Chapter I

INTRODUCTION

Preservation of pharmaceutical products is necessary to combat the effects of contaminating microorganisms. Products containing water in their formulation are liable to attack by such contaminants. No preservative ever approaches the ideal. All, without exception, have their limitations, and the choice must be made after consideration of the properties of the preparation to be preserved and of the suggested preservative.

The assessment of efficacy by simple microbiological techniques involving tube dilution (e.g. Minimal Inhibitory Concentration) can provide useful information on the effective concentration and antimicrobial spectrum of a preservative. However, when this preservative is incorporated in a cosmetic or pharmaceutical product, the available antimicrobial activity can be greatly reduced. The preservation of disperse systems presents particular and special problems. Bean and Heman-Ackah (1964) described that the activity of preservative in oil:water dispersion is dependent on their concentration in the aqueous phase and at the oil:water interface, both of which are controlled by the oil:water ratio. The activity is also governed by the oil:water partition coefficient which in turn is influenced by temperature (7).

In an emulsion or cream, any included preservative is distributed between the oil and water phases and it is only the concentration of free preservative in the water which is affective to microorganism. So whenever preservatives are more soluble in oil than water, enough quantity must be added to an oil:water system to obtain a sufficient concentration in the aqueous phase. Thus a knowledge of the partition coefficient of the preservatives is essential. Hibbot and Monks (1961) found that oil-water partition coefficient for anyone substance may have a very wide range depending on the nature of the oily phase (36).

Other important factor affecting the activity of preservative is the absorption of preservatives by microorganisms. Bacterial cells contaminating in the cosmetic or pharmaceutical products can absorb the preservative used in the formula. The portion, or all of biocides absorbed by cells are no longer effective in a system to act against other cells. This is particularly important where a high initial level of contamination is experienced and also where the preparation itself provides a good growth medium for such contaminants. Salton (1951) studied measurements of absorption and revealed that uptake often follows typical absorption isotherm (51). Few and Schulman (1953) found that cells sensitive to a particular bactericide may absorb several times as much as resistant organisms (30). Bean and Das

(1953) also found that measurement of uptake increases proportionally with bactericidal activity (9).

The activity of preservatives may be reduced when they are formulated with surface active agents. It is now accepted that nonionic surface active agents, which to a very large extent have replaced the anionics in pharmaceutical and cosmetic formulations, inactivated preservatives. Barr and Tice (1957) believed that inactivation is due to the formation of complex reactants (5). Equilibrium dialysis studies, utilizing semipermeable nylon membrane by Patel and Kostenbauder (1958), Deluca and Kostenbauder (1960), and Bahal and Kostenbauder (1964) showed the remarkable association of preservatives with the nonionic surface active agent (47, 26, 3).

To obtain the most effective value of preserving the product from microorganisms, so many factors must be studied and considered before making selection of the preservative and its concentration used in any given cosmetic or pharmaceutical formula. An attempt to provide the idea for selection of preservative and its concentration is the purpose of this project. The various parameters concenned with preservative evaluation are studied in these experiments.

Literature Survey

procedure employed

The Bacteriostatic Concentration or Minimal Inhibitory Concentration (MIC)

The Bacteriostatic Concentration or Minimal Inhibitory Concentration of a substance will be defined as the lowest concentration which prevents further growth of the organism.

Cook (1954) described three types of the traditional methods of ascertaining bacteriostatic strength, one is a liquid culture media and the other two are the plate method (21). In the first one, the bacteriostatic agent is diluted with a nutrient broth and then inoculated with a test organism and the culture is examined for growth turbidity after incubation. This method is the procedure of choice for ascertaining the strength of bacteriostatics to be incorporated in injections, multidose containers, and other pharmaceutical preparations. For the second method, an agar plate is seeded with a test organism and the size of zone of inhibition of growth caused by diffusion of the bacteriostatic from a cup or cylinder placed on the plate is measured. This method has been used in the assay of antibiotics. In the third one the bacteriostatic agent is incorporated in the solid medium, the positive or negative growth of a culture streaked on the surface is recorded. This method has

been used mainly in a qualitative pattern to ascertain the bacteriostatic spectra of various agent.

The Bactericidal Activity Concentration

Berry and Bean (1954) suggested that the frequently used methods for evaluation of bactericidal activity involve either the measurement of extinction time or the enumeration of surviving organisms after varying periods of exposure to bactericide (11).

In general, counting techniques are preferred for they can provide information on the velocity of the reaction over a wide range of the time-survivor curve, whereas extinction method yield information at just one point of the reaction and concerning only with the overall velocity of the complete bactericidal action. However, counting methods possess some obvious disadvantages just as tedious in performance, moreover such method could not be used in some cases, for example, where the test organisms themselves tend to aggregate into clumps or chains or where there is a tendency of the bactericide to agglutinate the organisms. Colony counts obtained from sample of agglutinated organisms would be meaningless.

Cook and Wills (1954) found that evaluation of bactericidal activity by the extinction method leads to a general economy in time and apparatus over counting methods. Besides

the evaluation of results is relatively simple and the results are rapidly read, so it is conceivable that this method might be applied to the routine testing of bactericidal activity (22). The extinction method of Berry and Bean (1954) is similar to the Rideal-Walker test (23) in principal techniques.

The Oil-Water Partition Coefficients

Oil-water partition coefficient of any substance is a ratio of equilibrium concentration of that substance in oil phase over such equilibrium concentration in aqueous phase.

A knowledge of the oil-water partition coefficients of preservatives is of value in studies of preservation for two principal reasons. Firstly, because it enables loss of preservative from the aqueous phase of emulsions which can be calculated and secondly because of the activity of preservative within a class is related to their oil-water partition coefficient value. Bean and Heman-Ackah (1964) stated that when the partition coefficient is less than 1.0, the majority of the preservative is in the aqueous phase and an increase in the oil:water ratio will increase the aqueous phase concentration. When the partition coefficient is greater than 1.0, most of the preservative is in the oil, and an increase in the oil:water ratio will reduce the concentration in the aqueous phase. It is only when the partition

coefficient is exactly 1.0, which is practically rare, the changing of oil:water ratio has no effect on the preservative concentration in either phase (7).

Hibbot and Monks (1961) found that, oil-water partition coefficients for anyone substance can have a very wide range depending on the nature of the oily phase (36). Furthermore, Bean et al (1965) described that for any oil, a given substance may show an equally wide range depending on oil: water ratio and temperature (8).

According to the partition law (41)

$$K_w^o = \frac{C_o}{C_w} \quad \text{---------- (1)}$$

where K_w^o = oil-water partition coefficient

C_o = equilibrium concentration of preservative in oil phase

C_w = equilibrium concentration of preservative in aqueous phase

In an oil-water dispersion where

C = overall concentration of preservative

V_o = volume of oil

V_w = volume of water

ϕ = V_o/V_w = oil-water ratio

The total weight of preservative in the dispersion

$$W = C\,(V_o + V_w)$$

since $C_o = K_w^o\, C_w$ by definition .

The weight of preservative in the oil phase

$$= C_o V_o = K_w^o\, C_w\, V_o$$

The weight of preservative in the aqueous phase

$$= C_w V_w$$

The total weight of preservative in the dispersion

$$C(V_o + V_w) = K_w^o\, C_w\, V_o + C_w\, V_w$$

$$= C_w(K_w^o\, V_o + V_w)$$

$$C_w = C\,\frac{(\phi + 1)}{(K_w^o\,\phi + 1)} \qquad \text{---------- (2)}$$

An oil-water partition coefficient may be determined according to equation (1) by dissolving a known weight of preservative in a known oil-water mixture and assaying the concentration partitioning into each of the two phases. In general, assays of the oil phase are often tedious, liable to inaccuracies and unnecessary. Provided the aqueous phase is assayed, K_w^o may be calculated by rearrangement of equation (2)

$$K_w^o = \frac{\frac{C}{C_w}\,(\phi + 1) - 1}{\phi} \qquad \text{------- (3)}$$

Absorption of Preservatives by Bacteria

Absorption of preservatives by microorganisms has been investigated by several workers. Salton (1951) related the bactericidal activity of cetrimide against Staphylococcus aureus to its uptake by the cells and revealed that the uptake often follows a typical absorption isotherm (52), whilst. Newton (1954) found that cell wall prepared from sensitive organisms absorbed polymyxin several times as much as those prepared from resistant organisms (45).

Few and Schulman (1953) found marked differences in the absorption isotherms for polymyxin-sensitive and polymyxin-resistant organisms (30). Bean and Das (1966) found that the uptake of preservative increases proportionally with bactericidal activity, that is the most active preservative in any class will be absorbed to the greatest extent (9). An increase in the concentration of a preservative leads to an increase in uptake until a plateau is reached or until the preservative solution reaches saturation, but the uptake pattern or absorption isotherm will vary from preservative to preservative and organism to organism.

Interaction between Preservatives and Emulsifying agent

The availability of free or unbound preservatives is of particular importance in the presence of all surface active agents. Micelle formation may occur in aqueous dispersions of these agents. At concentration above the critical

micelle level, the preservative may be solubilized by the surface active agent, as in the case of chloroxylenol in the presence of cetomacrogol studied by Mulley and Metcalf in 1956 (43). Although the solution may appear normal on visual inspection. Under these conditions, a preservative may be partitioned between the micellar and non-micellar phases so that the latter concentration is insufficient to prevent microbial growth. Allawala and Riegelman (1953) stressed the importance of considering the availability of drugs in the presence of surface active agents and emphasised that the absolute amount of a compound in a solution with a surface active agent was not a measured amount of the drug biological availability. They considered that only the drug present in the aqueous phase was biologically active (1).

In 1951, Gregg and Zopf reported that Tween 80 enhanced the antibacterial activityof hexachlorophene against Staphylococcus aureus (34). Evaluation was carried out by means of a cup-plate type assay. However, Lawrence and Erlandson (1953) found that Tween 80 caused a marked interference with the bacteriostatic effect of hexachlorophene against Staphylococcus aureus by utilization of a tube dilution test (38). A later report by the same authors indicated that Tween 80 also interfered the activity of a number of other antibacterial agents, the bacteriostatic efficiency of phenolic compounds was notably reduced.

Barker,de Kay and Christian (1956) evaluated the release of iodine and mercuric oxide from creams containing nonionic surface active agents. They found that the amount of medicament released was dependent on the nature and concentration of the nonionic substances used (4). Complex formation between iodine and various polyethylene glycols has been investigated by Guttman and Higuchi (35).

It is now well accepted that nonionic surface active agents which have replaced the anionics in pharmaceutical formulations can inactivate a wide variety of commonly used preservatives. The nature of interaction between preservative and surface active agent is much less certain. Barr and Tice (1957) suggested that the mechanism of interaction may be complex formation (5) or solubilization within the nonionic micelles.

Patel and Kostenbauder (1958) studied a quantitative evaluation of the degree of intermolecular association between p-hydroxybenzoic acid esters and Tween 80 by means of an equilibrium dialysis, employing a semipermeable nylon membrane, a relatively high degree of interaction has been observed, and the binding of the esters and Tween 80 has been found to be a function of both the concentration of unbound p-hydroxybenzoate and the concentration of Tween 80. At a concentration of 5% Tween 80, only 22% of the total methyl p-hydroxybenzoate and 4.5% of the total propyl p-hydroxybenzoate are present as unbound preservatives (47).

Equilibrium dialysis studies utilizing a semipermeable nylon membrane indicated a high degree of association and accompanying inhibition of quaternary ammonium germicidal activities such as cetylpyridinium chloride and benzalkonium chloride with nonionic surfactants such as Tween 80. Cetylpyridinium chloride was also found to bind with methylcellulose, but not with polyvinylpyrrolidone and Polyox (Deluca and Kostenbauder, 1960). Possible interaction of chlorobutanol and two aromatic alcohols, benzyl and phenylethyl, with Polysorbate 80, polyvinylpyrrolidone, and methylcellulose was investigated by Bahal and Kostenbauder (1964) using equilibrium dialysis method. All these common alcoholic preservatives were found to exhibit reversible association with Polysorbate 80. Only chlorobutanol was bound by polyvinylpyrrolidone, and none of the alcohols interacted with methylcellulose (3).

Microorganisms Used

Escherichia coli was described by Escherich in 1886. Its synonyms are Bacterium coli commune, Bacillus escherichii, Bacillus coli, Bacterium coli. Escherichia coli is referred as the "colon bacillus" because it is the predominant facultative species in the large bowel. Sear, et al (1949) found that at any particular time the Escherichia coli flora of the human intestinal tract consists of strains that persist

over relatively long periods of time, accompanied at a time with three or four other strains that maintain a tenure of a few days or a few weeks only. They called these two types of strains resident and transients, respectively. Though the resident strains may persist for many months, they eventually disappear to be replaced by other resident strains. The change of resident strain is sometimes accompanied by diarrheic attack. They may be associated with various disease syndroms (53). Besides gastrointestinal tract, they may be isolated from cases of infectious urinary tract, septicemia and endocarditis. Dulancy, et al (1935), Bray (1945), Kauffman and Dupont (1952), Bray and Beavan (1948), Taylor, et al (1949), Rappaport and Hening (1952), and Noyes, et al (1964) reported that the Enteropathogenic Escherichia coli was isolated from many cases of infantile diaarhea (27, 14, 37, 15, 57, 48, 46). The strains that produced infantile diarrhea have been found to be especially resistant to phagocytosis which led to the suggestion that antiphagocytic surface factors may be involved in their pathogenicity (24).

The envelopes of Escherichia coli B. consist of layers of mucopeptide, lipoprotein, and lipopolysaccharide. Weidel, et al (1960) studied on the mucopeptide portion of the envelope of Escherichia coli and stated that this layer is responsible for maintenance of cell shape (58). However, Weinbaum, et al (1967) suggested that there are at least two

components essential for maintenance of cell rigidity in Escherichia coli B. The first is the peptidoglycan (mucopeptide), which is susceptible to lysozyme. The second is a phospholipid which is either covalently linked to the mucopeptide or in closed association with it (59).

Salton and Horne (1951) and Murray (1957) reported the thickness of isolated cell wall and cytoplasmic membrane of Escherichia coli to be of the order of 100-150 Å (51) and a little thinner than 100 Å (44) respectively. Beckett and Robinson (1958) believed that the hexylresorcinol taken up by Escherichia coli is bound initially to the surface of the organism, and when a sufficient number of molecules are bound to form a closed packed monolayer, subsequent molecules penetrate the bacterial cell (10). Silver and Wendt (1967) proposed the mechanism of action of phenylethyl alcohol to Escherichia coli that the primary effect of the alcohol is a limited breakdown of the cell membrane, the inhibition of deoxyribonucleic acid synthesis and other cellular functions would then be secondary consequences of the alteration in the membrane structure (54).

Bayer (1967) studied the response of cell wall of Escherichia coli to a sudden reduction of the environmental osmotic pressure. The rate of survival after osmotic shocks was found to be dependent on the state of growth. When growing logarithmically, Escherichia coli was about 20 to

100 times more sensitive to an abrupt decrease of the environmental osmotic pressure than when it was in the stationary phase. The rigid layer of logarithmically growing cells showed abundant discontinuities and gaps in the distribution of its proteinaceous component, after exposure to osmotic shock, the gaps became wider. He concluded that these gaps might represent the sites where the rigid structure was opened enzymatically to allow for introduction of new building blocks in the older portions of the wall. As a consequence of this, the rigid layer should be mechanically weakened at these locations (6).

Pseudomonas aeruginosa was defined by Schroeter in 1872. Many synonyms are known such as Bacterium aeruginosum, Bacillus pyocyaneus, Pseudomonas pyocyanea. Its common name is "blue pus" organism, because it produces two pigments; a blue compound soluble in chloroform known as pyocyanin and a greenish substance soluble in water, but not in chloroform called fluorescin.

Pseudomonas aeruginosa is the bacterium most lethal to the human eye. Ayliffe et al (1966) found the pathogenicity of Pseudomonas aeruginosa to human eye occurred by the loss of eyes of six English patients from contaminated saline irrigant solution (2). This organism can occur in a variety of topical products and can be infectious to other areas of the human body. Bruch (1971) found that because of

its resistance to most antibacterial drugs it has become an increasingly important infectious agent for a variety of pathological processes in man including septicemia, endocarditis, skin infections, meningitis, gastroenteritis, osteomyelitis, arthritis, genito-urinary tract infections, and respiratory tract infections (18). Markley and Smallman (1968) reported that Pseudomonas aeruginosa is the most important pathogen for extensively burned patients (40). Infection of burned wounds by this organism is the major cause of sepsis and death in burned patients.

Cell wall of Pseudomonas aeruginosa which is a gram negative organism consists of mucopeptide, lipoprotein, and lipopolysaccharide. Eagon and Carson (1965) and Gray and Wilkinson (1965) reported that Pseudomonas aeruginosa dies rapidly in the presence of ethylenediamine tetraacetate (EDTA). Evidence was presented by both groups that the structural integrity of the cell wall is impaired. The former authours concluded moreover, that the binding of divalent cations is essential for the integrity of the cell wall of this microorganism (28, 33). Furthermore, Eagon et al (1965) reported that Ca^{++}, Mg^{++} and Zn^{++} are components of the cell wall of Pseudomonas aeruginosa (29). Evidence was presented by Carson and Eagon (1966) that the mucopeptide component is not solely responsible for the structural integrity of the cell wall of Pseudomonas aeruginosa (19).

There are difficulties associated with determining the growth rate of Pseudomonas aeruginosa because of its tendency to clump and also to form a pellicle. Brown and Richards (1964,a) reported that, the presence of Polysorbate 80 in the culture medium eliminated this effect. The reproducibility of the growth rate measurement has been established (16). Brown and Richards (1964,b) also found that, log phase culture of Pseudomonas aeruginosa in nutrient broth containing Polysorbate 80 was much less resistant to the action of benzalkonium chloride, chlorhexidine diacetate and polymyxin B sulphate than cells grown in plain broth (17). It seems likely that in all instances the presence of Tween 80 increased the rate of dispersion of slime from the surface of the actively dividing cells and this might rendered the organism more sensitive to chemical attack.

Staphylococcus aureus is described by Rosenbach in 1884. Staphylococcus aureus is the normal flora of the human skin and of the respiratory and gastrointestinal tracts, it is also found regularly in air and human environments. The organism can produce disease both through their ability to multiply and spread widely in tissue and through their production of many extracellular substances such as exotoxin, leukocidin, enterotoxin, coagulase etc. Certain strains of Staphylococcus aureus produce an enterotoxin which is a

common cause of food poisoning. Pathogenic Staphylcoccus aureus tend to be hemolytic, produce coagulase and yellow pigments and ferment mannitol. Nonpathogenic tend to be non-hemolytic, white, coagulase-negative and do not ferment mannitol.

In general, the bacterial cell is consist of a rigid cell wall which encloses the cytoplasm. Cell wall of gram positive organism such as Staphylococcus aureus is relatively thick, uniform dense layer, with the cytoplasmic membrane closely apposed to its inner surface. It composes of several amino acid, including D as well as L-isomers, and a few sugar, muramic acid, which was found to be the 3-0-lactyl ether of glucosamine. These components are arranged as a net work of the so called glycopeptide or mucopeptide layer , which is responsible for the rigidity of the bacterial cell wall. Dawson (1949) reported the thickness of isolated cell wall and cytoplasmic membrane of Staphylococcus aureus to be of the order of 150-200 Å and 50 Å thick respectively (25).

The cell wall of Staphylococcus aureus has been postulated to consist of a net work of chains and fibers with an effective pore diameter of about 10 Å , a hypothesis supported by the observation that dextran molecules (diameter about 25 Å) will not penetrate the structure, while smaller molecules rapidly penetrated to the cytoplasmic membrane.

Staphylococcus aureus is readily killed by most antiseptics and disinfectants at the appropriate concentrations in the absence of serum, pus or albuminous material, e.g. it is killed in a few minutes by 2 per cent phenol. Staphylococcus aureus strains are highly sensitive to most of the antibiotics used in therapy, but the resistant strains have become widespread as a result of the general use of antibiotics. The strains of Staphylococcus aureus resistant to benzyl penicillin are because they produce enzyme penicillinase, staphylococci resistant to other antibiotics probably arise by mutation.

Aspergillus niger the black aspergilli or Aspergillus niger group are the fungi which we commonly call black mold. The genus is widely distributed, most of air and soil seems to contain the conidia of these organisms. Asperigillus niger is often found on exposed foodstuffs, and causes decay. The organisms also cause considerable trouble as the common contaminants of cultures in bacteriological and mycological laboratories. Richards (1949) isolated and cultured Aspergillus niger from the glass surface of optical instruments (49).

Aspergillus niger is animal and human pathogen which causes a group of diseases collectively known as aspergillosis. The symptoms closely resemble those of tuberculosis, and it is probable that some doctors have mistakenly

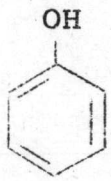
diagnosed this disease as tuberculosis. In addition to pulmonary aspergillosis, Aspergillus niger is most often seen as the causative agent for otomycosis.

Boll and Mirinoff (1950) found that all nonionics they tested, carbowax 1500 and some spans and Tweens possessed a stimulating action on the development of mold mycelia, they also observed that, with the exception of carbowax 1500, all nonionics tested inhibited the activity of antiseptic against Aspergillus niger (13). An incidental observation was also made by Gregg and Zopf (1951) that mold growth occurred in solutions of hexachlorophene containing Tween 80 on storage unless solutions were refrigerated (34).

Preservatives Used

Phenolic Group

a. Phenol is carbolic, phenic or phenylic acid; Phenyl hydroxide, Hydroxybenzene; Oxybenzene.



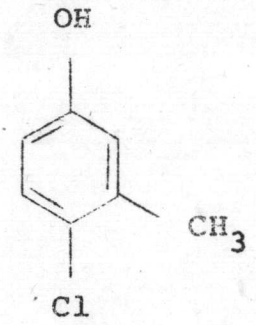
It is a colorless, acicular crystal or white crystalline mass, has a characteristic odor. Phenol gradually redden on exposure to light and air, hastened by presence of alkalinity. The molecular weight of phenol is 94.11, the

specific gravity is 1.071 and it boils at 182°C. Phenol congeals at 41°C and melts at 43°C. One gram dissolves in 15 ml water, very soluble in alcohol, chloroform, ether, volatile and fixed oils.

Phenol is caustic. It is used as a general disinfectant, 1% solution topically used as anesthetic for pruritic lesion, a pharmaceutical necessity as a preservative for injection etc. It has been widely used as a germicide and still be the standard for evaluation of other antiseptics. It has few legitimate uses in modern medicine.

Ingestion of even small amount may cause nausea, vomiting, circulatory collaspe, tachypnea, paralysis, convulsion, coma, greenish or smoky colored urine, necrosis of mouth and G.I. tract, icterus, death from respiratory failure, sometimes from cardiac arrest. Average fatal dose is 15 grams but death from 1.5 grams has been reported. Fatal poisoning may also occur by skin absorption following application to large area. Chronic poisoning with renal and hepatic damage may occur from industrial contact (56).

b. Chlorocresol (C_7H_7ClO) is 4-chloro-m-cresol; 4-chloro-3-methyl phenol; 3 methyl-4-chlorophenol; p-chloro-m-cresol; 6-chloro-m-cresol; 6-chloro-3 hydroxytoluene, 2-chloro-5-hydroxytoluene.



It is a colorless crystal, and odorless in pure form but usually a phenolic odor persists. It has the molecular weight of 142.58, melting point 64°C to 66°C, boiling point 235°C, and volatile in steam. One gram dissolves in 250 ml of water. Soluble in alcohol, ether, terpenes, fixed oils, chloroform, and aqueous alkali solution. Aqueous solution turns yellow on exposure to light and air. It is a powerful germicide of low toxicity, 0.05 to 0.1 percent was used as a bacteriostatic in parenteral solutions. It should not be used in preparations for intrathecally, intracisternally, or peridurally infections. A 0.2 per cent solution is used as a gargle. It also prevents mold growth in ophthalmic solutions during storage in a concentration of 0.03 per cent (20).

Organic acid

Sorbic Acid is 2,4-hexadienoic acid

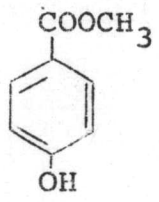
$CH_3CH = CHCH = CHCOOH$

It is a white crystalline solid, has a molecular weight of 112.12, melts at 134.5°C, and decomposes at 228°C. It is soluble in water to the extent of 0.16% w/w at 23.3°C. The solubility increases to 0.22% w/w in a phosphate buffer at pH 4.4 and to 1.02% w/w in a similar buffer at pH 5.9. Gooding (1945) reported that sorbic acid are effective fungistatic agents for foods and food wrappers (32). Sorbic acid has been found to be superior to sodium benzoate as a fungistatic agent in cheese and cheese products (Smith and Rollin, 55). It has also been stated that sorbic acid may be used with safety in pharmaceutical and cosmetic products.

Melnick, et al (1954) have presented a mechanism for mold inhibition by sorbic acid that an excess quantity of sorbic acid in a product will inhibit the dehydrogenase enzyme system in the molds, this important enzyme system is essential for mold propagation and even for survival (42). Sorbic acid is more effective in an acid medium, adjustment of pH on the acid side, therefore, might be expected to increase the effectiveness of this substance. It is practically nontoxic, may cause slight skin irritation.

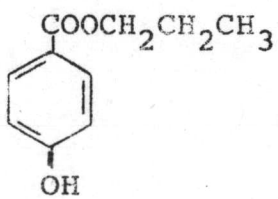
Organic Acid Ester

a. Methyl hydroxybenzoate ($C_8H_8O_3$) is para-hydroxybenzoic acid methyl ester; Nipagin M; Tegosept M; Methyl parasep; Solbrol; Methylparaben.



It is a colorless crystal or a white crystalline powder having a molecular weight of 152.14. It is odorless or has a faint characteristic odor and slight burning taste. Melting point 131°C, boiling point 270-280°C. One gram dissolves in 400 ml water, freely soluble in alcohol, acetone, ether. Methyl hydroxybenzoate is used as a preservative for galenicals in concentrations ranging from 0.05 to 0.25 per cent (51). It is also used in cosmetic preparations containing vegetable and animal fats and oils that are susceptible to decomposition.

b. Propyl hydroxybenzoate ($C_{10}H_{12}O_3$) is para-hydroxybenzoic acid propyl ester; Propylparaben; Nipasol; Chemocide PK; Propyl chemosept; Solbrol P; Propyl parasept.



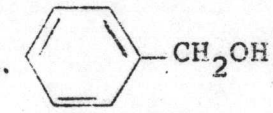
It is colorless crystals or a white powder; has a molecular weight of 180.20. It is odorless or has a faint odor, melting point 96-97°C. One gram dissolves in 2000 ml

water, it is soluble in alcohol, acetone, ether and oils. It is used for prophylaxis and treatment of fungus infections (56).

The antimicrobial activity of the p-hydroxybenzoic esters is directly proportional to the chain length, but solubility also decreases with increasing chain length, hence, in practice, the lower esters are commonly used.

Aromatic alcohols

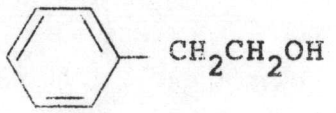
a. Benzyl alcohol (C_7H_8O) is phenylcarbinol; Phenylmethanol; α-hydroxytoluene



It is a colorless liquid with a sharp, burning taste and a faint aromatic odor. It has the molecular weight of 108.14 and has a refractive index of 1.5385 to 1.5405. Its specific gravity is between 1.040 and 1.050 at 25°C. One gram dissolves in about 30 ml water, 1.5 ml of diluted alcohol.

Benzyl alcohol is a local anesthetic by injection and by application to mucous membrane. For injection, it is administered in a 1 to 4 per cent solution. Externally, it is applied as an ointment or as a lotion of equal parts of benzyl alcohol, ethyl alcohol and water, to relieve cutaneous itching. In various injections it functions as a bacteriostatic agent (20).

b. Phenylethyl alcohol ($C_8H_{10}O$) is phenethyl alcohol; 2-phenylethanol

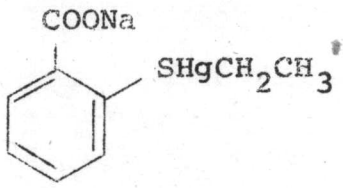


It is a colorless liquid with a rose-like odor and a sharp burning taste. It has molecular weight of 122.17, specific gravity between 1.017 and 1.020, and a refractive index at 20°C between 1.5310 and 1.5330. One gram dissolves in 50 ml of water, very soluble in alcohol, fixed oils, glycerin, and propylene glycol.

Phenylethyl alcohol is used as an antibacterial and preservative. Because of its pleasant oder, it has been employed as an ingredient in certain perfumes and flavors. The use of phenylethyl alcohol as a bacteriostatic agent was first studied by Lilley and Brewer (39), the results reported indicate that phenylethyl alcohol in relatively low concentration (1:400) exerts an effective inhibitory action on Gram negative bacteria.

Organomercurial

a. Thimerosal ($C_9H_9HgNaO_2S$) is sodium ethylmercurithiosalicylate; Merthiolate sodium; Merthiolate; Thiomersalate; Mercurothiolate; Merzonin; Mertorgan; Thiomersal; Merfamin.



It is a light cream-colored crystalline powder with a slight characteristic odor, having a molecular weight of 404.84. Stable in air, but not in sunlight. One gram dissolves in about 1 ml of water, and about 12 ml of alcohol.

Thiomersal has both bacteriostatic and fungistatic proporties. It is used as antiseptic for surface tissues in 1:30,000 to 1:1000 aqueous solution or 1:1000 tincture. Thiomersal is also included in the pharmacopoeia as a preservative for biological products at 0.01-0.02 per cent.

b. Phenylmercuric nitrate ($C_{12}H_{11}Hg_2NO_4$) is merphenyl nitrate; Phermernite; Phenmerzyl nitrate. Phenylmercuric nitrate is a mixture of phenylmercuric nitrate ($C_6H_5HgNO_3$) and phenylmercuric hydroxide (C_6H_5HgOH). It is a white crystalline powder, affected by light. Its molecular weight is 634.45, melting point 187-190°C. Soluble in about 1250 parts water, slightly soluble in alcohol and glycerin.

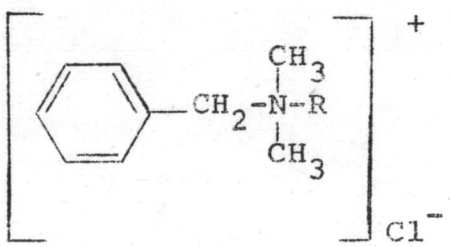
Organic mercurial compounds are use both in cosmetics and pharmaceutical preparations as preservatives. The British Pharmacopoeia (1973) permits the use of phenylmercuric nitrate as a bactericide for injections (0.001 per cent) and in heat sterilisation of injections (0.002 per cent). It is the

subject of monographs in the British Pharmaceutical Codex (1973) and is a listed preservative for eye drops, provided that they are not to be used over prolonged periods.

The organic mercurials do have obvious attractions as preservatives. They are active at high dilution, retain much of their potency in the presence of nonionic surfactants are relatively pH stable and resistant flora do not present a problem. However, the toxicity of these compound is of interest, mercury is a long-lasting poison which can damage the brain, central nervous system, kidneys and liver. The metal tends to accumulate in the environment which it may enter as industrial effluents or as agricultural fungicides.

Quaternary Ammonium

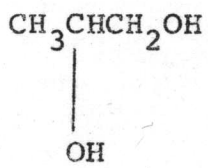
Benzalkonium chloride is alkyldimethyl benzylammonium chloride; Benasept or Zephiran chloride. It is a mixture of alkyldimethylbenzylammonium chloride of general formula $[C_6H_5CH_2N\ (CH_3)_2R]Cl$, in which R represents a mixture of the alkyls from C_8H_{17} to $C_{18}H_{37}$



Benzalkonium chloride is cationic surface active agent, very soluble in water, alcohol and acetone. It is useful for all-purpose local antibacterial agent for application to skin, tissues and mucous membrane. It has been used in 1:1000 tincture as the preoperative disinfection of unbroken skin or treatment of superficial injuries or fungus infections, 1:10000 to 1:2000 solutions are used for the preoperative disinfection of mucous membranes (20).

Aliphatic Alcohol

Propylene glycol ($C_3H_8O_2$) is 1,2-propanediol; 1,2-dihydroxypropane, Methyl glycol.

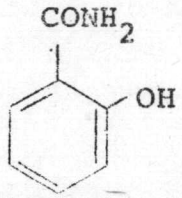


It is hygroscopic, viscous liquid, having the molecular weight of 76.09, and freezes at -59°C. Miscible with water, acetone and chloroform. Taking internally, propylene glycol is harmless, probably because its oxidation yields pyruvic and acetic acid. It has been used as solvent for pharmaceuticals, as inhibitor of fermentation and mold growth and as mist to disinfect air (56).

Aromatic amine

Salicylamide ($C_7H_7NO_2$) is O-Hydroxybenzamide; Samid; Acket, Algiamida; Cidal, Oramid; Panithal; Novecyl; Dolomide;

Salipur; Salrin; Salymid; Saliamin; Urtosa; Amid-Sal; Algamon; Benesal.



It is a white or slightly pink crystalline powder. It has the molecular weight of 137.13, and has the melting point of 140°C. Solubility in water at 30°C = 0.2%, at 47°C = 0.8% Soluble in hot water, alcohol, chloroform and ether. It has analgesic, antipyretic and antirheumatic action (56).

Chelating agent

Ethylenediaminetetraacetic acid disodium (EDTA disodium)

$C_{10}H_{14}N_2O_8Na_2$

It is a white powder, having the molecular weight of 372.25. It is used to prevent discoloration of the pharmaceutical preparations due to traces of metals, and to prevent oxidation catalyzed by trace metals in cosmetic creams or lotion containing unsaturated fatty acids and alcohols. EDTA may be used as an in vitro anticoagulant (20).