

Programme: Bachelor of Science (First Year)

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Unit IV: Microscopy and Principles of Staining

Module Name: Dye, Chromophore and Auxochrome group, Stains, Steps in Staining Process

Module No: 23

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Notes

Bright-field microscopes are probably the most common microscope found in research and clinical laboratories and certainly in teaching laboratories. However, many microbes are unpigmented and are not clearly visible because there is little difference in contrast between the cells, subcellular structures, and water. Hence, it is customary to resort to staining procedures. Staining process implies to adding colour contrast to the existing optical contrast and thereby greatly increasing the visibility of small structures by light microscopy.

Aims of staining:

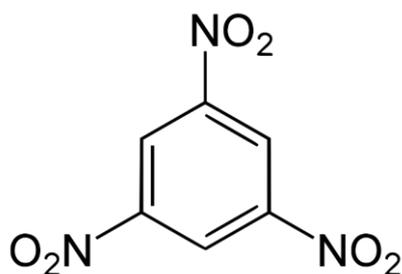
- To render the microscopic and semi-transparent bacteria visible by producing colour contrast.
- To reveal size and shape of bacteria.
- To study various internal and external structures of bacteria which help in identification and classification of bacteria.

Dye:

Any coloured compound that reacts with or is absorbed or dissolves in another phase and renders that phase coloured or an organic unsaturated cyclic compound (benzene ring) containing both chromophore and auxochrome group.

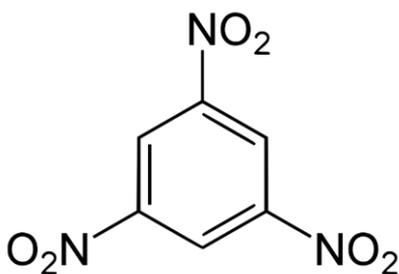
Chromophore group: Imparts colour to the dye. Benzene containing chromophore radicals are referred to as "Chromogens". Even though they it is coloured, it is not a dye. It possesses

no affinity to bind with fibres or tissues. To be a dye it must possess another auxiliary group called an "Auxochrome".



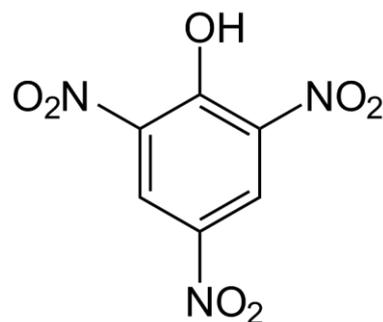
The nitro group of trinitrobenzene is a chromophore group and hence, it is a yellow coloured chromogen but not a dye. It does not dissociate electrolytically and is unable to form salt either with acid or base.

Auxochrome group: Capable of undergoing electrolytic dissociation and imparts salt forming and dyeing properties.



Trinitrobenzene

(Chromogen)



Picric Acid

(Dye)

Examples of Chromophore groups: Azo, Nitroso, Azoxy, Thiocarbonyl, Nitro, Imino etc.

Examples of Auxochrome groups: NR_2 , NHR , $-\text{NH}_2$, $-\text{OH}$, $-\text{OCH}_3$, I , Br , Cl etc.

Dyes that bind cells by ionic interactions are probably the most commonly used dyes. These ionizable dyes may be divided into two general classes based on the nature of their charged group.

1. Basic dyes: methylene blue, basic fuchsin, crystal violet, safranin, malachite green—have positively charged groups (usually some form of pentavalent nitrogen) and are generally sold as chloride salts. Basic dyes bind to negatively charged molecules like nucleic acids, many proteins, and the surfaces of procaryotic cells.

2. Acidic dyes: eosin, rose bengal, and acid fuchsin—possess negatively charged groups such as carboxyl ($-\text{COOH}$) and phenolic hydroxyl ($-\text{OH}$). Acidic dyes, because of their negative charge, bind to positively charged cell structures.

Dyes as Biological Stains

Stains: The dyes which are used for biological purpose and are highly purified.

Steps in Staining Process:

- A) Preparation of the film or smear
- B) Fixation of smear
- C) Staining of the smear

A) Smear Preparation:

Liquid culture: A loopful of liquid culture spread on clean glass slide.

Solid culture: A minute amount of the growth is first emulsified in a drop of distilled water.

B) Fixation

The stained cells seen in a microscope should resemble living cells as closely as possible. Fixation is the process by which the internal and external structures of cells and microorganisms are preserved and fixed in position. It inactivates enzymes that might disrupt cell morphology and toughens cell structures so that they do not change during staining and observation. A microorganism usually is killed and attached firmly to the microscope slide during fixation. There are two fundamentally different types of fixation. Heat fixation is routinely used to observe procaryotes. Typically, a film of cells (a smear) is gently heated as a

slide is passed through a flame. Heat fixation preserves overall morphology but not structures within cells. Chemical fixation is used to protect fine cellular substructure and the morphology of larger, more delicate microorganisms. Chemical fixatives penetrate cells and react with cellular components, usually proteins and lipids, to render them inactive, insoluble, and immobile. Common fixative mixtures contain components such as ethanol, acetic acid, mercuric chloride, formaldehyde, and glutaraldehyde.

Fixation is essential because:

- 1) Kills the bacteria
- 2) Increases the permeability
- 3) Unfolds globular proteins
- 4) Avoids shrinkage and swelling
- 5) Prevents autolysis
- 6) Makes cell rigid
- 7) Protects the structures
- 8) Avoids formation of artifacts

C) Staining of the smear

Performed by employing aqueous solutions of the dyes to fixed smear.