262 Zoo-continue

Fixation types

Lecture 3

From page 8 to 10

Dr. Nouf Alyami

1. Aldehydes

- Formaldehyde (formalin, when in its liquid form), paraformaldehyde, and glutaraldehyde.
- Tissues are fixed through cross-linking agents that react with proteins and nucleic acids in the cell (particularly lysine residues).
- Formaldehyde is a good choice for immunohistochemical studies.
- This fixative is used routinely for surgical pathology and autopsy tissues requiring hematoxylin and eosin (H and E) staining .

2. Glutaraldehyde

- Causes deformation of the alpha-helix structure in proteins, so it should not be used for immunohistochemistry staining.

- It fixes very quickly, which makes it an excellent choice for electron microscopic studies, it provides poor penetration.

- It gives very good overall cytoplasmic and nuclear detail and is prepared as a buffered solution (e.g., 2% buffered glutaraldehyde).

3. Oxidizing agents

- permanganate fixatives, such as potassium permanganate, dichromate fixatives (potassium dichromate), osmium tetroxide, and chromic acid.

- Causes cross-link proteins, they cause extensive denaturation.

4. Alcohols

- Alcohols are used primarily for cytologic smears.
- They are fast acting, cheap, and preserve cells through a process of dehydration and precipitation of proteins.
- Methanol has been shown to be effective during immunostaining .

5. Mercurials

- They contain mercuric chloride which is a known component in fixatives such as B-5 and Zenker's.

- These fixatives offer poor penetration and tissue hardness, but are fast and provide excellent nuclear detail, such as for visualization of hematopoietic and reticuloendothelial tissues (i.e., lymph nodes, spleen, thymus, and bone marrow).

- These fixatives must be disposed of carefully. Mercury deposits must be removed (dezenkerized) prior to staining, otherwise black deposits will occur in tissue sections .

6.Picrates

- include fixatives with picric acid, such as Bouin’s solution.
- This fixative provides good nuclear detail and does not cause much hardness.
- It is recommended for fixation of testis, gastrointestinal tract, and endocrine tissues.
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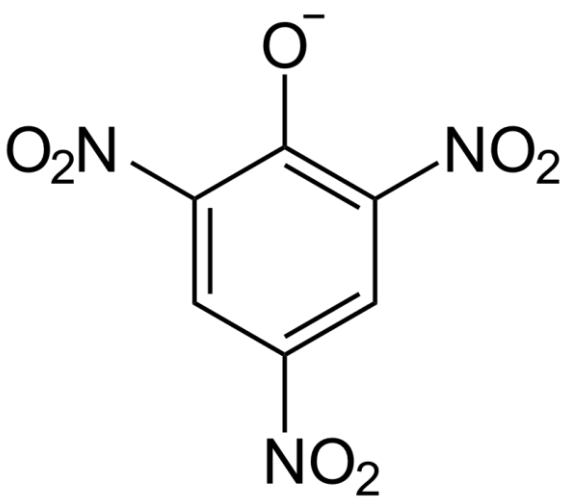
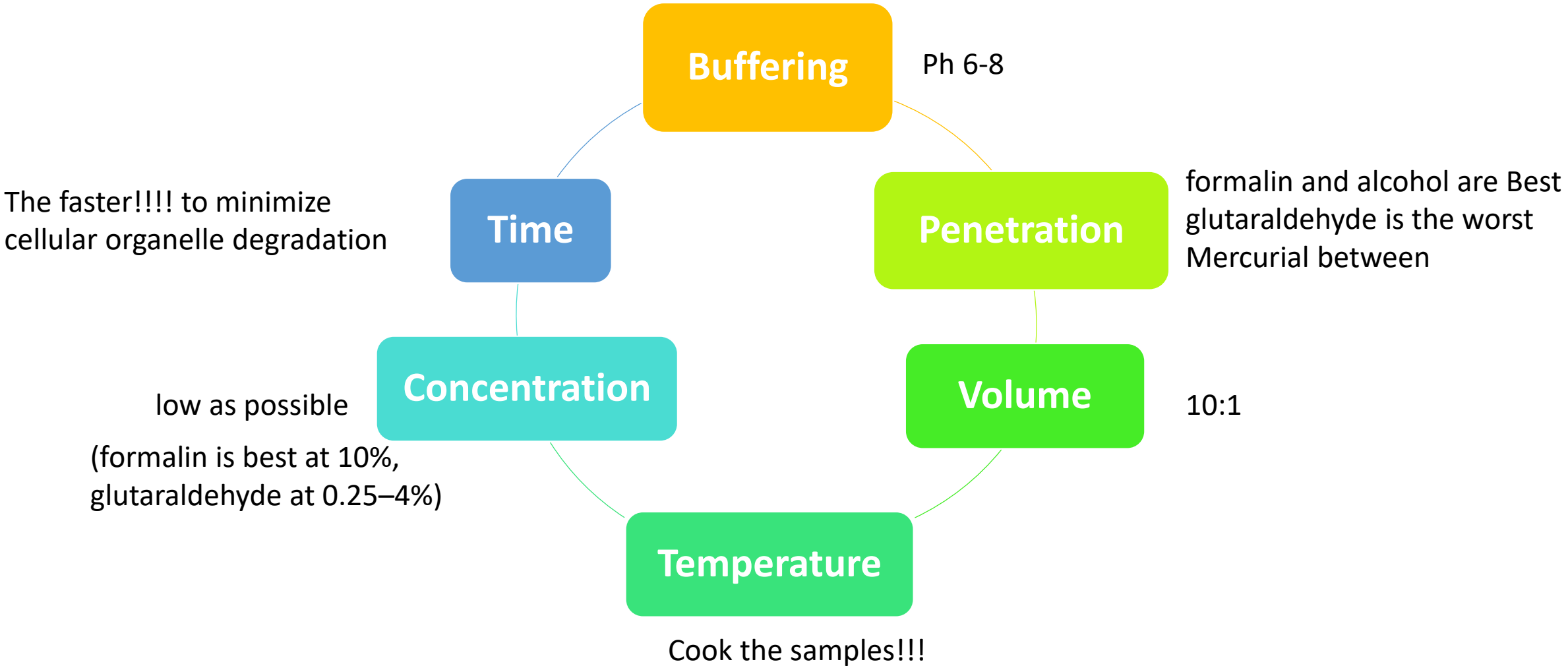
- This fixative has an explosion hazard in dry form, so it must be kept submerged in alcohol at all times.



Factors affecting fixation



Decalcifying agents

- Some animal tissues contain deposits of calcium salts (i.e., bone, teeth, and calcified cartilage) which may interfere with sectioning, resulting in torn sections and damaged blades.
- Calcium compounds must be chemically removed (usually with an acid).
- Cause minimal distortion to cells and connective tissue.
- Some typical decalcifying agents include, nitric acid, Gooding and Stewart's fluid, Rapid Bone Decalcifier (RDO), and chelating agents.