CHAPTER I

INTRODUCTION

1. Medicines for Oral Administration

Liquid medicines for oral use are dispersion of the therapeutically active drugs either solid or liquid in the liquid which is called the vehicles, The vehicle is usually aqueous, either water itself or an aqueous solution of a flavoring agent etc, Some vehicles possess therapeutic properties while other are pharmacologically inert, The medicines are of several types, and often have names indicative of their particular use or formulation e.g.a "linctus" is a thick syrupy product intended to be sipped and swallowed slowly so as to exert a soothing on the lining of the throat, An elixir is a sweetened and flavored preparation containing a potent or nauseating drug and may contain a high proportion of ethanol, glycerol or propylene glycol (1).

The substances used in liquid medicines may be incorporated as such e.g. Sodium Bicarbonate, Morphine Sulfate, or incorporated in form of a "galenical" or other preparation. Among the most common galenicals are tinctures, and fluid extracts, Other preparations of drug used to make medicines include syrup, spirit and solution,

Aqueous solution of solid, liquid or gases, are often contained sufficient ethanol to inhibit microbial growth (1,14).

The galenical and other preparations per se are usually resistant to microbial growth, either because of their ethanol content or their high osmotic pressure due to their sucrose concentration (syrup). However on dilution with water they frequently become ideal media for microbial growth (1,14). The addition of a relatively small amount of water may make all the difference. The B.P.C. 1963 states that the solutions of sucrose containing less than 65% w/w will ferment, where as those containing 66.7% w/w seldom permit microbial growth (14).

The simplest mixture that could be made consisting of a solution or suspension of a solid drug in water. In many cases such a preparation would not support microbial growth, and hence would not require the addition of a preservative. Certain drugs in aqueous solution, however will support microbial growth. Cocaine hydrochloride is an example (1). A few mixtures consist solely of a drug and water since it is frequently necessary to include other substances to make a stable and palatable product. The pH of a mixture may have to be adjusted so as to achieve optimal stability of the drug, or to obtain its optimal therapeutic effect; the resulting product may encourage microbial growth. If the drug has an unpleasant taste, it is usual to disguise this by the

addition of suitable flavoring or sweetening agents. Probably the most commonly used sweetening agents in pharmaceutical products are sucrose, sorbitol, glycerol, and propylene glycol (1,11).

Barr and Tice (1,16,19) reported on the inhibitory effects of sugars including dextrose, levulose and invert sugar. They found that 60% w/w invert sugar solution inhibited Aspergillus niger, but solutions containing 50% w/w of either levulose or dextrose was not stable. Solutions of the last two substances containing more than 55% w/w could not be made. It is evident, therefore that unless mixtures are made with pure syrup, or contain very high concentrations of glycerol or propylene glycol, the presence of a preservative in sweetened aqueous preparation is essential. They showed that various microorganism e.g. Pseudomonas aeruginosa, Aspergillus niger, Penicillium notatum & Camida albicans could grow in solutions and dispersions of such substances, and produce esterases which split the ester linkages. The adverse effects of the splitting of the surface active molecules, on the physical properties of the products are obvious as the need for inclusion of an effective preservative. Barr and Tice (1,16,19) described the results obtained when they screened over 50 antimicrobial substances and combinations for effectiveness in preserving the synthetic non-ionic surface active agents of the fatty acid ester types e.g. sorbitan esters and their polyoxyethylene derivatives, that are widely used in pharmaceutical products as emulsifying or solubilizing agents.

Among the effective preservatives were sorbic acid (0.1 to 0.2%), the phenylmercuric salts (about 0.01%) hexylene glycol 3.0% and benzalkonium chloride (0.1%), the phenols, were ineffective, probably due to complexation with the surface-active agent (1).

The physical stability of many suspensions and emulsions ---------- are enhanced by the presence of suspending or emulsifying agents e.g. acacia, tragacanth, they are carbohydrates and are liable to microbial attack. Various cellulose derivatives e.g. methyl cellulose are also used as suspending or emulsifying agent. Although these were formerly believed to be resistant to microbial growth by the studies of Musller and Deardorff (17,20), there is now evidence that under certain conditions suitable microorganisms do attack them and destroy their pharmaceutical usefulness. Moreover, methyl cellulose has been shown to form complexes with a number of common preservatives, notably the parabens (1,18).

1.1 Preservatives in common use

1.1.1. Benzoic Acid: benzoic acid is a useful preservative for mixture having a pH of 5 or less, About 0.1%w/v of the acid is sufficient to inhibit microbial growth, the acid is normally used in form of benzoic acid solution B.P.C. (containing 5.5% w/v benzoic acid and 75% propylene glycol in water (1).

1.1.2 Esters of para hydroxybenzoic Acid (Parabens):- these esters are tasteless, stable, and nontoxic but incompatible with certain substances used in medicines e.g. nonionic surface active agents. They are useful preservatives for many pharmaceutical products, especially syrups, and medicines containing syrups or gums. The potentiation of preservative action of parabens are depended on the addition of a small amount (2% or 5%) of propylene glycol (1).

1.1.3 Chloroform:- this has been widely used for many years as a preservative for oral medicines, because of its sweet and pleasant taste, the chief disadvantage of chloroform is its volatility, which leads to its loss from a medicine, unless the container is well closed About 0.15 to 0.25% chloroform is sufficient to inhibit or even kill bacteria and molds, but below 0.1% it is ineffective (1).

1.1.4 Sorbic Acid (2,4-hexadienoic acid):- this has been reported to be an effective fungistat for use in mucilages of vegetable gums, and in diluted syrup about 0.2% w/v will prevent the growth of most molds, It does not appear to be used much in the preservation of oral pharmaceutical products though it is used in various food stuffs (1,11).

1.1.5 Sulfur Dioxide and Sulfites:- sulfur dioxide, either as such or present in the form of sulfites, it can be used to preserve fruit syrups, and other preparations, it effective in preventing mold growth (14).

As to whether a particular formulation will, or will not, support microbial growth, only thorough testing in a laboratory will show. However, by a suitable choice of ingredients, galenicals etc., it may be possible to achieve the desired result without adding an antibacterial agent as such, this is the preferred procedure because it avoids the possibility of interaction between the antibacterial and the drugs, etc, present in the mixture. The use of a pleasantly flavored tincture in sufficient quantity to provide a final ethanol content of about 15%, should prevent microbial attack, especially if the pH is low (11,14).

Another important factor is the length of time, the preparation is required to "keep". Medicines made extemporaneously for a given patient are usually consumed within a few days and preservation of these is relatively simple. The problem becomes more difficult when a manufacturer wishes to prepare a product having a shelf-life of perhaps 2 years. The storage conditions required by the drug are also important. Some drugs are only stable in mixtures when refrigerated; such mixture would not require additional preservative (1).

The desirable properties of an antimicrobial for use in oral preparations include freedoms from an unpleasant taste and smell, in addition to the usual pharmaceutical requirements of compatibility and absence of toxicity (1,7,).

For the sakes of safety, and good sanitary quarantee for oral pharmaceutical preparations, the microbial contamination must be only non-pathogenic microorganisms, total microbial count must be in the limit specified for individual group of preparations, and absence of pathogenic microorganisms exactly, such as Escherichia coli, Salmonellae, Staphylococcus aureus, and Pseudomonas aeruginosa (11,14).

1.2 Microorganisms

1.2.1 Morphology and Characteristics: the staphylococci are ubiquitous pathogenic microorganism; they are the commonest cause of localized suppurative infections, and are among the longest recognized of the pathogenic bacteria (2,6,). They are cocci form tend to be much more uniform in size, staphylococci are consistently slightly less than 1 μ in diameter, typically are more nearly perfect spheres than many cocci, and tend to occur markedly in masses of cells. This is a consequence of cell division occurring in three planes, coupled with a tendency of the daughter cells to remain in close proximity to give the characteristic appearence. These irregular clusters are three-dimensional (3,4).

The staphylococci are relatively more resistant to heat, and to a certain extent disinfectants than the vegetative forms of most pathogenic bacteria, They are killed at 80' for 1 hour, and they are also resistant to drying, and may remain infectious for extended periods, They are able to grow in the presence of relatively high 10% concentration of sodium chloride (7).

As strongly gram-positive microorganisms, the staphylococci are sensitive to the bacteriostatic activity of triphenyl methane and other dyes, and are characteristically sensitive to those antibiotics effective on gram-positive bacteria, including penicillin and the broad-spectrum antibiotics (7,10).

The majority of the coagulase-positive pathogenic staphylococci are able to grow in the presence of tellurite, reducing it to give grayish-black colonies on tellurite containing mediums (2).

These staphylococci also often produce a zone of opacity in the medium around the colony when it contains egg yolk, This characteristic has been combined with tellurite reduction in the development of selective isolation medium, Mannitol and an acid-base indicator such as phenol red may be incorporated for differential purpose and isolation medium may be made selective by the inclusion of 10% sodium chloride (2,3).

They do not form spores; apparently do not have capsules except in the case of rare mucoid variants, are almost invariable and non-motile (4).

Growth on agar mediums are abundant, and the colonies are opaque, smooth and glistening, and are golden pigmented forms in appearance (4).

When cultured on blood agar plates, some of the staphylococci are β-hemolytic and others are non hemolytics. The typical pathogenic form is hemolytic Staphylococcus aureus. The staphylococci are not highly fastidious in their nutritive requirement, They grow readily on the usual meat extract-peptone medium, but they grow more profusely on blood agar commonly used for isolation of the pathogenic forms (21).

Growth and Environment

Nutrition, the nutritional requirements of Staphylococcus aureus are fairly complex and vary from strain to strain. The organism thus grows better in a complex medium than in a synthetic medium. All strains require a variety of amino acids and some vitamins including thiamine and nicotinic acid (9).

Physical parameters there are difference between strain to strain., as well as between growth media and foods, combination of two or more adverse factors will decrease the tolerance of the orgamism for either or both.

Temperature - Optimum 30-37°; Range 6-46°

Water Activity a_w - Range 0.999-1.86; Growth greatly reduced below 0.94.

O_2 Tension - Growth best in presence of O_2; facultative anaerobe

pH - Optimum slightly alkaline (7.0-7.5); Range 4.2-9.3

Particularly important is the low a_w at which growth occur. This characteristic is important in the ecology of Staphylococcus aureus and is the basis for some selective media (9).

All strains of Staphylococcus aureus are potential pathogens, and are stable association with man and may be found in many parts of his environmentals including air, dust,water, faeces, and on objects such as clothing and utensil handled by man (22).

Staphylococcus aureus causes several infections, such as various types of skin eruptions, and inflammations (boil, acne, stye etc.) and wounds infection, and can also cause respiratory infections, or may become established in the gut causing enteritis (20).

1.2.2 Family Enterobacteriaceae. Gram stains are negative. The microorganisms in this family are variable motile, absent of spores, and can break down carbohydrates by fermentation.

The Nomenclature of the Family Enterobacteriaceae

Tribe I	Escherichieae	
	Genus I	Escherichia
	Genus II	Shigella
Tribe II	Edwardsiellaeae	
	Genus I	Edwardsiella
Tribe III	Salmonelleae	
	Genus I	Salmonella
	Genus II	Arizona
	Genus III	Citrobacter
Tribe IV	Klebsielleae	
	Genus I	Klebsiella
	Genus II	Enterobacter
	Genus III	Pectobacterium
	Genus IV	Serratia
Tribe V	Proteeae	
	Genus I	Proteus
	Genus II	Providencia

Table extracted from Edwards & Ewing (1972) sets out the classification of the genera Salmonella & **Arizona** It is pertinent to mention here that organism belonging to the genus Arizona were originally included within the genus Salmonella, and were known as Salmonella arizona, its classification in the genus Salmonella was based upon the relationship of its H antigens to those of S.enteritidis

Edward and Ewing recognised only 3 species of Salmonella, these are Salmonella cholera-suis, Salmonella typhi and Salmonella enteritidis.

Escherichia coli-strains of Escherichia coli present in the gut of men and animals and are also widely distributed in the environment, All persons have a rich flora of Escherichia coli in the lower ileum and in colon, Escherichia coli are incriminated as a pathogen outside the gut, and particularly in the urinary tract and in wounds where the infection may be endogenous from the patient's own intestine or acquired from an exogenous source (19).

Escherichia coli appears to grow only as a parasite of men and animals mainly in the intestine. Being excreted in very large numbers in faeces, it comes to contaminate the environment including the soil very widely, and the bacilli may survive without growth for several days to a few weeks outside the body, When Escherichia coli is found in a water supply, it is considered

to indicate that the supply has recently been subjected to contamination with human or animal faeces (22).

Morphology and staining, Most strains are flagellate and fimbriate and a few capsulate.

Escherichia coli is a short, plump rod, 0.4 to 0.7 μ in width, and 1 to 4 μ in length, coccoid forms and short chains of organism, They are found often in exudates and young cultures, motility varies, greatly in different cultures, Some strain shows active motility while the other moves sluggishly, and some shows no motility, Spores are not formed, capsules occur in a small percentage of the strains. The organism are gram-negative, and stain uniformly with the usual aniline dyes, No characteristic of internal structure occur (6).

Most strain are killed in 15 to 20 minutes by a temperature of 60 °, but some survive the pasteurization process, and may spoil the color and flavor of substances. In water purification, chlorine in concentrations of 0.5 to 1 part per million is an effective bactericide for Escherichia coli and the various species of Salmonella and Shigella (8,10).

Culture characteristics. The colon bacilli are aerobic but facultatively anaerobic, grow in a medium consisting of inorganic salts, and ammonium salt and glucose, on agar plates surface

colonies appear within 12-24 hours, A size of 2 to 3 mm in 48 hours, The typical colony is low, convex, smooth and colorless but rather opaque with an entire edge. On blood agar a discoloration is produced in the medium immediately around the colony (12,13).

1.2.3 Salmonella

Morphology and Staining

Gram-negative rods, morphologically similar to Escherichia coli, and morphologically and serologically are related to the typhoid bacilli, motile usually, ferment dextrose usually with gas production. Do not liquefy gelatin or clot milk, or produce indole, aerobic, facultatively anaerobic, asporogenous, rod-shape bacteria, that grow well on artificial media, Nonmotile variants of motile species may also occur, motile forms are peritrichously flagellated. Nitrates are reduced to nitrite, and glucose is utilised fermentatively with the formation of acid and gas, The alginate is not liquefied (9).

The environmental factors that influence the growth, death and survival of Salmonellae are separated into the physical factor, temperature, pH, and chemical factors, including the presence of certain chemical and preservative such as nitrite and sulfite.

Temperature - The range of temperature that permits growth is between about 45-47°. with some slight variation dependent upon the serotype, the optimum temperature for growth is about 37° Salmonellae are not heat resistant, are easily killed by relatively low temperature.

pH - Salmonellae grow within the range of pH 4.4—9, and optimum growth is 6.5—7.5, at pH value lower than 4 and higher than 9 it dies slowly, although viability can be extended by freezing or freeze drying.

Water activity - The optimum a_w for growth rate varies with the serotype, generally is above 0.990, the minimum a_w for growth in liquid media is about 0.94 under favourable temperature and nutritional conditions.

Chemical environment-Chemical inhibitors which are quite active against Salmonella such as nitrite in the form of nitrousacid, undissociated form of volatile fatty acids, sorbic acid and sulphur dioxide (9).

Salmonellosis is an infection caused by organisms of the genus Salmonella, the principal site of infection in animal is the intestinal tract, and the primary route of infection is by ingestion of the organism (21).

1.2.4. Genus Pseudomonas aeruginosa.

Pseudomonas aeruginosa was isolated by Gessard in 1882 and named Bacillus pyocyaneus. This organism has been isolated from over 90% of samples of sewage, and from 11% of human fecal specimens (10).

Pseudomonas aeruginosa was classified according to Smith & Conant(Zinsser) as

Species:- Pseudomonas aeruginosa

Genus :- Pseudomonas

Family :- Pseudomonadaceae

Synonym:- Bacillus pyocyaneus,

Pseudomonas pyocyanea (3,8).

Pseudomonas aeruginosa are distributed widely in nature, and have been brought together in the genus Pseudomonas.

Pseudomonas aeruginosa and Pseudomonas pseudomallei are only species pathogenic for man and caused pyocyanosis (18).

Morphology and characteristics of Pseudomonas aeruginosa

Pseudomonas aeruginosa is an organism of low pathogenicity for healthy subject, but an important pathogen in patients or tissues with poor resistance. It causes infection of burns, wounds, skin, urinary tract, respiratory tract, ear, eye, and meninges, and has a tendency to invade the blood stream (17,18).

Pseudomonas aeruginosa has responded poorly to antibiotic therapy, and becomes resistant to antibiotics even more readily than Escherichia coli and Klebsiella, sometime it may appear upon surgical dressings (7,21).

Pseudomonas aeruginosa is gram negative, bacillary in shape, small, slender rod about 1 to 2 μ in length and 0.5 μ in width, usually active motile, This microorganism possesses one to three polar flagella, it is nonsporogenous and non-encapsulated, Stains readily with aniline dyes, the colonies are large and spreading, edges are irregular, and the consistency butyrous (4,6).

Culture characteristics:-Pseudomonas aeruginosa is aerobic, excellent growth is obtained on all the usual laboratory media, over a temperature range 5° to 42° with an optimum at 37° after 24 hours incubation, large, soft, smooth, grayish, spreading colonies are produced which may become confluent and cover the entire surface of the medium.

The soluble pigment produced by the organism is diffuse into the medium to produce a bright green fluorescent color, which becomes darker after several days(13).

One of the most distinctive characteristic of Pseudomonas aeruginosa is its production of a bluish green soluble pigment, which does not color the colonies or other mass of growth, but diffuses into the medium, two pigments are formed actually, one pyocyanin is deep blue in color and can be extracted from aqueous solution by chloroform, and the other a yellowish green fluorescent piement is soluble in water, but not in chloroform. Thay are both oxidation products of colorless precursors. Pyocyanin is formed only by Pseudomonas aeruginosa, but the fluorescent pigment is formed by several other species of Pseudomonas. These pigments may occour separately or together. Pyocyanin is of interest in that it is a phenazine compound (2,4).

About 4% of culture encountered in routine clinical work fail to produce pigment, but even these can be identified by their odor and biochemical reaction. Gelatin is liquefied rapidly, and a pellicle is formed on liquid media, hydrogen sulfide is produced, but urea is not split and indol not formed, false positive reactions for indol may occur with Ehrlich's reagent, but not with that of Kovac (20).

Pseudomonas aeruginosa produces a small amount of acid from a number of carbohydrates, but prefers to obtain its carbon and nitrogen from peptone, which results in an alkaline reaction that neutralizes the acid produced from the carbohydrates. On blood agar the typical pigment is not seen even when produced. The colonies of Pseudomonas aeruginosa sometimes discrete more often spread over the plate like a swarming Proteus, but their presence can be suspected from the characteristic aromatic grape like odor (13,16).

Resistance: Pseudomonas aeruginosa is killed by heating at a temperature of 55° for one hour, but is somewhat more resistant to the usual chemical disinfectants than other gram negative bacilli. The organism is resistant to Penicillin and most of the broad spectrum antibiotics. Polymyxin B is most effective followed in order by Neomycin, Oxytetracycline, and Streptomycin (5,21).