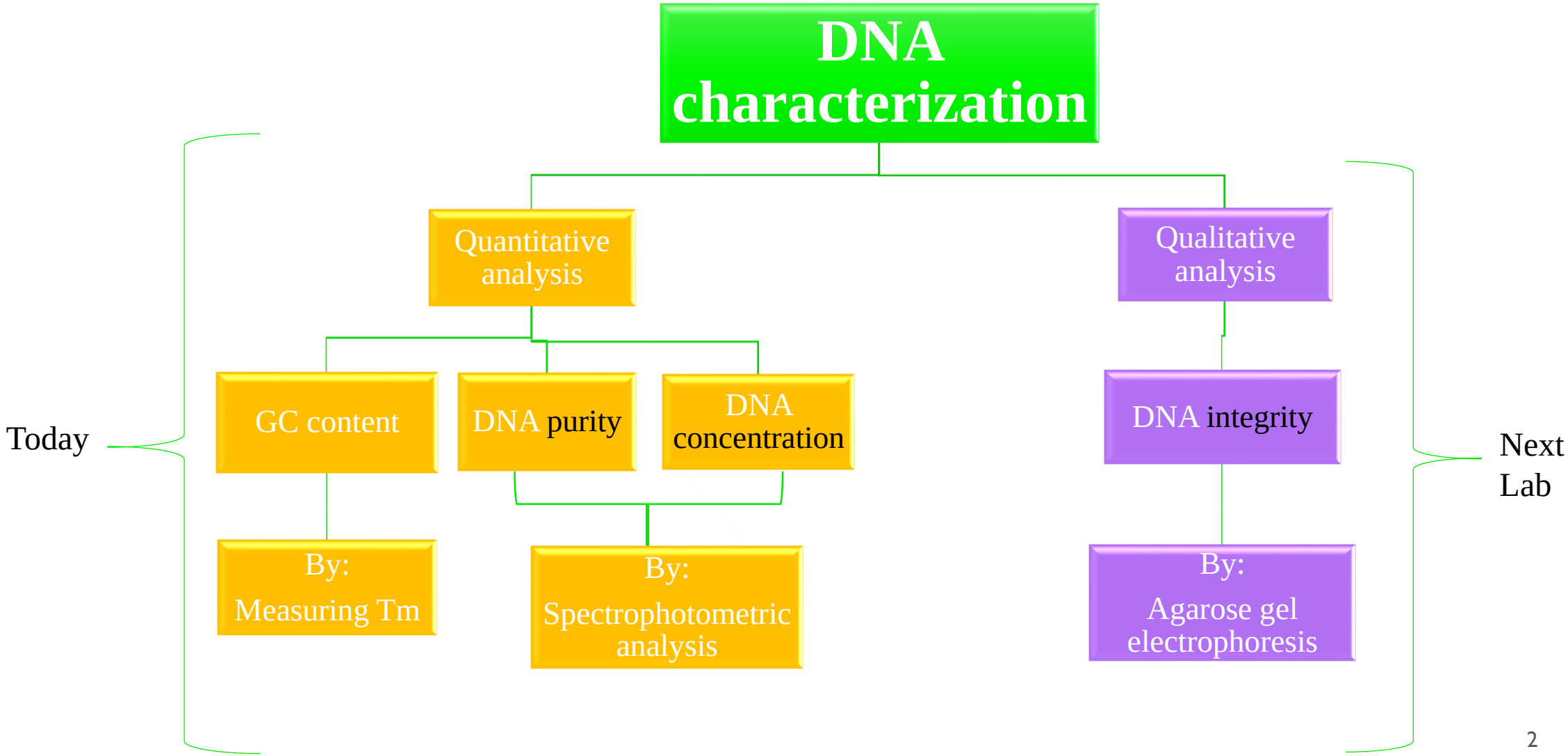
# Characterization of DNA by Spectrophotometric Assay and Melting Temperature (Tm)

# After DNA isolation:



# Characterization of extracted DNA by spectrophotometric assay:

1. DNA concentration.
2. DNA purity.

} **By measuring the absorption of ultraviolet light (UV).**

- DNA has maximum absorption at **260nm**. WHY?

# 1st. UV for quantification of nucleic acid concentration :

- Is determined by measuring absorbance at **260 nm.** WHY?
- At 260 nm double-stranded DNA has specific absorption coefficient of 0.02 $(\mu g/ml)^{-1}cm^{-1}$.
- So:

➔**Concentration of DNA=** $(A_{260} / \varepsilon L)$ x Dilution Factor (DF).

Beer-Lambert Law:

$A = \varepsilon cl$

# 2nd. DNA purity:

**1. To detect nucleic acid purity from proteins contamination:**

➔ **Calculate $A_{260}/A_{280}$** WHY?

- Highly purified DNA samples have a $A_{260}/A_{280}$ nm ratio of (**1.8-1.9**).

➔ What if the ratio is below 1.8? What that means?

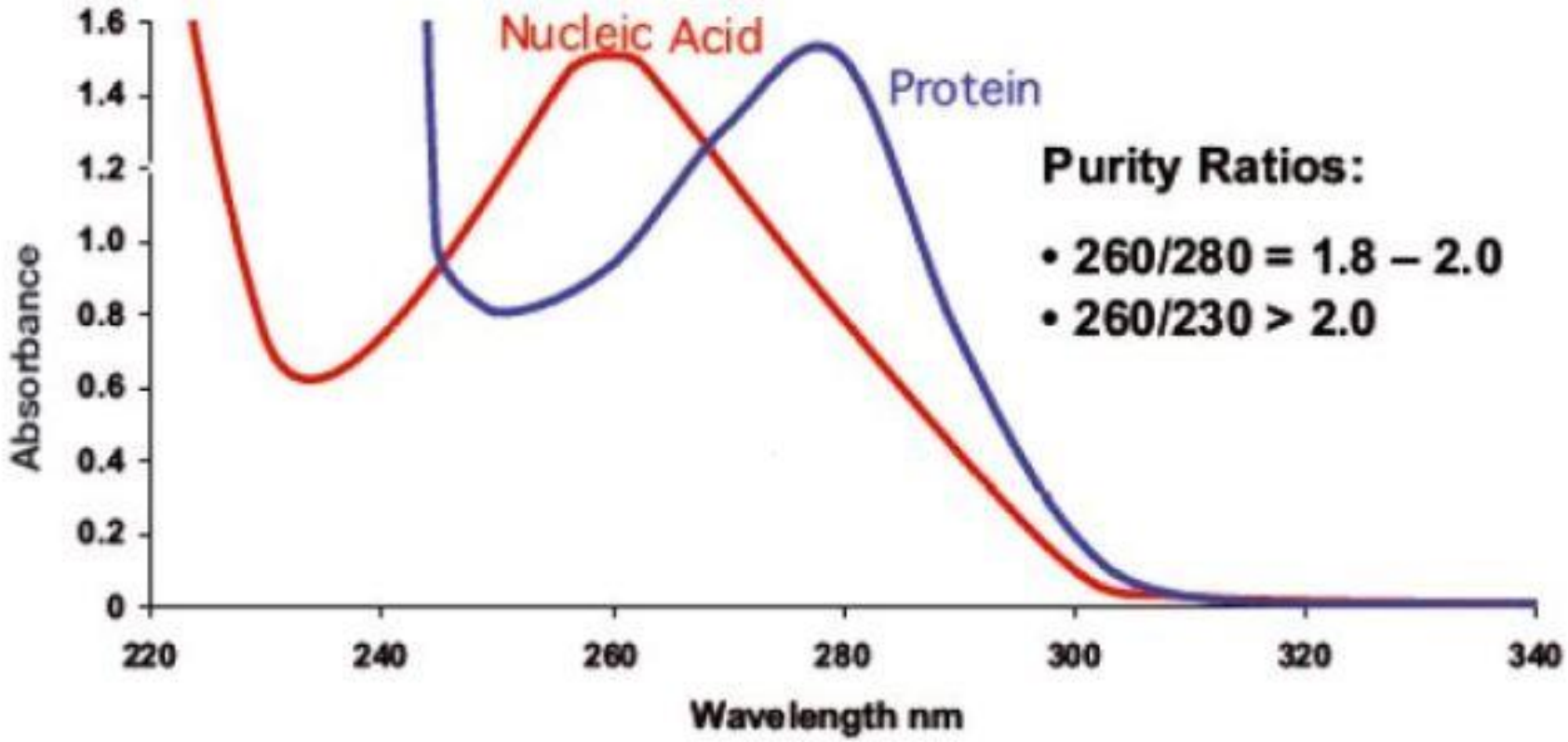
➔ What if the ratio is higher than 1.9? What that means?

**2. To detect nucleic acid purity from carbohydrates, peptides, ethanol or any organic compounds:**

➔ **Calculate $A_{260}/A_{230}$** WHY?

- Purified DNA samples have a $A_{260}/A_{230}$ nm ratio of (**2-2.2**).

# DNA and protein absorption spectrum:




**Purity Ratios:**

- **260/280 = 1.8 – 2.0**
- **260/230 > 2.0**

***What is the effect of the contaminants on DNA concentration?**

*** What if the samples contaminated by proteins or organic compound?**

# 3ed. GC content:

- The two strands of a DNA molecule can be dissociated ("melted") into single strands by **heat or altered pH**, which breaks the hydrogen bonds between complementary bases (A═T and G≡C).

➔ What that process called ?

- Hyperchromic and hypochromic effect.

- Melting temperature profile.

- The melting temperature (Tm) is the temperature at which **50% of the DNA is unpaired** (denatured).

➔Is the Tm same for all DNA molecules ? WHY?

➔What is the important of knowing Tm of DNA?

# DNA melting curve:

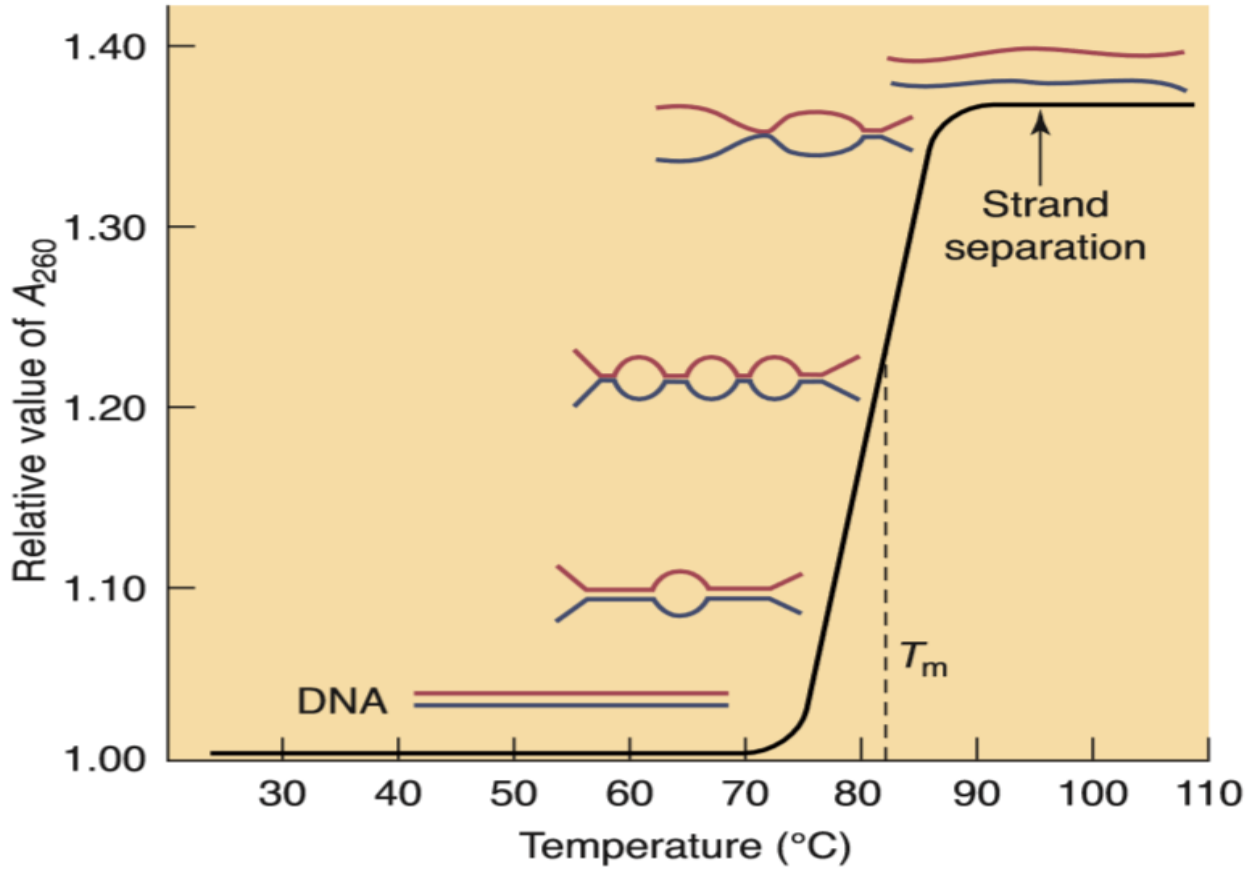



**FIGURE 4.4 DNA melting curve.** A melting curve of DNA showing $T_m$ (the melting temperature) and possible molecular conformations for various degrees of melting.

# 3ed. GC content:

- GC content can be calculated by generating Tm profile (DNA melting curve).

$$\%(G+C) = 2.44\ (Tm - 69.3)$$

# Relationship between Tm and GC%:

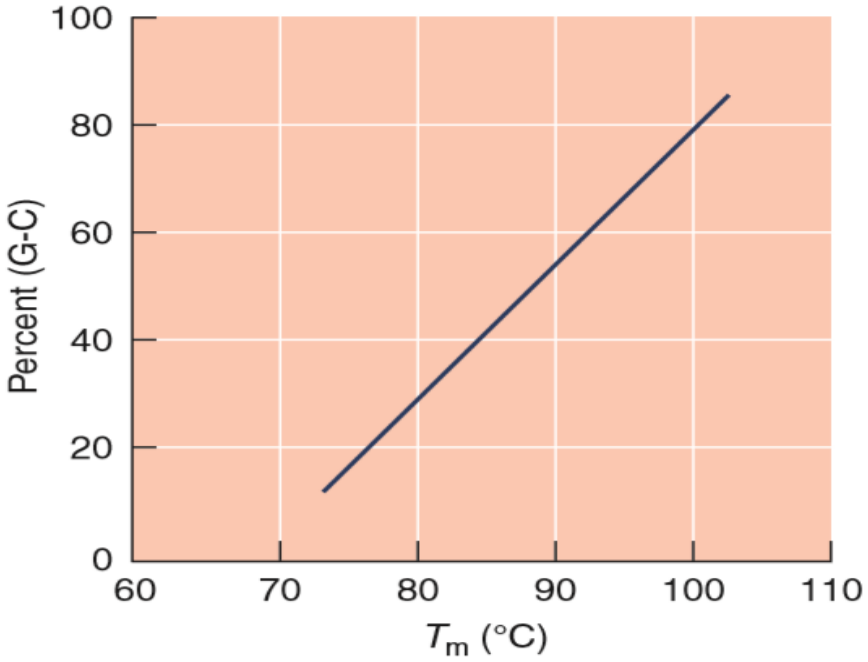
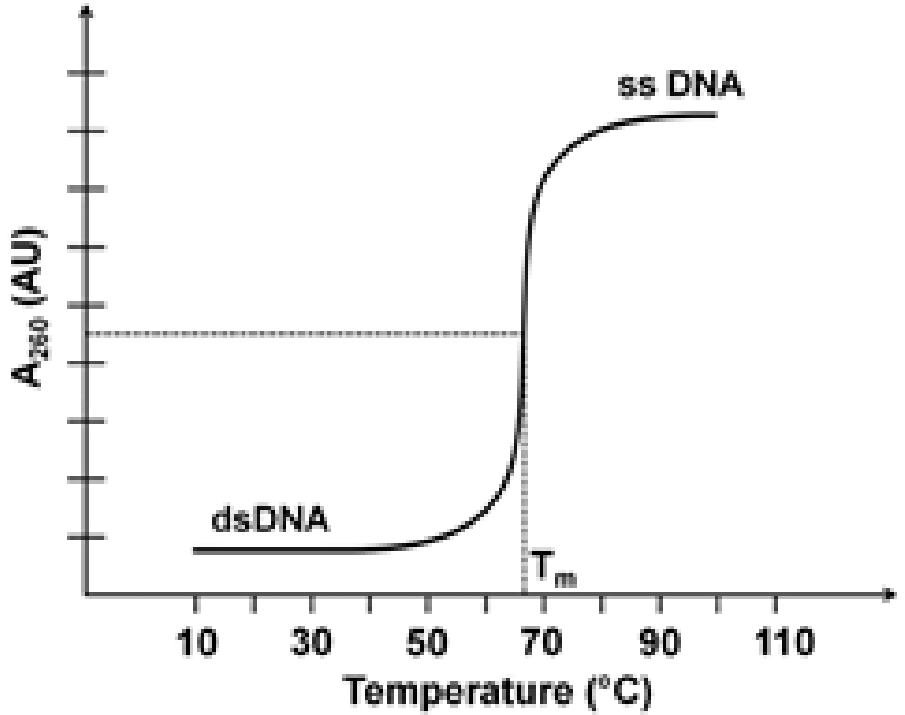



**FIGURE 4.5 Effect of G-C content on DNA melting temperature.** $T_m$ increases with increasing percent of G + C.




What do you notice about the GC content in relation to Tm?

# Practical Part

# Aim:

- Determination the concentration and purity of extracted DNA using UV spectrophotometer.

- Determination of DNA melting temperature and GC content percentage.

# Principle:

- DNA and proteins have a maximum absorbance at 260 and 280 respectively.
- dsDNA will be separated to ssDNA by heat (denaturation).
- O.D at 260 nm will increase during denaturation... Why?
- Temperature for midpoint of denaturation gives Tm.
- The DNA of each species has a specific denaturation curve.. Why?

# Results:

## 1. Characterization of DNA by Spectrophotometric Assay:

| Wavelength (nm) | Absorbance of DNA | |
|---|---|---|
| | Blood | Plant |
| 230 | | |
| 260 | | |
| 280 | | |

➔ Find out the concentration of DNA using the following equation:

**Concentration of DNA= (A260 / ε L) x Dilution Factor.**

➔ Determine the purity of the DNA.

# Results:

## 2. Melting Temperature of DNA:

| Temperature (°C ) | DNA Absorbance at 260 nm | |
|---|---|---|
| | Blood | Plant |
| 25 | | |
| 50 | | |
| 60 | | |
| 70 | | |
| Boiling | | |

➔Plot the value of absorbance vs. temperature and calculate the Tm for sample DNA.

➔Find out the GC content of your sample using the following formula:

**Percent of G + C = (Tm - 69.3) x 2.44**

# Home Work:

- Watch the following videos :

- https://www.youtube.com/watch?v=wXiiTW3pflM
- https://www.youtube.com/watch?v=U2-5ukpKg_Q

➔ What is agarose gel electrophoresis?