



## **SOME COMMON PRESERVATIVES, STAINS AND REAGENTS**

Whenever a part of an organism is removed or an aquatic organism, as for example, the amoeba kept without water, they soon disintegrate, dry or putrefy by the action of bacteria. In order to study these, they need to be kept in a condition as close to normal as possible or in other words preserved and fixed. In many Biology practicals, for example cytochemistry about which you learnt in chapter 31 and many physiology experiments, chemicals are required. Some such chemicals preserve and specifically dye certain parts of the cell while others are used as solutions. In this lesson you shall learn about some preservatives, stains and reagents.

In the laboratory study of biological tissues and other materials, various types of chemicals are needed for specific results. Such chemicals are mainly the preservatives, stains and some other reagents used in various experiments. Some such chemicals are being explained in this lesson.



### **OBJECTIVES**

After completing this lesson you will be able to :

- *differentiate between reagents, stains and preservatives;*
- *describe methods of staining;*
- *list the commonly used preservatives, stains and reagents;*
- *state the chemical composition of Bouin's fluid, Carnoy mixture, Leishman's stain, Safranin, Acetocarmine, Methylene blue, Iodine solution, Benedict's solution, Fehling's solution, Ringer's solution, FAA (Formalin Acetic Acid-Alcohol) solution;*
- *describe the uses of above mentioned solutions and stains.*



### 33.1 PRESERVATIVE, STAINS AND REAGENTS

Whenever a tissue is outside the body, it needs to be placed in a fixative for its preservation. It is later stained and studied in details because (a) a fixed specimen is like the original specimen and (b) it can remain in the undeformed state for a long time; (c) and also it can be stained to differentiate its parts.

Fixative is a chemical which maintains the equilibrium (balance) of the cell inclusions so that cell gets preserved in a condition close to normal. Fixation also renders the material suitable for staining.

Let us first define the above mentioned three terms to know the difference between them.

#### Preservative

Preservative is a chemical which is used to fix (to maintain) the tissues of plants and animals for a long time so that decomposition does not take place.

#### Stain

Stain is a chemical (natural or synthetic) which imparts colour to the cell or part of it. It enables different components to differentiate more clearly than in the unstained object. Example Safranin is a stain which colours xylem tissues pink.

#### Reagents

Reagent is a substance that takes part in chemical reactions or biological processes. It is used to detect substances present in the cell. Example Iodine solution is used for detecting starch.

### 33.2 PRESERVATIVES OR FIXATIVES

Chemicals are used to kill, preserve and fix plant/animal tissues and specimens in such a way that they retain their original shape, form size and structure. These make the tissues hard and prevent them from decaying. A fixative must penetrate rapidly the tissue removed from the body. Some of the preservatives are given below alongwith their composition :

- Bouin's fluid** : This preservative or fixative is yellow in colour and penetrates rapidly in the tissues, for making histological preparation.

#### Composition :

Saturated aqueous picric acid	-	75 ml
Formalin (40% Formaldehyde)	-	25 ml
Glacial Acetic Acid	-	5 ml



**Notes**

**2. Carnoy's fluid :** It penetrates rapidly and gives excellent nuclear fixation.

**Composition :**

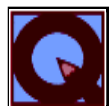
- Absolute Alcohol - 60 ml
- Chloroform - 30 ml
- Glacial Acetic Acid - 10 ml

It is prepared fresh. Care is to be taken as it is highly poisonous and inflammable.

**3. Formalin Acetic Acid Alcohol (F.A.A.) :** This is or a very good fixative and tissues may be left in it for a long period without any harm.

**Composition**

- 50% Alcohol - 100 ml
- 40% Formaldehyde - 6.5 ml
- Glacial Acetic Acid - 2.5 ml



**INTEXT QUESTIONS 33.1**

1. Define the terms :
  - (i) Preservative .....
  - .....
  - (ii) Stain .....
  - .....
2. State the composition of Carnoy's fluid.
  - .....
3. How is Bouin's fluid more advantageous than other preservatives?
  - .....

**33.3 STAINS**

There are different stains for study of different biological materials. Stains are dyes which react with the components of the cell to give the component a particular colour. These dyes may be synthetic chemicals or obtained from plants or from animals e.g. carmine is derived from the cochineal insect. Staining can be done in several ways.

**1. Single Staining :** Where only one stain is used giving a single colour to the tissue e.g. Acetocarmine stains both the nucleus and the cytoplasm pink.



Notes

2. **Double Staining** : Where **two** stains are used, each stains a specific area or the particular cell organelle e.g. Methyl green which stains nucleus green is used with Pyronin which stains the cytoplasm pink.
3. **Multiple Staining** : **More than two** stains are used in the preparation of slide of tissue or organelle. Each stain will colour only the specific organelle of the cell e.g. Triple Mallory stain.
4. **Vital staining** : stains such as the Janus Green B which stains mitochondria is used to colour living cells. Such stains which do not kill the cell, do not require prior fixation and impart colour to a specific part are termed vital stains (vita = live).

Some of the stains are given below :

1. **Leishman's Stain** : It is a readymade double stain, used for staining blood films. It gives blue colour to the nucleus and pink to the cytoplasm.

#### Composition

Leishman stain powder - 15 g  
A ethyl alcohol (solvent) - 100 ml

For good results this stain is kept in dark coloured bottle.

2. **Safranin** : It is used as a general stain for plant tissues. The stain may be prepared both in water as well as in 90% alcohol depending on the requirement.

#### Composition

Safranin powder - 1 g  
Distilled water - 100 ml

It is a synthetic dye which gives pink or red colour to the object stained.

3. **Acetocarmine** : It is mainly used to stain chromosomes in the study of cells.

#### Composition

Glacial acetic acid - 45 ml  
Carmine powder - 2 g  
Distilled water - 55 ml

4. **Methylene blue** : This stain may be used both as aqueous or alcoholic stain. It is a basic stain and so mainly stains acidic parts such as DNA of the nucleus and fungal bodies. Methylene blue is a vital stain

#### Composition

Aqueous Methylene blue :

Methylene blue - 100 mg  
Distilled water - 100 ml


**Notes**

The stain is dissolved in distilled water

Alcoholic Methylene blue :

Methylene blue	- 0.3 g
95% Ethyl alcohol	- 30 ml
Distilled water	- 100 ml

This stain is prepared by adding 30 ml of saturated alcoholic solution of methylene blue (0.3 gm of it to 30 ml of 95% ethyl alcohol) in 100 ml of distilled water.

**33.4 REAGENTS**

There are different reagents which are used to test the different substances present in certain solutions. Some of them commonly used in a biology laboratory are given below :

- 1. Benedict's Solution :** It is used for the test of sugar.

**Composition**

Copper sulphate	- 1.7 g
Sodium citrate	- 17.3 g
Sodium carbonate (anhydrous)	- 10.0 ml
Distilled water	- 1000 ml

Dissolve 17.3 g sodium citrate and 10 g of anhydrous sodium carbonate in 600 ml of distilled water. Filter the solution. Simultaneously prepare copper sulphate solution. Add this solution slowly to the previous filtered solution, constantly stirring it. Add enough distilled water to make a total of 1 litre.

If to a solution containing glucose, Benedict's is added and warmed a brick red precipitate forms.

- 2. Fehling's Solution A and B :** It is also used for testing of sugar. It is commonly purchased ready made from the market.

**Composition**
**Fehling's solution A**

Copper sulphate	- 34.6 g
Distilled water	- 500 ml

**Fehling's solution B**

Sodium hydroxide	- 175 g
Sodium potassium tartarate	- 173 g
Distilled water	- 500 ml

When testing for sugar, equal amounts of Fehling's solution A and Fehling's solution B are added to the solution which is to be tested. Results are the same as that with Benedicts



Notes

- 3. Iodine Solution :** It is commonly used for testing starch. As such it is brownish in colour.

**Composition**

Iodine	- 0.3 g
Potassium iodide	- 1.5 g
Distilled water	- 100 ml

Iodine added to starch turns the starch grains or starch solution, dark blue.

- 4. Ringer's Solution :** This solution is isotonic to that of tissue that is when tissue is placed in Ringer's no osmotic changes occur. It does not spoil quickly and living material can be placed in it for observation in normal living state.

**Composition**

Potassium chloride	- 0.42 g
Sodium chloride	- 9.0 g
Calcium chloride	- .24 g
Sodium bicarbonate	- .20 g

**INTEXT QUESTIONS 33.2**

- Mention the use of
  - Ringer's solution .....
  - Leishman's stain .....
- Write the full form of F.A.A.  
.....
- Write the composition of
  - Iodine solution .....
  - Carnoy's fluid .....

**WHAT YOU HAVE LEARNT**

- Preservative is a substance or method which prevents decay and decomposition of an organism or its parts.
- Stain is a chemical which colours tissue or its parts.
- Different types of preservatives are used for different experimental material and for different purposes.
- Various types of stains are used for various tissues or cellular components.
- Staining may be single, double or multiple. Vital staining stains living organisms and cells.
- Different types of reagents are used for different experiments.

**Notes****TERMINAL QUESTIONS**

1. Define the term reagent
2. What is meant by (i) Double staining and (ii) Multiple staining?
3. Mention the use and the composition of Bouin's fluid.
4. Mention the components of F.A.A.
5. Which tissue is normally stained by Leishman's stain?
6. Name any one stain used generally in biology laboratories.
7. Give the composition of Fehling's Solution A and B. Mention the substance that can be tested by Fehling's reagent.
8. Mention the use of Ringer's solution.

**ANSWERS TO INTEXT QUESTIONS**

- 33.1**
1. (i) Preservatives are chemicals used to kill and fix the tissues such that they retain their original form, size and structure or preservatives maintain, plant and animal tissues without decomposition for a long time.  
(ii) Stain is a chemical which imparts colour to the cell or its parts.
  2. Abs. alcohol 60 ml, chloroform 30 ml, glacial acetic acid 10 ml
  3. Penetrate into tissues rapidly.
- 33.2**
- (i) cells of tissue kept in Ringer's solution retain their normal shape as no osmotic changes take place.  
(ii) It is used to stain blood cells – nucleus becomes blue and cytoplasm becomes pink in colour.
  2. Formalin Acetic Acid Alcohol.
  3. (i) Iodine, Potassium Iodide and Distilled water.  
(ii) Absolute alcohol, Chloroform and Glacial Acetic Acid.