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Fixation and different types of fixatives: Their role and functions: A review

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Abstract
Fixation is a complete and complex physiochemical process wherein cells and tissues are chemically fixed for further analyses. Fixatives are used for Solidification, Hardening, Optical differentiation, prevention of autolysis and tissue putrefaction. Various Types of fixatives (Simple, Compound, Microanatomical, Histochemical and Cytological) are used in Histology and Histopathology which includes Mercuric Chloride, Osmic Acid, formaldehyde, glutaraldehyde, osmium tetroxide, glyoxal, picric acid, Zenker's fluid and so on. Due to limited availability of the material related to the topic a need was felt to write a review article so as to make the Researchers, Students. pathologists and laboratory technicians familiar with the basic Concept of fixation and fixatives.

Keywords: fixation, fixatives, role, functions

Introduction

Fixation is a complex series of chemical events which brings about changes in the various chemical constituents of cell like hardening, however the morphology of a cell and structural details are preserved. Tissue fixation is of utmost importance soon after the removal from the body it will undergo
degenerative changes due to autolysis so that the morphology of the individual cell will be lost. Autolysis is a combination of post-mortem changes due to rupture of cell homeostasis that leads to uncontrolled water and electrolytes dynamics in and out of the cell and of alteration of enzymatic activity. These changes are favourable conditions for bacterial and fungal growth and ultimately result in complete destruction of tissue structures. To halt autolysis, tissues should be preserved in an appropriate fixative that permanently cross-link its proteins and stabilize it. The process of autolysis virtually begins immediately after death. Therefore, rapid and adequate fixation after sampling is essential. This can be achieved by immersion of the tissue sample in an adequate volume of fixative solution. Fixatives bring about crosslinking of proteins which produces denaturation or coagulation of proteins so that the semifluid state is converted into semisolid state; so that it maintains everything *in vivo* in relation to each other. Thus, semisolid state facilitates easy manipulation of tissue. Fixatives can be classified in different ways, as shown below.

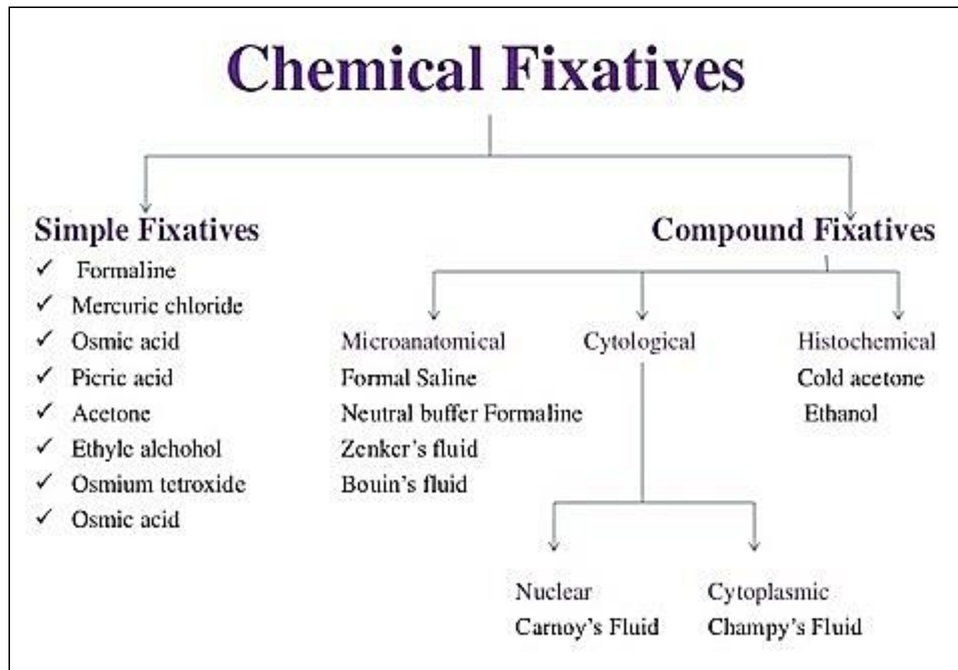

Chart depicting various types of Chemical Fixatives

Functions of Fixatives

If a fresh tissue is kept at room temperature it will become liquefied with a foul odour mainly due to action of pathogens i.e.; putrefaction and autolysis so the first and fore most aim of fixation is:

1. To preserve a tissue live and prevent from post mortem changes like autolysis (lysis or dissolution of cells by enzymatic action probably as a result of rupture of lysosomes) and putrefaction (breakdown of tissue by bacterial action often with formation of gas).
2. Preservation of chemical compounds and microanatomic constituents so that further histochemistry is possible.
3. Solidification: Converts the normal gel form into a sol form. The semifluid consistency of cells (gel) is changed into an irreversible semisolid consistency (solid).
4. Hardening: Easy manipulation of soft tissue like brain, intestines etc. is possible and maximally explored through Hardening via fixatives.
5. Optical differentiation: It alters to varying degrees the refractive indices of the various components of cells and tissues so that unstained components are more easily visualized than when unfixed.
6. Effects of staining by certain fixatives intensifies the staining character of tissues.

Factors Affecting Fixation and Fixatives

Length of Fixation

Different tissues require different time span for fixation depending on the size of the tissue (Thinner tissues require less time than thick tissues). If fixation period is prolonged, it will result in tissue brittleness due to over–cross-linking. If fixation time is shortened, it will result in less penetration of fixatives in tissues and cross-linking will not occur. Optimally overnight fixation is preferred for maximum tissue samples.

Concentration

Different fixatives have different ideal concentration that are determined experimentally. if concentration of a fixative is low prolonged time is needed for fixation and vice versa for high concentrations of a fixative resulting in damage to cellular structures as well as obliterated enzyme activities.

Temperature

With increase in temperature to an optimum degrees (37°c-45°c) rate of fixation is increased, otherwise it will cause autolysis. If temperature is decreased, diffusion rate will also get decreased, resulting in extended penetration time.

Size

Tissue thickness is one of the important factors for fixation. 4- to 6-mm thick specimen is best suited and preferred size for good penetration. If the sample size is too large, it is difficult for a fixative to penetrate and to reach the deeper part of the tissue, and thus resulting in autolysis.

Various Fixatives Used in Histopathology

Simple fixatives

(I) Formaldehyde

Formaldehyde was discovered in 1859 by Butlerov. In 1889 Trillat was the first who manufactured formaldehyde commercially as industrial reagent. In 1892, Ferdinand Blum recognized that formalin could give benefit when used as a fixative. The most routinely used solution for fixation of tissue is 10% formalin solution v/v-is nothing but an aqueous suspension of formaldehyde. In 10% neutral buffered form, formaldehyde is found to be the most commonly used fixative in pathology. Reaction between the formaldehyde and macromolecules of tissue seems to be complex. Formaldehyde reacts with nucleic acids as well as proteins, and it penetrates between nucleic acids and proteins and forms stabilized shell of nucleic acid-protein complex. As compared with other fixatives, formaldehyde causes lesser tissue shrinkage, with exceptions being acetone and ethanol. Formaldehyde seems to harden tissue more when compared with other fixatives. The lipids are conserved, but carbohydrates are not fixed by formaldehyde. Formalin comprises 37 to 40% formaldehyde

and 60 to 63% water by weight. After continuous storage for long periods, accumulations of white deposits are observed in the solution. These are the precipitates of paraformaldehyde. By storing formalin at low temperature, these white deposits can be avoided. Also, 10% methanol may be added into the formalin to minimize the polymerization reaction that produces paraformaldehyde precipitate. It also contains a slight amount of formate ions. These are obtained from Cannizzaro reaction. In this reaction, two molecules of formaldehyde react together. One molecule condenses to form methanol and second molecule gets oxidized to form formic acid. The solution is acidic in reaction because of formic acid, but acidic nature of solution can be counterbalanced with incorporation of magnesium carbonate in little proportion. it is very cheap as compared to other fixatives in market, penetrates rapidly. Formalin is not a fixative of choice for carbohydrates as it partially preserves Glycogen. Some enzymes but not all can be demonstrated in formalin fixed tissues. It has no effect on neutral lipids but Complex lipids are completely fixed. A fat may be demonstrated in frozen section after fixed in a formalin fixative. Pure formalin is not a satisfactory fixative as it over hardens the tissue. A 10% dilution in tap water or distilled water is necessary. If kept standing for long period it gets oxidized to formic acid and forms a brown pigment in tissues called as Artifact, so it should be neutralized by addition of phosphates or calcium carbonate. There is a greater danger of explosion if concentrated Formalin is neutralized. Precautions are very much needed as Formalin on prolonged exposure can cause dermatitis. Its vapour may damage the nasal mucosa and cause sinusitis. Time required for fixation at room temperature is 12 hours for small biopsies and 4- 6 hours.

(II) Ethanol and Methanol

Ethanol (CH_3CH_2OH) and methanol (CH_3OH) are considered to be coagulants that denature proteins. They replace water in the tissue environment disrupting hydrophobic and hydrogen bonding thus exposing the internal hydrophobic groups of proteins and altering their tertiary structure and their solubility in water. Methanol is closer to the structure of water than ethanol so ethanol interacts more strongly with hydrophobic areas than methanol. Fixation commences at a concentration of 50 – 60% for ethanol and >80% for methanol. Ethanol is sometimes used to preserve glycogen but will cause distortion of nuclear and cytoplasmic detail. Methanol is commonly used as a fixative for blood films and 95% ethanol is used as a fixative for cytology smears but both alcohols are usually combined with other reagents when used as fixatives for tissue specimens. They penetrate slowly, hardens and shrinks the tissue efficiently and penetrates rapidly in presence of other fixative hence in-combination with other fixatives e.g., Carnoy's fixative is used to increase the speed of tissue processing. Ethanol is used to detect certain enzymes as it preserves some proteins in relatively un-denatured state so that it can be used for immunofluorescence or some histochemical methods. Ethanol dissolves fats and lipids being a good fat solvent. Methyl alcohol is used for fixing blood film smears and bone marrow smears.

(III) Acetone

Acetone (CH_3COCH_3) has a similar action to alcohol and has been used as a fixative and dehydrant for tissue processing, particularly rapid hand-processing of small specimens. It is widely recommended for fixation as part of the histochemical demonstration of enzymes where it is generally used cold (4 °C). It is an effective lipid solvent with a rapid action which can make tissues very brittle. Because it is highly volatile and flammable it is generally not used on automatic tissue processors. Acetone should not be used on some tissue processors because it will adversely affect seals and other components of the equipment. Cold acetone is sometimes used as a fixative for the histochemical demonstration of some tissue enzymes like phosphatases and lipases.

(IV) Mercuric Chloride ($HgCl_2$)

Mercuric chloride ($HgCl_2$) was one of the first reagents used for tissue fixation. Although the mechanisms by which it fixes tissue are not fully understood it is known to react with amines, amides, amino acids and sulfhydryl groups, the latter being prominent in its reaction with cysteine where it is thought to produce cross-links. It is a powerful protein coagulant which leaves tissue in a state which produces strong staining with acid dyes. It reacts with phosphate residues of nucleic acids and effectively fixes nucleoproteins. It is for this reason that it is the major component in formulated fixatives such as B-5 and Helly's fixatives recommended when high quality nuclear preservation is required (e.g., bone marrow trephines). These fixatives have slow penetration capacity, so the thickness of the specimens being fixed by mercuric fixatives should be thin. Disadvantages of Mercuric chloride as a fixative are that apart from the corrosive nature of mercuric chloride are, mercury is highly toxic, can be absorbed through the skin and is a cumulative poison, tend to penetrate poorly and if fixation is prolonged tissues become very hard and are prone to shrinkage during processing. In recent years a number of metal salts have been introduced as substitutes for mercuric chloride including salts of zinc and barium. Zinc chloride and zinc sulphate have been accepted fairly widely as being suitable and there are now many proprietary B-5 substitutes available. Some of the characteristics of Mercuric chloride as a fixative are as:

- It is rarely used alone because it causes shrinkage of the tissue.
- It brings about precipitation of the proteins which are required to be removed before staining by using potassium iodide in which they are soluble.
- Thick tissues more than 4mm thick are not fixed properly as it hardens the tissue at the peripheries whereas the Centre remains soft & under fixed.
- It penetrates rapidly without destroying lipids but ineffective on carbohydrates
- Mercuric chloride treatment brings out more brilliant staining with most of the dyes.
- Mercuric chloride fixed tissues contain black precipitates of mercury which are removed by treating with 0.5% iodide solution in 70% ethanol for 5-10 minutes, sections are rinsed in water, decolourized for 5 minutes in 5% sodium thiosulphate and washed in running water.

(V) Picric acid

Picric acid or trinitro phenol ($C_6H_2(NO_2)_3OH$) is a yellow crystalline substance that should be stored wet with water to avoid the risk of explosion. Distilled water can be added if it

appears to be drying out. For fixation it is always used in combination with other agents Bouin's and Hollande's solutions. It is also used as an acid dye in several stains (e.g.; in Van Gieson's solution for staining muscle). It imparts a yellow colour to tissues during fixation and because of its acidic nature residual picric acid should be washed from tissues with 70% ethanol before processing.
Being a coagulant fixative, it changes the charges on the ionisable side chains of proteins and disrupts electrostatic and hydrogen bonds. It forms salts (picrates) with basic groups of proteins causing coagulation. It does not to fix lipids and most carbohydrates but fixes glycogen. Picric acid hydrolyses nucleic acids so it should be avoided if DNA or RNA are to be examined. A little bit of shrinkage occurs during the processing of tissues fixed in picric acid containing reagents and that is the reason it is not used alone It penetrates well and fixes rapidly. It precipitates proteins and combines with them to form picrates some of the picrates are water-soluble so must be treated with alcohol before further processing where the tissue comes into contact with water.

(VI) Potassium dichromate
Potassium dichromate ($K_2Cr_2O_7$) works as a non-coagulant unless it is used at pH < 3.4 – 3.8, where it reacts like chromic acid, as a coagulant. It is a component of several compound fixatives like Zenker's and Helly's solutions. It oxidises the proteins with the interaction of reduced chromate ions forming some cross-links, the extent being determined by the pH of the fixative. Chromium ions react with carboxyl and hydroxyl side chains of proteins leaving amino groups available and thus favours staining with acid dyes. Chromate will react with unsaturated lipids rendering them insoluble, and thus is a good fixative for mitochondria. It is normally recommended that tissues fixed in a chromate containing fixative are thoroughly washed in water prior to processing to avoid the formation of insoluble chromates. Traditionally dichromate containing fixatives were used in histochemical methods for the amine containing "chromaffin" granules of endocrine tissues. It fixes the cytoplasm without precipitation. Valuable in mixtures for the fixation of lipids especially phospholipids, phosphatides and mitochondria.

(VII) Osmium tetroxide
Osmium tetroxide is type of fixative that is water soluble and also soluble in nonpolar solvents. Osmium tetroxide seems to react with proteins side chains that cause cross-linking. The reactive groups of osmium tetroxide include various groups such as disulphide, carboxyl, hydroxyl, sulfhydryl, amide, and so on. During fixation by osmium tetroxide, either due to slow rate of reaction or due to restricted penetration of osmium tetroxide into tissue, large amounts of carbohydrates as well as proteins are eradicated. For electron microscopic studies, osmium tetroxide is used as secondary fixative, and it also performs well as stain and imparts contrast when observed under electron microscope. Osmium tetroxide is also helpful for staining of lipids in frozen sections. It is observed that fixation by osmium tetroxide causes swelling in tissue, which can be decreased by adding sodium chloride or calcium chloride to fixatives. It is traditionally sold as crystalline solid that is sealed in glass ampule. It is seen that osmium tetroxide crystals convert from solid state to vapor state. Continued exposure to osmium tetroxide vapours can cause deposition into cornea, which eventually leads to blindness Osmium tetroxide (OsO_4) is a highly volatile, toxic, crystalline solid, soluble in polar and non-polar solvents. It fixes those substances having unsaturated bonds of lipids and phospholipids as it is one of the few fixatives that stabilises lipids and fats e.g., Myelin. During fixation process it is reduced to oxides which are deposited as black and insoluble particles in tissues, particularly on membranes. As osmium is a heavy metal it scatters electrons and thus adds electron density to the electron microscope image. It can also be used as an *en bloc* stain for demonstrating lipids (particularly myelinated nerve fibres) at the light microscope level and thus used in Electron Microscopy.

(VIII) Acetic acid
Acetic acid (CH_3COOH) is considered as a noncoagulative fixative agent. It acts by causing nuclear proteins coagulation. Incidentally, it stabilizes and assists to prevent nucleic acids loss. Acetic acid, when combined with ethanol, is used as an effective Cytological fixative that helps in conservation of nucleic acids, but if it is used singly, it results in swelling of cells, so used with other fixatives and to counter the shrinkage caused by other ingredients such as ethanol. Time required for fixation by acetic acid is less as penetration of acetic acid is faster into tissues penetrates rapidly and is a coagulant in action with nucleic acids but generally does not fix proteins. It is incorporated in compound fixatives to help prevent the loss of nucleic acids.

(IX) Glutaraldehyde (C5H8O2)
Glutaraldehyde was found in 1963 by Sabatini et al as particular fixative for ultrastructural researches. Glutaraldehyde comprises two aldehyde groups that are divided by three methylene bridges. Although penetration rate of glutaraldehyde is found to be slower when compared with formaldehyde, glutaraldehyde is more effective cross-linker for proteins than formaldehyde and it also inhibits enzyme activity. When polymerization of aqueous solution of glutaraldehyde occurs, it forms oligomeric and cyclic compounds, and also forms glutaric acid by oxidation. For stability, it requires pH of 5 and storage at 4°C.19,20 At room temperature, glutaraldehydes are not able to cross-link the nucleic acids. Glutaraldehyde preserves the ultrastructure of the tissue, thereby it is used in electron microscopy studies, but owing to poor penetration and overhardening properties, it is not used as tissue fixatives for light
microscopy. On exposure to oxygen, glutaraldehyde becomes unstable and breaks down with decrease in pH. Glutaraldehyde can act as sensitizer, and its exposure may result in respiratory tract, skin, and digestive tract irritation It is an amine reactive crosslinker in proteins, employed alone or mixed with Formaldehyde to stabilise human cells. It is also used alone or in combination with osmium tetroxide to crosslink and stabilize cells and membrane lipids for electron microscopy.

Compound fixatives
These are the mixtures of a number of fixatives combined in a definite proportion so that the disadvantage of one is reduced by use of another fixative and require a lesser amount of time for fixation. For example, Susa fluid, Carnoy's fluid, Bouin's Fluid, Formal saline, buffered formalin etc. All these compound fixatives have their own

advantages and disadvantages. There are three broad types of compound fixatives. Microanatomical, Cytological and Histochemical fixatives.

A. Micro anatomical fixatives are aimed at preserving anatomy of the tissues accurately and preserving the relationship of tissue layer and large cell aggregations. These fixatives are used for routine work of normal and histopathological studies. For example, buffered formalin, Zenker's fluid, Bouin's fluid etc.

1. Bouin's fixative

It is known as noncoagulant picrate fixative solution and was explained by Pol Andre Bouin in 1897. Bouin's fixative is considered as good fixative for conserving delicate as well as soft tissue structures. The major portion of Bouin's fixative contains picric acid with little quantity of acetic acid as well as formaldehyde. In the samples that have to be undertaken in situ hybridization, Bouin's solution cannot be used because it decreases the severity of hybridization. Chemical Composition of Bouin's fixative is:

a) Saturated aqueous picric acid = 75ml
b) Formalin = 25ml
c) Glacial acetic acid = 5 ml

This fluid Penetrates rapidly and evenly and causes little shrinkage. It is an Excellent fixative for carbohydrates (glycogen), testicular and intestinal biopsies because it gives very good nuclear details, in testes it is used for detection oligospermia (low sperm count) and infertility studies.

2. Buffered formalin

The fixative is a good routine fixative. This solution is hypotonic in buffer ions and has a pH of 6.8. Specimens should be fixed for 1-4 hours minimum, longer for larger specimens. Formalin fixation is thought to form between the aldehydes and the proteins, creating a gel, thus retaining cellular constituents in their *in vivo* relationship. Once properly fixed, the tissue should be able to withstand the subsequent stages of tissue processing or staining. It has been used as a popular general-purpose histological fixative to fix tissues. Chemical composition is as

a) Formalin = 10ml
b) Acid sodium phosphate (monohydrate) = 0.4 gm
c) Anhydrous disodium Phosphate = 0.65 gm
d) Water = 100 m

3. Buffered formal sucrose

This is an excellent fixative for the preservation of fine structure of phospholipids and some enzymes. It is recommended for combined cytochemistry and electron microscopic studies. The pH of this solution should be close to pH 7.4, but does not have to be too precise. It is unlikely to produce formalin pigment, unless very bloody tissues are stored in it for a long time without changing the solution, an unlikely situation. Formal sucrose gives more protection to cytological components and has been recommended as an initial fixative for electron microscopy, if used at 4°C. Otherwise, it is used for phospholipids or whenever greater physical protection is needed for the tissue. It is not usually recommended as a routine formalin fixative as it is basically formalin in a light sugar syrup.

If used for electron microscopy, the fixative is applied at 4 °C for an appropriate time, then transferred to the secondary fixative. Otherwise, as with the other formalin fixatives, it should be applied overnight as a minimum, days being better.

Chemical composition is as:

a) Formalin = 10ml
b) Sucrose = 7.5 gm.
c) M/15

4. Heidenhain Susa

It is a decalcifying solution that derives its name from the initial letters of sublimate (mercury bichloride saturated in 0.6% NaCl) and saure (trichloroacetic and glacial acetic acid), key ingredients of the fixative together with formalin, all in aqueous solution. It is considered a good general-purpose fixative, with rapid penetration. It is an excellent fixative for routine biopsy work and allows brilliant staining with good cytological detail. Gives rapid results and has even penetration with minimum shrinkage. Tissue has to be left in it for over 24 hours. Tissue should be treated with iodine to remove mercury pigment impurity if any. Chemical composition is as:

Mercuric chloride = 4.5gm
Sodium chloride = 0.5 gm.
Trichloroacetic acid = 2.0 gm.
Acetic acid = 4.0 ml
Distilled water to = 100 ml

5. Zenker's fluid

A fixative solution containing corrosive mercuric bichloride, potassium bichromate, sodium sulfate, glacial acetic acid, and water; the sodium sulfate is frequently omitted. A mercury precipitate is left in the tissues and is troublesome to the inexperienced microscopist unless removed by an iodine solution. The most widely used variations of this fixative are the modifications by Maximow, by Helly, and by Custer, in which formalin replaces the acetic acid. It is a good routine fixative and gives fairly rapid and even penetration. It is not stable after the addition of acetic acid hence acetic acid (or formalin) should be added just before use. Washing of tissue in running water is necessary to remove excess dichromate.

Chemical composition is as:

Mercuric chloride = 5gm
Potassium dichromate = 2.5 gm.
Sodium sulphate = 1.0 gm
Distilled water to = 100 ml
Add immediately before use Glacial acetic acid: 5 ml

6. Zenker formal (Helly's fluid)

Helly's fluid or Zenker-formol fixative or formol-Zenker solution is a histologic fixative consisting of Zenker's fluid in which the glacial acetic acid is replaced by formalin. The concentration of formalin used varies between 5 and 10%: the most widely used formula consists of 9 parts Zenker stock solution and 1part neutral formalin (Zenker-Helly-Maximow). This is an excellent cytological and tissue fixative. It is used for bone marrow spleen and blood containing organs. As with Zenker's fluid it is necessary to remove excess dichromate and mercuric pigment. Chemical composition is as:

Mercuric chloride	=	5 gm.
Potassium dichromate	=	2.5 gm.
Sodium sulphate	=	1.0 gm.
Distilled water to	=	100 ml

Add formalin immediately before use 5 ml

7. Gendre's fluid
This is an alcoholic Bouin solution that appears to improve upon aging. It is highly recommended for the preservation of glycogen and other carbohydrates. After fixation, the tissue is placed into 70% ethanol. Residual yellow colour should be washed out before staining. Chemical Composition is:

Saturated picric acid in 95% alcohol	=	80ml
Formalin	=	15ml
Glacial acetic acid	=	5ml

B. Cytological fixatives are used to preserve the constituent elements of the cells. According to their mode of action, they can be further subdivided into two groups:

- **Nuclear fixatives:** These are the fixatives that primarily fix the nuclear components of the cells. For example, Carnoy’s fluid, Clarke’s fluid etc.

1. Carnoy's fluid
It is rapid acting, gives good nuclear preservation, and retains glycogen. Nissl’s granules and glycogen are preserved. It lyses erythrocytes and dissolves lipids and can produce excessive hardening and shrinkage. Chemical composition is:

Absolute alcohol	=	60ml
Chloroform	=	30ml
Glacial acetic acid	=	10 ml

2. Clarke's fluid
Has been used on frozen sections and smears. Can produce fair results after conventional processing providing fixation time is kept very short. Preserves nucleic acids, but lipids are extracted. Rapid, good nuclear fixation and good preservation of cytoplasmic elements. It is excellent for smear or cover slip preparation of cell cultures or chromosomal analysis. Tissues can be transferred directly into 95% ethanol. Fixation time is 3-4 hours. Chemical composition is:

Absolute alcohol	=	75 ml
Glacial acetic acid	=	25 ml

3. New Comer's fluid. Newcomer's is a fixative containing isopropanol, propionic acid, and dioxane that is recommended as a substitute for Carnoy’s fixative in preservation of chromatin. It is also useful for fixing polysaccharides. When fixing, small pieces of tissue should be used. Fixation is complete in12-18 hours.
Isopropanol = 60 ml
Propionic acid = 40ml
Petroleum ether = 10 ml.
Acetone =10 ml.
Dioxane =10 ml.

Cytoplasmic fixatives: These are the fixatives that primarily fix the cytoplasmic components of the cell. For example, Champy’s fluid, Zenker’s fixative etc.

1. Champy's fluid
The solution is stable for some time. It is suitable for use when cytological preservation is the main interest. This fixative cannot be kept hence prepared fresh. It preserves the mitochondrial fat and lipids. Its Penetration power is poor and uneven. Tissues must be fairly small, preferably 1-2 mm thick and never more than 3 mm. Pieces more than 2 millimetres should be washed overnight after fixation and should be fixed within 24 hours. Aftertreatment the specimen should be Washed in running tap water overnight to remove chromium and osmium compounds. Chemical composition is:

Chromium trioxide	=	1g
Potassium Dichromate	=	3g
Osmium tetroxide	=	1g
Distilled water	=	250ml

2. Formal saline
This mixture of formaldehyde in isotonic saline was widely used for routine histopathology prior to the introduction of phosphate-buffered formalin. It often produces formalin pigment. Fixation time is 12 to 24 hours. Chemical composition is:

40% Formaldehyde	=	100ml
Sodium Chloride	=	9g
Distilled water	=	900 ml

3. Formal Calcium
Recommended for the preservation of lipids especially phospholipids. Fixation time is 12 to 24 hours. Chemical composition is:

40% Formaldehyde	=	100ml
Calcium Chloride	=	10g
Distilled water	=	900 ml

Histochemical fixatives
They are used for the Histochemical studies of the tissues where the minimum or no changes in the components to be demonstrated are required. for example, Buffered formalin or vapor fixatives include Formaldehyde vapour, Acetone, Acrolein etc.

1. Acetone
Acetone (CH_3COCH_3) has a similar action to alcohol and has been used as a fixative and dehydrant for tissue processing, particularly rapid hand-processing of small specimens. It is widely recommended for fixation as part of the histochemical demonstration of enzymes where it is generally used cold (4°C). Acetone is a histochemical fixative agent used in histopathology. It acts as an efficacious lipid solvent that results in tissue brittleness. Apart from tissue fixation, they are primarily used as an agent for dehydration in tissue processing. Because of extremely volatile as well as flammable nature, they are not recommended for use in automatic tissue processor.

2. Acrolein /chromyl chloride
It used at 37 °C for 1-2 hours. Acrolein was introduced by Luft as a primary fixative agent, and it is a three carbon αβ unsaturated monoaldehyde. Acrolein provides magnificent preservation of structural detail and conserves the virus antigenicity. It is also known as acrylic aldehyde. It reacts with macromolecules that result in formation of cross-links

that are reversible. Acrolein is not commonly used because it is unstable at alkaline pH and forms insoluble polymers. Acrolein is highly reactive and is found to penetrate tissues rapidly. Acrolein fixatives are chiefly used in enzyme histochemistry.

3. Formaldehyde Vapour

It is obtained by heating paraformaldehyde at temperature between 50° and 80 °C. Blocks of tissue require (3-5) hours whereas section require (½-1) hours.

Conclusion

Fixation is a key step in histology and histopathology procedure. Each and every fixative has its own advantage and disadvantage. Various different fixatives perform various functions, and various factors such as size, concentration and temperature etc have direct effects on fixation procedure.

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