Antimicrobial Terminology

- Antiseptic : Agent which either kills or inhibits growth of microorganism e.g bacteria, fungus etc.
- Germicide : Agent which kills microorganisms e.g bactericide, fungicide etc

  Those which does not kill but inhibit growth of microorganism are bacteriostat, fungistat.
- Disinfectant : kills microorganisms but used on inanimate objects e.g. instruments, equipments, rooms
- Sterilization : use of a disinfectant or other procedure (e.g heat) to render an object completely free of microorganisms.

Mechanism of action of Inorganic Antimicrobial agents

Inorganic antimicrobials act by 3 ways :

1. **Oxidation**
2. **Halogenation**
3. **Protein precipitation**

based on the primary chemical interactions or reactions that occur between the agent and the microbial protein.

This results in the death of the microbe or inhibition of its growth.

Site of action of inorganic antimicrobials are nonspecific i.e. it will interact with all proteins in a similar fashion and in high concentrations and will affect both microbial and host proteins.

Oxidation

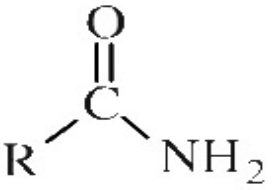
- Nonmetals and certain types of anions function through oxidative mechanisms.
- Examples are hydrogen peroxide, metal peroxides, permanganates, halogens (e.g chlorine and iodine) and oxo-halogen anions.
- Oxidative action of these compounds involves the reducing groups present in most proteins e.g the sulfhydryl (-SH) group in cysteine.
- Illustration of reaction between oxidizing antiseptic and a sulfhydryl-containing protein.

Oxidation

- The formation of the disulphide bridge will alter (change) the conformation (shape) of the protein.
- Thereby alters the function of the protein.
- The overall change or destruction of the function of the protein is responsible for the ultimate destruction of the microorganism.
- Chemical result of oxidizing the protein is destruction of the antimicrobial agent.

Halogention

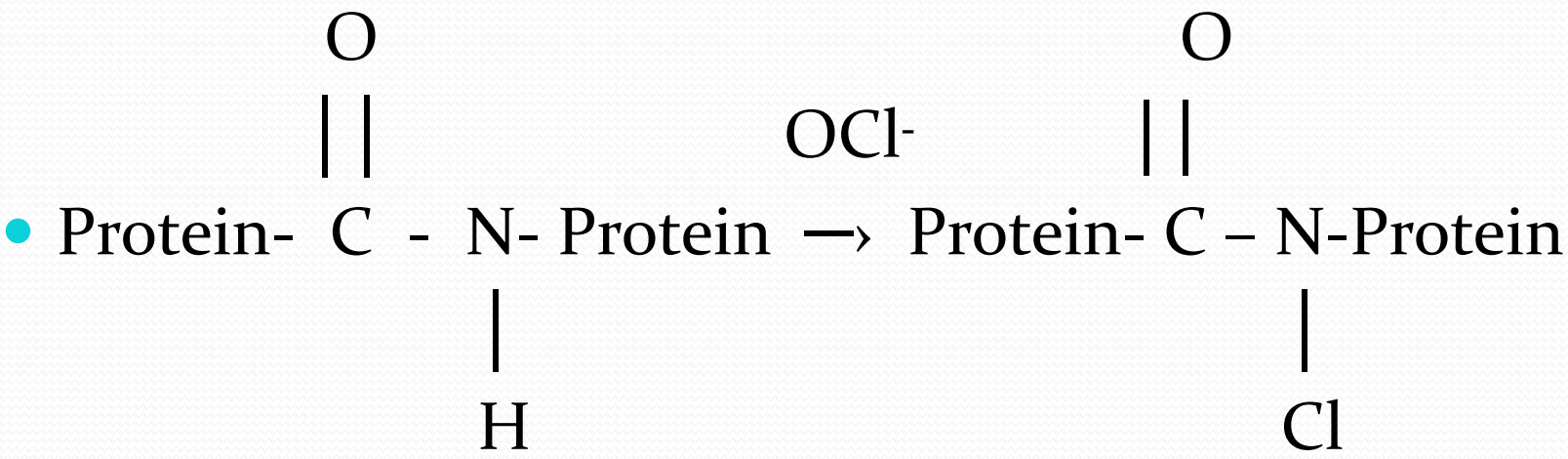
- This is a reaction occurring with antiseptics of the hypohalite type i.e. Hypochlorite, OCl^-
- Since these types of compounds can serve as reagents in the chlorination of primary and secondary amides, e.g



it is expected that a similar reaction can take place under appropriate conditions with the peptide linkage between the amino acid groups comprising the protein molecule.

Halogention

Example of the reaction is shown below :



Halogention

- This reaction is ultimately destructive to the function of proteins.
- The substitution of the chlorine atom for the hydrogen produces changes in the forces (hydrogen bonding) that is responsible for the proper conformation of the protein molecule.
- Changes in the conformation result in destruction of function of proteins.

Protein Precipitation

- This type of mechanism involves the interaction of proteins with metallic ions having large charge/radius ratios or strong electrostatic fields.
- This property is available in transition metal cations e.g. Cu(II), Ag(I), Zn(II).
- Al(III) due to its charge and small ionic radius is also an effective protein precipitant.
- Except alkali and alkaline earth metals, most metal cations will demonstrate protein precipitant activity.

Protein Precipitation

- The nature of the interaction is complexation of the metal ion with various polar groups on the protein that act as ligands.
- The complexation of the metal ion with the protein results in radical changes in properties of the protein or protein precipitant.
- Certain metals have affinity for particular enzymes.
- The metal 'ties up' important functional groups at the active site on the enzyme by forming the complex.
- Increasing the concentration of the metal cations can increase antimicrobial and astringent properties.

Protein precipitation

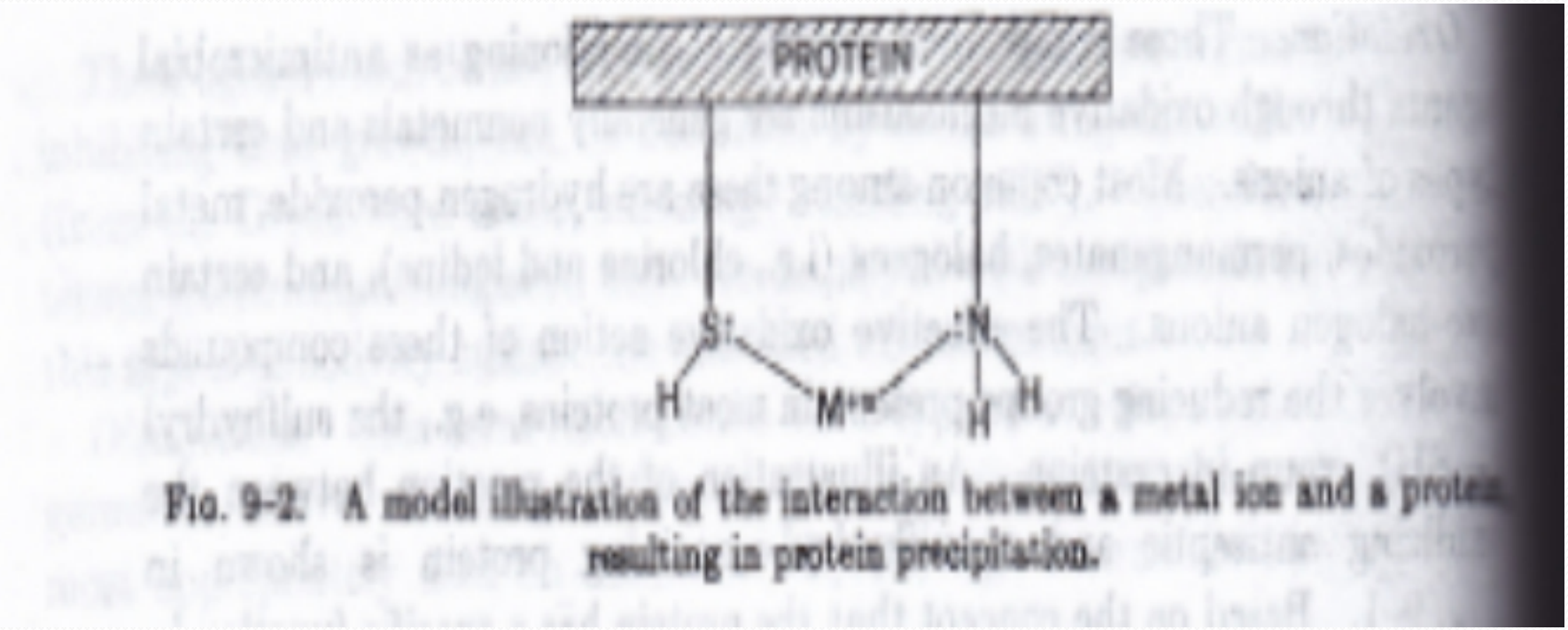


FIG. 9-2. A model illustration of the interaction between a metal ion and a protein resulting in protein precipitation.

Astringents

- The application of a very dilute solution of a metal cation to tissue primarily involves a local or surface protein precipitant action.
- This activity is known as astringent.
- As it is a surface phenomenon it does not usually result in the destruction of host tissue.
- Its effect can be observed or felt when applied to skin or mucous membranes.
- The effect can be described as a 'shrinkage' or 'firming' of the tissue.

Uses of astringents:

- used as styptics to stop bleeding from small cuts as astringents cause constriction of capillaries and small blood vessels
- to reduce the volume of exudates from wounds and skin eruptions.
- as antiperspirants in deodorants due to their ability to constrict pores and destroy microorganisms that produce body odors
- may stimulate growth of new tissue when applied topically to wounds.
- higher concentrations can provide corrosive effect on contact with tissue that can be used to remove undesirable tissue .e.g warts.

Control of antimicrobial/astringent action

The concentration of protein precipitants needed for astringent activity is higher than that needed for antimicrobial activity.

Very high concentrations can produce an irritant action and corrosive action.

- For water soluble compounds control of activity is done by making solutions of a appropriate concentrations for the desired use.

- The concentrations will depend on the area of use - e.g higher concentrations may be used on skin than on eye.

Control of antimicrobial/astringent action

- Soluble compounds may be controlled by placing them in a vehicle/ solutions (e.g glycerin or polyethylene glycol) that will slow their release to the site of action.
- Complexation with a ligand (e.g. Povidone-Iodine) also provides controlled release.
- Other compounds may be synthesized in an insoluble form and used in suspensions, creams or ointments for their antimicrobial action and slow release of the agent.

Oxidative Antimicrobial Agents

1. Hydrogen Peroxide Solution USP XVIII

In 100 ml, it contains not less than 2.5 g and not more than 3.5 g of hydrogen peroxide.

Uses :

- Used as a mild oxidizing antiseptic in contact with open or abraded tissue, exposing the chemical to the enzyme, catalase. Catalase causes decomposition of H_2O_2 to water and oxygen.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

- The release of oxygen provides mechanical cleansing action to remove dirt, bacteria, debris from the surface of the wound.

- When diluted with one part of water it can be used as a gargle or mouthwash for the treatment of throat and mouth infections.

Potassium Permanganate USP XVIII ($KMnO_4$)

- Odorless, dark purple crystalline compound
- Soluble in water
- When heated at 240° C, it decomposes with the liberation of oxygen, leaving manganese oxide (MnO_2) and potassium manganate (K_2MnO_4).
- Strong oxidizing agent both in dry state and in solution
- $2KMnO_4 + H_2O = 2MnO_2$ (brown ppt)$+ 2KOH+3[O]$
- Great care must be taken in handling it as dangerous explosives may occur if it is brought in contact with organic or readily oxidizable substances, either in solution or in dry state.

Potassium Permanganate USP XVIII ($KMnO_4$)

- Acid solutions of potassium permanganate reduces the permanganate ion (MnO_4^- / Mn^{+7}) to the manganous ion with the evolution of oxygen.

$$2KMnO_4 + 3H_2SO_4 \longrightarrow K_2SO_4 + 2MnSO_4 + 3H_2O + 5[O]$$

- Neutral or alkaline solution produces a brown precipitate of manganese dioxide (MnO_2).

$$2KMnO_4 + H_2O \longrightarrow 2MnO_2 + 2KOH + 3[O]$$

- The antibacterial action is dependant upon its oxidation of protein or other bioorganic substances. The oxygen released is the effective agent.

Potassium Permanganate USP XVIII ($KMnO_4$)

Uses:

- Used for both their antibacterial and antifungal actions
- Used externally in concentrations ranging from 0.2% to about 0.006%.
- Used for skin infections (dermatitis) caused by bacteria and fungi and for poisoning produced by plant and animal toxins.
- Used in the treatment of vesicular (small blisters/raised areas containing fluids) stage of eczema, tinea pedis (fungal infection of the foot).

Sodium Hypochlorite Solution N.F. XIII (Dakin's solution)

- NaOCl is very unstable and usually found only in solutions
- Contains not less than 4% and not more than 6% by weight of NaOCl
- Clear, pale greenish yellow liquid having an odor of chlorine
- Has an alkaline pH coloring red litmus blue
- Not suitable for application to wounds since its alkalinity and oxidizing action is too strong for use on tissues
- In addition, the solution dissolves blood clots and delays healing
- A diluted form is known as Labaraque's solution and consists of Sodium Hypochlorite Solution that has been diluted with an equal volume of water (approx. 2.5% NaOCl)
- This solution is used a disinfectant on inanimate (nonliving) objects.

Sodium Hypochlorite Solution N.F. XIII (Dakin's solution)

Uses:

- Useful as a disinfectant and laundry bleach
- Effective as an germicidal agent that can be used to disinfect areas , instruments and utensils exposed to pathogenic microorganisms
- Diluted form is used as a mouthwash

Disadvantages :

- Prolongs clotting time, can lead to secondary hemorrhage
- Instability of solution requires storage in tight, light-resistant containers and avoidance of excessive heat.

Iodine Preparations and Compounds :

- **Iodine Solution N.F XIII**
- **Iodine Tincture U.S.P. XVIII**
- Both the solutions contain the same concentration of ingredients.

- In each 100ml they contain not less than 1.8g and not more than 2.2g of iodine (I) and not less than 2.1g and not more than 2.6g of sodium iodide (NaI).

- They differ only in the nature of the solvent i.e. – Iodine solution is aqueous, having being prepared with purified water, and Iodine Tincture contains approximately 50% alcohol as the final solvent.

Uses of Iodine Tincture and Iodine Solution :

- Effective topical antiseptic agents
- Used as antiseptics on the skin prior to surgery – Iodine Tincture is more suitable for this purpose as the alcohol can improve the penetration of the iodine due to a wetting or spreading effect and also provides additional antibacterial effect.
- Can be diluted with water for applications to wounds and abrasions – Iodine solution is preferred since the alcohol in the tincture is very irritating to the open tissues and causes stinging when applied to wounds.

Povidone-Iodine N.F. XIII (Aerosol and Solution)

- Povidone-Iodine is a complex of iodine with Povidone N.F XIII which is a polymer also known as polyvinylpyrrolidone or PVP.
- Both the aerosol and solution contains not less than 85% and not more than 120% of the labeled amount of iodine.
- Acid to litmus
- Soluble in water and in alcohol and insoluble in organic solvents.
- The Povidone- Iodine solution is a transparent liquid having a reddish brown color and a pH of not more than 6.

Povidone-Iodine N.F. XIII (Aerosol and Solution)

- Povidone-Iodine is a member of a class of compounds referred to as iodophors.
- Iodophors are complexes of iodine with carrier organic molecules serving as a solubilizing agent. These complexes slowly liberate iodine in solution.

Advantages of Povidone-Iodine complex over elemental iodine solutions:

- Nonirritating effect on tissues
- Its comparatively low oral toxicity
- Water solubility
- Low iodine vapor pressure making it stable to possible iodine loss
- Nonstaining and can be washed clear from skin & clothing.

Povidone-Iodine N.F. XIII (Aerosol and Solution)

Uses:

- Lack of tissue irritation makes it useful for application to sensitive areas and mucous membranes
- Recommended for surgical scrubs and preoperative antisepsis of the skin
- Used in gargles and mouthwashes

Examples : **Betadine solution** (concentrations of 0.1% to 1% of available iodine).

Protein Precipitant Antimicrobial Agents

1. Silver Nitrate U.S.P. XVIII

Properties:

- Colorless or white crystals which become gray or grayish black on exposure to light in the presence of organic matter
- Very soluble in water, sparingly soluble in alcohol and freely soluble in boiling alcohol (What are the different levels of solubility???)
- pH of 1% solutions is between 4.5 and 6 and must be clear or colourless
- Solutions of silver nitrate in concentrations between 0.5 and 1% are used as antibacterial agents.

Silver Nitrate U.S.P. XVIII

- When a protein solution is treated with a solution containing a soluble silver salt, a heavy precipitate is formed involving a complex interaction between the silver ions and protein.
- This type of reaction is the basis for the antimicrobial action of the silver compounds.
- The protein precipitant action of silver ion is not selective i.e. it will precipitate both bacterial and human proteins.
- Silver ion precipitation of protein involves interactions between the cation and various polar groups on the protein molecule e.g -SH, -COOH, $-NH_2$ and heterocyclic residues e.g histidine.

Silver Nitrate U.S.P. XVIII

- When applied to tissue in a concentration of 0.1% silver the activity is rapidly bactericidal.
- **Sustained action**: A bactericidal action continues after the initial application due to the slow production of silver ions from the silver proteinate and silver chloride.
- This sustained action at the tissue levels has given rise to colloidal products of silver proteinate and halide as antibacterials.

Silver Nitrate U.S.P. XVIII

- Silver has **oligodynamic action**, meaning it is active in small quantities.
- Silver preparations are bacteriostatic at concentrations of silver ion below that required for protein precipitation.
- Solutions of silver nitrate are bacteriostatic at concentration of 1:30,000 and bactericidal at 1:4000 in the presence of organic matter.
- Irritation of the skin becomes a factor at concentrations above 1:1000.
- Extended use of silver preparations causes a darkening of the skin due to the deposition of free silver below the epidermis a condition known as **argyria**.

Silver Nitrate U.S.P. XVIII

Uses:

- Employed as an antibacterial in solutions ranging from 0.01 to 10% (higher concentration present astringent and irritant properties to the tissues).
- Used in concentration of about 1:10,000 on sensitive membranes e.g. irrigation of the urethra and bladder.
- Used in treatment of infected ulcers in the mouth (10% solution of silver nitrate).
- Silver Nitrate Opthalmic solution U.S.P XVIII is a 1% solution for instillation into the eyes of newborn babies.
- Used as a prophylactic measure against infections caused by gonococcal organisms.

Silver Nitrate U.S.P. XVIII

- 0.5% aqueous solution of silver nitrate is used in the form of wet dressing on burned areas of patients suffering from third-degree burns.

- This form of therapy involves the mechanism of sustained action in that the silver ions are precipitated by tissue protein and chloride ion. The antibacterial activity is then dependant upon a low but minimal concentration of ions in equilibrium with the insoluble forms.

- This is a good initial treatment due to both the antibacterial effect and the reduction in fluid evaporation and the heat loss produced by the wet dressing.

- Side effects of this treatment: electrolyte imbalance due to precipitation of chloride such as low serum sodium, metabolic acidosis and diarrhea.

Astringents

Aluminium Chloride N.F. XIII

- White or yellowish white, deliquescent, crystalline powder
- Nearly odorless
- Very soluble in water, freely soluble in alcohol and soluble in glycerin (*What are the different levels of solubility???*)
- Acid to litmus in aqueous solutions

Uses: local external astringent and mild antiseptic used in aqueous solutions in concentrations ranging from 10 to 25%. Initially employed for use as antiperspirant but was found to be too irritating.

Soluble Zinc Compounds

Zinc Chloride U.S.P XVIII

Uses :

- Zinc chloride is used for the activity of zinc ion which is a very strong protein precipitant.
- The compound is a powerful astringent in solution and mild antiseptic.
- The strong astringent properties makes it useful as an escharotic (an agent that causes the sloughing of tissue, thus helping in the formation of scar tissue to improve healing).
- It is applied as a solution containing from 0.5% to 2% of zinc chloride.
- The lower concentration may be applied to mucous membranes and can be used as a nasal spray to aid drainage from infected sinuses.
- It is applied topically (10% solution) as a desensitizer of dentine.

Zinc Sulphate U.S.P.XVIII

- Colorless, transparent prisms or as small needles.
- May also be a granular, crystalline powder.
- Very soluble in water, freely soluble in glycerin and insoluble in alcohol.
- Acid to litmus

Uses :

- Used externally as an ophthalmic astringent (Zinc Sulphate Ophthalmic Solution U.S.P.XVIII) in 0.25% aqueous solution. The acidic nature of the solution requires buffering. The pH should be between 5.8 and 6.2. It is usually applied in 0.1 ml doses to the conjuctiva.
- Used internally as an emetic (1 to 2 g doses in a 1% solution).

White Lotion N.F. XIII

Preparation :

- This is a topical preparation prepared by adding a solution of Sulfurated Potash (40g in 450 ml of water) slowly to a solution of Zinc Sulphate (40g in 450 ml of water) and then adding water to a volume of 1000 ml.
- When completed the resulting product is a suspension of zinc sulphides.

$$K_2S + ZnSO_4 \longrightarrow ZnS + K_2SO_4$$

- The order of addition of the compounds is very important. If the order is reversed, the hydroxide ions in the Sulfurated Potash cause the formation of basic zinc salts and zinc hydroxide, rather than zinc sulphides.
- The precipitate tends to become lumpy upon standing so the suspension should be freshly prepared and shaken thoroughly before using.

Uses:

- used topically for the effects of sulphide ion and the astringent action of the zinc ion.
- Has been used in the treatment of acne vulgaris, seborrhea dermatitis and other dermatological problems.