Stains

Crystal violet:

It also called as Gentian violet. It is basic in nature. It has triamino - diphenyl - methane group. Its solubility in water is 1.68% and in alcohol 13.87%.

Crystal violet can be used as 1%. solution in Distilled water. Through this solution has long shelf life, for certain cytological investigations freshly prepared stain is preferred. The draw back with crystal violet is that while dehydrating with alcohol, the stain also removed Therefore, it is advisable to use mordants such as clove oil. Alternatively, this stain can also be prepared by dissolving in clove oil.

Crystal violet - 1gm

clove oil - 100ml.

Safranin

Safranin is a basic stain. It has Azin group. Its solubility is 5.4% in water and 23.4% in alcohol. Safranin is the most widely used stain in botanical microtechnique.

It is a general stain, imparts colour to lignified, cutinized, suberised and chitinised structures and parts of cells such as chromosomes, nucleolus and centrosomes.

Contrary to its certified low solubility in alcohol, it dissolves fairly well in absolute alcohol.

2.25% gm of safranin O powder can be dissolved in 225 ml of 95% Ethyl alcohol and be kept as stock solution. when needed, a required portion is to be diluted with Distilled water just before use. If this dilution still appears to be too strong, it can further diluted using 50% alcohol.

Johansen's Method.

Safranin - 4 gm

Methyl cellosolve - 200 ml

After the stain has dissolved completely following chemicals to be added into it

95% alcohol - 100 ml

Distilled water - 100 ml

Sodium acetate - 4 gm

Formalin - 8 ml

⇒

Cotton blue in lactophenol

This is acidic in nature. It is prepared 1% in 70% alcohol.

Anilin blue is also known as cotton blue, Starid blue, china blue, and so on. Both alcoholic and aqueous acidic solution of Anilin blue are in use.

Anilin blue dissolved in Lacto-phenol is very ideal to stain structures such as fungal mycelia, pollen tubes etc. It can be prepared as follows.

phenol - 100ml
Glycerine - 100ml
Lactic acid - 100ml
Distilled water - 100ml
Anilin blue - 4gm

⇒ ~~The~~ acetate ~~added~~ intensifies the stain. Formalin act as a mordant. slides can be kept in this stain solution for 24-48 hrs. It imparts very sharp differentiation and brilliant contrast

Generally safranin over stains the preperation. Therefore differentiation and moderating are very important steps while using this stain. Using 95% alcohol, acidified with picric acid at dehydrating step gives very good differentiation.

Excess stain is to be washed off at once using water. Otherwise, reddish precipitates are formed which reduces the quality of the micropreperation.

Acetocarmine

Female insects of the species *Coccus cacti* live on the plant *Opuntia coccinellifera* is dried and powdered. From this powder a yellow colour matter is seperated. When alum is added to this, a dark red stain called Carmine is obtained. Carmine mixed with acetic acid ~~and as~~ to form acetocarmine. This stain ~~used to stain particularly for~~ out as a specific stain for chromosomes.

To prepare acetocarmine ~~stain~~, 100 ml of 45% acetic acid is heated in a beaker to boiling point. ~~A portion~~ 2gm of carmine is added to the boiling acetic acid. The beaker is removed from the heater and the remaining portion of carmine is added to the beaker and stirred with a glass rod. The solution is allowed to cool ~~down~~ and ~~then~~ filtered. To this few drops of ferric acetate dissolved in glacial acetic acid (are added) to change the colour to wine red. The aceto carmine solution thus prepared can be stored in a refrigerator for quite long

Methylene blue:

New Methylene blue stain is used for reticulocyte staining and counting Procedures. It can also be used to examine cytology specimens.

* Dissolve 0.5g powdered new methylene blue into a solution containing 99 mL 0.85% saline and 1 mL 40% formalin.
* Filter the stain solution and store it in a brown bottle.