1 = 25000 µL

2) What should 250 µL of water weigh ? 0.25 grams

1 gram → 1000 µL
"x" ? gram → 250 µL

x = 250 / 1000 = 0.25 gram

3) Is the following set of measurements **precise** ? YES , (NO)
Is the following set of measurements **accurate** ? (YES) , NO

Using a P-1000 micropipette attempting to measure 500 µL the mass of water equalled :
(note the grams measurement)

لازم 1 من كل 5 مرات

((0.551 g , 0.525 g , 0.448 g , 0.476 g , 0.502 g))

الحل

Average = 500.4 g

$$SD = \sqrt{\frac{\Sigma(x_i - \bar{x})^2}{n-1}} = \sqrt{\frac{(0.551 - 500.4)^2 + (0.525 - 500.4)^2 + (0.448 - 500.4)^2 + (0.476 - 500.4)^2 + (0.502 - 500.4)^2}{5 - 1}}$$

= 0.04034 g

لأنه دخلت عندي القيم بال gram ، وبده بال mg الأخ 🙂
لذلك SD = 40.34 mg
هاي إلي رح نستخدمها
على الآله الحاسبه أسرع 🙂

$$\% \text{ accurate} = \frac{(\text{mean volume} - \text{nonimal volume})}{\text{nonimal volume}} \times 100\% = \frac{500.4 - 500}{500} \times 100\% = 0.08\% \quad (\text{accurate})$$

لأنه القيمة بالجدول 1.60 لل accurate
وأنا عندي القيمة طلعت 0.08
طيب ال 0.08 أقل من 1.60 <-- لذلك بنحكي إنه accurate

$$\% \text{ precise} = \frac{\text{sample standard deviation}}{\text{mean volume}} \times 100\% = \frac{40.34}{500.4} \times 100\% = 8.06\% \quad (\text{not precise})$$

لأنه عندي بالجدول القيمة 0.60 لل precision
وأنا طلع الجواب معي 8.06
هسى ال 8.06 أعلى من ال 0.60 لذلك بنحكي إنه not precise .

4) Why should you only use degassed water for this experiment ?
(إجابات فقترحه)

- That it's to remove gas / pores in the tip .
- That it's to for calibration .

الآن رح تحكي عن ٣ صفحات موجودين بالمانيوال كتير حكي حكي - اقرأهم وخذ المفيد منهم و بس 🙂 مش هذيك الأهميه بصراحه غير كم معلومة 🙂

How to use a Micropipette (Operation of the Microliter Pipette) :

(1) Set the volume by turning the volume adjustment knob at the end of the pipette until the correct volume shows on the indicator.
أضبط الحجم من خلال دوران ال volume adjustment knob حتى تصل إلى الحجم المطلوب الذي يظهر على الشاشة عندك

Note : Never go above or below the range of the pipettor ! Know these ranges at all times.
لا تذهب أبدا أسفل أو أعلى نطاق ال pipette

(2) Attach a new disposable tip to the pipette shaft . Press firmly with a slight twisting motion.
Make sure you are using tips of the correct size for each pipette.
أضغط بقوة بحركة ملتوية خفيفة [باختصار أضغط عليه على الاخر]
تأكد من أنك تستخدم أطراف بالحجم الصحيح لكل pipette .

(3) to aspirate the liquid in the tip, press the plunger to the first stop. This part of the stroke is the calibrated volume displayed on the digital volume indicator. Do not press the plunger all the way down, or you will draw up too much solution.

لسحب السائل إلى الطرف [tip] . اضغط على المكبس حتى المحطة الأولى
هذا الجزاء من الحد هو الحجم المعاير المعروض على المؤشر الحجم الرقمي
لا تضغط على المكبس حتى النهاية . وإلا ستسحب الكثير من المحلول .

(4) Holding the microliter pipettor vertically immerse part of the disposable tip into the sample.
أمسك ال microliter pipettor بشكل عامودي ، وأغمس جزاء من الطرف المقابل في العينة .

(5) Allow the push-button to return slowly to the up position. Never let it snap up !
(If it does happen, tell a TA so that the microliter pipettor may be dismantled and cleaned to prevent corrosion and the contamination of your succeeding samples).
حتى يتم تفكيك ال microliter pipettor وتنظيفها لمنع تآكل وتلوث العينات الدلية

Do this slowly and keep the tip submerged in the solution to prevent any air bubbles from entering the tip-this will mess up your volume measurement.
إفعل ذلك ببطء وحافظ على بقاء الطرف مغمور في المحلول لمنع أي فقاعات هواء من الدخول الطرف (tip)
لأنه وجود الفقاعات رح تخرب القراءات للحجم التي سيتم أخذها .

(6) Wait a few seconds to ensure that the full volume of sample is drawn into the tip.

(7) Withdraw the tip from the sample liquid . You should observe the liquid in each type of tip with each pipettor so that you can become aware if there is a significant problem with the pipettor. This is an incredibly important part of the technique and becoming efficient at pipetting small volumes.
يجب أن تراقب السائل في كل نوع من ال tip مع كل pipette حتى تتمكن من ادراك ما إذا كانت هناك مشكلة كبيرة في ال pipette .
يعد هذا جزءا مهما للغاية من التقنية ويصبح فعالا في سحب الأحجام الصغيرة .

(8) To dispense the liquid, touch the tip end to the sidewall of the receiving vessel and depress the plunger slowly to the first stop.
Wait two seconds.Then press the plunger to the second stop (the bottom stroke), expelling any residual liquid in the tip.
لطرد السائل ، إجعل ال tip ملامس طرف الجانبي ال receiving vessel وإضغط على المكبس ببطء حتى ال first stop .
وإنتظر ثانيتين ، ثم إضغط على المكبس (plunger) للوصول إلى ال second stop ... و اطرد اي سائل متبقي في الطرف .

(9) With the plunger fully depressed, withdraw the microliter pipettor from the vessel carefully, with the tip sliding along the wall of the vessel.

(10) Allow the plunger to return slowly to the up position.
أسمح للمكبس (plunger) بالعودة ببطء إلى الوضع الطبيعي

(11) Discard the tip. You want to use a different tip each time you are gathering/dispensing different materials.
If you don't do this, your concentrations of solutions will be inaccurate, and as a result, so will your data.
إرمي ال tip [من خلال الضغط على ال tip ejector] [لانه بتحتاج كل شوي بغير ال tip لوحده جديده عند إستخدام المواد] و أنت تقوم بجمع وتوزيع المواد المختلفة .
إذا لم تقم بذالك فسيكون تركيز المحاليل إلى بتشتغل فيها غير صحيحه وغير دقيقة ؛ ونتيجة لذالك ستكون بياناتك ايضا غير دقيقة وغير صحيحة .

Note : To prevent liquids from being drawn الإنجراف into the microliter pipettor shaft pipette slowly and never invert وعدم القلب or lay microliter pipettor on its side with liquid in the tip وعدم وضعها على جانبها مع وجود سائل في طرفها .

Position 1 is where the pipette is at rest

Position 2 (First Stop) is reached by pushing down on the plunger until resistance is met

Position 3 (Second Stop) is reached by pushing down from position 2

The plunger will stop at two different positions when it is depressed.

سيتوقف المكبس - plunger - عند موضعين مختلفين عندما يكون مضغوط

The first of these stopping points is the point of initial resistance and is the level of depression that will result in the desired volume of solution being transferred.

The second stopping point is when the plunger is depressed beyond the initial resistance until it is in contact with the body of the pipettor.

At this point, the plunger cannot be depressed further.

This second stopping point is only used for the complete discharging of solutions from the plastic tip.

◇◇◇

Safeguards for a pipette (INFORM YOUR INSTRUCTOR IMMEDIATELY) الإجراءات الوقائية

Each micropipette is very expensive
To keep these pipettors functioning properly it is important that they be handled with care.
Please follow these rules to keep from breaking the micropipettes

الحفاظ على عمل هذه ال pipette بالشكل الصحيح ، من المهم أن يتم التعامل معها بعناية يرجى
إتباع هذه القواعد لتجنب كسر ال micropipettes.

{1} Never adjust the volume beyond the range of the micropipettor.
no micropipette should be adjusted below zero µl.
the P20 should never be adjusted above 20µ , the P200 over 200 ul and the P1000 over 1ml.

{2} Never force the volume adjustor dial. If the knob becomes difficult to adjust it probably means that you are exceeding the limits for the pipette or the pipette is damaged. Please report the problem to the instructor or TA .

إذا أصبح من الصعب ضبط المقبض (adjustor) ، فربما يعني ذلك أنك تجاوزت حدود ال pipette أو تلفها

{3} **Keep the pipette clean and unclogged at all times.**
If you feel excessive resistance when you depress or release the plunger , then the pipette is clogged and needs to be cleaned .

{4} **Do not drop the micropipettes.**

لا تسقطها على الأرض لأنه وقتها رح تخرب عندي ال micropipettes

{5} **Always use a smooth motion when using the pipetters.**
This will help give you accurate measurements and also prevent breakage of pipettes.
سيساعدك هذا في إعطائك قياسات دقيقة وأيضا منع كسر ال pipettes

{6} **Always choose to appropriate size pipette for the volume you are measuring .**

{7} **Always dispose of tips in appropriate waste containing .**

◇◇◇

Procedure (Calibrating and Using a Micropipette and the Mass of Water)

In this experiment you will learn to use the adjustable micropipettes of various sizes and measure their accuracy, precision, and calibration .

Pipette a known volume of degassed water onto a balance and weight it .
With the digital top loading balance in our laboratory.

[1] Place a weighing dish on a balance and tare it

[2] Pipette 750 μL using the correct model of pipette 4 times into the weigh dish and record the mass

[3] Pipette 50 μL using the correct model of pipette 4 times into the weigh dish and record the mass.

[4] Pipette 10 μL using the correct model of pipette 4 times into the weigh dish and record the mass.

[5] To save time and materials, just tare the balance between each addition of the defined volume of water.
make sure that the balance shows 0.000g before adding any additional water.

[6] Record these values in the report sheet table and determine the average and standard deviation .
If your value is accurate and precise as determined by the standard values,
you will have successfully completed the exercise .

Pipetting accurately and precisely is a major component to getting good data in this course .

سيعوضك الله عن كلّ هذا الحمل الذي حملته وحدك في هذا الطريق ، عن كلّ هذا التعب الذي قاومته دون أن تتكلم ،
سيعوضك الله لأنك رضيت في وقت لم يكن فيه الرضا عليك سهلًا 🌺

Experiment 4
Qualitative Determination of Protein and amino acids

البروتين هو عنصر غذائي مهم ضروري للبقاء على قيد الحياة
هم مكونات مهمه في الخلايا وبالتالي موجود في جميع الأجسام الحية

Protein is an important macronutrient essential for survival . They are an important constituent of cells and hence are present in all living bodies .

تحتوي الخلايا على أكثر من 3000 بروتين مختلف تلعب مجموعة متنوعة من الأدوار الهيكلية والوظيفية في الخلية

Cells contain more than 3000 different proteins that play a variety of structural and functional roles in the cell .

10 - 35% of calories should come from protein . Protein is found in meats , poultry , fish , meat substitutes , cheeses , milk etc . They contain carbon , hydrogen , oxygen , nitrogen , phosphorus and Sulphur in some cases .

Proteins are large biological molecules of alpha aminoacids .

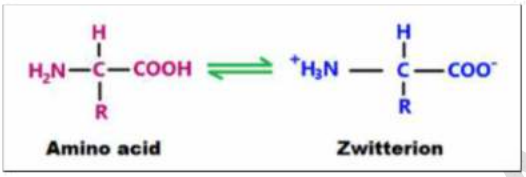
10-35%: من السعرات الحرارية يجب أن تأتي من البروتين . يوجد البروتين في اللحوم والدواجن والأسماك .
بدائل اللحوم والأجبان والحليب وما إلى ذلك . تحتوي على الكربون والهيدروجين والأكسجين والنيتروجين . الفوسفور والكبريت في بعض الحالات.

Amino acids are the main building blocks of proteins .
They are relatively small molecules that characterized by presence of α-amino group , carboxylic group and a unique R group .

إنها جزيئات صغيرة نسبيًا التي تتميز بوجود مجموعة α-amino ومجموعة الكربوكسيل ومجموعة R الفريدة

the amino group is attached to alpha carbon , which are crystalline in nature and exist as zwitterions (it has both positive and negative)

المجموعة الأمينية مرتبطة بال alpha carbon ، وهي بلورية بطبيعتها وتوجد على شكل zwitterions (لأنها تحتوي على كلا الشحنتين ال positive charge القادمه من ال amino group وال Negative charge القادمه من ال Carboxyl group)



على الرغم من أن الأحماض الأمينية تشترك في ال Structure بشكل عام ، إلا أنها تختلف في القطبية والحجم الذوبان والشحنة الكهربائية

Although amino acids share the general structure , they differ in the polarity , size solubility and electrical charge .

Amino acids form a specific type of linkage known as **peptide linkage** , amino acid molecules undergo a condensation reaction .

تشكل الأحماض الأمينية نوعًا محددًا من الروابط المعروفة باسم ال peptide linkage ، جزيئات الأحماض الأمينية تخضع لتفاعل التكثيف أي زيادة التركيز (condensation) . ال condensation يحدث لما يترابط بين 2amino acids (مجموعة α-carboxyl من حمض أميني واحد ومجموعة α-amino من أخرى).

the condensation of the **α-carboxyl group** of one amino acid and an **α-amino group** of another .

يتم تصنيف المنتجات المتكونه على أنها :
اعتمادًا على عدد جزيئات الأحماض الأمينية المشاركة في ال condensation reaction .

The products formed are classified as ;
depending on the number of amino acid molecules involved in the condensation reaction .

Dipeptide : they are the products formed by the condensation of two molecules of alpha-amino acid .

Tripeptide : they are formed by the condensation of three molecules of alpha amino acid .

Polypeptide : If a large number of molecules of amino acids combine , the formed product is called a polypeptide .

تختلف البروتينات بشكل أساسي عن بعضها البعض في تسلسل الأحماض الأمينية.
يوجد حوالي 20 من الأحماض الأمينية بالطبيعة .

Proteins differ primarily from each other in their amino acid sequence .
There are about 20+ amino acids here .

Some amino acids are not produced by the body and are delivered by diet . They are called amino acids , which are essential .

بعض الأحماض الأمينية ليست فنتجه من خلال الجسم ويتم الحصول عليها من خلال النظام الغذائي
او الوجبه الغذائية (diet) تسمى بال essential (اي ضرورية او أساسية) .
11 non-essential amino acids
9 essential amino acids.

Detection of Proteins : Detection based on the following :

1. **Presence of peptide bond :** Biuret reaction عن طريق إستخدام ال
2. **The nature of protein** (coagulation by heating , precipitation by strong acid and heavy metals)
3. **Presence of certain amino acids** : color reactions of amino acids

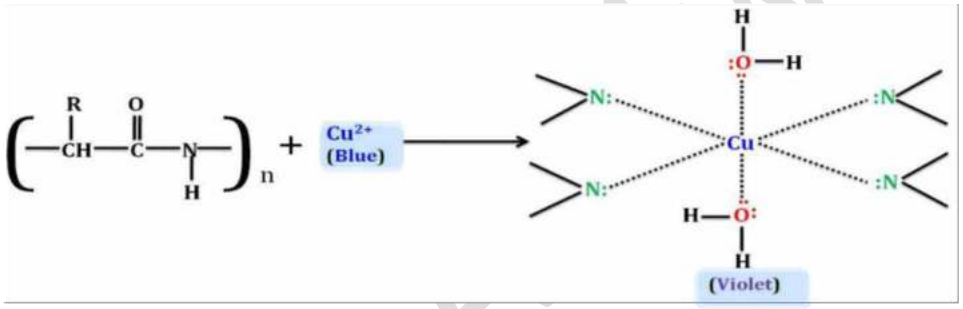
Qualitative test

Protein

A. Biuret test This test is used to detect the presence of peptide bond **(not less than two peptides)**.
When treated with copper sulphate solution in presence of alkali (NaOH or KOH),

protein reacts with copper (II) ions to form a violet coloured complex called biuret .

This test is applied to gelatin , casein & albumin .



B. Heat Denaturation Proteins are unequally stable فستقرة بشكل غير فتساوي when heated in aqueous solution , fractionation can be achieved by controlling heating .

The presence of a substrate , a product , or an inhibitor often stabilizes a protein against heat denaturation .

Denaturation consists of the unfolding polypeptide chain and loss of the compact structure.

It is usually **irreversible** and can result from the application of heat , extremes of pH or the action of detergent.

Denaturation therefore frequently causes precipitation of the protein.

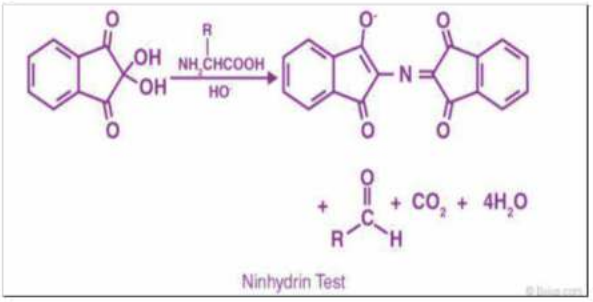
Amino acid

A. Ninhydrin Test

Ninhydrin is a powerful oxidizing agent عامل مؤكسد قوي ,
it reacts with **α-amino acid** to **decarboxylate the amino acid**
yielding an intensely **colored blue-purple product , CO2 , water & the corresponding aldehydes .**

Ninhydrin yields a similar blue-purple reaction upon reaction with primary amines and ammonia .

The reagent also reacts with amino acids such as **proline** to yield a **yellow to red product .**



This test give a positive result by only amino acids & proteins which **contain free – NH2 groups (α amino group)** in their structure .

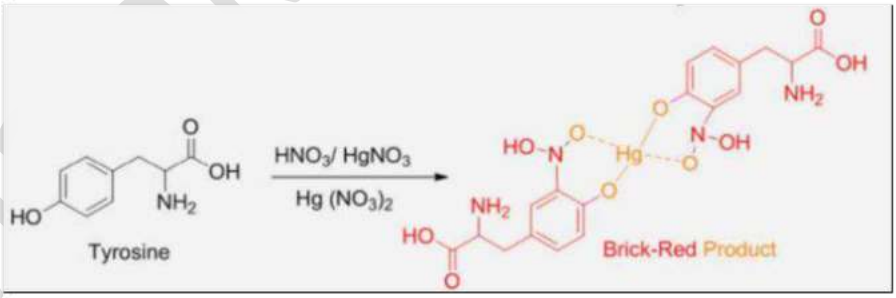
Amino acid residues for which good color tests have been achieved are **arginine , cysteine , proline , hydroxyproline , tryptophan & tyrosine .**

The colour intensity is proportional to the concentration of amino acid
i.e. ammonia from the amino groups.

B. Millon's Test

☆ Millon's reagent is a concentrated HNO3 reagent that dissolves mercury ذائب في الزئبق .

☆ The test by Millon is specific to phenol that contains structures.
Specific for tyrosine (tyrosine is the only common phenolic amino acid) and proteins containing it .



A red precipitate or a red solution is regarded as a positive test as a consequence of the reaction .

Inorganic salts in large amounts interfere تتداخل with the reagent by precipitating mercury .

A yellow HgO precipitate is NOT a positive response , but it usually shows that the solution is too **alkaline** .

☆ This test can be **applied to tyrosine , phenylalanine .**

C. Hopkin's Cole Test

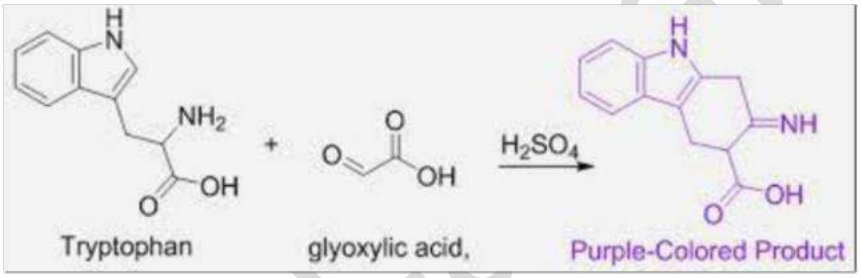
Hopkin's Cole test is **a specific test used for the detection of indole ring** and thus , **tryptophan in proteins .**

The test is also termed as ' glyoxylic acid test '
as the reagent contains glyoxylic .

Glyoxylic acid is prepared from glacial acetic acid by being exposed to sunlight acid .

In the presence of **concentrated H2SO4** , the **indole tryptophan group reacts with glyoxylic acid** to give a **purple color** .

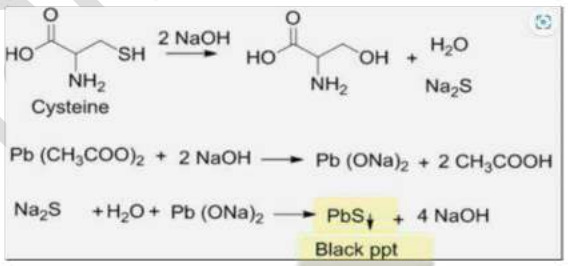
The H2SO4 added to the reagent helps to stabilize the glyoxylic acid & prevent its decomposition and the release of carbon dioxide.



D. Lead – sulfur test

Sulfur containing amino acids containing sulfur , S-S group in cysteine , and **S-H group like cysteine & cystine**

on heating with **KOH , a black lead sulphide precipitate is formed** by reacting with **lead acetate .**

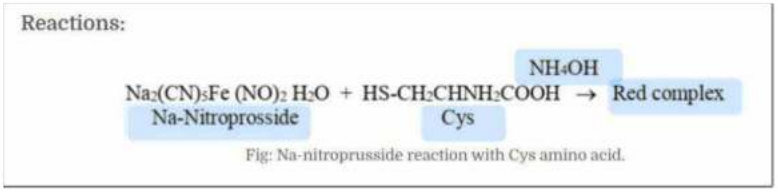


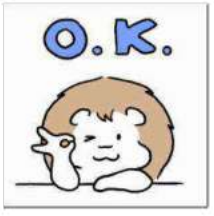
E. Nitroprusside's test

The nitroprusside's test is specific for **cysteine** , the only amino acid containing **a sulfhydryl group (–SH)** .

The group reacts with nitroprusside in an alkaline solution to yield a red complex .

a free sulfhydryl group (-SH) that is able to react with the nitroprusside in the presence of excess ammonia (NH4OH) عند وجود فائض منه





Practical work

A. Biuret test:

1. Place 15 drops of each sample (unknown) test solution in a separate test tube.
2. add 5 drops of 20% NaOH and mix.
3. Add 2 drops of 0.2% CuSO4 solution, warm if necessary

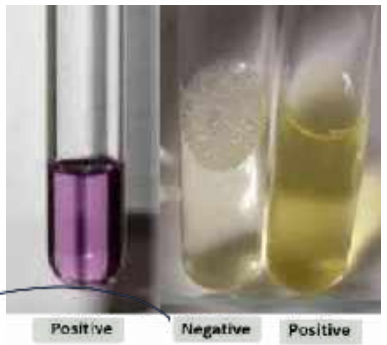
B. Heat denaturation:

Take 5 ml of each sample (unknown) test solution in a separate test tube, heat to boiling.
What do you observe?

في هذه الجزئية من التجربة ، الألوان ما رح تكون كتير واضحه احيانًا
فعادي ممكن بيكون ال test tube الذي يحتوي على ال aminoacids بيكون لونه شفاف وما فيه أي ترسبات ، مش شرط بيصير اللون على أبيض وغيوم

C. Ninhydrin Test:

1. Take 2 ml of each sample solution in a separate test tube.
2. Add 0.5 ml of 0.1 % Ninhydrin reagent the test tubes.
3. Place the test tubes in the water bath for 5 minutes and then allow cooling to room temperature.
4. Observe the formation of a deep blue/purple colour indicates the presence of amino acids.



Result:

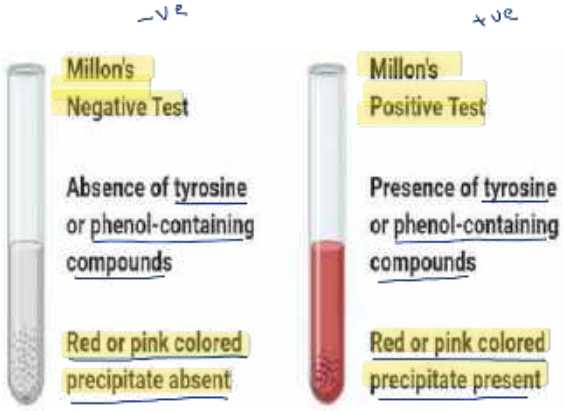
- ✓ blue-purple reaction
- ✓ yellow or yellow to red color

Interpretation

1. This reaction suggests that amino acids, other amines, and ammonia are present in the test material.
2. Negative: not a.a , not protein
3. Positive purple: aa or protein with free amino gp
4. Positive yellow: proline aa or hydroxyproline.

تفسير interpretation :
1] هذا التفاعل يوحي بأنه عندي الأحماض الأمينية ، و الأمينات والأمونيا موجودة في الاختبار مادة.
2] بالنسبة للنتيجه السلبيه Negative : تعني انه لا يوجد amino acid ، و ما عندي بروتين ولا حتى عندي proline .
3] اللون البنفسجي الإيجابي Positive : تعني إنه عندي (alpha amino acid) amino acid & protein which contain free NH2 groups
4] اللون الأصفر الإيجابي positive : تعني إنه عندي proline or hydroxyproline
(اللون الناتج هون إما yellow or yellow to red)

D. Millon's test

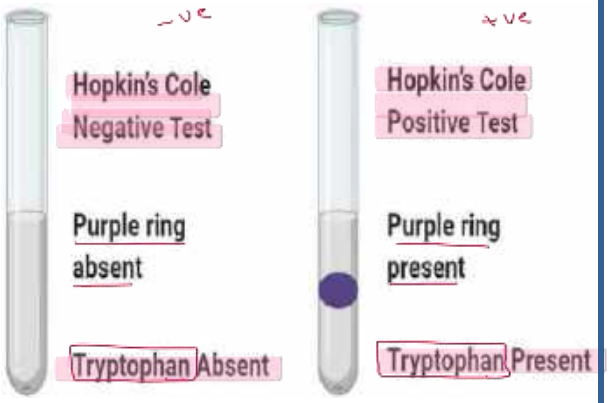


1. Take 2 ml of each sample solution or unknown solution is taken in a seperate test tube.
2. To this, about 2 ml of Millon's reagent is added. Observe the formation of red brick precipitate
3. if red colored precipitate is not observed immediately. the test tubes are then kept in the water bath for about 2 minutes The tubes are then observed for the formation of the colored precipitate.

Positive result: A positive result in the Millon's test is demonstrated by the formation of a red brick or pink colored precipitate. This indicates the presence of tyrosine or tyrosine containing protein.

Negative result: A negative result in the Millon's test is demonstrated by the absence of colored precipitate in the test tube. This indicates the absence of tyrosine or tyrosine-containing protein.

E. Hopkins-Cole test



1. In two separate test tubes, take 2 ml of Hopkins-Cole reagent and 2 ml of each sample liquid are taken.
2. To this, concentrated H_2SO_4 is added along the sides of the test tube held at a slanting position. Two distinct layers of liquid are to be formed without mixing. (*Note: Mouth of the tube must point away from the face, as we should be careful while adding the sulfuric acid.*)
3. The test tube should be observed for the formation of a purple colored ring at the interface of two layers.

Interpretation of result

Positive result: A positive result is represented by the formation of a purple-colored ring at the junction of two layers. This indicates the presence of tryptophan-containing proteins.

Negative result: A negative result is represented by the absence of a purple-colored ring in the test tube. This indicates the absence of tryptophan-containing proteins.

F. Lead sulfur test

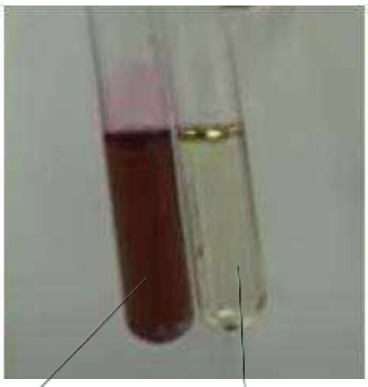
1. In two separate test tubes, place 2 ml of sample solution add 2 ml of 20% NaOH and boil for a minute.
2. Cool it and add a drop of 20 % lead acetate solution.
3. Observe the formation of black lead sulfide precipitate.

Note: Carry out this test in the hood if possible.

Positive test: Formation of black precipitate indicate the presence of sulfur-containing amino acid.
Negative test: No formation of black precipitate indicates the absence of sulfur-containing amino acid.

G. Nitroprusside test:

1. In two separate test tubes, place 2 ml of sample solution.
2. Add 0.5 mL nitroprusside solution and shake thoroughly.
3. Add 0.5 mL of 20% Sodium hydroxid .
4. Observe the color change.



Result interpretation
Positive test: Formation of red color indicate presence of cysteine.
Negative test: No formation of red color indicates absence of cysteine.

Experiment 5

Quantitative Determination of protein concentration by Spectrophotometry

→ To detect , quantify a particular type of molecule , it is necessary to have a method or procedure for measuring it .

للكشف عن نوع من الجزيء وتحديد كميته ، من الضروري أن يكون لديك طريقة أو إجراء لقياسه

→ This procedure is called an " assay " an example of which is Spectrophotometric qssays .

→ The purpose of the protein assay (quantification) الغرض أو الهدف من فحص البروتين
is to determine the amount or concentration of a specific protein
or an array of different proteins in a sample . أو مجموعة بروتينات مختلفة في عينة

☆ **Isolating & detecting protein** ... used for many clinical (العمليات السريرية)and
research processes (العمليات البحثيه) or in a clinical laboratory as part of a disease diagnosis
أو في العيادات المُختبرية كجزاء من تشخيص المرض
(to quantify loss of protein , level of certain enzymes or antibodies) .

→ There are many different types of protein assay , and variations upon the major techniques .

Here we provide a simple overview of colorimetric reagent protein assay .

الجهاز ال spectrophotometry :

يساعد ع قياس تراكيز ال molecules التي تسمى بال spectrophotometeric assays .

فكرة الجهاز انه في عندنا ضوء ، ويمر هذا الضوء من خلال العينه .
هاذ الضوء الداخل عند عبوره العينة (التي هي عبارة عن مواد كيميائية و molecules وهاذه ستعمل ع إمتصاص جزاء من الضوء الداخل، ويعبر من خلالها الضوء الخارج الذي يسمى بال transmitter light) .

☆ يتم التعامل مع ال light بالعاده ع إنه energy wave وهذه ال wave لها
طول موجي نعبر عنه بال Lambda ويقاس بوحده Lnm وهوه عباره عن (nanometre = 10^9)

☆ الآن إنتقال الطاقة من الضوء لل photon ، تسمى هذه العملية بال absorption .
كما قلنا سابقاً، العينة تحتوي ع مواد كيميائية و molecules والتي تعمل ع إمتصاص الضوء ، وإمتصاصها للضوء بيعتمد على خصائصها الفيزيائية والكيميائية لهذه الماده .

- يعني كل ماده عندنا موجوده بتقدر بتمتص الضوء على wavelength (اي طول موجي) مُعين .
بالعاده بيكون إلها ال strongest photon / wavelength
وهاي ال wavelength تسمى بال Lambda max .

- هسى ممكن بتكون الماده عندي شفافه بالعينة ، بهاي الحاله بقدر بضيف ماده ثانيه او Reagent ثاني
هوه الي اله ال Lambda max او هوه الي بيستطيع ان يمتص الضوء بعد ما تفاعل مع المادة الي بدي أحللها .

☆ الآن كل ال Biochemical assays for biomolecules سواء كان protein or carbohydrates or lipid
بالعادة بتعتمد على ال ultraviolet - visible spectrophotometer .

" بتجربتنا اليوم بدنا نستخدم ال ultraviolet - visible spectrophotometer أو الذي يُختصر بال UV spectrophotometer "

ال Range for UV normally يمتد (extends) from 100 to 400 nm هاذ الطول الموجي تبعها .
ال visible range تقريباً (approximately) from 400 to 800 nm .

حيث أن اللون الأحمر بيبلش بالطول الموجي 400nm وعند ال 800nm بنكون وصلنا ال Violet color.

☆ زي ما حكينا عن الجهاز انه رح يمر الضوء عندي من خلال عدسه فحدبه (Collimator Lens) وبيمشي الضوء الفجمع (Monochromator أحادي اللون) ليصل للمنشور الثلاثي (Prism or grating) الذي يعمل على تحليل الضوء الأبيض - الضوء المرئي حسب الأطوال الموجيه لألوان الطيف السبعه (Visible range 🌈) .

الجهاز يسمح بإختيار وتحديد الطول الموجي الي بدنا نقيس عليه التركيز بالزبط 👌😉 Lambda max
وزي ما حكينا الضوء الخارج منه يسمى بال transmitter light .
وبعد ذالك هاذ ال transmitter light بيروح لل Photocell detector الي بتقيس كم صار عندنا إمتصاص للضوء بالزبط ، وبتطلع قيمة على شاشة الجهاز .

نجي على الجد هسى🧐📚

Spectrophotometry :
A spectrophotometer is an instrument (أداة) that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution.

With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected .

Light is often treated as energy wave غالبا ما يتم التعامل مع الضوء على أنه موجة طاقة
The wavelength is expressed by lambda λ . And measured in nanometre = 10^{-9} m

The transfer of energy from a photon to a molecule is absorption .
Light absorption may result **directly** from the intrinsic chemical properties of the molecules of interest .

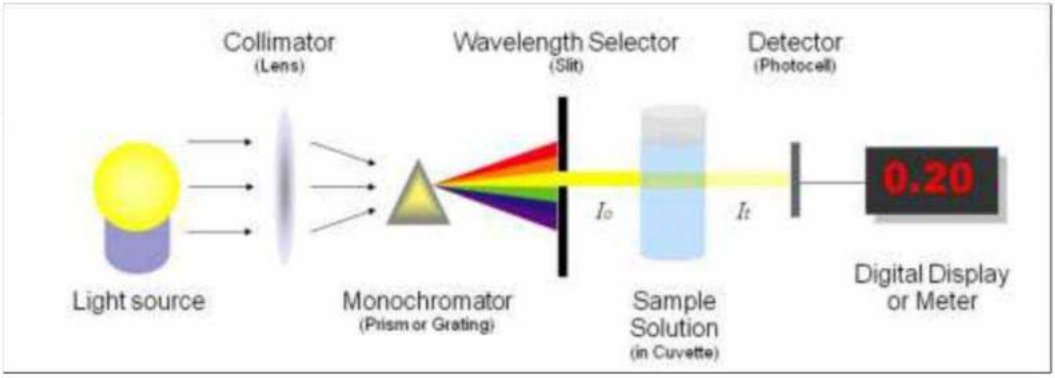
Alternatively بدلًا من ذلك , they may occur **indirectly** as **a result of treating the molecules of interest with other compounds which react with them to create new chemicals that exhibit absorption** .

The wavelength at which a substance
has its strongest photon absorption (highest point along the UV spectrum) is called Lambda max (λ max).

Biochemical assays for biomolecules are usually **based on absorption in the UV or visible region** .
In this experiment we will use the ultraviolet-visible spectrophotometer (figure 1),

the UV range normally extends from 100 to 400 nm ,
with the visible range from approximately 400 to 800 nm (red to violet wavelength range).

Figure 1:
Components of
spectrophotometer



Beer-Lambert Law 📌

الكمية الإجمالية للضوء الممتص عند أي طول موجي معين (الامتصاصية) هو تحددها ثلاثة عوامل :

The total amount of light absorbed at any particular wavelength (the absorbance) is determined by three factors :

خصائص امتصاص لجزيئات ال interest

(1) the absorption characteristics of the molecules of interest
(2) the pathlength or distance through which the light must travel
(3) the concentration of the absorbing molecule.

طول المسار أو المسافة التي يجب أن يمر الضوء من خلالها

These factors are summarized in the following expression , which is called **the BeerLambert law** :

$$A = \log_{10} I_o/It = E \times C \times L$$

Where **A** is the absorbance of the solution
I_o is the intensity of the incident light الضوء الداخل عندي
It is the intensity of the transmitted light
E is the molar extinction coefficient
c is the concentration of the absorbing solute
L is the pathlength of the light

The concentration of the solute (c) is usually expressed in **moles/liter (M)** and **the pathlength of light (l)** is expressed in **cm** .

The molar extinction coefficient (E) is an intrinsic characteristic of each molecule at a particular wavelength.

It is numerically defined as the absorbance of a 1.0 M solution of the molecule of interest in a 1.0 cm light path .
Because **E** has **the units of liter/(cm x mole)** , **absorbance itself is a parameter with no units** .

The larger the value of E , the more a compound absorbs at a particular wavelength .

The equation for Beer's law is a straight line with the general form of **y = mx + b**

Beer's Law : **A = (E l) C**

The general form : **y = (m) x + b**

where **the slope = m = (El)**

In this case , the absorbances of different concentrations of a standard substance is plotted against their concentrationsto generate a standard " calibration " curve.

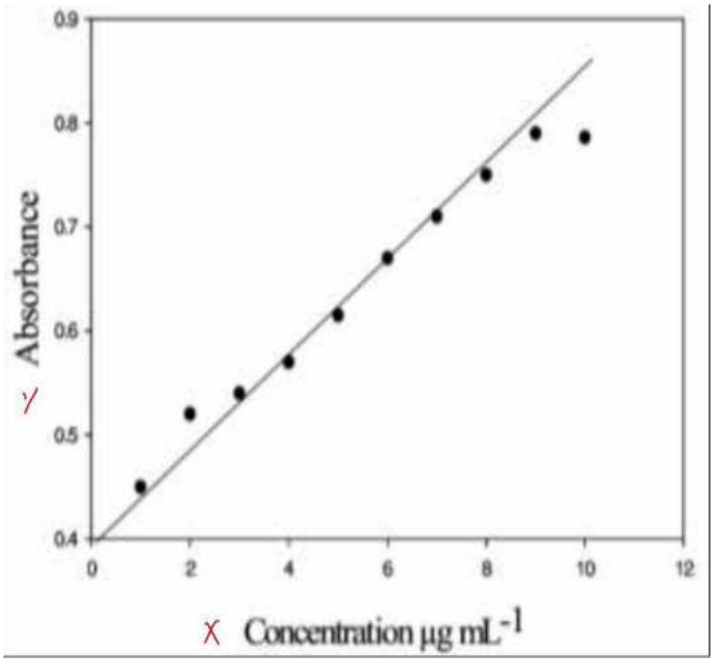
A calibration curve (a standard curve) is a general method for determining the concentration of a substance in an unknown sample

by comparing the unknown to a set of standard samples of known concentration (for protein assay , sugar assays , and various types of assays).

You can find **the slope & y-intercept** of the linear graph **to get the linear equation .**

By measuring the absorbance of the sample of unknown concentration ,
you can apply it to the equation to find the concentration (there is an example afterward).

يُفضل آخذ النقاط أن القيم على على الخط
حتى يروح يوخذ القيم اكثر من الخط المستقيم



Calibration curve of absorbance against concentration using Beer's Lambert law.

نظريًا ، يجب أن يكون تقاطع Y صفرًا
لل the Beer's Lambert law ؟

Theoretically the Y-intercept should be zero for the Beer's Law Plot ?

لأنه عند التركيز الصفري ، لا يوجد مركب
مذاب في المحلول ،

Because at zero concentration , there is no compound dissolved in solution ,
and the spectrophotometer is zeroed using the blank before taking the measurements .

و يتم وصف مقياس الطيف
الضوئي باستخدام ال blank قبل أخذ القياسات

All light absorption measurements are made relative to **a blank solution** which contains all the components of the sample solution except the substance being analyzed
But practically , you may have y-intercept ,

يتم إجراء جميع قياسات امتصاص الضوء بالنسبة إلى ال blank solution يحتوي على
جميع مكونات محلول العينة باستثناء المادة التي يتم تحليلها لكن عمليًا ، قد يكون لديك تقاطع y ،

if the plot is not linear or if the y-intercept deviates substantially from the origin , it indicates that the standards were improperly prepared , that there is an unknown interference in the sample .

إذا لم يكن الرسم البياني خطيًا أو إذا كان تقاطع y ينحرف بشكل كبير عن الأصل ، فإنه يشير إلى
أن القعاييره كانت غير صحيحة ، أن هناك تداخلا (interference) غير معروف في العينة

If we have two concentrations of the same solution ,
then they have the same molar extinction coefficient (E) ,
and the length path (l) is 1 cm in most spectrophotometers .

إذا كان لدينا تركيزان من نفس المحلول ، فسيكون لهما
نفس ال molar extinction coefficient (E) ، وطول
المسار (l) هو 1 سم في معظم أجهزة قياس الطيف الضوئي

$$A = (El)C$$
$$(El) = A/C$$

- E x l (1st concentration) = E x l (2nd concentration)
- E 1 = E2
- A /C 1 = A / C2

Therefore the absorbance / concentration relationship
can be applied using the same linear equation we got for the standard .

Example ✍

A calibration curve was generated for a set of standard concentrations (mg / ml).
The slope for the line is 0.13 and the y-intercept is 0.018

$m = E * L$

using beers law ,
calculate the concentration of an unknown sample , given that its absorbance was 0.5

$$y = (m)x$$
$$A = (El)C$$

slope = m = E * L = 0.13

y- intercept = b = 0.018

absorbance = y = 0.5

c = x = standard concentration = ?! (mg / mL)

$y = A + b$

$y = (E * L * C) + b$

$\boxed{y = (m * c) + b}$

-8 الكل

$0.5 = (0.13 * x) + 0.018$

$-0.018 \qquad -0.018$

$0.4892 = 0.13 * x$

$x = \frac{0.4892}{0.13} = 3.763 \text{ mg/ml}$

Colorimetric reagent protein assay

These assays are useful to quantify the amount of protein in a given sample .These are fast and easy to perform , and do not require complex or hazardous chemicals .

In these assays , a reagent that specifically absorbs a specific amount of light is attached to a specific protein , and then the amount of light is measured .

Two main types of colorimetric reagent protein assay :

[1] Copper chelation and detection of the reduced copper

Bicinchoninic acid (BCA) assay

Compatible متوافق with samples containing detergents

Incompatible غير متوافق with reducing agents ; chelators

Assay range : 20 to 2,000 µg/mL

sample volume : 25 µL

Incubation time فترة الحضانة & temperature : 30 min at 37°C

Assay measurement (absorbance maximum wavelength -λ max) : 540 nm

[2] Protein - dye binding and associated colour change

Bradford (Coomassie dye)

Compatible with samples containing buffer salts , metal ions , reducing agents , chelators.

Incompatible with samples containing detergents.

Assay range : 100 µg/ml - 1,500 µg/ml

sample volume : 20 µL

Incubation time & temperature : 5 - 10 minutes at room temperature

samples should not be incubated longer than 1 hr at room temperature.

Assay measurement (absorbance maximum wavelength -λ max) : 595 nm

■ Bradford assay principles

Use of Coomassie G-250 Dye in a colorimetric reagent for the detection and quantitation of total protein was first described by Dr. Marion Bradford in 1976. It is a colorimetric , spectrophotometric quantitative assay to measure protein concentration.

■ Chemistry of Bradford , Coomassie - based protein assays

In an acidic environment , proteins bind to coomassie dye . This results in a spectral shift from the brown form of the dye to the blue form . The optimal wavelength to measure the blue color from the Coomassie dye-protein complex is 595 nm.

Development of color in Coomassie dye-based protein assays has been associated with the presence of certain basic amino acids-primarily arginine, lysine, and histidine in the protein .

Van der Waals forces & hydrophobic interactions also influence dye-protein binding .

The number of Coomassie dye molecules bound to each protein is approximately proportional to the number of positive charges found on the protein .

هون في هذه الحاله لل ٣ مواد ما بنستخدم الصبغة عليهم

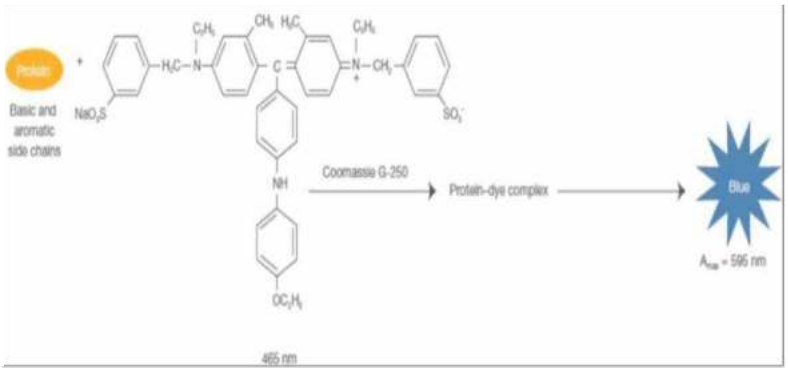
Free amino acids , peptides, and low molecular weight proteins do not produce color with Coomassie dye reagents . In general , the mass of a peptide or protein should be at least 3,000 Dalton for quantification with this reagent.

لأنه لازم البروتين إلّي عندنا أو البيتيد يتكون كتلة المولية أقل من 3000 دالتون .
بالعادة ال Basic amino acids بيحملو الشحنة الموجبة

Bovine serum albumin (BSA)

which is used to generate the standard curve has a molecular mass of 66.5 KDalton and is used because it is widely available in high purity and it is inexpensive .

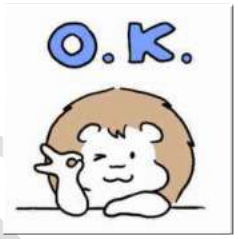
Figure : Reaction schematic for the Coomassie dye–based Bradford protein assays.



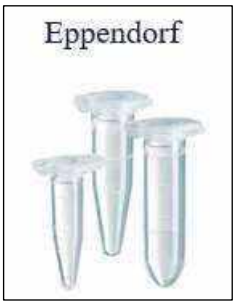
The main disadvantage of Coomassie based protein assays is their incompatibility with surfactants/detergents at concentrations routinely used to solubilize membrane proteins.

In general , the presence of a surfactant in the sample, even at low concentrations, causes precipitation of the reagent.

In addition , the Coomassie dye reagent is highly acidic, so proteins with poor acid - solubility cannot be assayed with this reagent.

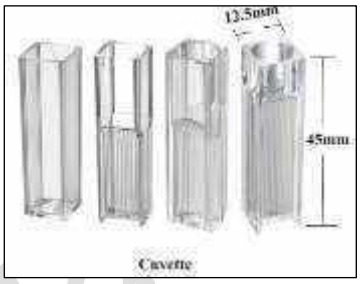


Experimental procedure:



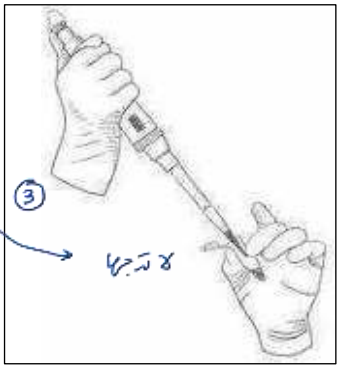
Materials

- Unknown sample
- Micropipette (P-1000, P-20)
- Tips
- 6 Eppendorf's
- Cuvette
- Distilled water
- BSA standard 5 (125 µg/ml)
- BSA standard 4 (250 µg/ml)
- BSA standard 3 (500 µg/ml)
- BSA standard 2 (1000 µg/ml)
- BSA standard 1 (1500 µg/ml)
- Spectrophotometer



Method

1. Label each Eppendorf with the concentration of the standard/ sample on the cap or the lateral side. Then:
2. Add 20 µl of each standard to 1000 µl Bradford reagent in an Eppendorf and mix by flipping upside down (do not shake vigorously and cause bubbles, it will interfere with your measurements).



Assay range (sample volume), 100 µg/ml-1,500 µg/ml (20 µL).
Ration of sample to Bradford reagent is 1:50
(20 µl sample: 1000 µl Bradford reagent)

Eppendorf	Bradford reagent	Standard/sample
1	1000 µl	20 µl standard 5 (125 µg/ml)
2	1000 µl	20 µl standard 4 (250 µg/ml)
3	1000 µl	20 µl standard 3 (500 µg/ml)
4	1000 µl	20 µl standard 2 (1000 µg/ml)
5	1000 µl	20 µl standard 1 (1500 µg/ml)
6	1000 µl	20 µl Unknown sample

2. Incubate for 5-10 minutes at room temperature.
3. Switch the instrument at least 5-10 minutes before use to allow it to stabilize.
4. Select the λ max.
5. Place the blank solution (or water) in the cuvette, so that the instrument is zeroed.
   - Make sure the clear faces of the cuvette are in the light path and that the outside of the cuvette is dry and clean.

- Handle cuvettes only by the top edge of the ribbed sides (finger print affects the measurement). All solutions should be free of bubbles.

6. Place the sample (standard/unknown) in the allocated place in the spectrophotometer.
7. Measure absorbance of standards and unknowns at 595nm, using water as a blank (when measuring by spectrophotometer, use 800 µl of the sample/standard or more in plastic cuvettes, using less volume will result in bad absorbance numbers- sample cuvette must be half/two third full)
8. Measure absorbance of the unknowns at 595nm similar to standard procedure.
9. Record your measurements.

➢ For standard samples: you can use one plastic cuvette multiple times without washing if you work from low to high concentration. Otherwise, you must use a new cuvette each time or wash the cuvettes for reuse to get the blue out.

يمكنك استخدام cuvette بلاستيكي واحد عدة مرات بدونه غسل إذا كنت تعمل من التركيز المنخفض إلى العالي

يجب عليك استخدام cuvette جديد في كل مرة أو اغسل ال cuvettes لإعادة استخدامها لإخراج اللون الأزرق

➢ If your unknown colour is more intense than the higher standard, you should dilute it in water• It is advisable to make several different dilutions of your unknown sample (1 in 2 or 1 in 4 dilution) so that it falls within the standard range of the assay. When analysing the data, you have to multiply the concentration you got after dilution with the dilution factor:

➢ concentration of the unknown= concentration after dilution x dilution factor.

➢ YOU DO NEED TO RUN STANDARDS EVERY TIME! The reagent's response will change even after one day.

إذا كان لون الألوان أكثر كثافة من ال higher standard ، فيجب عليك ان تخففه في الماء . من الأفضل عمل عدة تخفيفات مختلفة من ال unknown sample (1 في 2 أو 1 من 4 تخفيف) بحيث تقع ضمن نطاق الفحص المعياري .

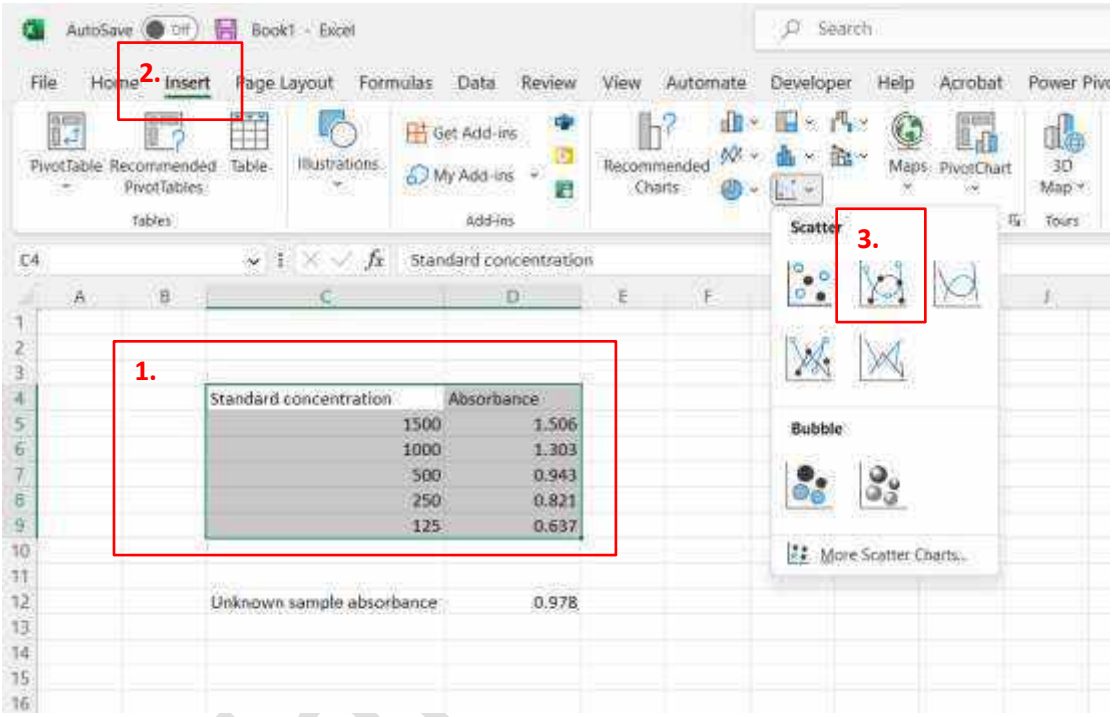
🤡 من غشى فليس منا

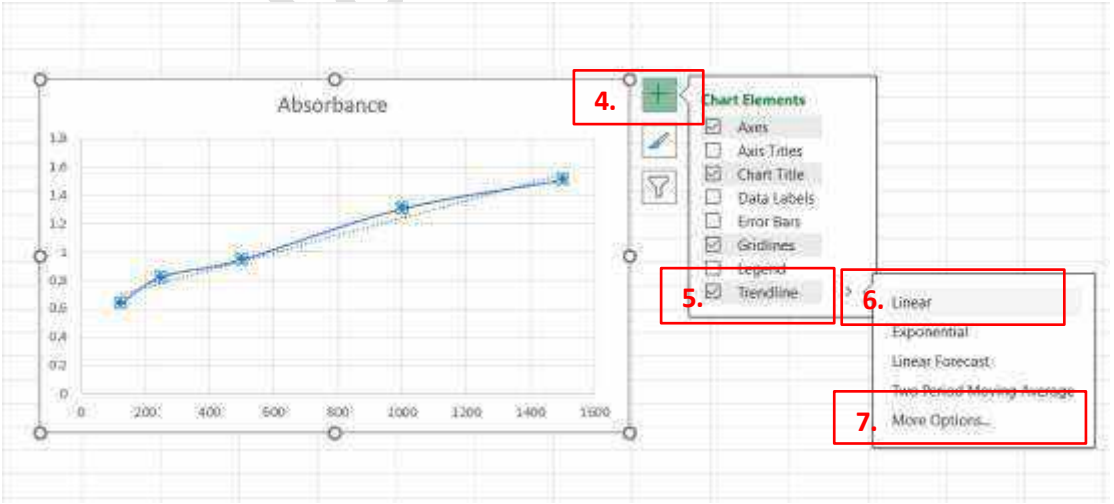
Data analysis:

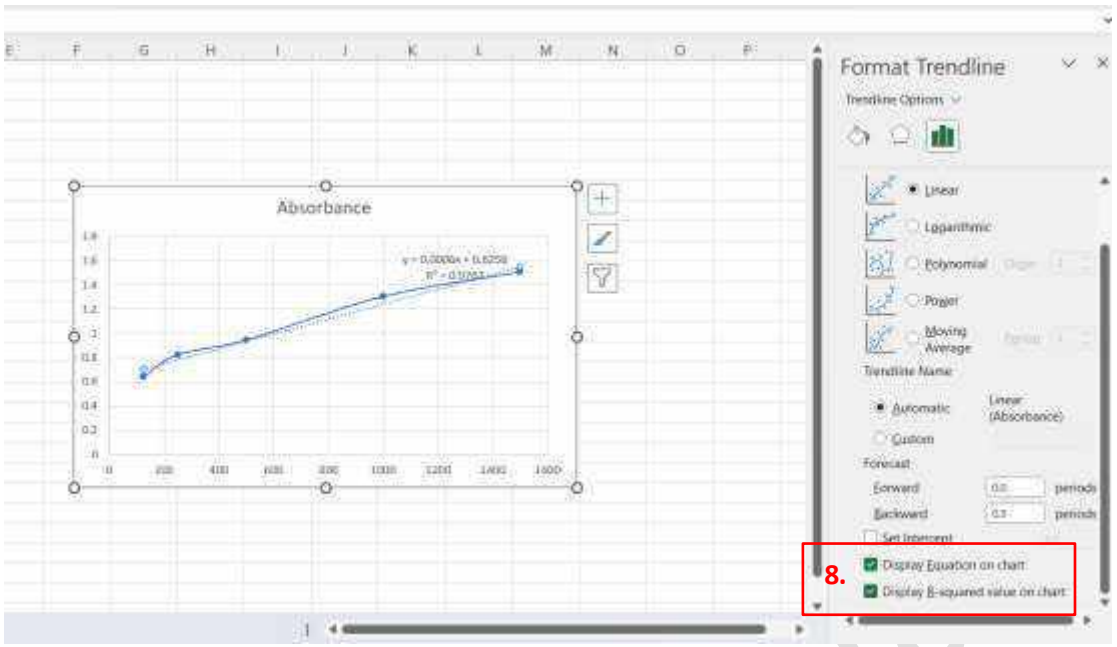
Draw a standard curve, and find the linear equation for your curve, R^2 value, and calculate your unknown concentration.

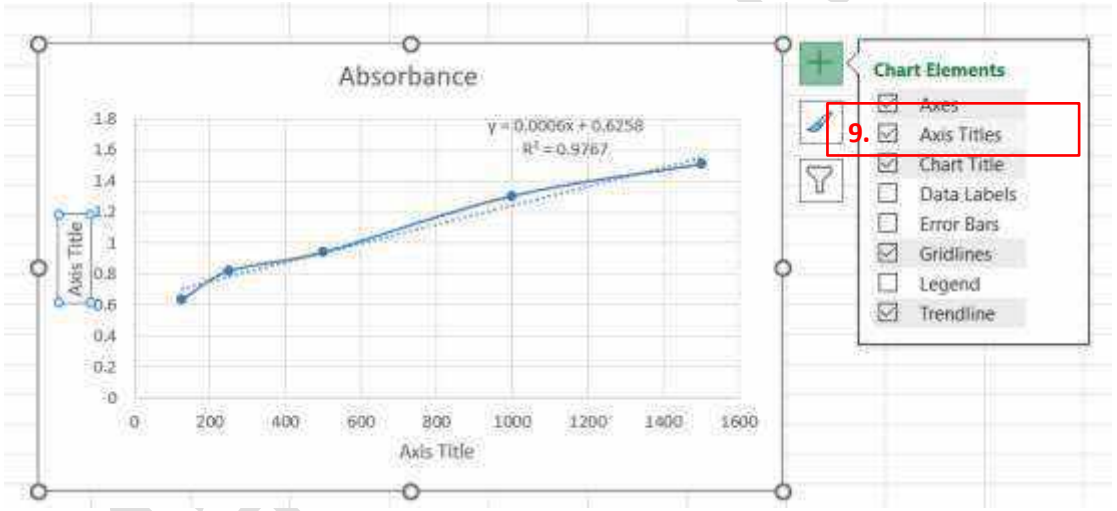
• Create a calibration/standard curve graph of bovine serum albumin (BSA) protein concentration vs. absorbance using Bradford method.

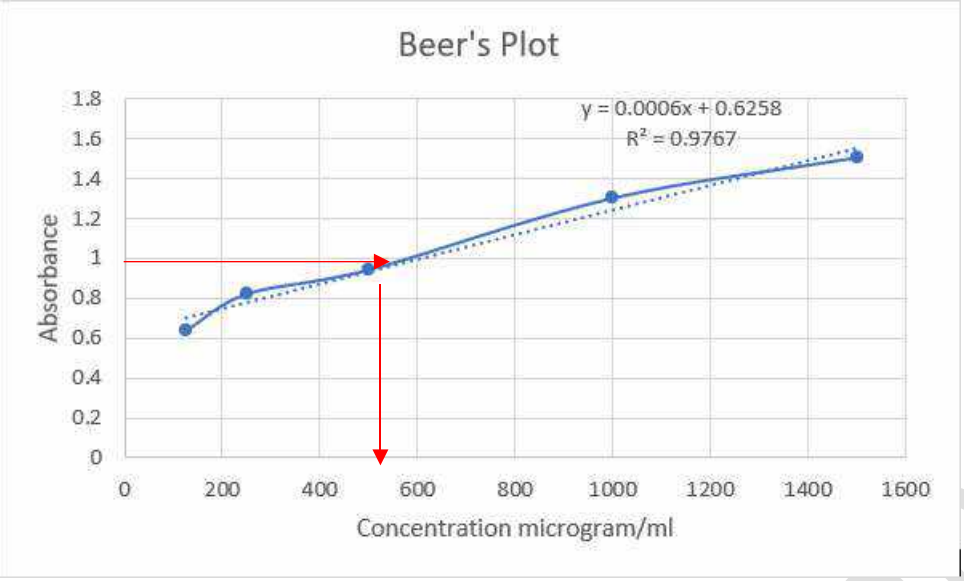
1. Enter the recorded measurement you obtained in the lab as in the image below, then click insert, choose scatter and click on the second option (scatter with smooth line and marker).
2. Double click on the line generated and choose the +, choose trendline, and then click on the linear option, then more options
3. On the right panel click on: Display equation on chart and display R-squared value on chart. R^2 value should ideally be above 0.9.
4. Edit y and x-axis and the plot title,
5. Print the form and submit it with your report. From the absorbances of the unknowns, find the protein concentration using the linear equation generated.






Absorbance
y = 0.0006x + 0.6258
R² = 0.9767
Format Trendline
Trendline Options
Linear
Logarithmic
Polynomial
Power
Moving Average
Trendline Name
Automatic
Linear (Absorbance)
Custom
Forecast
Forward
Backward
periods
Display Equation on chart
Display R-squared value on chart
8.


Absorbance
y = 0.0006x + 0.6258
R² = 0.9767
Axis Title
Axis Title
Chart Elements
Axes
Axis Titles
Chart Title
Data Labels
Error Bars
Gridlines
Legend
Trendline
9.



Unknown absorbance = 0.978

$y = 0.0006x + 0.6258$, $R^2 = 0.9767$

$0.978 = 0.0006x + 0.6258$

$0.978 - 0.6258 = 0.0006x$

$X = 587$ microgram/ml

مدى تقارب القراءات خ

كلما كانت أقرب من 1 كلما كان شغلي صحيح .
وكلما كانت قيمته أقرب للصفر معناته شغلي غير دقيق .

Experiment 6
Isolation of casein from milk and its evaluation

Milk is the most nutritionally complete food found in nature .
All kinds of milk , human or animal , contain vitamins (principally thiamine , riboflavin , pantothenic acid , and vitamins A , B12 , and D), minerals (calcium , potassium , sodium , phosphorus , and trace metals), proteins (mostly casein), carbohydrates (principally lactose), and lipids (fats).

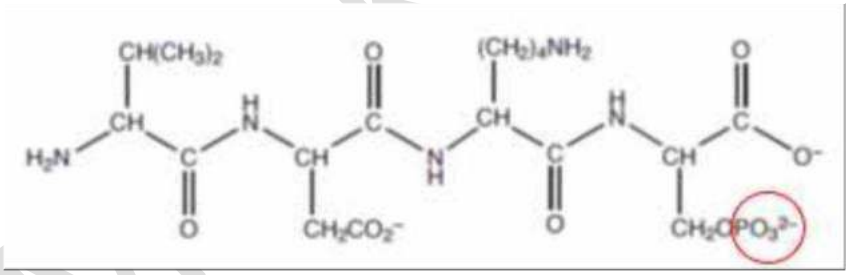
The amounts of these nutrients present in different types of milk differ greatly .
Cows milk & goats milk are almost identical in every respect .

Human milk contains less than half of the proteins and minerals of cows or goats milk , but almost 1.5 times as much sugar .

Casein the main protein in mammalian milk ,
is a phosphoprotein , meaning that phosphate groups are attached to the hydroxyl groups of some of the amino acid side - chains .

Casein **exists in milk as the calcium salt , calcium caseinate .**

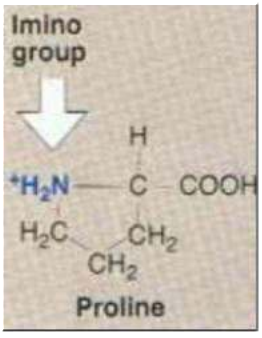
➢ The negatively charged phosphate groups are balanced by positive calcium ions and are responsible for the high nutritional calcium content in milk .



➢ **Almost all essential amino acids are found in casein protein in high proportion .**

➢ It contains a fairly high number of proline residues , which do not interact . There are also no disulfide bridges .

As a result , it has relatively little tertiary structure .



➢ Amino group of proline is found in a ring (immino group) preventing free rotation and thus limiting the formation of tertiary structure .

Calcium caseinate is actually a mixture of at least three similar proteins which differ primarily in molecular weight and the amount of phosphorus groups they contain .

Alpha - & beta - casein have molecular weights in the 25,000 range and possess about 9 & 4-5 phosphate groups per molecule , respectively.

They are both insoluble in water .

Kappa - casein has a molecular weight of about 8,000 kd and possesses 1-2 phosphate groups per molecule .

It is responsible for solubilizing the other two caseins (alpha & beta caseins) in water by promoting the formation of micelles.

》 **Casein protein is found in milk as a suspension called " Casein Micelles ".**

في تناقض / إختلاف حاد بالنسبة لل surfactant micelles ، فإن الجزء الداخلي من casein micelle رطب بدرجة عالية
(يعني إنه الجزء الداخلي لل surfactant micelles مش رطب كتير زي ال casein micelle) 🧐
الكازين في ال micelles يتم تجميعه معًا بواسطة أيونات الكالسيوم والتفاعلات الكارهة للماء .

Casein micelles show only limited resemblance with **surfactant - type micelle** in a sense that the hydrophilic parts reside at the surface and they are spherical .

However, in sharp contrast to surfactant micelles , the interior of a casein micelle is highly hydrated .

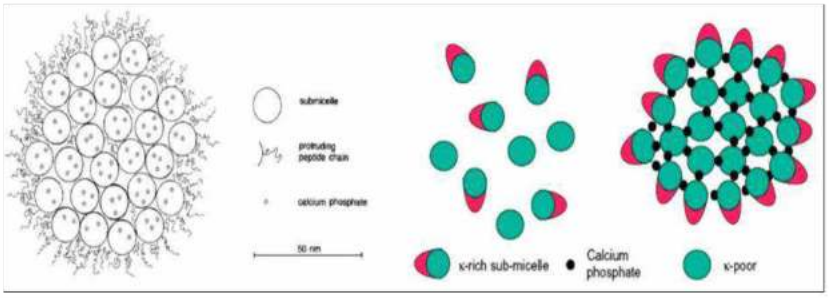
The caseins in the micelles are held together by calcium ions and hydrophobic interactions .

Casein micelles are considered as colloidal particles formed by casein aggregates wrapped up in soluble **κ- casein molecules** .

The outer surface of the micelle are rich with **κ- casein** , has protruding hairs of negative charges which induce a steric repulsion between micelles , thus preventing them to coagulate and hence stabilizes the milk.

لها شعيرات بارزة من الشحنات السالبة التي تُحفز التنافر الفراغي بين ال micelles

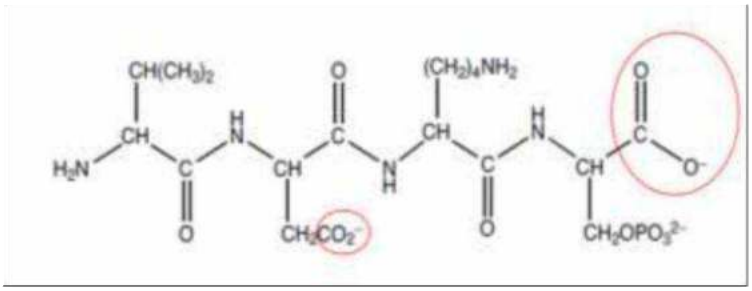
وبتالي تمنعهم من التخثر وتساعد على إستقرار الحليب



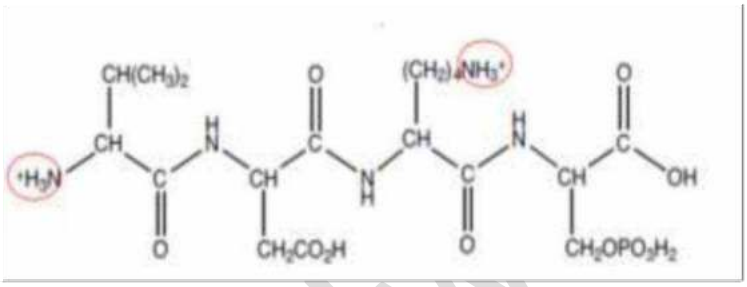
Casein and pH

At high pH , casein will have a net negative charge due to ionization of its acidic side chains (- COO -). Because casein is ionized at high pH values , it is soluble in dilute sodium hydroxide solution .

》 Casein is readily dispersible in dilute alkalis and in salt solutions such as sodium oxalate and sodium acetate .

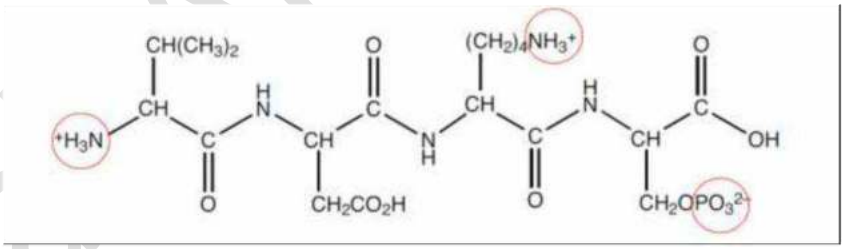


At low pH , casein will have a net positive charge due to protonation of its basic side chains (-NH3+). Because casein is ionized at low pH values , casein is also soluble in strongly acidic solutions .



At intermediate pH values , casein will contain an equal number of positively and negatively charged groups and the protein will have a net charge of zero .

Casein is insoluble in neutral solutions because it is not charged under these conditions .



The solubility of a protein is usually at a minimum at its isoelectric point (IP).
The isoelectric point is defined as the pH at which a protein has a net charge of zero .

For casein , due to the attached phosphate groups , the isoelectric point is close to pH = 4 [ranges from 4.6 - 4.8].

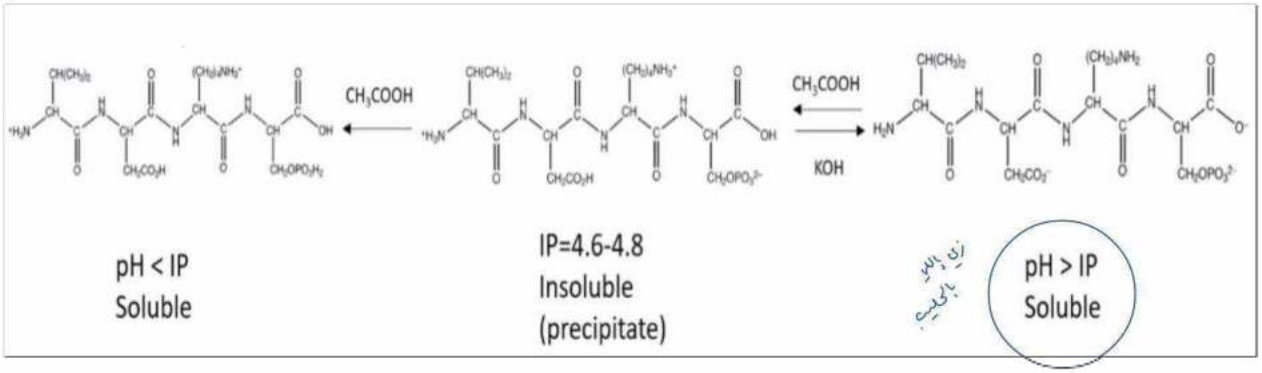
《 Casein isolation 》

Since milk's pH is 6.6 (which is higher than the isoelectric point of casein)

, so casein has a negative charge in milk ; which induce a steric repulsion between micelles , thus preventing
them from coagulation and hence stabilizes the milk .

To precipitate the casein → acetic acid is added dropwise until the isoelectric point is reached .

Note : if excess CH3COOH is added the precipitate redissolves .



الكازين غير قابل للذوبان في الإيثانول وهي خاصية تستخدم لعزله عن الدهون غير المرغوب فيها

Casein is insoluble in ethanol which is a property used to isolate it from the unwanted fat .
Unlike many proteins , casein is not coagulated by heat . على عكس العديد من البروتينات ، لا يتخثر الكازين بالحرارة

Ether is also used in casein isolation to remove impurities & to remove any traces amounts of remaning water (drying).

Casein has many uses including إستخدامات الكازين

Paint

دهان الكازين عبارة عن وسط سريع الجفاف وقابل للذوبان في الماء يستخدمه الفنانون منذ القدم المصرية مرات كشكل من أشكال الطلاء الحراري
، وكان يستخدم على نطاق واسع من قبل الرسامين التجاريين مثل المادة المفضلة حتى أواخر الستينيات عندما أصبح الكازين مع ظهور طلاء الأكريليك أقل شعبية.

Casein paint is a fast - drying , water - soluble medium
used by artists since ancient Egyptian times as a form of tempera paint ,

and was widely used by commercial illustrators as the **material of choice** until the late 1960s when , with the advent of **acrylic paint** , casein became **less popular** .

Glue

مواد لاصقة أساسها الكازين ، مصنوعة من الكازين والماء والجير المطفأ وهيدروكسيد الصوديوم كانت شائعة في الأعمال الخشبية ، بما في ذلك الطائرات

Casein - based glues , formulated from casein , water , hydrated lime and sodium hydroxide were popular for woodworking , including for aircraft .

Cheese making

Cheese consists of proteins and fat from milk , usually the milk of cows🐄 , buffalo🐃 , goats🐐 , or sheep 🐑.
It is produced by coagulation of casein .

Typically , the milk is acidified and then coagulated by the addition of **rennet** مادة تُستخرج من الغشاء الفبطن للعجل , containing a proteolytic enzyme , typically obtained from the stomachs of calves .

The solids are separated and pressed into final form .
During the process of clotting , milk-clotting **proteases** act on the soluble portion of the caseins, κ-casein , thus originating an unstable micellar state that results in clot formation .

When coagulated with **chymosin** , **casein is sometimes called paracasein** .

Chymosin is an aspartic protease that specifically hydrolyzes
the peptide bond in Phe105 - Met 106 of κ- casein , and is considered to be the most efficient protease for the cheesemaking industry .

Calf rennet , which consists of over 90% chymosin , is commonly used in
cheese industries for the curdling of milk .

Whey protein = watery substance = milk serum

British terminology , on the other hand , **uses the term caseinogen for the uncoagulated protein & casein for the coagulated protein .**
As it exists in milk , it is a salt of calcium .

Cow's milk 🥛🐄 contains several proteins . Casein is one of the major proteins - around 80%.
When casein is removed from milk , the remaining proteins are known as whey proteins 20%.

Whey is a **watery substance (milk serum),** It consists of several globular proteins (beta lactoglobulin , alpha lactalbumin). **Casein** doesn't have a good tertiary structure .

Further whey contains lactose , vitamins and minerals in contrast to casein على عكس الكازيين

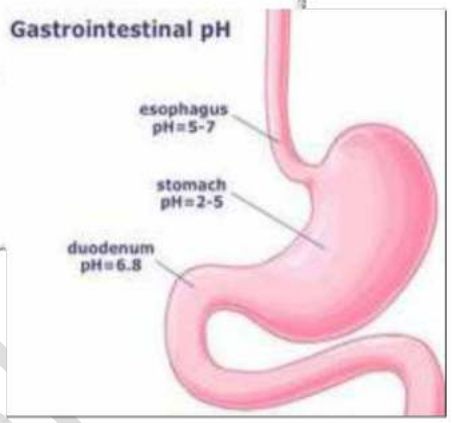
Protein Supplement

An attractive property of the casein molecule is **its ability to form a gel or clot in the stomach** , which **makes it very efficient in nutrient supply .**

The clot is able to provide a sustained slow release of amino acids into the blood stream , sometimes lasting for several hours .

Often casein is available as **hydrolyzed casein**,
whereby it is hydrolysed by a **protease such as trypsin**.

Hydrolysed forms are noted to taste bitter and such supplements are often refused by infants and lab animals in favour of intact casein.



Procedure:

Material:

1. 100 mL of Milk (10% w/v)
2. Acetic acid (CH_3COOH) (10% v/v), dropper.
3. Ethanol (95%v/v) (flammable)
4. Diethyl ether (highly flammable)
5. Thermometer 100°C (ethanol or mercury thermometer).
6. Hot plate.
7. Graduated cylinder.
8. Beaker.
9. Volumetric flask.
10. funnel
11. Spatula.
12. Glass rod.
13. Muslin cloth
14. Filter paper
15. Watch glass.

Method:

1. Weigh an empty 250 ml beaker,then place 100 ml of milk and weigh the milk
2. warm the milk to 40°C on a hot plate.
3. Once temperature is reached, remove the beaker containing milk from the hot plate and start adding acetic acid slowly (drop by drop) to the milk while stirring with a stirring rod.
   Note: never transfer the acetic acid all at once.
4. keep adding acetic acid drop by drop until milk clots starts to form and the casein in precipitated (~2 ml CH_3COOH). This point means that you have reached a pH of 4.6-4.8
5. . Leave to stand for 5 min and filter/decant through muslin cloth (you may need to wait for 10-15 minutes for the filtration to be completed).
6. Collect the casein precipitate and transfer into a beaker using spatula. And suspend the precipitate in 50 ml mixture of equal volume of ethanol and ether (1:1), mix by

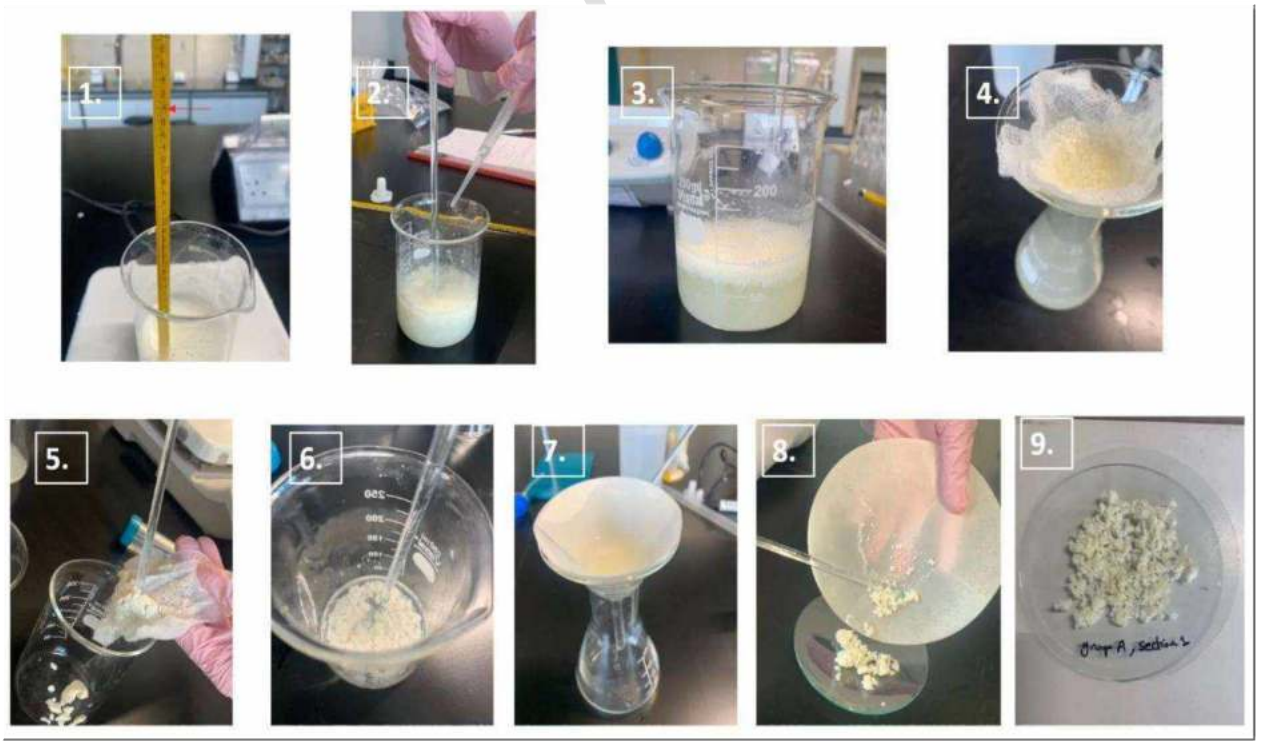
glass rod so that the extra fat may come down to the solvent system. Note: Be very careful not to spill the precipitate.

7. filter the precipitate again using filter paper. Wait until the solution is totally filtered.
8. Once all the solvent has been removed from casein. Remove the powder and spread out on a watch glass (or petri dish, weighing boat) until it is completely dry (usually overnight).
   Note: label your watch glass/weighing boat with your group name and section number.
9. Weigh your sample and calculate the percent yield you obtained (Normal Range 3-5 %)

$$\%casein = \frac{gram\ casien}{grams\ of\ milk}\ X\ 100$$

$$\%casein = \frac{5}{105}\ X\ 100$$

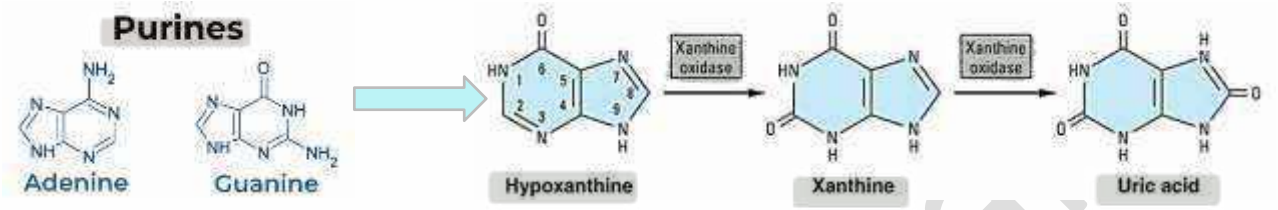
% casein= 4.7%



☆ ال Xanthine oxidase (XO) هو شكل من أشكال xanthine oxidoreductase ، وهو نوع من الإنزيم الذي ينشأ أنواع الاكسجين التفاعلية.
هذه الإنزيمات تحفز أكسدة hypoxanthine إلى xanthine و يمكن أن يحفز كذلك أكسدة ال xanthine إلى uric acid .
تلعب هذه الإنزيمات دورًا مهمًا في عمليات الأيض
لل purines في بعض الأنواع ، بما في ذلك البشر .

Experiment 7
Xanthine oxidase with allopurinol-IC50 protocol

Xanthine oxidase (**XO**) is a form of **xanthine oxidoreductase**, a type of enzyme that generates reactive oxygen species. These enzymes catalyse the oxidation of hypoxanthine to xanthine and can further catalyse the oxidation of xanthine to uric acid. These enzymes play an important role in the catabolism of purines in some species, including humans.



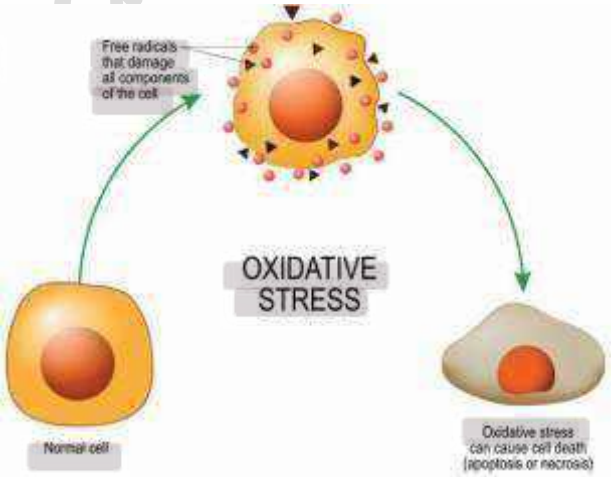
The following chemical reactions are catalyzed by xanthine oxidase:

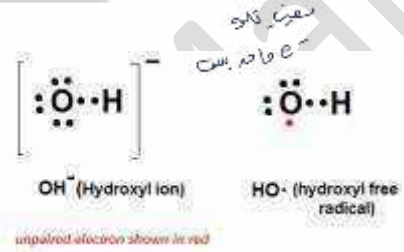
hypoxanthine + H_2O + O_2 ⟷ xanthine + H_2O_2
xanthine + H_2O + O_2 ⟷ uric acid + H_2O_2

☆ يعمل كعنصر مهم من المصدر البيولوجي
لل oxygen derived free radicals وبتالي يساهم في
الأضرار التأكسدية للأنسجة الحية.

Xanthine oxidase (XO) serves as an important biological source of oxygen-derived free radicals that contribute to the oxidative damage of living tissues. Free radicals are atoms or groups of atoms that have a single unpaired electron. They are unstable molecule that is made during normal cell metabolism. Free radicals can build up in cells and cause damage to other molecules, such as DNA, lipids, and proteins. This damage may increase the risk of cancer and other diseases.

Free Radical



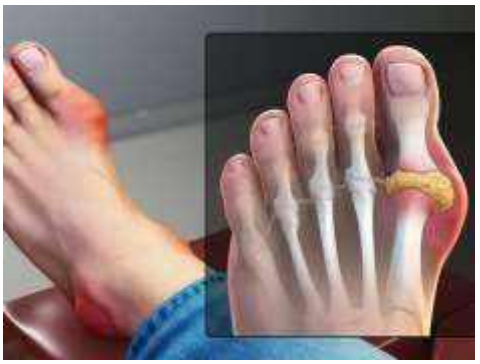


☆ الجذور الحرة FREE RADICAL : هي الذرات أو مجموعات الذرات التي
لها single unpaired electron. هم جزيء غير مستقر التي يتم إجراؤها
أثناء التمثيل الغذائي الطبيعي للخلايا

Gout-النقرص

☆ ال free radical يمكن أن تتراكم الجذور في الخلايا وتسبب أضرارًا للجزيئات الأخرى ،
مثل ال DNA والدهون والبروتينات. هذا ال damage قد يؤدي إلى زيادة خطر الإصابة بالسرطان و الأمراض الأخرى .

XO is involved in the medical condition known as gout, which is characterized by hyperuricemia that leads to uric acid deposition in the joints resulting in painful inflammation. Hyperuricemia, which is present in 5–30% of the general population, seems to be increasing worldwide and is considered an important risk factor in serious disorders, e.g. renal failure.



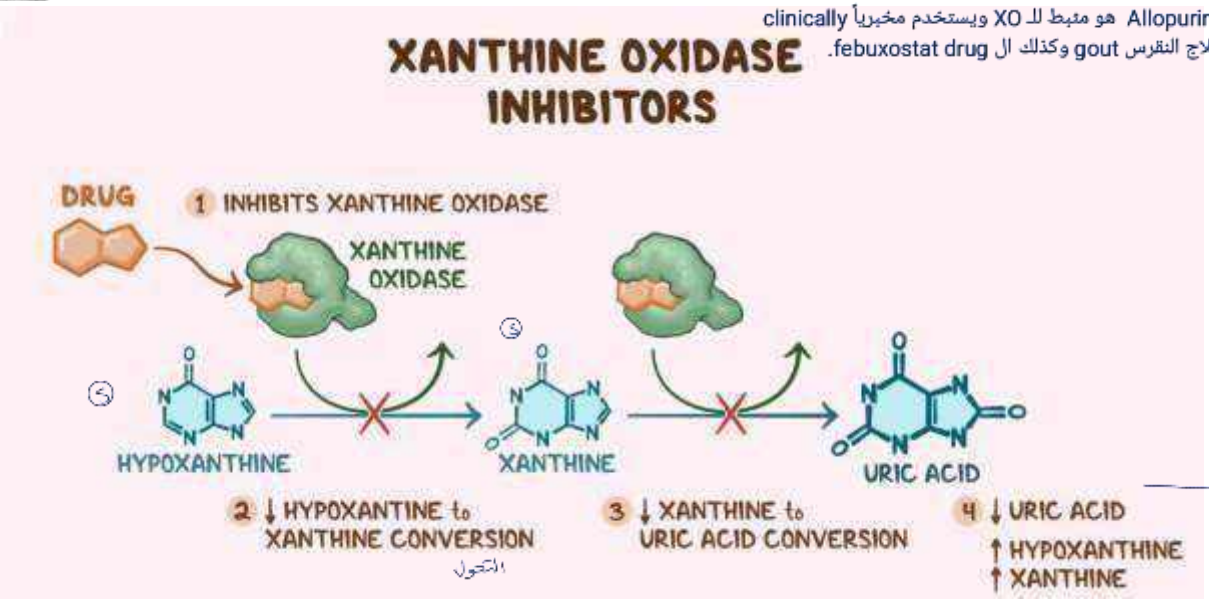
☆ ال xanthine oxidase تُساهم في الحالة الطبية المعروفة باسم النقرس Gout ، وهو يتميز بزيادة ال uric acid بالدم الذي يؤدي تراكمه الي تولد ضغط في المفاصل مما يؤدي إلى
التهاب مؤلم . زيادة حمض يوريك الدم ، التي تتواجد في 5-30٪ من عامة السكان ، على ما يبدو تتزايد في نطاق أوسع وتعتبر عامل خطر مهم في الاضطرابات الخطيرة ،
على سبيل المثال الفشل الكلوي renal failure .

☆ أدوية حمض اليوريك - Uricosuric drugs التي تزيد من ال urinary excretion من ال uric acid ، أو مثبطات ال XO التي تمنع الخطوة النهائية في التصنيع الحيوي لحمض اليوريك ، يمكن أن يخفض تركيز حمض اليوريك في البلازما ، وهي كذلك يستخدم بشكل عام لعلاج النقرس .

Uricosuric drugs which increase the urinary excretion of uric acid, or XO inhibitors which block the terminal step in uric acid biosynthesis, can lower the plasma uric acid concentration, and are generally employed for the treatment of gout.

Allopurinol is an inhibitor of XO and used clinically in the treatment of gout as well as febuxostat drug

☆ ال Allopurinol هو مثبط لل XO ويستخدم مخبرياً clinically في علاج النقرس gout وكذلك ال febuxostat drug.



Assay procedure:

1. The xanthine oxidase activity will be assayed using xanthine as substrate and utilizing a plate reader.
2. In a 96 well plate, add 50 µl of allopurinol (concentration as written in the table), 95 µl of 1X Phosphate buffer and 50 µl of xanthine substrate (0.6mM). (Final volume per well is 200 µl) (prepare the blank and enzyme only wells as per the numbers in the table below)

Well no.	1	2	3	4	5	6	7	8	9
Phosphate Buffer	150µl	145µl	95 µl	95 µl	95 µl	95 µl	95µl	95µl	95µl
Xanthine substrate (0.6mM)	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50µl	50µl	50µl
Allopurinol	-----	-----	50 µl 40ng/ml	50 µl 400ng/ml	50 µl 2µg/ml	50 µl 6µg/ml	50 µl 40µg/ml	50 µl 50µg/ml	50µl 100µg/ml
Xanthine oxidase enzyme	-----	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl	5µl	5µl

3. Preincubate for 10 min at 25°C
4. Start the reaction by the addition of 5 µl ml of XO enzyme (0.1U/ml in phosphate buffer).
5. Incubate the reaction at 25°C for 30 min and measure the absorbance against phosphate buffer as blank at **295 nm** using **Biotek plate reader.**

6. Select the xanthine oxidase protocol from the software and make sure the settings are correct and validated (set the plate layout according to the wells used)

Calculation

Calculate % inhibition for each concentration of allopurinol using endpoint absorbances. Make sure to blank out your Absorbance reading-by subtracting the absorbance of blank. (show your calculation):

- $\%in\ \ ibition = 100 - [\frac{A1 - A\ (blank)}{A_{\circ} - A\ (blank)}] * 100$

Where A1 is the activity of the enzyme in presence of plant extract, $A_{\circ}$ is the absorbance in absence of the plant extract (activity of the enzyme alone).

For example; allopurinol (40 ng/ml → the final conc. in the well is 10 ng/ml as the inhibitor is diluted by 1 in 4 in each well) and the % inhibition is calculated as follow
Blank Abs=0.18075, $A_{\circ}$=4.473 and A1=2.4159.

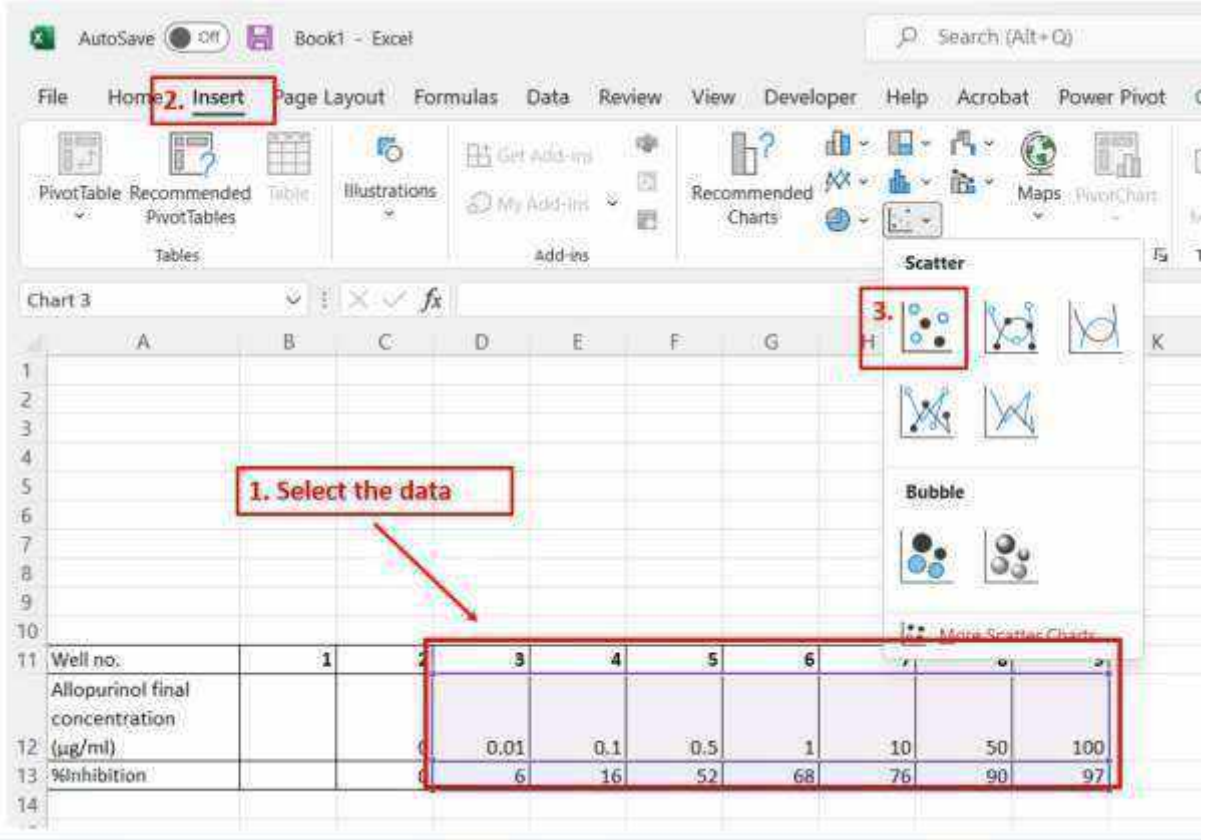
Well no.	1	2	3	4
Phosphate Buffer	150µl	145µl	95 µl	95 µl
Xanthine substrate	50 µl	50 µl	50 µl	50 µl
Allopurinol	-----	-----	50 µl 40ng/mL	50 µl 400ng/ml
Xanthine oxidase enzyme	-----	5 µl	5 µl	5 µl
Final allopurinol concentration per well.	-----	Zero		
Absorbance (30 minutes)	Blank Abs 0.1807	Ao 4.473	A1 2.4159	
%Inhibition			47.92	

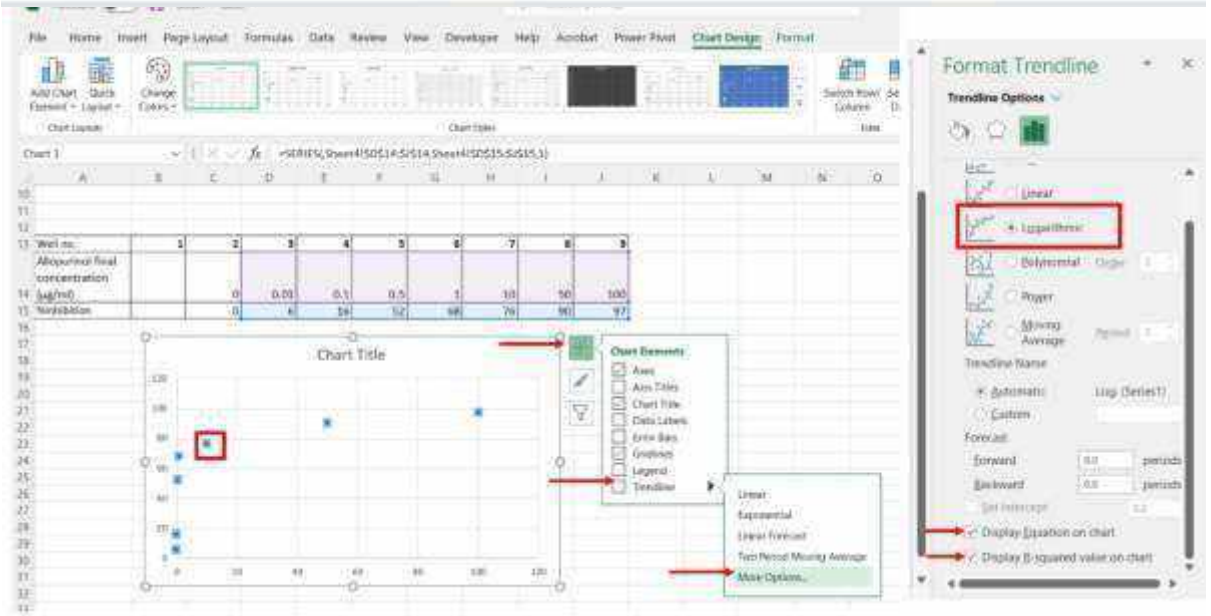
$\%in\ \ ibition = 100 - [\frac{2.4159 - 0.18075}{4.473 - 0.18075}] * 100$

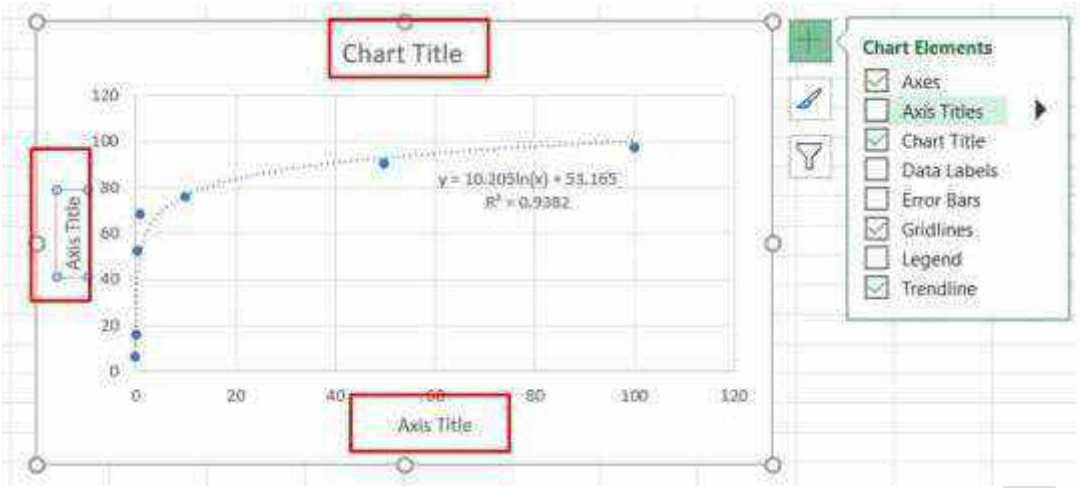
= 100 -52.07

= 47.92%

Draw graph using excel, by following the steps below:







How to determine the IC50?

IC50 is the half maximal inhibitory concentration

- For example: maximum inhibitory concentration was found to be 97% as in the table below:

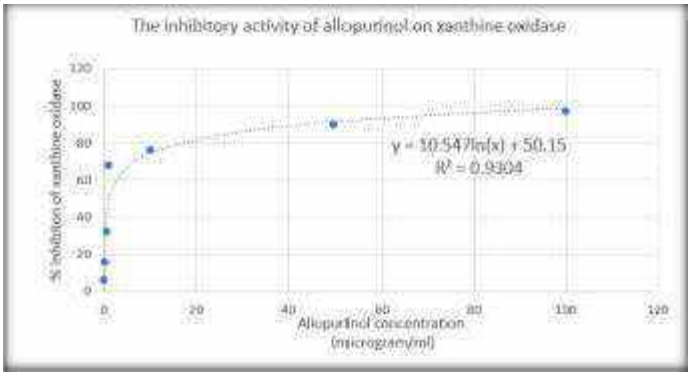
Well no.	1	2	3	4	5	6	7	8	9
Allopurinol final concentration (µg/ml)		0	0.01	0.1	0.5	1	10	50	100
%Inhibition		0	6	16	32	68	76	90	97

- Then IC50 for this example will be the concentration which gives an inhibition value of 48.5%

(النصف)

Apply the value on the equation obtained

- y = 10.547ln(x) + 50.15
- 48.5= 10.547ln(x) + 50.15
- Ln(x)= $\frac{48.5-50.15}{10.547}$
- Ln(x)= -0.15644
- X= $e^{-0.15644}$
- X=0.855181 µg/ml is the IC50 in this example.



$48.5 - 50.15 = 10.547 \ln(x)$

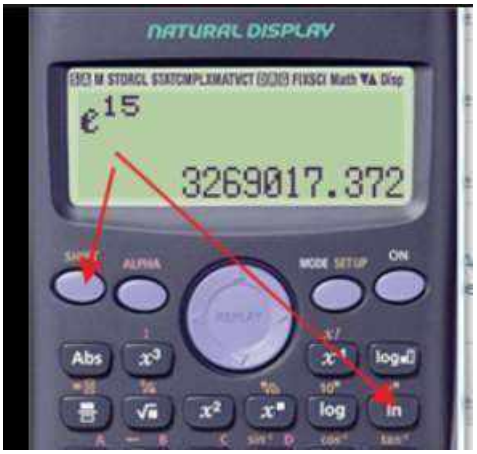
$-1.65 = 10.547 \ln(x)$

$\ln(x) = \frac{-1.65}{10.547} = -0.156443$

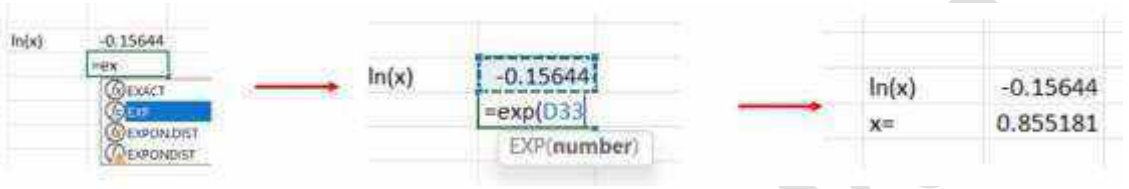
$\ln(x) = -0.15644$

How to find anti-ln

- On calculator: press shift then → press ln



- On excel: find the exponential function and open a bracket then select the cell which has the number and close the bracket again, press enter to obtain the value.



Experiment 7

Xanthine oxidase with allopurinol-IC50

Student name:	Section no.:
1.	Group no.:
2.	report mark/10
3.	...../ 10
4.	
5.	
6.	
7.	

Objectives:

Results: Fill in the table below manually or using excel.

Well no.	1	2	3	4	5	6	7	8	9
Phosphate Buffer	150µl	145µl	95 µl	95 µl	95 µl	95 µl	95µl	95µl	95µl
Xanthine substrate (0.6mM)	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50µl	50µl	50µl
Allopurinol	-----	-----	50 µl 40ng/ml	50 µl 400ng/ml	50 µl 2µg/ml	50 µl 6µg/ml	50 µl 40µg/ml	50 µl 50µg/ml	50µl 100µg/ml
Xanthine oxidase enzyme	-----	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl	5µl	5µl
Final concentration of allopurinol per well (µg/ml).	-----	Zero	0.021	0.21	1.052	3.957	21.052	26.315	52.631
Absorbance (30 minutes)	Blank Abs 0.02	0.52	0.49	0.43	0.35	0.3	0.19	0.17	0.07
%Inhibition		0	6	18	34	44	66	70	90

1. Calculate the final concentration of allopurinol per well (µg/ml).

Stock concentration

40ng/ml :

$$C_1 = \frac{40 \times 10^{-9}}{10^{-6}} = 40 \times 10^{-3}\ \mu m/ml = 0.04\ \mu m/ml$$

$$0.04 * 50 = C_2 * 95$$

$$C_2 = 0.02105\ \mu g/ml$$

$V_1 = 50$

$V_2 = 95$

$C_1 = 0.4$ mm/mL

- **400ng/ml**

$0.4 * 50 = C_2 * 95$

$C_2 = 0.2105$ µg/mL

- **2µg/ml:**

C_1

$2 * 50 = C_2 * 95$

$C_2 = 1.0526$ µg/mL

- **6µg/ml:**

$6 * 50 = C_2 * 95$

$C_2 = 3.1579$ µg/mL

- **40µg/ml:**

$40 * 50 = C_2 * 95$

$C_2 = 21.0526$ µg/mL

- **50µg/ml**

$50 * 50 = C_2 * 95$

$C_2 = 26.3157$ µg/mL

- **100µg/ml**

$100 * 50 = C_2 * 95$

$C_2 = 52.6315$ µg/mL

2. Calculate the %xanthine oxidase inhibition for each allopurinol concentration (show detailed calculation).

Allopurinol conc. (1):

$$\% \text{ inhibition} = 100 - \left[\frac{A_1 - A(\text{blank})}{A_0 - A(\text{blank})}\right] * 100\%$$

$$100 - \left[\frac{0.49 - 0.02}{0.52 - 0.02}\right] * 100\% = 100 - 94 = 6\%$$

Allopurinol conc. (2)= % inhibition =

$$100 - \left[\frac{0.43 - 0.02}{0.52 - 0.02}\right] * 100\% = 100 - 82 = 18\%$$

Allopurinol conc. (3)= % inhibition =

$$100 - \left[\frac{0.35 - 0.02}{0.52 - 0.02}\right] * 100\% = 100 - 66 = 34\%$$

Allopurinol conc. (4)= % inhibition = $100 - \left[\frac{0.3 - 0.02}{0.52 - 0.02}\right] * 100\% = 100 - 56 = 44\%$

Allopurinol conc. (5)= % inhibition = $100 - \left[\frac{0.19 - 0.02}{0.52 - 0.02}\right] * 100\% = 100 - 34 = 66\%$

Allopurinol conc. (6)= % inhibition = $100 - \left[\frac{0.17 - 0.02}{0.52 - 0.02}\right] * 100\% = 100 - 30 = 70\%$

Allopurinol conc. (7)= % inhibition = $100 - \left[\frac{0.07 - 0.02}{0.52 - 0.02}\right] * 100\% = 100 - 10 = 90\%$

3. Fill the table given above and plot the final concentration (i.e diluted) vs % inhibition and determine the IC50 value (show detailed calculation of IC50).

(Print the figure with Y and X axis labelled and add it to the report)

$\frac{60}{2} = 30$

4. From the figure shown below calculate the IC50 for this xanthine oxidase inhibitor?

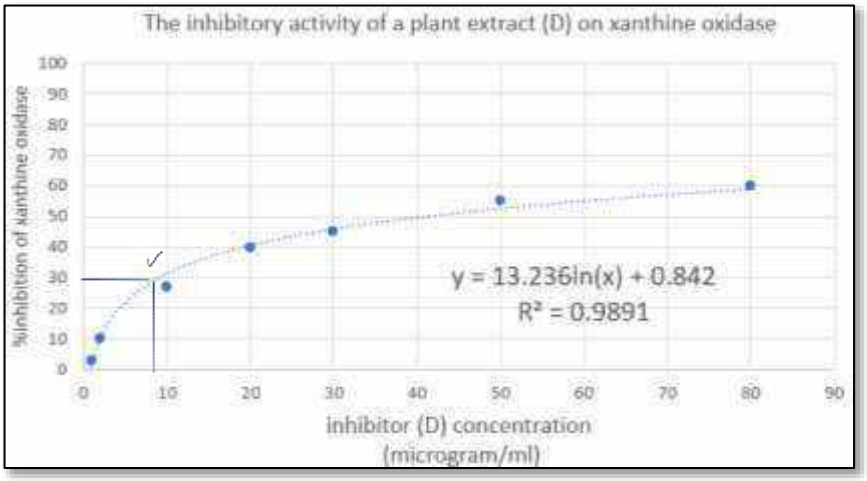
$y = 13.236 \ln(x) + 0.842$

$30 = 13.236 \ln(x) + 0.842$

$\ln(x) = 2.2029$

$x = e^{2.2029}$

$x = 9.05122$ mg/ml is the IC 50.



Experiment 8
Qualitative determination of Carbohydrates

This experiment is intended to introduce you to one of the three major classes of macronutrients found in food , carbohydrates .

To identify carbohydrates (sugars) as being polyhydroxylated aldehydes and ketones .
Mono or di or polysaccharides and their chemical characteristics .

You will also learn a variety of ways to categorize carbohydrates and several tests used in the analysis of carbohydrates .

Carbohydrate is the primary source of energy for brain , retina شبكية العين and erythrocytes and so the glucose concentration requires precise regulation that is accomplished by many metabolic processes and interrelated with several glands and organs .

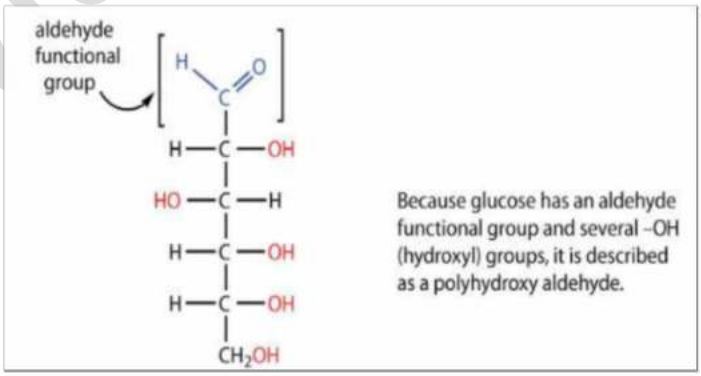
If any disturbance اضطراب occurs to the glands or organs , a loss of glycemic control will occur which results in the formation of a pathological state .

However , **the diabetes mellitus** is the result of glucose metabolism abnormality , and is often has different causes , which include genetic , autoimmune and other causes .

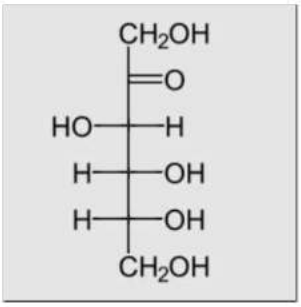
All carbohydrates consist of carbon , hydrogen , and oxygen atoms and are polyhydroxy aldehydes or polyhydroxyketones or are compounds that can be broken down to form such compounds.

Examples of carbohydrates include **glucose , ribose , fructose (fruit sugar) , sucrose (table sugar) , lactose (milk sugar) ,maltose (malt sugar) , galactose , starch , and structural materials such as cellulose .**

The molecular formula for a carbohydrate can be considered as $C_n(H_2O)_n$, where n is the number of carbon atoms . Example , because its formula is $C_6H_{12}O_6$, glucose was once thought to be a " carbon hydrate " with the structure $C_6(H_2O)_6$.



سكر احادي Hexose



Fructose has a ketone group , so it is considered a polyhydroxy ketone .

Classification according to Molecular size or Complexity :

A- Simple Carbohydrates often referred to as simple sugars or monosaccharides , contain one unit of saccharide and cannot be broken down into smaller carbohydrates.
such as glucose , fructose , galactose , and mannose .

Monosaccharide can be also classified according to different factors as follow :

a. Classification according to Number of carbon atoms

1. **Triose C3 sugars**
   example : dihydroxyacetone & glyceraldehydes .

2. **Tetrose C4 sugars**
   example : D - erythrose , D - threose & D - erythrulose .

3. **Pentose C5 sugars**
   example : nucleic acids (DNA & RNA)
   and coenzymes NAD , Ribose that is a structural element of ATP

   and **flavor proteins :** Arabinose , ribulose & xylose .

4. **Hexose C6 sugars**
   example : glucose , fructose .

b. Classification according to Functional group

1. **Aldose :** sugars having an aldehyde functional group R-HC=O
   example : glucose

2. **Ketose :** sugars having a ketone functional group R2-C=O
   example : fructose

c. Classification according to Reactivity in Redox Reactions

1. **Reducing sugars** oxidized by Tollens' reagent or Benedict's or Fehling's reagents .

   Examples are glucose , fructose , glyceraldehydes , lactose , arabinose & maltose .

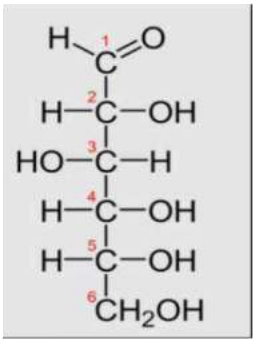
2. **Non-reducing sugars** not oxidized by Tollens' reagent or Benedict's or Fehling's reagents .

   Example : sucrose , starch .

For example على سبيل المثال ,

Glucose is an **Aldose** { it contains an **Aldehyde functional group** }
& **Glucose** is also a **Hexose** { it containts **six carbon atoms** }

Therefore لذلك ,
Glucose is considered / classified as an Aldohexose ,
because it contains six carbon atoms & an Aldehyde functional group .



	Aldoses	Ketoses
($C_3H_6O_3$) Trioses	Glyceraldehyde	Dihydroxyacetone
($C_4H_8O_4$) Tetroses	Erythrose	Erythrulose
($C_5H_{10}O_5$) Pentoses	Ribose	Ribulose
($C_6H_{12}O_6$) Hexoses	Glucose	Fructose

B- Complex Carbohydrates

Those containing more than one group of saccharides are complex carbohydrates .

Through a process known as hydrolysis , complex carbohydrates can be broken down into smaller carbohydrate units .

1. Disaccharides contain two units of monosaccharide, Hydrolysis can break disaccharides into two monosaccharide units and lose one molecule of water .

such as :

- **Maltose** two glucose residues in 1, 4 linkages .
- **Lactose** can be found in milk .
  On hydrolysis , it produces D-galactose and D-glucose .

  it is a reducing disaccharide .

- **Sucrose :** Cane sugar (table sugar) or sucrose is a disaccharide of fructose and glucose .

2. Oligosaccharides have 3-6 units of monosaccharides , Oligosaccharides can be broken by
hydrolysis into 3-6 monosaccharide units and lose one or more water molecules .

3. Polysaccharides (hundreds or thousands of units) , Polysaccharides can be broken into 7 or more units of monosaccharides by hydrolysis and one or more molecules of water can be lost .

Such as :

- **Starch** is a storage form of energy in plants .
It contains two polymers composed of glucose units: amylose (linear) and amylopectin (branched) .

The complete hydrolysis of starch yields , in successive stages , glucose :

starch → dextrins → maltose → glucose

In the human body , several enzymes known collectively as amylases degrade starch sequentially into usable glucose units .

- **Glycogen** is a storage form of energy in human and animals .
It is a branched polymer composed of glucose units .
It is more highly branched than amylopectin .

هو شكل من أشكال تخزين الطاقة في الإنسان والحيوان
إنه بوليمر متفرع تتكون من وحدات الجلوكوز , إنه أكثر تشعبا من الأميلوبكتين

glycogen is found as granules in liver and muscle cells .

الجليكوجين وجدت كحبيبات في خلايا الكبد والعضلات

When fasting , animals draw on these glycogen reserves during the first day without food to obtain the glucose needed to maintain metabolic balance .

عند الصيام , تعتمد الحيوانات على الجليكوجين الإحتياط خلال اليوم الأول بدون طعام للحصول على الجلوكوز اللازم للمحافظة عليه التوازن الأيضي

- **Cellulose** is a structural polymer of glucose units found in plants .
It is a linear polymer with the glucose units linked through β-1,4-glycosidic bonds .

《 Qualitative tests for carbohydrates 》

There are several difficulties in their qualitative as well as quantitative analysis when analyzing a sample containing a mixture of carbohydrates , particularly sugar .

These problems are attributed to their structural and chemical resemblance , as well as their stereoisomerism .

تعزى هذه المشاكل إلى تشابهها الهيكلي والكيميائي ، وكذلك الفراغية.

It is therefore necessary to determine , during biochemical investigations , whether or not a given sample contains carbohydrates .

In order to establish the presence or absence of a sugar or a carbohydrate in a sample , several rapid tests are available .

Such tests are based on specific typical color reactions for their group .The sensitivity of these tests can be confirmed by using different concentrations of sugar solutions (0.1 - 1 percent).

[A] General tests for carbohydrates

For **all carbohydrates** , whether free or in combined form , it is a group test . It is routinely used to detect the presence of carbohydrates , despite its limitations .

In order to detect the presence of carbohydrates in a solution , the most common tests used are :

----------[Molisch's Test]----------

The response is based on the fact that H_2SO_4 concentrated catalyzes the dehydration of sugars to form furfural (from pentoses) or hydroxymethyl furfural (from hexoses).

تعتمد الاستجابة في الحقيقة على أن تركيز H_2SO_4 يحفز dehydration of sugars تجفيف السكريات إلى شكل furfural (من pentoses) أو hydroxymethyl furfural (من hexoses).

These furfurals then condense to give a purple or violet colored product with sulfonated alpha - naphthol .

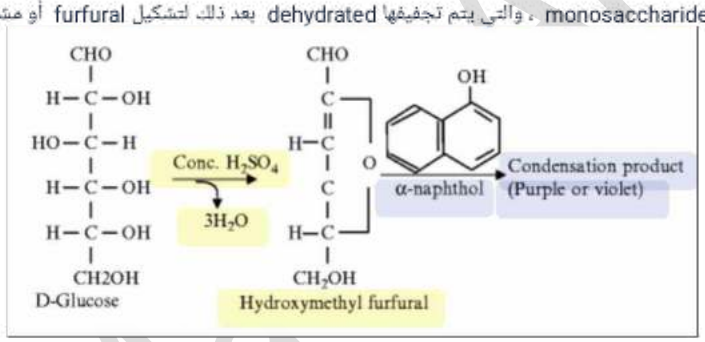
A +ve reaction is also provided by polysaccharides and glycoproteins .

تم هذه ال furfurals يتكثف condense لاعطاء منتج ملون أرجواني أو بنفسجي purple or violet colored product مع Sulfonated alpha naphthol .

والتفاعل +Ve يتم توفيره أيضًا بواسطة ال polysaccharides والبروتينات السكرية glycoproteins .

The acid first hydrolyses it into monosaccharides , which are then dehydrated to form furfural or its derivatives , in the case of the carbohydrate being a poly or disaccharide .

يتحلل hydrolyses الحمض acid أولاً إلى السكريات الأحادية monosaccharides ، والتي يتم تجفيفها dehydrated بعد ذلك لتشكيل furfural أو مشتقاته ، في حالة ال carbohdrate كونها poly or disaccharide .



Molisch test give a positive result with all carbohydrates 🙂

[B] Differentiation test of Carbohydrates

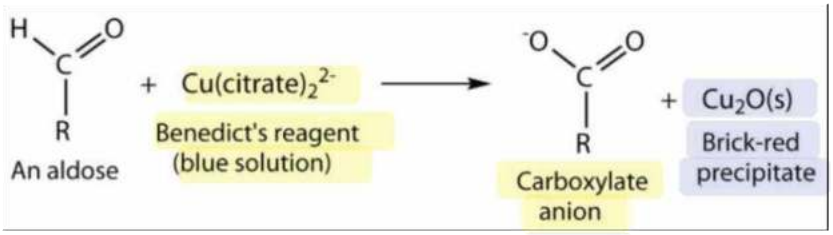
----------[Benedict's Test]----------[1]----

Benedict's test determines whether a monosaccharide or disaccharide is **a reducing sugar** .
To give a positive test , the carbohydrate must contain either a free aldehyde group or a ketone group .

Benedict's reagent is an alkaline solution containing cupric ions , which **oxidize the aldehyde to a carboxylic acid** . In turn, the cupric ions are reduced to cuprous oxide , which forms a red precipitate .

Give positive result with all reducing sugar as glucose , fructose , lactose , maltose , galactose ,

except إستثناء for sucrose & starch .



[Barfoed's Test] [2]

Consist of cupric ion in acidic solution (acetic acid). This test is performed **to differentiate between mono & disaccharide reduction .**

Monosaccharides are more reactive reducing agents than disaccharides
and thus react in about 1-2 minutes , while it takes 7-12 minutes for the reducing disaccharides to get hydrolysed and then react in the acidic solution .

Therefore, **it is possible to detect the difference in property reductions .**
It gives positive red pricipitate result with **glucose and fructose and other monosaccharides .**

[Seliwanoff's Test] [3]

This test is **a keto hexose - specific** timed colour reaction . It is therefore used for the differentiation of aldoses from ketoses .

Dehydration is carried out in the presence of concentrated HCl or H2SO4 with resorcinol ,
ketohexoses to yield 4-hydroxy methyl furfural more rapidly than aldohexoses .

In addition , these furfural derivatives condense **to form a red coloured complex with resorcinol .**

It gives positive result with fructose . And in prolong heating it will give a positive result with sucrose also due to hydrolysis to fructose and glucose , **while other sugar gives a negative result** .

[Iodine test] [4]

Iodine forms color complex with starch , glycogen and dextrins . The color varies according to the chain length and the size of the molecule .

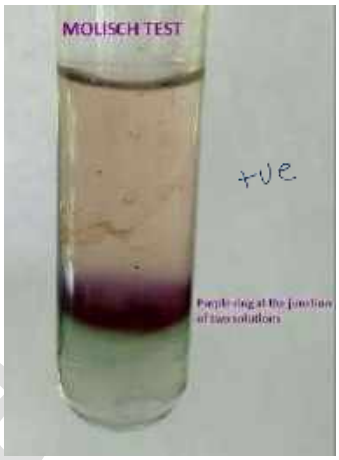
Starch gives blue color , glycogen gives purple while **dextrines forms red to colorless complexes .**
The complexes with iodine are very unstable . The iodine is readily removed from these compounds by alcohol , NaOH , Na2S2O3 or heating .
This indicates the weak interaction between iodine and these compounds .

بإختصار بمجرد إضافة اليود الى ال sugar سنلاحظ تكون complex فقط مع ال polysaccharides
وبتالي سيعطي +Ve مع ال Starch , glycogen & dextrines وطبعاً كل واحد منهم بيعطي لون شكل وبيختلف عن الآخر

Practical part:

1. Molisch's test:

Procedure



1. In a test tube, add 2 ml of test solution (unknown)
2. Add 2 drops of Molisch's reagent and mix by gentle shaking
3. Tilt the tube and carefully pour 1 ml conc. H_2SO_4 along the side of the tube (no mixing)
4. Violet ring at the junction of the two liquids indicates a positive result.

مظهر الحلقة الأرجواني أو البنفسجي يؤكد الوجود من الكربوهيدرات

Note: *The appearance of purple or violet ring confirms the presence of carbohydrate.*

Interpretation: The formation at the junction of two layers of a **purple or violet ring or zone** indicates the presence of **carbohydrates**.

تشكيل حلقة أو منطقة أرجوانية أو بنفسجية
عند تقاطع طبقتين يشير إلى وجود الكربوهيدرات .

Precautions:

محلول alpha-naphthol غير مستقر ويجب أن يكون fresh ✨

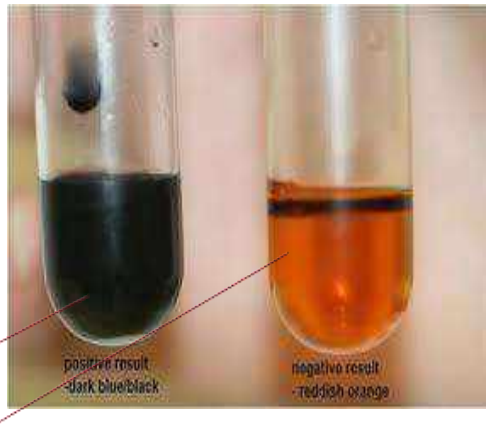
(i) The solution of alpha-naphthol is unstable and should be made fresh.

(ii) Conc.H_2SO_4 should be carefully added along the sides of the test tube, causing the contents of the tube to be minimally disturbed.

2. Iodine test:

1. To two ml of your test solution (unknown), add few drops of diluted HCl
2. Add 2 drops of iodine solution
3. **A blue color** indicates positive result
4. Heat the colored solution and observe the disappearance of the color

Iodine test give + result (blue color complex) with starch as a polysaccharides.



positive result
dark blue black

Negative result
reddish orange

3) اللون الأزرق يدل على نتيجة إيجابية
4) قم بتسخين المحلول الملون وراقب اختفاء اللون

يعطي اختبار اليود نتيجة +Ve (مركب أزرق اللون)
مع النشا ك polysaccharides .

3. تسخين الأنبوب في حمام مائي مغلي لمدة 5 دقائق
4. تتم الإشارة إلى النتيجة الإيجابية من خلال التغيير في اللون أو تكوين مادة راسب اللون الأصفر إلى الأحمر القرميدي ، اعتمادًا على التركيز من الجلوكوز أو السكر المختزل في المحلول .

3. Benedect's test

1. In a tube add 5 ml of benedict reagent
2. Add 8 drops of your test solution (unknown) and mix
3. Heat the tube in boiling water bath for 5 min
4. Positive result is indicated by the **change in color or the formation of a precipitate yellow to brick red color**, depending upon the conc. of the glucose or reduced sugar in the solution.

ملاحظة : ظهور الترسبات الحمراء يؤكد وجود الكربوهيدرات.

Note: *The appearance of red precipitate confirms the presence of carbohydrates.*

3. ضع أنابيب الاختبار في حمام مائي وأرصد الوقت عندما الترسب يظهر .

4. Barfoed's test

1. Add 1 mL of the test solution (unknown) to a test tube.
2. Add 5 ml Barfoed's reagents to test tube and mix
3. Place the test tubes in a water bath and observe the time when **a precipitate** appears.

تشير الرواسب الحمراء الرقيقة إلى وجود إختزال في السكاريد الأحادي في القاع أو جوانب الأنبوب.

Thin **red precipitates** indicate the presence of a **reduction of monosaccharide** at the bottom or sides of the tube.

Positive Barfoed's test for monosaccharides: glucose, fructose.

Note:The boiling should not be prolonged beyond 1-2min, otherwise the disaccharide reduction will respond to this test as well.

لا ينبغي إطالة فترة الغليان لأكثر من دقيقة إلى دقيقتين ، وإلا فإن تقليل ثنائي السكاريد سوف تستجيب respond لهذا الاختبار أيضا .

5. Seliwanoff's test

1. Add 1 mL of the test solution (unkown) to about 2 mL of the Seliwanoff reagent
2. Heat the test tube in boiling water bath for 10-20 min or just heat to boiling.

-ve

+ve

A positive reaction is indicated by **a deep red color and formation of brown precipitate.**

The presence of **ketohexose** indicates the appearance of **a red colour (fructose).**

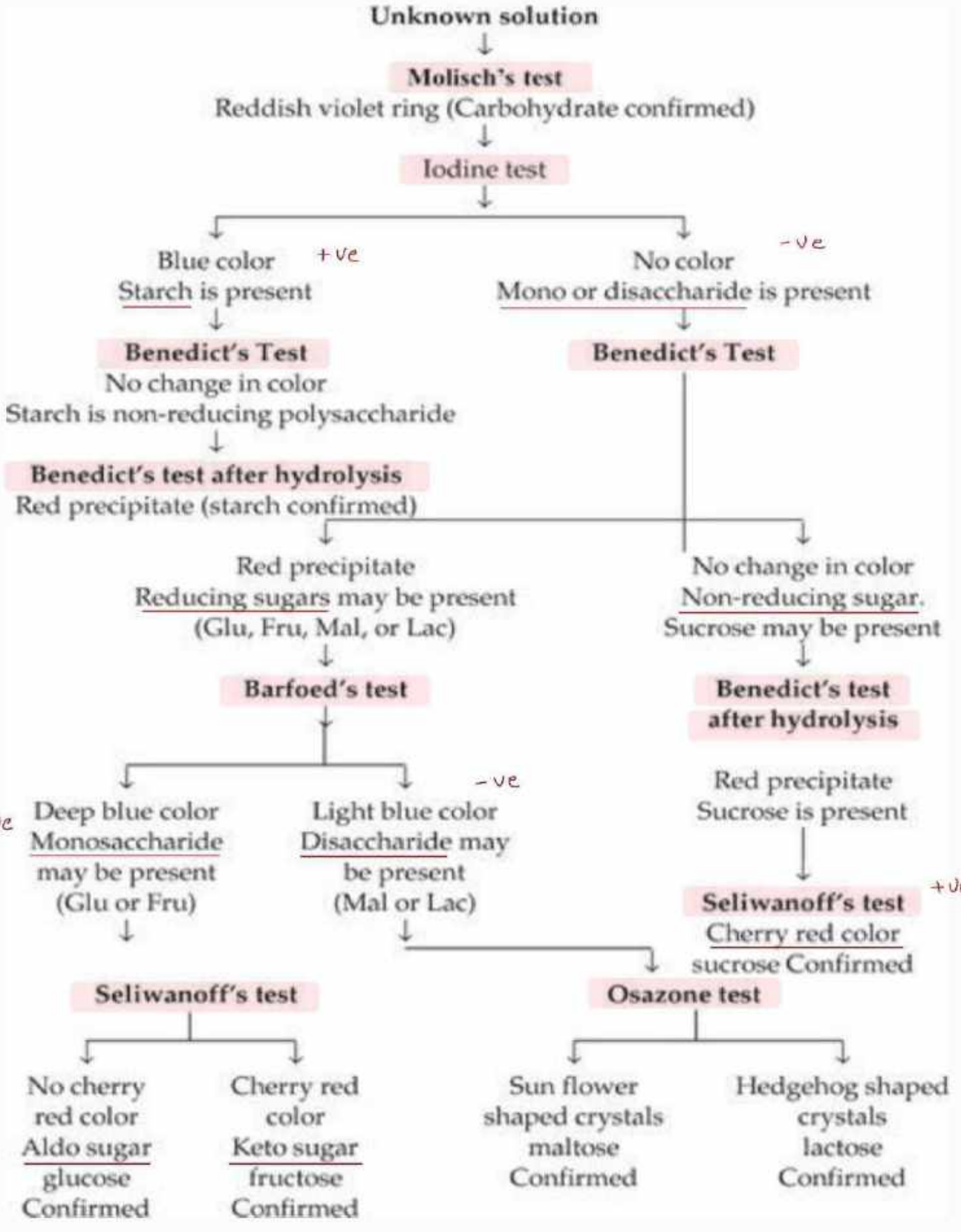
2. سخن أنبوب الاختبار في حمام مائي مغلي لمدة 10-20 دقيقة أو مجرد تسخينها حتى الغليان.

يشار إلى التفاعل الإيجابي من خلال اللون الأحمر الغامق وتشكيل راسب بني .

يشير وجود ketohexose إلى ظهور اللون الأحمر (سكر الفاكهة)

Summary

Biochemical test	Glucose	Fructose	Maltose	Lactose	Sucrose	Starch
Molisch	Positive	Positive	Positive	Positive	Positive	Positive
Benedict	Positive	Positive	Positive	Positive	Negative	Negative
Barfoed	Positive	Positive	Negative	Negative	Negative	Negative
Seliwanoff	Negative	Positive	Negative	Negative	Positive	Negative
Iodine	Negative	Negative	Negative	Negative	Negative	Positive


Unknown solution
Molisch's test
Reddish violet ring (Carbohydrate confirmed)
Iodine test
Blue color
Starch is present
+ve
No color
Mono or disaccharide is present
-ve
Benedict's Test
No change in color
Starch is non-reducing polysaccharide
Benedict's test after hydrolysis
Red precipitate (starch confirmed)
Benedict's Test
Red precipitate
Reducing sugars may be present
(Glu, Fru, Mal, or Lac)
No change in color
Non-reducing sugar.
Sucrose may be present
Barfoed's test
Benedict's test after hydrolysis
Red precipitate
Sucrose is present
+ve
Deep blue color
Monosaccharide
may be present
(Glu or Fru)
-ve
Light blue color
Disaccharide may
be present
(Mal or Lac)
Seliwanoff's test
Cherry red color
sucrose Confirmed
Seliwanoff's test
Osazone test
No cherry
red color
Aldo sugar
glucose
Confirmed
Cherry red
color
Keto sugar
fructose
Confirmed
Sun flower
shaped crystals
maltose
Confirmed
Hedgehog shaped
crystals
lactose
Confirmed


O.K.

Experiment 9
Qualitative Determination of Lipids

هي مجموعة واسعة من الجزيئات التي تحدث بشكل طبيعي والتي تشمل الدهون والشمع (نباتي و الحيوان) ، ال sterols ، الفيتامينات التي تذوب في الدهون ، monoglycerides ، ال diglyceride ، الدهون الثلاثية triglyceride ، ال phospholipids ، و إلخ ...

LIPIDs are a broad group of naturally-occurring molecules which includes fats , waxes (plant and animal), sterols , fat-soluble vitamins , monoglycerides , diglycerides , triglyceride , phospholipids , and others .

وهي تشمل الدهون والزيوت الصالحة للأكل (مثل الزبدة وزيت الزيتون وزيت الذرة) ، وهي في المقام الأول الدهون الثلاثية.
الفسفوليبيدات (على سبيل المثال ، Lecithin) ، وهي مهمة في بنية الخلية و عمليات الأيض .

They include the fats and edible oils (e.g., butter , olive oil , corn oil), which are primarily triglycerides ; phospholipids (e.g., lecithin), **which are important in cell structure and metabolism .**

تشمل وظائف الدهون تخزين الطاقة ، وتحتوي الدهون على أكثر من ضعف الكثير من الطاقة (السعرات الحرارية) لكل وحدة وزن مثل الأخرى (البروتينات والكربوهيدرات).

The functions of lipids include storing energy , lipids contain more than twice as much energy (calories) per unit of weight as the other two (proteins and carbohydrates).

وتعمل كمكونات هيكلية لأغشية الخلايا . الدهون لها تطبيقات في صناعات مستحضرات التجميل والأغذية وتكنولوجيا النانو

signaling , and acting as structural components of cell membranes . Lipids have applications in the cosmetic and food industries , and in nanotechnology .

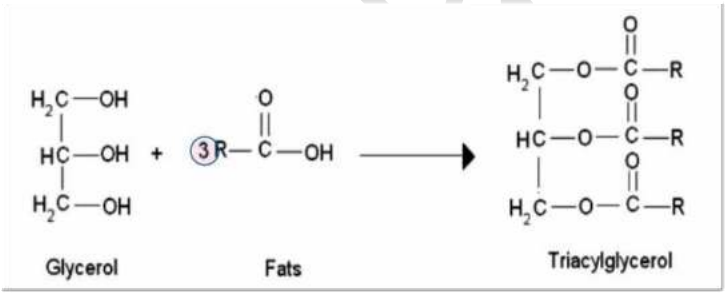
A fat molecule consists of two kinds of parts : a glycerol backbone & three fatty acid tails .

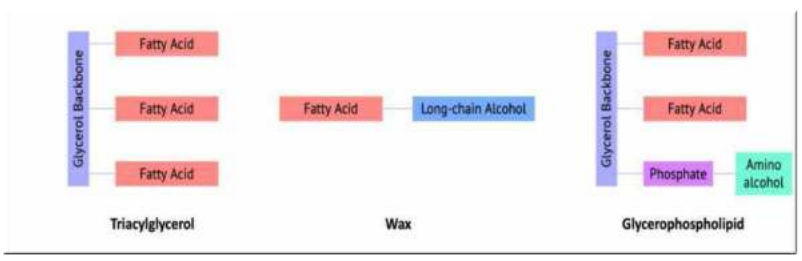
Glycerol is a small organic molecule with three hydroxyl (OH) groups , while a fatty acid consists of a long hydrocarbon chain attached to a carboxyl group .

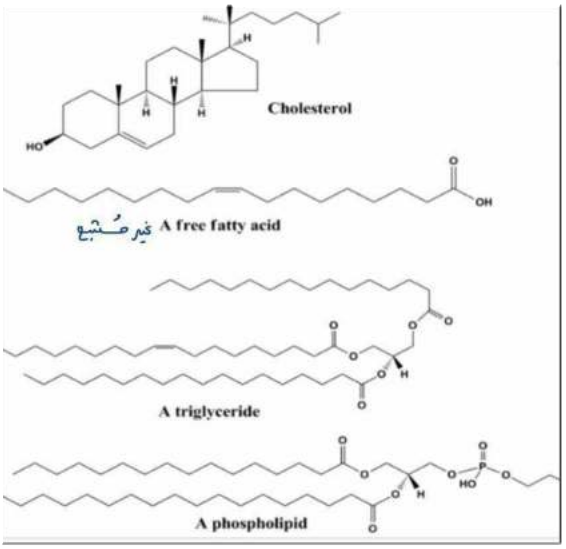
ال Glycerol هو جزيء عضوي صغير به ثلاث مجموعات هيدروكسيل (OH) ،
بينما يتكون حمض دهني لسلسلة هيدروكربونية طويلة متصلة بمجموعة كربوكسيل.

A typical fatty acid contains 12–20 carbons , though some may have as few as 4 or as many as 36 .

يحتوي الأحماض الدهنية النموذجية typical fatty acid على 12-20 الكربون ،
على الرغم من أن بعضها قد يحتوي على أقل من 4 أو ما يصل إلى 36







Classification of lipids :

1. Simple lipids are esters of fatty acids with various alcohols

e.g., **fats** (esters of fatty acids with glycerol)
and **waxes** (esters of fatty acids with higher molecular weight of monohydric alcohols).

2. Complex lipids are esters of fatty acids containing groups in addition to an alcohol and a fatty acid

e.g., **phospholipids** or **glycolipids**

☐ **Phospholipids** yield in addition to alcohol and fatty acids , phosphate and a nitrogenous base like choline , ethanolamine , etc.

Specialized lipids called phospholipids are major components of the plasma membrane .
Lecithin's & cephalous are representatives of the phospholipids .

☐ **Glycolipids** contain carbohydrates

☐ **Sulpholipids** contain sulphate

☐ **Lipoproteins** are combinations of lipids with proteins.

There are **four major classes of circulating lipoproteins , each with its own characteristic protein and lipid composition** . They are هم :

- **chylomicrons**
- **very low density lipoproteins (VLDL)**
- **low density lipoproteins (LDL)**
- **high density lipoproteins (HDL)**

3. Derived lipids When both simple and compound lipids combine and undergo the process of hydrolysis , **the produced chemical is known as the derived lipids** .

Derived lipids include **cholesterol** , **carotenes** , **steroids** and **prostaglandins** , **fat soluble vitamin (A , K , E , D)**, and **hormones** .

They have the common property of being :

(1) Relatively insoluble in water .
Lipids are hydrophobic " water - fearing ".

(2) Soluble in nonpolar solvents such as **ether & chloroform** .

Fatty acids is a carboxylic acid with an aliphatic chain (12 - 20 carbon), which is either saturated or unsaturated .

1. Saturated If the aliphatic chain contains no double bond .

Saturated fatty acids tails are straight , so fat molecules with fully saturated tails can pack tightly against one another . This tight packing results in fats that are solid at room temperature .

most of the fat in butter is saturated fat . Some of **the most common fatty acids are palmitic acid & stearic acid** .

Palmitic has 16 carbon atoms & stearic has 18 carbon atoms . both are saturated fatty acids .

2. Unsaturated if it contains one or more double bond . If there is just one double bond in a fatty acid , it's **Monounsaturated (MUFA)** while if there are multiple double bonds , it's **Polyunsaturated (PUFA)**.

fats with unsaturated tails tend to be liquid at room temperature .

Another class of fatty acids which is considered as PUFA that deserves mention includes the omega - 3 & omega - 6 fatty acids .

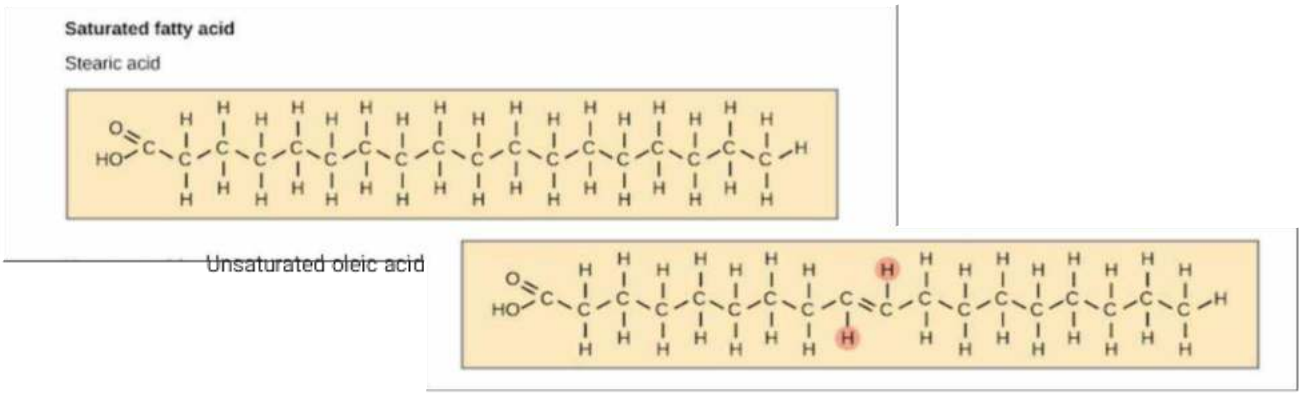
while omega - 9 is **MUFA** .

The names omega - 3 , - 6 or - 9 refer to the locations of the first double bond in the three different fatty acid molecules .

classified as essential fatty acids and must be obtained from a person's diet . Some fish , such as salmon , and some seeds , such as chia and flax , are good sources of omega - 3 fatty acids .

Omega - 6 found in Sun flower seed & Olive oil
while avocado & almond اللوز is rich with omega - 9 .

Fats have more saturated fatty acids whereas oils have more of unsaturated ones .



Saponification is a process of converting esters into soaps and alcohols by the action of aqueous alkali

(for example , aqueous sodium hydroxide solutions).

Soaps **are salts of fatty acids salts** . Sodium stearate is **a typical** soap .

Triacylglycerol can be hydrolyzed into their component fatty acids and alcohols (أي أنها يتم) **they are broken down by the addition of a water molecule .**

In the body , enzymes known as hydrolases carry out this hydrolysis .

This reaction can also be carried out in the laboratory by a process called saponification , where **the hydrolysis is carried out in the presence of a strong base (such as NaOH or KOH).**

" كل شيء يسير لصالحك مهما بدا في نظرك سلبيا فالله يدبر الأمور بحكمة تتخطى فهمك "

التجربة ما كملتها - تكملتها عليكم 😉✨