Chapter ... 15

PRESERVATION OF PHARMACEUTICAL PRODUCTS

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- *The Importance of Preservatives*
- *Factors which affect on Preservative Efficacy*
- *Principle of Preservative Efficacy test for Microbial Stability*

15.1 INTRODUCTION

An antimicrobial 'preservative' may be included in a formulation to further reduce the risk of spoilage and to kill any contaminant remaining in a non-sterile medicine after manufacturing. Ideally, preservatives should be able to kill all microbial contaminants rapidly not be an irritant or toxic to the patient, stable and effective throughout the life of the medicine. They should be selective in reacting with the contaminants and not the ingredients of the medicine. The most active antimicrobial agents are often generally non-selective in action, inter-reacting significantly with formulation ingredients and patients. A number of microbiologically effective preservatives used in cosmetics are reported to cause significant incidences of contact dermatitis.

The correct approach to preservation has as its foundation in two important principles. The first of these is that the addition of a preservative to a product must not be done to mask any deficiencies in the manufacturing procedures and the second is that the preservative should be an integral part of the formulation, chosen to afford protection in that particular environment.

The use of a single preservative to protect a pharmaceutical preparation may be unrealistic. Increasing attention has focused upon the use of mixtures of preservatives and the addition of various potentiators to achieve better results.

Preservatives are widely employed in pharmaceutical dosage forms such as emulsions, suspensions, semisolids, parenteral preparations etc.

A single preservative is not suitable for preservation of all pharmaceutical formulations. The selection of a preservative system must be made on an individual basis, using published information and microbiological studies. Combination of two or more preservatives are used to extend the range and spectrum of preservation. Preservative Germall 115 has antibacterial activity but combined with parabens, shows antibacterial as well as antifungal activity. Combination of antimicrobial preservatives may exhibit synergy. Synergy is exhibited when a combination of two compounds exerts a greater inhibitory effect than the simple additive effect of the two compounds against the single microorganism. Eye drops and contact lens solutions include phenylethyl alcohol and phenoxetol in conjunction with benzalkonium chloride to widen the antimicrobial spectrum. Frequently, a combination of two or more esters of parahydroxy-benzoic acid is used to achieve the desired antimicrobial effect. Methyl parahydroxybenzoic acid and propyl parahydroxybenzoic acid are often used together in a ratio of 10 to 1, respectively. An effectively designed preservative system must retain its antimicrobial activity for the shelf-life of the product.

15.2 CHEMICAL PRESERVATIVES

The main function of antimicrobial preservative is to prevent the growth of unwanted microorganisms in pharmaceutical preparations. Different physical methods are used for protection of pharmaceutical formulations from microorganisms. These methods include sterilisation, pasteurisation, unfavourable pH, low temperature, minimum nutrient content etc.

Antimicrobial preservatives can be classified into four major groups such as acidic, neutral, mercurial and quarternary ammonium compounds. A list of commonly used chemical preservatives in different dosage forms is given in Table 15.1.

The concentration of preservative required in an emulsion depends to a large extent on its ability to interact with microorganisms. Microorganisms can reside in the water or the lipid phase or both, the preservative should be available at an effective level in both phases. Chemical preservatives for semisolids must be carefully evaluated for their stability. Plastic containers may absorb the preservatives and therefore decrease the quantity available for inhibiting or destroying the microorganisms responsible for spoilage. Multiple dose eye drops contain an effective antimicrobial preservative system which ensure to maintain sterility during use. Chloroform is the most widely used preservative in oral formulations. Syrups can be preserved by the maintenance of a high concentration of sucrose as part of the formulation. Preservatives are widely employed in cosmetic preservatives for lotions, creams and shampoos.

Table 15.1: Preservatives used in pharmaceutical formulations

Formulation	Preservative	Concentration (% w/v)
Tablets	Methyl paraben	0.1
Injections	Phenol	0.2 – 0.6
	Cresol	0.2 – 0.5
	Benzyl alcohol	1.0 – 2.0
	Thiomersal	0.01
	Methyl Hydroxybenzoate	0.1
Eye drops	Benzalkonium chloride	0.01
	Phenylmercuric nitrate	0.002
	Chlorhexidine acetate	0.01
Liquids/mixtures	Bronopol	0.02
	Alcohol	15 – 20
	Methyl paraben	0.1
	Chloroform	0.25
	Benzalkonium chloride	0.005 – 0.02
	Chlorocresol	0.1
Semisolids	Chlorocresol	0.2
	Dichlorobenzyl alcohol	0.1 – 0.2
	Cetyltrimethyl ammonium bromide	0.05 – 0.1

15.3 FACTORS AFFECTING PRESERVATIVE EFFICACY

A wide range of antimicrobial preservatives are available for the preservation of pharmaceutical formulations. The preservative must prevent accidental contamination and serious or even dangerous decomposition of the product throughout its accepted strange life. The major reasons for a preservative not attaining an effective concentration in the aqueous phase is its interaction with emulgents, solubility in oil, suspended solids, interaction with the container or pH of the formulation.

1. Interaction with formulation components:

Hydrocollids such as methylcellulose, polyvinylpyrrolidone, alignates and tragacanth can interact with preservatives and diminish their activity. Many emulgents are used in pharmaceutical preparations to produce elegant applications. Interactions may occur between preservatives and the emulsified oil phase and with emulgent molecules or micelles. Nature of oil, oil water ratio, type of concentration of emulgent, influence the concentration of preservative needed to protect the system. Many tablet additives cause

problems in tablet preservations due to their interaction with added preservatives. Therapeutically active ingredients (sulphadimidine, kaolin, magnesium trisilicate) in the form of suspended solids also reduce preservative concentration by absorption.

2. Properties of the preservatives:

The distribution of the preservative must be homogeneous and more solubility in the bulk phase is preferable in a multi-phase system. Some chemicals such as chlorobutol may hydrolyse on storage if the pH is unfavourable. Preservatives may react with substances leached from the container and lose its antimicrobial activity.

3. Effect of containers:

Formulations packed in glass containers can be expected to retain their preservative content if the closure is airtight. Preservatives may penetrate through the plastic container and interact with it. Rubber also reacts with many preservatives but is still used for teats and closures. Containers or closures may cause contamination of pathogens. Screw-capped containers and corks are a common source of mould spores.

4. Type of microorganisms:

Plant products may contain pathogenic microorganisms from the soil e.g. *Clostridium* species, *Bacillus anthracis*. These soil microorganisms can cause spoilage of pharmaceutical products. Soil organisms are common in dust which may gain access to a preparation during processing or packaging. Many products prepared from animal sources may contain pathogens like *Salmonella typhi*. Spores of tetanus and gas gangrene have been isolated from gelatin.

5. Influence of pH:

Adjustment of the pH of a solution may affect the chemical stability and the activity of the preservative. Benzoic acid (weak acid preservative) mainly requires to be predominantly in an undissociated form in order to exert antimicrobial activity. This act has a pka value of 4.2, an ambient pH, below this is needed for efficient preservative activity. The majority of preservatives are less dependent upon pH, although cationic active quarternary ammonium compounds are more active at high pH values.

15.4 EVALUATION OF MICROBIAL STABILITY: PRESERVATIVE EFFICACY TEST

This test is applied to the formulated medicine in its final container to determine whether it is adequately protected against microbial spoilage. The test is provided to demonstrate, for multiple dose parenteral, otic, nasal, oral, topical and ophthalmic products made with aqueous bases or vehicles. The effectiveness of any added antimicrobial preservatives, the presence of which is declared on the label of the product concerned. The tests and standards apply only to the product in the original, unopened container in which it is supplied by the manufacturer.

Medium: For the initial cultivation of the test microorganisms, use soyabean casein digest agar medium or any other medium not less nutritive than the said medium.

Choice of test microorganisms and innoculum preparation: The intention is to use microorganisms which are likely to arise in the raw materials used in the product and which occur in the manufacturing environment and represent a particular health hazard if they grew in the product. A preservative should be active against as wide a range of microorganisms as possible hence the choice should be of both Gram-positive and Gram-negative bacteria, yeasts and moulds in the IP test. The test microorganisms used for preservative efficacy tests are *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404. The microorganisms used in the test should not be more than 5 passages made from the original culture, to prevent any phenotypic changes in the strains.

Fresh stock culture of each test microorganism is subcultured on the surface of soyabean casein digest agar medium. Incubate the bacterial cultures at 30 to 35°C for 18 to 24 hours and incubate the cultures of *Candida albicans* and *Aspergillus brasiliensis* to 20 to 25°C for 48 hours and 7 days respectively. Using sterile saline solution, harvest the bacteria and *Candida albicans* and dilute suitably with sterile saline solution to bring the count to about 1×10^8 CFU per ml. Similarly, harvest *Aspergillus brasiliensis* culture with sterile saline solution containing 0.05% w/v of polysorbate 80 and adjust the spore count to about 1×10^8 CFU per ml with sterile saline solution. Alternatively, the stock culture micro-organisms may be grown in a suitable liquid medium and the cells may be harvested by centrifugation, washed and resuspended in sterile saline solution to give the required microbial or spore count.

Procedure: Innoculate each original product container or product tube with one of the standardised microbial suspension using a ratio equivalent to 0.1 ml of inoculum suspension to 20 ml of product and mix. The final concentration should be between 1×10^5 and 1×10^6 microorganisms per ml of product. Determine the number of viable microorganisms by the plate count method in each inoculum suspension and from these calculate the initial concentration of microorganisms per ml of product being examined.

Incubate the inoculated containers or tubes at room temperature. Determine the viable count by the plate count method at 7, 14, and 28 days subsequent to inoculation. Calculate the percentage of reduction in CFU per ml for each organism at the stated test intervals and express the changes in terms of percentage of initial concentration.

Interpretation of results: The preservative is effective in the product examined if:

(i) For parenteral, ophthalmic, sterile nasal and otic preparations:

(a) The concentration of viable bacteria is not more than 10% of the initial concentration at 7 day and not more than 0.1% of the initial concentration at 14 day and there is further decrease in count at 28 day.

(b) There is no increase in yeast and mould count at 7, 14 and 28 day from the initial count.

(ii) For topical preparations made with aqueous base, non-sterile nasal preparation and emulsions:

(a) The concentration of viable bacteria is not more than 1% of the initial concentration at 14 days and there is further decrease in count at 28 day.

(b) There is no increase in yeast and mould count at 14 and 28 day from the initial count.

(iii) For oral preparations:

(a) The concentration of viable bacteria is not more than 10% of the initial concentration at 14 days and there is further decrease in count at 28 day.

(b) There is no increase in yeast and mould count at 14 and 28 days from the initial count.

QUESTIONS

(A) Objective type questions:

1. Define the terms:
   (a) Preservative
   (b) Bacteriostatic
2. List different chemical preservatives.

(B) Short answer questions:

1. Explain in short 'Preservative Efficacy Test'.
2. What is preservative efficacy? Explain.

(C) Long answer questions:

1. How will you evaluate microbial stability of formulation? Why are combined preservatives used in many pharmaceutical formulations?
2. Discuss the factors which effect on preservative efficacy.

(D) Multiple choice questions:

1. Glycerol may be used as a preservative upto a percentage of _____ .
   (a) 15 (b) 50
   (b) 10 (d) 1
2. Test microorganisms used for preservative efficacy test are all of the following except _____.
   (a) *Staphylococcus aureus* (b) *Bacillus subtilis*
   (c) *Aspergillus niger* (d) *Candida albicans*
3. _____ test is used for detection of adequate protection of formulation from microbial spoilage.
   (a) Preservative efficiency (b) Sterility
   (c) Pyrogen (d) Endotoxin