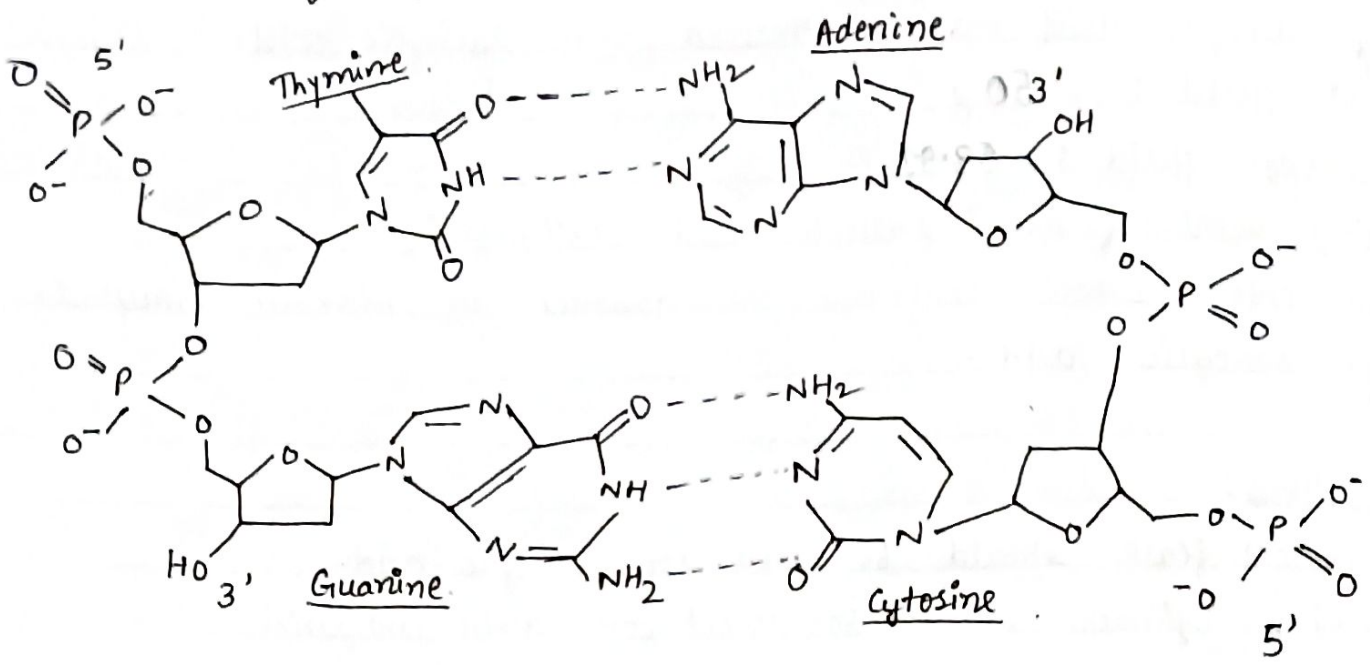
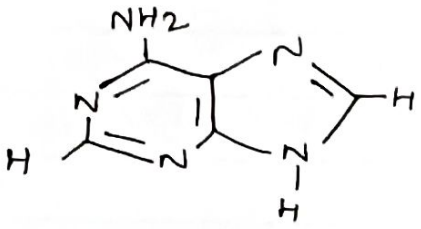
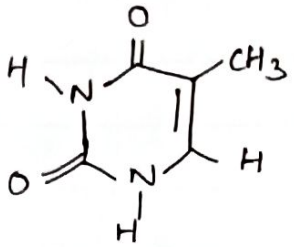
Structure of DNA:



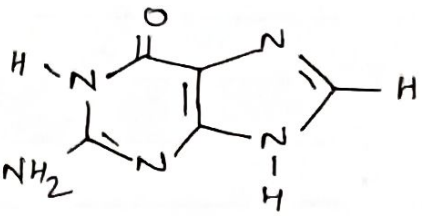
DNA Bases:



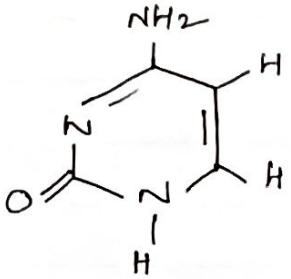
Adenine (A)



Thymine (T)



Guanine (G)



Cytosine (C)

Date 05.01.2022

Expt. No. 4

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Aim: Extraction and Identification of DNA

Apparatus Required: Beaker, standard flasks, hooked glass rods, test tube, dropper, UV visible spectrometer.

Chemicals Required: Onion/ peas, Sodium chloride, SDS solution, papain extract, ethanol, Tris EDTA (TE) buffer solution, diphenyl amine reagent.

Principle: DNA or deoxyribonucleic acid contains all genetic information necessary for growth, functioning and reproduction of almost all living organisms. DNA molecules consist of two biopolymer strands coiled around each other to form a double helix. Chromosomal DNA, exists in well known X shape and is bound by proteins into a supercoil.

In the structure of DNA, two strands are twisted around a common axis. There are 10 bases per complete twist. The helix is right-handed. Hydrogen bonding is present between complementary bases:- A bonded to T by two hydrogen bonds; C bonded to G by 3 hydrogen bonds.

First we blend the tissue with NaCl. The cell membranes are lipid and protein in composition. Homogenisation breaks the cell wall, cell membrane and nuclear membrane to allow the release of DNA. SDS is then added to the homogenised material. SDS causes the lipids and proteins to precipitate. Addition of a protease enzyme denature and detach the proteins clinging to DNA. Addition of DNA and ethanol results in separation of DNA from all other cellular material and DNA moves towards the liquid interface.

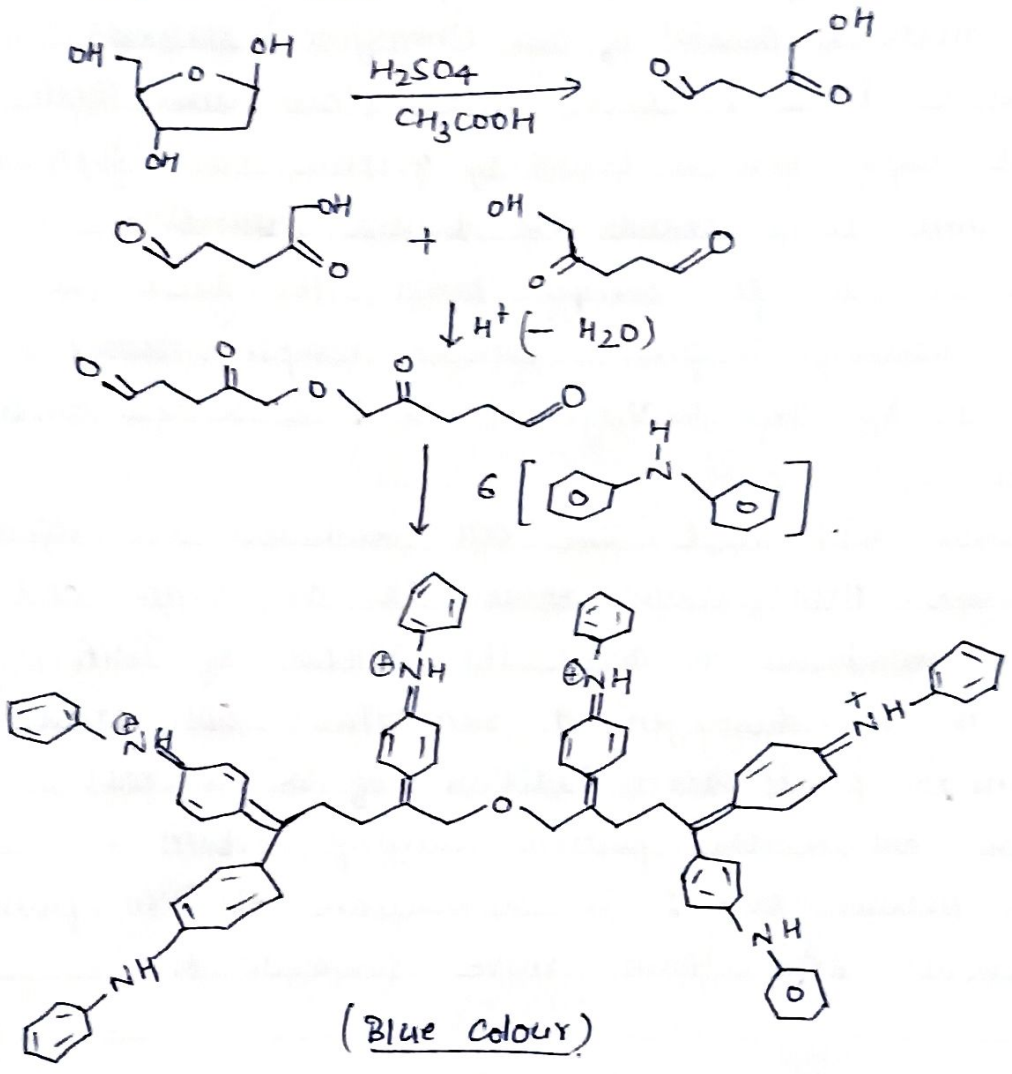
Teacher's Signature

Observation Table:

Wavelength (in nm):	Absorbance
260nm	1.650
280 nm	0.870

$$\text{Ratio} = \frac{1.650}{0.870} = 1.896$$

Diphenylamine Test:



Procedure :

(a) Extraction of DNA

1. Take 10 ml of peas extract in boiling tube and add 1.5 ml of the SDS solution and gently swirl. Let it stand for 10 minutes in ice.
2. Add 5-6 drops of papain extract and stir gently.
3. Now hold the boiling tube at an angle and pour very slowly 24 ml of ice cold ethanol down the wall of test tube so that it forms a layer above the extract layer.
4. Allow the boiling tube to stand straight for a few minutes.
5. Some stringy white substance comes in the alcohol layer. This is DNA.
6. use hooked glass rod and place it such that its end is just below the alcohol layer. Now try to spool the DNA out of the tube.

(b) Identification of DNA : Diphenylamine Test.

1. In a test tube, add small amount of crude DNA and 2 ml. of 4% solution of sodium chloride. Add 2 ml of diphenylamine reagent and mix.
2. Place the test tube in boiling water bath for one hour and record changes. The solution turns blue.

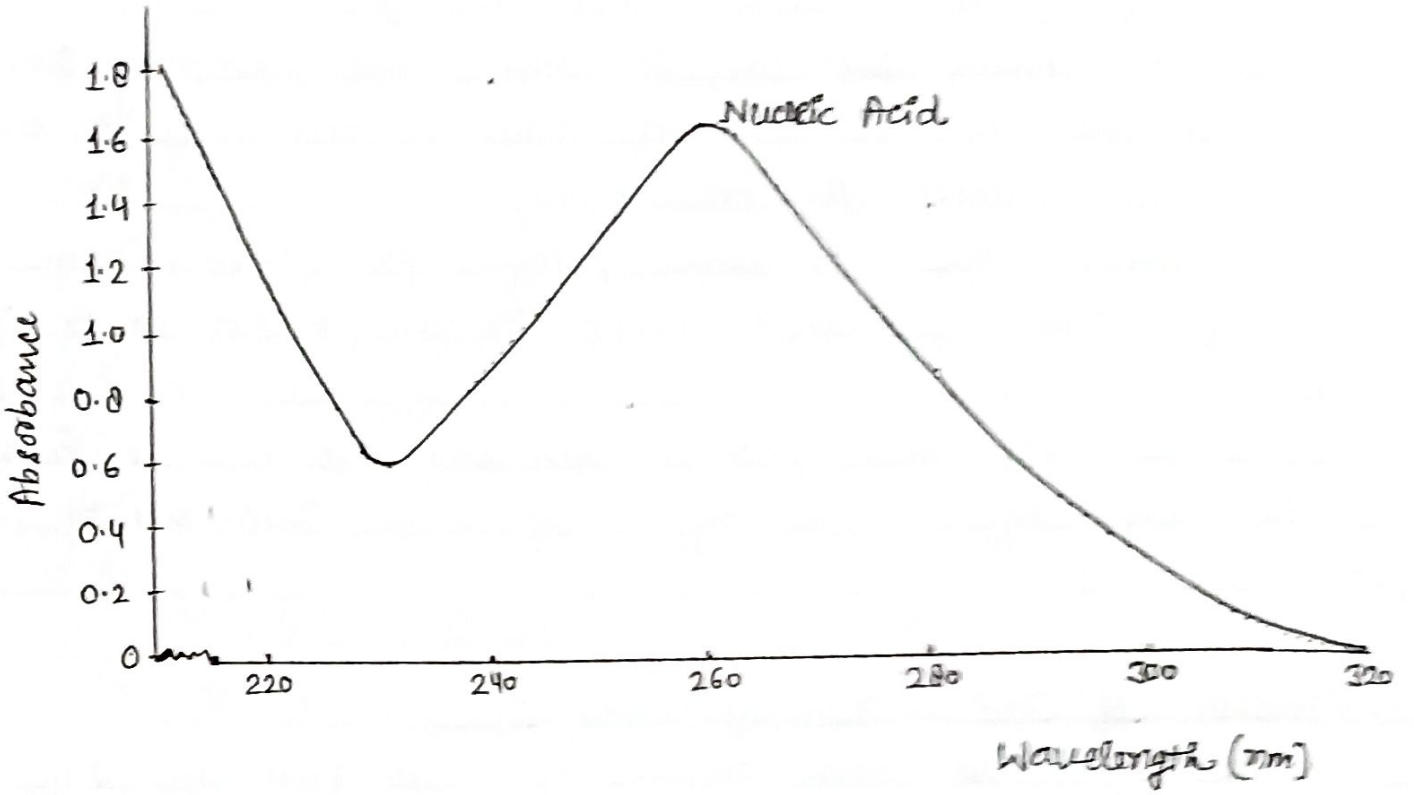
(c) UV - Vis Absorption

Dissolve DNA in 2-3 ml of TE buffer solution and determine the ratio of absorption at 260 & 280 nm.

Observations :

Diphenylamine Test: The reaction of diphenylamine with deoxyribose

Teacher's Signature _____


Nucleic Acid
Absorbance
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
220
240
260
280
300
320
Wavelength (nm)

sugar produces a blue coloured complex.
The DNA sample is boiled under extremely acidic conditions; this causes depurination of DNA followed by dehydration of deoxyribose sugar into a highly reactive ω-hydroxylevulinylaldehyde. The reaction is not specific for DNA and is given by 2-deoxypentoses in general. The ω-hydroxylevulinyl aldehyde, under acidic conditions, reacts with diphenylamine to produce a blue coloured complex that absorbs at 595 nm.
RNA willn't undergo this reaction.

UV Visible Spectroscopy:
DNA can be identified by its UV absorption at 260 nm.

Results:
(1) Diphenylamine reagent: colour change from colourless to blue.
(2) Absorption at 260 nm: 1.65
(3) Absorption at 280 nm: 0.87
(4) Ratio of absorption: 1.896