1. **Purpose of fixation**

In performing their protective role, fixatives denature proteins by coagulation, by forming additive compounds, or by a combination of coagulation and additive processes. A compound that adds chemically to macromolecules stabilizes structure most effectively if it is able to combine with parts of two different macromolecules, an effect known as cross-linking. Fixatives have different actions e.g. crosslinking, precipitative, coagulative etc. They also have different penetration rates, usually measured in depth penetrated per mm per hour. Normally recommended fixing is in 20x the volume of fixative to tissue and fix for consistent periods of time- for example, samples of 0.5 cm thick are fixed in at least one dimension for 24 hours in Neutral Buffered Formalin, or for 6 hours in Bouins fluid. Then samples are transferred into 70% ethanol (the first stage in many routine paraffin processing protocols) prior to processing. Fixation of tissue is done for several reasons. One reason is to kill the tissue so that postmortem decay (autolysis and putrefaction) is prevented. Fixation preserves biological material (tissue or cells) as close to its natural state as possible in the process of preparing tissue for examination. To achieve this, several conditions usually must be met.

First, a fixative usually acts to disable intrinsic biomolecules—particularly proteolytic enzymes—which otherwise digest or damage the sample.

Second, a fixative typically protects a sample from extrinsic damage. Fixatives are toxic to most common microorganisms (bacteria in particular) that might exist in a tissue sample or which might otherwise colonize the fixed tissue. In addition, many fixatives chemically alter the fixed material to make it less palatable (either indigestible or toxic) to opportunistic microorganisms.

Finally, fixatives often alter the cells or tissues on a molecular level to increase their mechanical strength or stability. This increased strength and rigidity can help preserve the morphology (shape and structure) of the sample as it is processed for further analysis.

Fixation improves the optical differentiation of the tissues. This is actually done by altering the refractive index by varying degrees. This is of real importance since the refractive indices of some of the cellular components are so close to that of the surrounding structure which renders them invisible in the living state when examined under the microscope.

Even the most careful fixation does alter the sample and introduce artifacts that can interfere with interpretation of cellular ultrastructure. A prominent example is the bacterial mesosome, which was thought to be an organelle in gram-positive bacteria in the 1970s, but was later shown by new techniques developed for electron microscopy to be simply an artifact of chemical fixation. Standardization of fixation and other tissue processing procedures takes this introduction of artifacts into account, by establishing what procedures introduce which kinds of artifacts. Researchers who know what types of artifacts to expect with each tissue type and processing technique can accurately interpret sections with artifacts, or uchoose techniques that minimize artifacts in areas of interest.

  **2. Compound fixatives and their compositions**

**Bouin Solution**

Composition;

- 37-40% Formaldehyde

- Picric Acid

- Glacial Acetic Acid

**Gendre solution**

Composition;

- 37-40% Formaldehyde

- 95% alcohol saturated with picric acid

- Glacial acetic acid

**Hollande solution**

Composition;

- 37-40% Formaldehyde

- Distilled water

- Picric acid

- Copper acetate

**Zenker and Helly solution**

Composition;

- Mercuric Chloride

- Distilled water

- Potassium Dichromate

- Sodium Sulfate

**Orth solution**

Composition;

- 37-40% Formaldehyde

- Distilled water

- Potassium Dichromate

- Sodium Sulfate