INVESTIGATIONS ON THE MICROBIAL CONTAMINATION OF FOLIC ACID TABLETS DURING MANUFACTURING

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I hereby declare that this thesis has been composed by myself and that it is a record of my own research work. It has not been accepted in any previous application for higher degree. All quotation marks and the sources of information are specifically acknowledged by means of references.

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DATE: 22nd March 1985

DEDICATION

This work is dedicated to the two women who had played the most significant roles in my life: My mother, Madam Adisetu who is the human architect of my present status; and my wife, Habibat who has now taken over that role.

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ABSTRACT

The microbial contamination levels at various stages of tablet manufacturing were monitored, using Folic acid tablets.

The raw materials for the production of Folic acid tablets were examined for general microbial contamination, especially fungal and bacterial organisms. The bacterial contaminants were differentiated by their morphological characters. Tests were also carried out to detect the presence of specific pathogens, namely Escherichia coli, Staphylococcus aureus, Salmonella species and Pseudomonas aeruginosa, using appropriate diagnostic media. Both the Wet-Granulation and Direct Compression methods of tablet production were used. The tablets were subsequently subjected to quality control tests to assure compliance with Official Standards. The microbial contamination levels and types at the various stages of tablet production in each tabletting method were determined. Tablets were produced under two different environmental conditions; a Teaching Laboratory and a local Pharmaceutical Manufacturing Laboratory. Microbiological analysis were similarly carried out. The effect of the quality of water used and the drying temperatures on the microbial contamination levels of the wet granules were also investigated.

The results of this investigation indicated that the bacterial counts of the tablet products were influenced by the following factors:

(a) the microbial flora and level of the starting raw materials. Raw materials of natural origins were more

highly contaminated than those of semi-synthetic and synthetic ones;

(b) the level of microbial contamination of the wet granules. The microbial contamination levels of the initial mixing stage was greatly increased during the wet mixing stage of the Wet-Granulation method, due to incorporation of binder solution, that was in itself highly contaminated.

(c) the temperature of drying the wet granules. The high level of microbial contaminants in wet granules was substantially decreased when dried at 50°C, while drying at lower temperature of 28°C encouraged bacterial proliferation.

(d) the tablet production environment. The higher microbial contamination levels of environment B gave tablets of higher microbial contamination levels compared with tablets produced in a lower microbial contamination environment A.

Tablets produced by the Direct Compression method gave lower level of microbial contamination than those of wet granulation method. All the tablets produced complied with official microbial limits except the batch produced by the wet granulation method and dried at 28°C.

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CHAATER ONE

INTRODUCTION

Microbial contamination refers to the presence of micro-organisms as foreign bodies in preparations where their presence is undesirable. While some pharmaceutical preparations such as parenterals and Ophthalmic products are required to be sterile, others which are not introduced directly into the blood circulation are not expected to meet this sterility requirement. The latter group are generally referred to as Non-sterile Pharmaceutical Preparations. This category includes:-

(a) Oral preparations e.g. tablets, capsules, syrups, oral powders, gargles and mixtures.

(b) Topical preparations- Ointments, lotions, creams and solutions meant to be applied to abraded skin.

It has long been appreciated that to produce satisfactory preparations which would not show signs of spoilage, constant attention must be paid to formulation, high standards of cleanliness and good hygiene both during manufacture and subsequent dispensing.

Parenteral preparations, by virtue of their route of administration are required to be sterile, otherwise they could easily cause diseases. A number of incidents involving infections of the eye due to the application of various eye preparations in Sweder, Britain and United States of America (Kallings et al. 1966, Ayliffe et al. 1966 Remington (1970)), led to the recommendation that all preparations meant for application to the eyes have to be sterile. This is necessary when it is realised that apart from the self-irrigation mechanism through the lachrymal glands, the eye as it is, has virtually no defence mechanism against invading organisms.

Recently, there have been increasing numbers of reports of drug-borne infections recorded in the medical press from widely separated areas of the World. Kallings et al. (1966), traced an outbreak of Salmonellosis to tablets containing a drug of natural origin, to the presence of large numbers of Enterobacteria in some synthetic drugs. Infections associated with the use of thyroid tablets and carmine dye Capsules have also been reported in the United States of America and Britain (Kunz and Ouchterlong 1955, Kormany et al 1967). These and many other documented reports (Table 1.1) prompted the need to establish

microbiological Standards for pharmaceutical products in many countries. The establishment of such standards become more necessary when the following points are considered.

(a) An empty, resting stomach has a pH of 1 - 2 which is bactericidal. This is raised to pH 4 – 5 in the presence of food at which the bactericidal activity of gastric fluid is greatly reduced. Further down the stomach is the small intestine which is slightly alkaline, devoid of any antibacterial activity and has even been known to favourably support the proliferation of bacteria.

(b) The presence of a large number of non-pathogens would tend to exhaust the body's defence armamentarium, thereby lowering the body's resistance, as a result of the fact that the body's defence, especially the white blood cells, can not differentiate between pathogens and non-pathogens. In large numbers, the non-pathogens can colonise the intestine from which some may migrate to other regions, thereby becoming an infection risk. Moreover, some of the non-pathogens by their products of fermentation, can

Table 1.1: CONTAMINANTS FOUND IN PHARMACEUTICAL PRODUCTS

YEAR	PRODUCT	CONTAMINANT
1907	Plague Vaccine	Cl. tetani
1943	Flourescin eye-drops	Ps. aeruginosa
1946	Talcum Powder	Cl. tetani
1948	Serum Vaccine	Staph. aureus
1955	Chloroxylenol disinfectant	Ps. aeruginosa
1966	Thyroid tablets	Salm. muenchen
1966	Antibiotic eye ointment	Ps. aeruginosa
1966	Saline solution	Serratia marcescens
1967	Handcream	Klebsiella pneumoniae
1967	Carmine powder	Salm. cubana
1969	Peppermint water	Ps. aeruginosa
1970	Chlorhexidine-cetrimide antiseptic solution	Ps. cepacia
1972	Intravenous fluids	Pseudomonas, Erwinia and Enterobacter spp.
1972	Pancreatin Powder	Salm. agona
1977	Contact lens solution	Serratia and Enterobacter Spp.
1981	Surgical dressings	Clostridium spp.

Adapted from Pharmaceutical Microbiology, Edited by W.B. Hugo and A.D. Russel, 3rd Edition, 1985 Blackwell Scientific Publications, Chap 18, pg 356.

cause stomach discomfort e.g. carbon dioxide and gas production by saprophytic organisms. Also, some hitherto non-pathogens have now been recognised as pathogens. For example, Aspergillus species by its production of aflatoxin, is now implicated in the increased number of malignant tumors. This Aspergillus species is common in groundnuts and coconuts.

(c) The toxins produced by some bacteria especially the exotoxins have been shown to be highly poisonous in small amounts and do easily overcome the body's defence mechanism. Botulinium toxin produced by Clostridium botulinium and toxins produced by Clostridium welchii are often the cause of various degrees of headache, vomiting, diarrhoea, paralysis and even death; symptons which are frequently associated with food poisoning.

The knowledge that drug-borne infection may have serious effects on debilitated patients and even on normal healthy individuals prompted the Pharmaceutical Society of Great Britain in 1971 and the Public Health Laboratory Services in 1972, to set up Working Parties to investigate the numbers and

types of organisms in medicaments given to patients, paying attention to the following areas:-

(a) In Manufacturing;

(i) raw materials,

(ii) finished products in their final containers;

(b) In Hospitals and in General practice Pharmacies:

(i) products as received from manufacturers,

(ii) products after repeated dispensing from bulk packs and on hospital wards.

The problem of microbial contamination could have been solved by specifying that all pharmaceutical products shall be sterile and dispensed in sealed unit-dose packs. This, however is not a practical proposition because of the cost and the production problem involved. Accepting the alternative of allowing a lowlevel of microbial contaminants for some pharmaceutical preparations raised certain control problems. This control requirements is subject to the following considerations:-

(a) The variety and level of microbial contaminants in various pharmaceuticals and also the significance of their presence.

(b) The source of such microbial contaminants

(c) The hazards that can be associated with their presence

(d) The measures that can be taken to reduce the population of the micro-organisms or to prevent their proliferation when the preparation is being dispensed in a general practice pharmacy, in the hospital or when being used in the hospital ward or by a patient in his home.

1.1 TYPES OF MICROBIAL CONTAMINANTS

Many of the studies on microbial contamination of pharmaceuticals had initially been based on two approaches, namely (a) total number of microbial contaminants (bacteria, mould and fungi); and (b) identification of the contaminants based on colonial and cell morphology and biochemical tests. In 1971, the report of the Pharmaceutical Society Working Party revealed that the quantity of viable organisms isolated from various pharmaceutical products ranged from 0 to 10^6 per gram or mililitre (ml). Reports by other workers went further to classify and identify the contaminants. Beveridge ((1971), and Bowman et al. (1971, 1972) included the following among the contaminants:- Bacillus species,

Penicillium Chrysogenum, Other Penicillium species, Aspergillus niger, Yeast, Pseudomonads beside Ps.aeruginosa, Staph. aureus, Staph. epidermidis, Coliforms (E. coli in few cases), and rarely Salmonella species. In the report by the Public Health Laboratory Services Working Party 1972, 567 distinct bacterial types were identified in the 393 samples of various pharmaceutical preparations. Details of the report show that the most frequent isolates were coagulase negative Saphylococci and Micrococci, followed by Pseudomonads and related oxidase positive gram negative bacilli. Other organisms often isolated but in low numbers include Flavobacterium species, Achromobacter species, Cladosporium species, Alcaligenes and Leuconostic species. Results of the investigations of various other workers revealed that the type and quantity of contaminants in most cases were invariably dependent on the type of preparations (White et al (1968) and Bowman (1969), Beveridge and Hope 1971). Mixtures are more heavily contaminated than liquid extracts, containing Pseudomonads and Staphylococcus species, while aromatic waters have high concentration of Pseudomonads. Yeast and mould are common contaminants of creams and ointments.

1.2 SOURCES OF MICROBIAL CONTAMINATION

The quality of Pharmaceutical products is greatly influenced by the environment in which they are manufactured, the raw materials used, the condition of the equipment used in making the product, the personnel involved in the production, the packaging materials and other factors. The level of microbial contamination of the final product may thus represents the contaminants from these various sources.

1.2.1 RAW MATERIALS

Contaminants found in finished products have in many cases been traced to the use of contaminated raw materials. According to current Good Manufacturing Practice (GMP) in manufacturing, processing, packing or holding for drugs, "representive samples of components liable to microbial contamination (e.g. of animal and botanical origin) shall be subjected to microbiological tests. Such samples shall contain no micro-organisms which are objectionable in view of the intended use". Included in the list of raw materials are: the active ingredients, the diluents, other adjuvants and vehicles. An extensive and varied microbial flora is usually associated with untreated raw materials

which are derived from natural sources. Products such as dessicated thyroid, pancreas and cochineal which are derived from animal sources are easily contaminated with animal-borne pathogens such as E. coli and Salmonella species (Kunz and Ouchterlong (1959), and kormany et al 1967). For products of botanical origin, gram negative bacteria such as Erwina and Pseudomonald species and the gram positive, spore-forming bacteria (Bacillus and Lactobacillus species), mould and non-mycellated Yeast are commonly implicated. Investigations carried out established the fact that raw materials of natural origin are more heavily contaminated than inorganic and lastly semi-synthetic and synthetic components (Westwood, 1971). Those introduced by sewage includes the animal borne pathogens and Salmonella species especially in gums (acacia and tragacanth) and digitalis. Kaolin, talc, magnesium and aluminium salts including other inorganic components from the soil have also been implicated as sources of contamination in their final products. White et al (1968), showed that the use of grossly contaminated powder in the formulation of tablets and ointments, resulted in highly contaminated product. Beveridge and Todd (1973) showed that contamination of B.P.C. antacid mixtures

by E. coli and P.aeruginosa was due to the type of interraction between these organisms and the *trisilicate* component. This type of interraction also occurs with kaolin and bentonite and it has been attributed to electrostatic attractions between the negatively charged cell surfaces and the positively charged edges of the clay crystals. Additives are sometimes of greater risk than the active ingredients as sources of contamination. Wilson (1972) showed that the growth of Ps.aeruginosa in Peppermint Water B.P. was due to nutrients leached from talc with which the peppermint oil was initially triturated before dilution to make the solution. Kallings et al. (1968) found that coliform contamination of tablets of barbiturates and tranquillizers were due to the Starch used in the formulations.

1.2.2 WATER

The greatest potential source of contamination to pharmaceuticals is water, which is used in the formulation of many products as well as for various washing and cooling processes. The microbial flora of water depends on the quality of the raw water and any processing it received and the distribution system. Fresh water generally contains micro-organisms,

usually of the non-pathogenic types such as Flavobacterium, Alcaligene species, Chromobacterium and some Pseudomonads. These organisms are non-exacting in their nutritional requirements. The dissolved organics such as tannins, dead plants and animal cells in water due to soil and sewage, is responsible for such additional bacteria as Bacillus, Enterobacteria, Streptococcus and Clostridium species. Water from such sources as streams, ponds, surface water are more heavily contaminated than those from running rivers. Water from deep wells are relatively pure due to the filtration by the different strata of the soil. Rain water is inevitably contaminated as it descends from the atmosphere where it comes in contact with contaminated air. Distilled and de-ionized waters are usually made use of in the Pharmaceutical Industry. De-ionized water is inevitably contaminated as it flows from the ion-exchange column which is easily contaminated because it is not easily accessible to washing and cleaning, hence acting as a reservior of bacteria. Distilled water is usually free from micro-organisms and contamination occurs as a result of a fault in the cooling system, the storage used or distribution system. In the report by the Pharmaceutical Society

Working Party (1971), it was shown that of the B.P.C. mixtures prepared, water contributed the highest number of viable organisms. The storage period is the stage at which distilled water is usually heavily contaminated. Favero et al. (1971), reported a thousand fold increase in the number of Ps.aeruginosa in distilled water stored in a tank at 37°C for 24 hours, and more at 25°C. Bacteria may gain access to water distribution system through any outlet such as cork or tap and hose pipes which are not regularly disconnected and disinfected. Reservior of infection may build up in any unused sections of pipeline as well as in booster pumps and water meters.

1.2.3 AIR AND ENVIRONMENTAL CONDITIONS

The environment under which pharmaceutical products are made can also influence the level of microbial contamination. The sort of hygiene, cleanliness, or movement allowed into the place influences the final microbial count of the product. Air by itself can not support bacterial growth since it lacks the natural environment for the growth and reproduction of bacteria such as adequate moisture and nutrients in a form that can be utilized. However, it does act as a vehicle, carrying

micro-organisms from one area to another especially the spore-bearing Bacillus, and Clostridium species; Staphylococcus, Streptococcus and Corynebacterium species, moulds, mucur and yeast.

The microbial flora of air depends on the sort of activities that is carried out in that environment. An area containing working machinery and active personnel have higher microbial count than one with a still atmosphere, more in dusty than in clean rooms. The air microbial flora also fluctuates with the seasons. The dusty harmattan season aid the spread of bacteria, but less in wet season where the atmosphere is more humid and damp.

microbial flora of air can also be increased by handling of contaminated products during.dispensing, blending and their addition to formulations. In particular, the use of starches and sugar in the dry state increases the mould count of air.

Some packaging components especially those of card and paperboard have a high microbial flora of both mould and bacteria and this is often reflected in high air counts around cartoning and packaging machines.

Microbial flora varies from one place to another. Whitby and Rampling (1972) found Ps. aeruginosa in sinks, floors, mops in Pharmacy and wards of hosoitals but none in corresponding areas in domestic homes. Bair d and Shooter (1976) and Baird et al (1976) traced Ps. aeruginosa contaminated medicaments to Pharmacy environment at which the preparation was made. The sort of environment sometimes dictates the type of micro-organisms present in that environment. When swabs are taken to compare the bacterial flora of a road surface with a ward floor, the road swabs look very dirty but cultures on blood agar incubated at 37°C usually yields a scanty growth of organisms, harmless to man, compared with heavy growth from hospital wards, laden with Staph. aureus (Joan-Stokes and Ridgway 1980). In the buildings where manufacturing takes place, moulds are commonly found especially on walls and ceillings. They are common in poorly ventilated buildings with painted walls. The organisms derive their nutrients from the plaster on which the paints has been applied, thus a hard gloss finish is better than a soft matt. Cracks on floors and dust collecting traps such as windows and other edges and overhead pipes are common reserviors for bacteria.

1.2.4 EQUIPMENT

It is quite obvious that certain amount of specialized production equipment is necessary in the manufacture of a satisfactory product Each piece of equipment used to manufacture or pack medicaments has its own peculiar area where microbial growth may be promoted. Traces of product left in the transfer pipes or indentations or scratches on the inner surfaces of kettles can bring about rapid multiplication of organisms resulting in contamination of subsequent baiches of the product. Portions of equipment that do not make themselves available to cleaning can easily become infective sites. For instance, Basset (1971) traced an outbreak of pneumonia in the newborn to the use of Pseudomonas contaminated suction apparatus. Joints and cross connections in pipe often provide a site for microbial growth. Morse et al. (1967), traced the occurrence of septicaemia due to Klebisiella pneumoniae to a hand cream dispenser, used in dispensing the drug.

1.2.5 PERSONNEL

The microbial flora of air and the environment at which pharmaceutical production takes place is greatly affected by the personnel involved at the

various stages of production. Micro-organisms can be transferred from the operator to pharmaceutical products. The skin is rich with various types of bacteria. Body tissues are continuously disintegrating. It has been estimated that at least 6.0 gm of dead skin material is sloughed off daily from an adult. This means that a lot of bacteria is shed into the environment daily. Of the natural skin flora, Staph. aureus is perhaps the most undesirable organism. It is common on the hands and face, not easily eliminated by washing since it resides in the deep layers of the skin. Other commonly present types are Sarcina species and diphtheroids. It is known that:

(i) Man is intimately associated with bacteria,

(ii) Different areas of the skin carry different types and numbers of microbes. Gram negative rods such as Alcaligene species are resident in most regions of the skin while lipophilic yeast is present in the fatty and waxy secretions of the skin.

(iii) Each individual is different and the daily microbial profile may fluctuate,

(iv) Coughs and sneezes are prolific sources of air contamination, especially droplet infectious organisms. Talking has been shown to increase the microbial flora of a clean enclosed environment.

Bacteria other than the natural skin flora may be transferred from the operator as a result of poor personal hygiene. This include feacal organisms from theanal region, and those from wounds and abscesses. Open wounds usually support the growth of pathogens such as Staph. aureus, Streptococcus pyogenes , Micrococci, ∝-haemolytic and non-haemolytic Streptococci, enterococci, and in rare cases, Clostridium species. These infections of the wound may occur without showing any clinical manifestations, normally associated with such bacteria. Organisms from nasal passages often transmitted are Staph. aureus, limited quantity of Staph. albus and Saprophytic Streptococci of the viridans group.

1.2.6 CONTAINERS AND PACKAGING COMPONENTS

The containers in which product are packed can very much introduce microbes to its contents. The microbial flora of packaging material is dependent

(iv) Coughs and sneezes are prolific sources of air contamination, especially droplet infectious organisms. Talking has been shown to increase the microbial flora of a clean enclosed environment.

Bacteria other than the natural skin flora may be transferred from the operator as a result of poor personal hygiene. This include feacal organisms from theanal region, and those from wounds and abscesses. Open wounds usually support the growth of pathogens such as Staph. aureus, Streptococcus pyogenes , Micrococci, ∝-haemolytic and non-haemolytic Streptococci, enterococci, and in rare cases, Clostridium species. These infections of the wound may occur without showing any clinical manifestations, normally associated with such bacteria. Organisms from nasal passages often transmitted are Staph. aureus, limited quantity of Staph. albus and Saprophytic Streptococci of the viridans group.

.2.6 CONTAINERS AND PACKAGING COMPONENTS

The containers in which product are packed can very much introduce microbes to its contents. The microbial flora of packaging material is dependent

upon its composition and storage conditions. Packaging components made of glass, metal and plastic usually have low microbial contents due to lack of nutritive sources for bacterial growth. They however become contaminated sometimes due to their transportation in cardboard boxes or during storage with spores of moulds such as Aspergillus, Penicillium, Cladosporium and Bacillus species. Packaging materials made of cellophane, cellulose acetate, polyvinyl chloride, Polyethylene and polypropylene usually have low microbial levels. However, cardboard and paperboard boxes unless treated, carry mould spores and such bacteria as Bacillus and Micrococcus species. Closure liners of pulpboard or cork unless treated specially with preservatives are good sources of contamination for liquid and semi-solid preparations. Containers and closures stored in damp conditions or coming in contact with sugar, proteins and glycerol will become susceptible to microbial attack. In a report by the Public Health Laboratory Services Working Party (1971), it was shown that every type of organisms was more common in preparations dispensed in Pharmacy containers than in the original

Manufacturer's pack. The containers usually brought by patients for filling of their prescription are sometimes not properly washed and are contaminated prior to containment of preparations.

1.2.7 STORAGE OF DRUGS

The Storage condition at which the raw material and final products are subjected to is very important. This is more so for hygroscopic substances. The minimum water activity Aw, for Osmophilic yeast is 0.60, spoilage moulds 0.80, and most spoilage bacteria 0.71. Storage of pharmaceuticals in damp conditions will exceed this Aw and thus enhance bacterial growth. Also, some packaging used for raw materials such as unlined paper sacks, when Stored in damp condition, will absorb moisture and be subjected to microbial deterioration and contaminate the contents. While the microbial count may remain stationary or even decrease in some solid preparations such as tablets during the period of Storage, it may likely increase in others such as mixtures, lotions and creams few days on Storage and later level off. (Khante 1979). The prevailing situation however depends on the constituents and type of the pharmaceutical product concerned.

1.2.8 DISPENSING OF THE PRODUCT AT THE PHARMACY

Microbial contamination is frequent with products such as ointments and creams that are dispensed from wide-mouthed, multi-dose containers. Morse et al (1967) traced the occurrence of Septicaemia due to Klebsiella pneumoniae to a handcream dispenser in which the cream is dispensed with.

1.2.9 IN-USE CONTAMINATION

Higher rates of contamination are often associated with products after opening and use. This is more so with drugs dispensed from bulk containers in hospital wards and pharmacies, as compared with those in use in the general community. There are fewer opportunities for contamination in homes since smaller quantities are used by individual patients. Hugo and Wilson (1970), and and Harte et al (1978), showed a high proportion of eye-drop and eye ointment preparations which had been contaminated during the period of use.

1.3 HAZARDS ASSOCIATED WITH MICROBIAL CONTAMINATION

The capacity of bacteria to initiate disease in a host i.e. pathogenicity is dependent on many factors. Bacterial pathogenicity requires the attributes of transmissibility, infectivity and virulence. The pathogenicity is often related to the dosage of the pathogens, its phase of growth and its endowment with virulence factors. Infectivity of bacteria i.e., the ability to penetrate the body's first line of defence, is in turn dictated by the host's degree of immunity. The second factor - transmissibility which is the ability to spread from one host or reservior to another involves the potential to grow profusely in cavities and fluids and get disseminated in large numbers to sites of infection. In effect, it is dose related. Virulence is the third factor that influences the pathogenicity of bacteria. Epidemiological data showed that within a microbial family, there are differences in virulence, thus Shigella dysentariae, group one and in particular type I causes more serious dysentary than the group 4 (Shigella Sonnei). Among these factors, it is the dose of the ingested organism and its virulence that had been areas of focus in microbial contamination

and the basis by which microbial limits with respect to control has been and is being set in various literatures.

Investigations conducted on Salmonella and Pseudomonas species showed that a host needs to ingest approximately 1.5×10^5 of Salmonella organisms before showing clinical symptons of illness while 10^6 of Ps.aeruginosa need to be ingested before its colonisation of the bowel. (Buck and Cooke, 1969). Infact, each bacterium has its own level at which it causes illness. Also, this critical microbial level is significantly reduced in the presence of trauma, foreign bodies or if accompanied by drugs having a vasoconstrictive action. Reports of serious infections associated with the use of non-sterile pharmaceutical products abound. Most of the implicated contaminants in the products are Ps. aeruginosa, Salmonella species, Staph. aureus and E. coli. Though some of these bacteria are not really highly pathogenic, they however serve as an indicator of feacal contamination e.g. E.coli, implying unhygienic production conditions. The presence of pathogenic organisms such as Ps.aeruginosa in cosmetics, creams, and lotions, especially in solutions of disinfectants

have been implicated in the cause of various infectious conditions ranging from minor skin infections to loss of sight and fatal Septicaemia. In Sweden, 1964, (Kallings et al 1966), eight cases of severe eye disorders were reported in workmen treated with a Ps. aeruginosa contaminated antibiotic ointment. As a result of the infection, one patient lost an eye and the other seven had reduced visual acuity. In another documented reports (Remington, 1970), rive out of eighteen corneal ulcers in a hospital, loss of four eyes in an industrial infirmary and loss of sight in two dogs were all traced to Flourescin and Eserine Eye drops, which were found to be contaminated with Ps. aeruginosa. Noble and Savin (1966), Baird and Shooter (1976), also reported Ps. aeruginosa infections due to use of contaminated creams. Ayliffe et al (1965) and Basset (1971), traced the following disease conditions: Meningitis, pneumonia, respiratory tract infections, urinary tract infections, Septicaemia, arterial thrombosis and wound infections to the use of Ps. aeruginosa contaminated products and surgical equipments. Bacteremia had also been caused by Ps. thomasii due to contamination of autoclaved fluids. [Phillips et al (1972), and Lapage (1973)_7.

Many cases of Salmonellosis had also been reported as a result of ingestion of tablets contaminated with Salmonella species. Presence of Salmonella species can cause typhoid and paratyphoid fever. Thirty cases of salmonellosis due to Salmonella cubana occurred in hospitals in the United States of America, following the use of infected carmine dye capsules used in the investigation of intestinal abnormalities (Kormany et al 1967). Salmonellosis had also been reported to be caused by the use of infected dried yeast used in tube-feeding and also in case of contaminated thyroid capsules (Kunzand Ouchterlong (1955). Glencross (1972) reported the case of infection of two babies by Salmonella species following the use of infected pancreatin in the treatment of fibrocytic diseases of the pancreas which resulted in the hospitalization of one of the babies for treatment. Peppermint water-flavoured antacid mixtures contaminated with Ps. aeruginosa had also been found to establish bowel infections.

Between 1960 and 1964, documented cases of food poisoning in the Food Industry showed that the causative agents in most instances were Staph. aureus and Salmonella typhi. In an investigation conducted in Sweden 1964, (Kallings et al 1966), apart from the

high numbers of reported contaminated batches, an acute gastroenteritis caused by Staphylococcus enterotoxin was also reported. Though Staph. aureus may not affect normal persons, it can cause infections in the very young, the aged and in people with impaired immunological defence mechanisms. Although most strains of Staph. aureus are human pathogens, the virulence of particular strains varies due to a single 'phage type. The main reason why Staph. aureus is a danger are the number of cocci inevitably present in wards and its ability to survive and remain virulent in an environment laden with antibiotics.

The level of microbial contamination in a product can also influence the physico-chemical degradation in pharmaceutical products. The degradative versatility of microbes is such that a great majority of organic compounds can be chemically modified at a fast rate under mild conditions when compared to those reactions initiated by non-biologic process. Pseudomonas organisms are not solely a health hazard. They represent a potential economic loss in terms of product spoilage. They have been responsible for the degradation of Shampoos, facial lotions and sun screen preparations. (Tenenbaum 1967).

They attack hydrocarbons, including petrolatum and utilize alkanes and aromatics such as hexane and benzene (Rogoff 1962). They produce lipases and oxidise fatty acids as well as a variety of carbohydrates (Sultzer (1961), Alford and Pierce (1963)). In addition, they produce protolytic and and amylotic enzymes. Many of the thickening and suspending agents used in pharmaceutical formulations are subject to depolymerisation by micro-organisms, and this greatly affects their specific properties. Some therapeutic agents are acted upon by microbes to influence their degradation. For example, aspirin is easily affected to yield irritant salicylic acid. Gross microbial contamination of emulsions have led in some instances to phase separation (Hugo and Russel 1980). Rancid smell and objectionable tastes of some products are often due to microbial contamination.

One of the greatest hazards of microbial contamination is in the case of potable water. Sometimes, the growth of micro-organism lead not only to a change in water quality but to biodeterioration of the material concerned. Ten year inspection of two underground service reserviors constructed in 1961 revealed that the polyurethane jointing material

has been colonized and partially destroyed by fungi to such an extent that some leakages has occurred (Burman and Colbourne, 1977). Of all the plumbing materials, plasticized polyvinyl chloride has been particularly susceptible to microbial attack, leading to potable water quality deterioration.

1.4 OBJECTIVE AND SCOPE OF THIS INVESTIGATION

This investigation is undertaken to monitor the microbial contamination levels from the raw materials, through various stages of tablet production, using folic acid under different production environment and employing different tablet production methods. At the end, the quantitative contribution of each factor and the collective contribution of the factors mentioned above among others, to the final microbial contamination levels of the tablets will be obtained. It is expected that the result of this investigation would provide a basis for recommending some remedial steps to local manufacturers.

CHAPTER TTWO

GENERAL PROCEDURES OF TABLETTING

Compressed tablets are solid dosage forms prepared by compaction of a formulation containing the drug(s) and certain excipients selected to aid the processing and improve the properties of the product. The term 'Compressed tablet' usually refers to a plain, uncoated tablet for oral ingestion prepared by a single compression. Tablets are the most widely used of all pharmaceutical oral dosage forms for the following main reasons;-

(i) Convenience of administration,

(ii) Stability over extended period of time,

(iii) ease of administration,

(iv) Cheapness with regards to manufacture, and

(v) high degree of accuracy in delivery of its intended dose. Tablets come in different forms or shapes to fulfil different functions. The variety includes buccal, chewable, effervescent, layer, film coated and sugar coated tablets.

2.1 TABLET PRODUCTION IN A TABLET PRESS

Tablet formulation and design may be described as the process whereby the formulator insures that the correct amount of drug in the right form is delivered at or over the proper time at the proper rate and in the desired location, while having its chemical integrity protected to that point. Tablets are made by compression of a solid formulation containing a drug or drugs and excipients on a tablet press.

The tablet press consist of a set of die and an upper and lower punches with facilities for a hopper which provides for holding and feeding the granulation, a feeding mechanism for feeding the granulation to the die-cavity, provision for placement of punches and dies, and cam tracks for guiding the movement of punches. Tablet presses are of two types - the single station press and the multistation rotary press. Each tablet press depending on its design can produce at rates ranging from a few numbers to few thousand tablets per minutes.

The first stage in the compression process is the filling cycle during which the lower punch is lowered to a preset point to form a cavity in the die to

provide a volume corresponding to the correct fill weight for the tablet. The feed shoe is then pulled out of the way, and the upper punch descends into the die-cavity to compress the tablet. Next, the lower punch is raised level with the surface of the die table so that the feedshoe can eject the tablet as it comes over the die for another fill cycle.

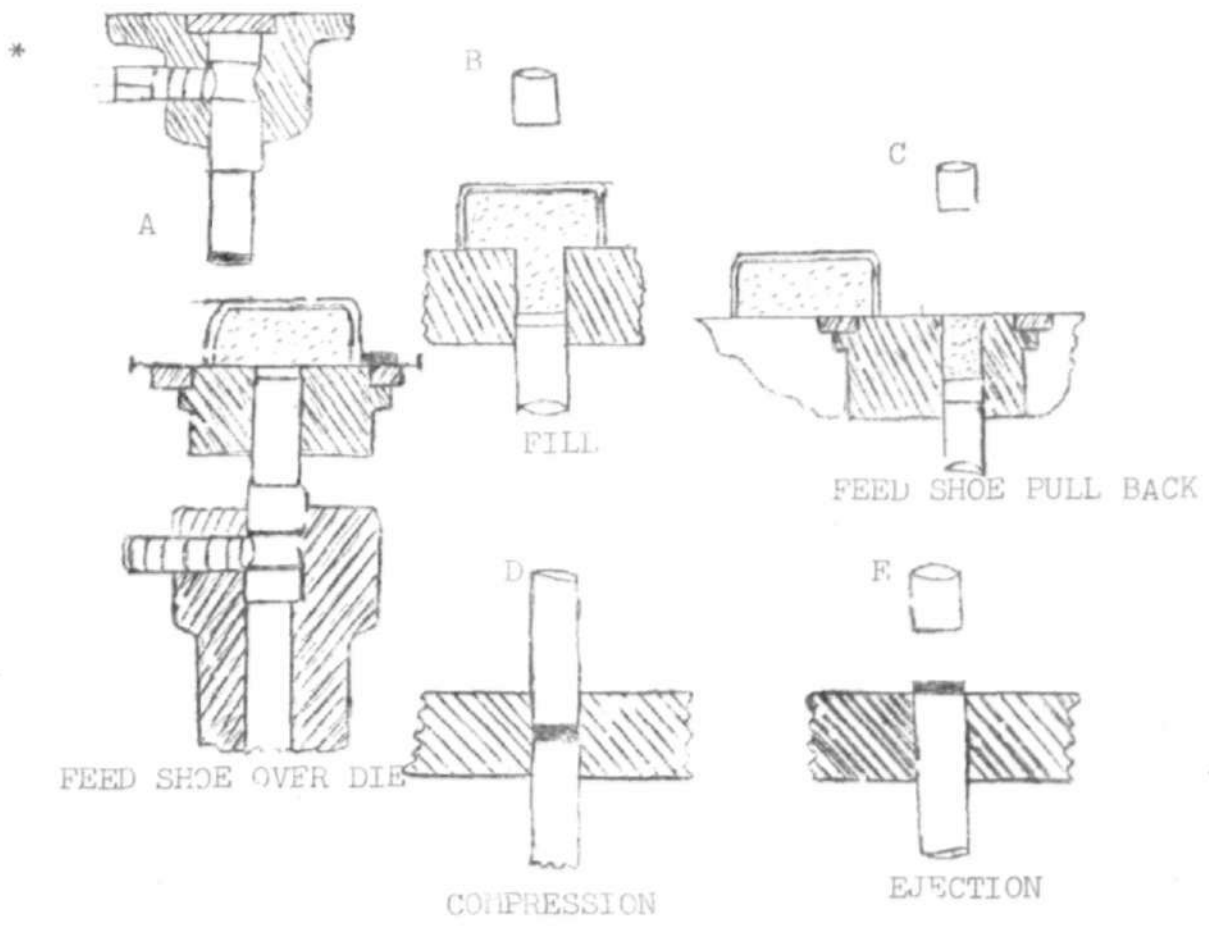


FIG. 2.1: Schematic diagram of the Compression Process in a Single-Punch Tablet-Press.

*Adapted from 'Pharmaceutical Dosage Forms: Tablets, Volume 1. 1980, Ed. H. A. Lieberman & Lachman pg. 110

The granules for filling into the die-cavity must satisfy three main requirements:-

(i) Good flow properties for precise volumetric feeding of the material to the die-cavity;

(ii) Compressibility to form the compact; and

(iii) Adequate lubricant properties for ejection of the tablet.

Because most of or all of the active ingredients for one reason or the other lack the above properties, excipients which will impact such lacking properties are added.

2.2 TABLET EXCIPIENTS

A tablet formulation contains a number of excipients beside the active ingredients. Each excipient is selected to meet or satisfy the needs of processibility and product use. The commonly used excipients include fillers, binders, glidants, lubricants and disintegrants, and in some cases flavours, sweetners and colours.

2.2.1 FILLERS

Fillers or diluents are used to increase the bulk of the tablet to give adequate size for a formulation to be compressed into tablets. Fillers also improve the flow and binding properties of the formulation. It is essential that diluents be inert and stable. Among the insoluble fillers are Calcium Sulfate, Dicalcium phosphate, Calcium Carbonate, Starch, Microcrystalline cellulose and modified Starches. The soluble fillers include lactose, sucrose, Mannitol and sorbitol. The choice of a diluent depends on the desired property of the produced tablet and the inherent property of the active ingredients.

2.2.2 BINDERS

Binders are those substances that are incorporated into formulations to increase the hardness of the resulting compact. They control to a great extent, the granulation particle size, uniformity, adequate hardness, ease of compression and the tablet general quality. Binders are either sugars or natural gums of polymeric substances. They include polyvinyl alcohols, gelatin, tragacanth, agar, acacia, ethylcellulose, methylcellulose, polyvinylpyrrolidine, starches and polyacrylamides.

They are used in concentration ranges of 2 - 10 % w/w as aqueous pastes or aqueous solutions.

2.2.3 LUBRICANTS

These are included in the formulation to ease tablet ejection from the die, to prevent sticking of the tablets to the punches and also to reduce tool wears and tears especially of the dies and punches. The particle size, the type and the stage of production at which it is incorporated, are very critical to the performance of its function. Lubricants coat the tablet granules. A general approach is to add the lubricant to the dry granulation just before compression. Lubricants may be hydrophobic, for example, oils and fats which are the most widely used, or soluble for example, those for Effervescent tablets. Lubricants are added at a concentration range of 0.5 - 5 % w/w and they include Stearic acid, Magnesium Stearate, Zinc stearate, Calcium Stearate, Polyethylene glycol, Sodium benzoate, Sterotex, Sodium lauryl sulphate, light Mineral oil, hydrogenated vegetable oils and Polyoxyethylene monostearate.

2.2.4 DISINTEGRANTS

Disintegrants are added to tablets to cause the break-up of tablets into individual granules when placed in an aqueous environment. A disintegrant can further causes break-up of the granules into powder particles from which the granulation was prepared. Disintegrants are either added externally in which case, it is added to the dry granules just prior to compression or internally, in which case, it is added before wet-mixing. Some formulators use a combination approach. The disintegrating property in most cases is due to its ability on contact with water to swell, hydrate, change in volume or position or react chemically to cause disruptive changes within the tablet matrix. Commonly used disintegrants are Starch, Microcrystalline Cellulose, Veegum, Bentonite, Alginic acid, Kaolin, Explotab and acid-base combinations in concentrations of 5 - 20 % w/w.

2.2.5 GLIDANTS

Glidants are those substances that are added to cohesive powders and granulations to reduce their interparticulate friction. They therefore improve flow from the hopper into the die-cavities, hence ensuring proper and uniform fill of the die. In general,

hydrophillic glidants are more effective on hydrophillic powders than on hydrophobic powders and vice-versa for hydrophobic glidants. For any particular system, there is usually an optimum concentration above which the glidants may start to act as antiglidants. This optimum, among other things depend on the moisture level in the sample. Some of the glidants in use are Starch, talc, the Stearates, Magnesium oxide, Magnesium carbonate, calcium sillicate and silica aerogels at concentration ranges of 0.2 - 10 % w/w depending on each glidant selected. The sillica type glidants are the most effective. The effectiveness of glidants can be evaluated either using the angle of repose or timing the time it takes a measured quantity of sample to pass through an orifice of known diameter.

2.2.6 COLOURS

Colours, in addition to aethestic value help to distinguish one product from another during product manufacture. Besides, it help, the patient to identify particular medicaments. A selection is made from the list certified by the Food and Drug Administration (FDA). Colouring agents can be added during the wet mix or incorporated through a specially designed spray drier.

(c) Preparation of binder solution.

(d) Mixing of binder solution with powder mixture to form a wet mass.

(e) Coarse screening of wet mass using 6 - 12 mesh size screens.

(f) Drying of moist granules.

(g) Screening of the dry granules through 14 - 20 mesh screen.

(h) Mixing of screened granules with lubricants and disintegrants.

(i) Tablet compression.

These various steps involved in the wet granulation process have significant effects on the tabletting properties of the resulting granulations. Wet granulation method has a number of advantages.

(i) The cohesiveness and compressibility of powders is improved leading to application of lower pressures for compression, thus resulting in improvement in tooling life and machine wear.

(ii) It is the recommended method of granulation for high dose drugs having poor flow or compressibility properties since through it, both properties are adequately improved.

(iii) It provides good distribution of colour additives and uniform content for soluble low dosage drugs as these can be added in the binder solution before incorporation.

(iv) It prevents segregation of components of a homogenous powder mix during processing, transferring and handling.

(v) It improves the dissolution rate of hydrophobic drugs if proper choice of solvent and binder is made.

Wet granulation, however, has its drawback and limitations. The greatestof its drawbacks is its cost. It is expensive in terms of labour, time, equipment, energy and space requirements. The use of soluble dyes in wet granulation can be a disadvantage as it often causes migration of the dyes during the drying cycle. Another inherent limitation of wet granulation is that any incompatibility between formulation components will be aggravated by the granulating solvent which brings them into close contact.

2.4 DIRECT COMPRESSION METHOD

Direct compression usually involves relatively simple steps of blending and compression. It offers increased product stability, reduces cost, has fewer operations, fewer pieces of equipment, fewer labour force and much shorter time (from days to few hours) to do the required work and in most cases with better products. For instance, time for preparation is reduced to a point where finished tablets can be produced in the laboratory in 15 - 20 minutes. The elimination of heat and moisture in direct compression is also a great advantage since the hazards associated with them can not arise. Also, fewer chemical stability problems are encountered with the direct compression process. During the quality control, since only one substance is examined (rather than one made of many components), many of the difficulties which may be associated with improper or inadequate blending of various additives are obviated. Changes in dissolution profiles are less likely to occur in tablets made by direct compression than in wet granulation method.

In most cases of direct compression, the original drug is compressed without change (chemical or physical), so that during the subsequent interparticulate break-up, the basic drug particles

are available in the form in which they were originally introduced, thus approximating to conditions of maximum drug availability and absorption. This unique biological availability potential is the chief function and major advantage of the direct compression process.

In direct compression, the problem of formulation is a blunt one, as starting materials are often unsuitable. The majority of active constituents for tabletting lack sufficient bulk satisfactory for compression, and adequate flow properties. The addition of direct compression vehicles which might impact lacking properties to such powders are therefore of prime importance. For direct compression venicle to be able to perform its desired function, the following are its desired properties:-

(i) High flowability or fluidity

(ii) High compressibility and reworkability over several cycles without loss of fluidity or compressibility.

(iii) Physiological inertness and compartibility with virtually all active ingredients, and non-interference with the biologic availability of the active constituents.

(iv) Stability to air, moisture and heat, and chemical and physical stability on aging.

(v) High capacity, that is, the amount of the active ingredient it can carry and still be compressible.

(vi) The bulk density is critical as there is no densification stage in direct compression.

(vii) Beside the above properties, it should be tasteless, colourless, inexpensive, good mouth feel, good hardness-pressure profile, accept colour uniformly and have particle size range equivalent to most active ingredients.

There is no vehicle that meets all the above requirements, the choice of one or a mixture of some solely depends on the formulator, the characteristic of the active ingredients and the use to which the resulting tablet is to be directed. The two most critical considerations for the choice of a vehicle for direct compression are the fluidity and compressibility. The direct compression vehicles most commonly used are:- Spray-dried lactose, Emdex, Emcompress (unmilled dicalcium phosphate), Fast-Flo lactose, Anhydrous Lactose, Sta-R_x 1500 Starch, DT-Pac, Nu - Tab and Microcrystalline Cellulose PH 101 and PH 102.

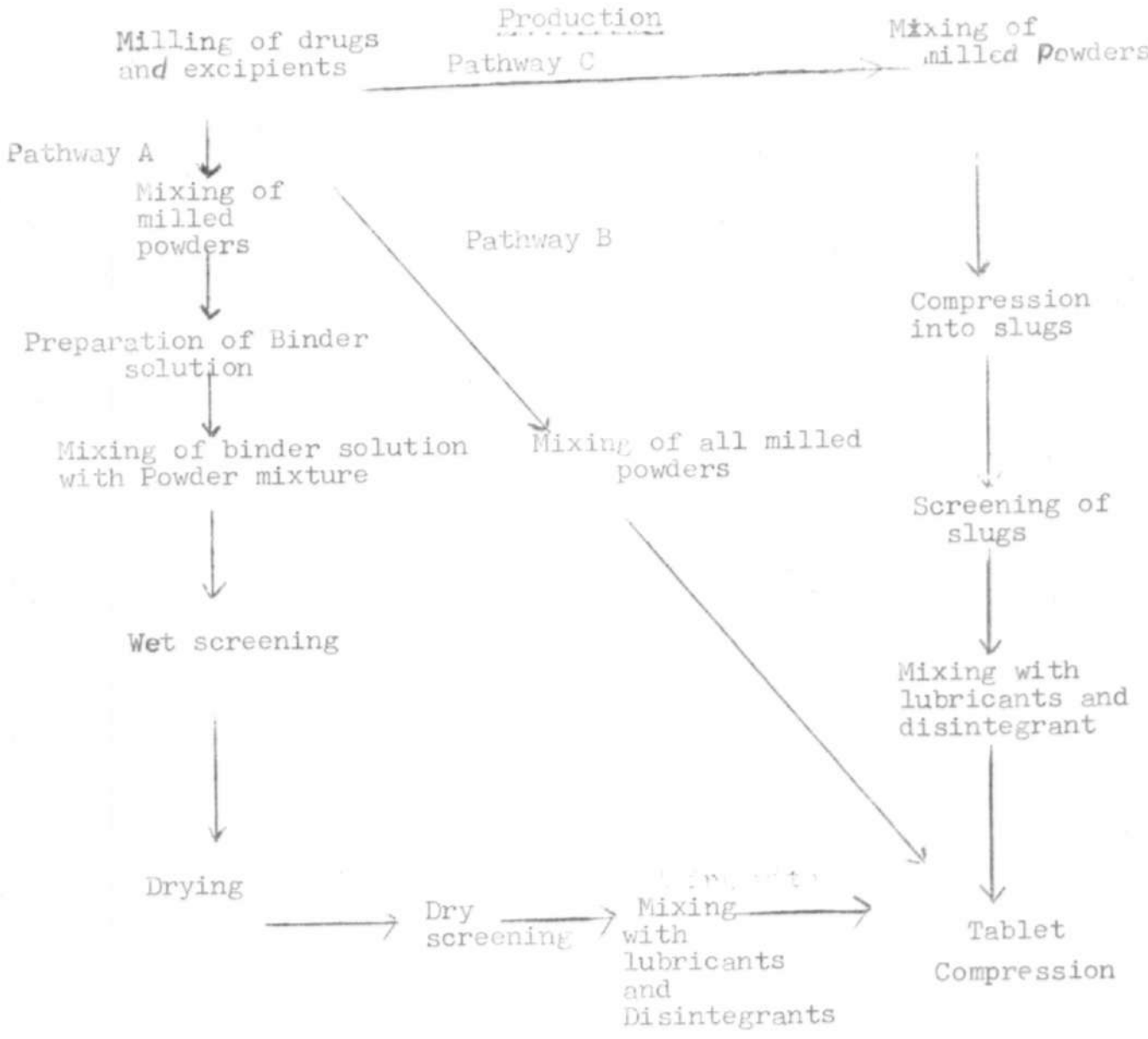
Other necessary excipients in direct compression are lubricants and glidants. Colours may be added if necessary.

The direct compression process involves basically three steps:-

(i) Milling of the drugs and excipients
(ii) Mixing of all ingredients
(iii) Tablet compression.

Despite its appeal, the direct compression process should not be considered an easy one, it has its problems and drawbacks. It is not easily applicable for tablet production of high dose drugs which have inherent poor fluidity and compressibility, since the quantity of fillers needed to be added in such a case is very limited. Proper blending in case of low-dose drugs is another limitation, and related is the proper dispersal of colours in the tablet matrix. The physico-chemical properties of individual ingredients such as particle size, fluidity and moisture are likely to be more critical in tablets produced by direct compression than those prepared from granulations.

FIG 2·2: Diagramatic representation of the methods of Tablet



Pathway A - Wet Granulation method

Pathway B - Direct Compression method

Pathway C - Dry granulation method.

2.5 DRY GRANULATION METHOD

Dry granulation refers to the granulation of a powder mixture by compression, without the use of heat and moisture. Though it is the least desirable of the three methods of tablet production, it is however the method of choice in the following cases:-

(a) In moisture sensitive and or heat sensitive materials which because of its inherent properties and dose can not be tabletted by direct compression.

(b) For anhydrous soluble materials that tend to set when wet.

Some of its disadvantages are:-

(i) It requires a specialized heavy-duty press to form slugs.

(ii) A Pressure-roll press such as the Chilsonator can not be used with insoluble drugs since this may retard dissolution rate.

(iii) It does not permit uniform colour distribution as can be achieved with wet granulation where the dye can be incorporated in the binder solution.

(iv) The process tend to create more dust than in wet granulation, thereby increasing the potential for cross contamination.

The dry granulation process entails the following steps:-

Milling of drugs and excipients

Mixing of milled powders

Compression into large, hard tablets, to make slugs,

Screening of slugs

Mixing with lubricants and disintegrants

Tablet Compression.

The slugging process is the most critical stage. There are many factors which influence how well a material may slug and subsequently affect the properties of the resulting slugs. These are:-

(i) Compressibility or cohesiveness of the material;

(ii) Compression ratio of the powder, which is exposed as the ratio of the depth of die fill to slug thickness,

(iii) Density of the powder;

(iv) Machine type - Single punch or rotary, and Machine size;

(v) Punch and die sizes and clearance;

(vi) Slug thickness; and

(vii) Speed of compression and pressure used to produce the slugs.

The excipients used in dry granulation are same as those used in the other two processes. However, the powder flow properties do not have to be as good as in direct compression, so also is the compressibility since higher compaction pressures are used.

2.6 DESIRABLE PROPERTIES OF A GOOD TABLET

Whichever method of manufacture that is used, the resulting tablets must have satisfactory properties such as:-

(i) The tablet must be sufficiently strong and resistant to abrassions during handling such as manufacture, packaging, transportation and use. This is determined by hardness test and friability test.

(ii) The drug in the tablet must be bioavailable which is determined by disintegration and dissolution tests.

(iii) The tablet must be uniform in weight and in the drug content of individual tablets. This is measured by tablet weight variation and content uniformity tests.

(iv) The tablet must be elegant in appearance and have the characteristic colour, shape and other markings that identify the product.

(v) The tablet must retain all of its functional attributes such as drug stability and efficacy.

The properties of a tablet are affected by both the formulation and the method of manufacture, and between these two factors, there is a high degree of interrelationship. A suitable formulation is critical to the manufacture of satisfactory tablets, but the formulation must be designed according to the needs, advantages and limitation of the manufacturing method and equipment used. Since tablet manufacture involves the processing of powders, a high degree of control of the temperature and humidity of the work area is necessary.

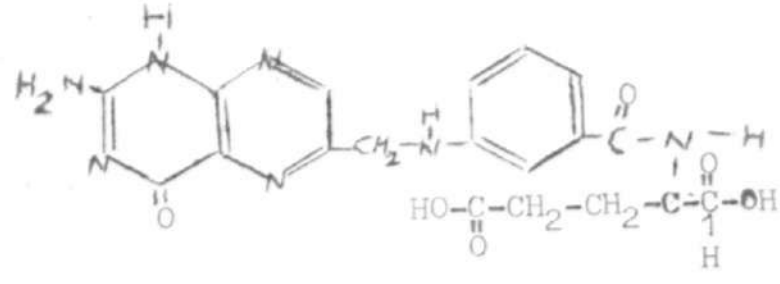
CHAPTER THREE

MANUFACTURING OF FOLIC ACID TABLETS

3.1 PHYSICO-CHEMICAL PROPERTIES AND USES OF FOLIC ACID

The knowledge of the physico-chemical properties of the active ingredient in a preparation is important as this forms one of the basis for the choice of formulation and manufacturing procedure to employ.

Folic acid is a member of the vitamin B group, present as free compound or in combination with several L(+) - glutamic acid moieties, in peptide linkages, in liver, meat, spinach, yeast and green leafy vegetables. It is also produced synthetically. It has the chemical structure given below:



N-P-(2-Amino-4-hydroxypterid-6-ylmethylamino) benzoyl-L (+)- glutamic acid. $C_9H_{19}N_7O_6$ = 441.4.

Folic acid occurs as an orange-yellow, tasteless, almost odourless, microcrystalline powder containing 5 - 8.5 % of water. It is insoluble in most organic

solvent and cold water, sparingly soluble in boiling water, soluble in alkali and acidic solutions. It is incompatible with oxidising and reducing agents, and ions of heavy metals. Solutions of folic acid are easily inactivated by ultra-violet light. Folic acid should be stored in air-tight containers, protected from light.

Folic acid is used in the treatment of nutritional macrocytic anaemia, megaloblastic anaemia, anaemia of pregnancy, in pellagra, spruce and pernicious anaemia. It is necessary for the normal production of red blood cells, including maturation of megaloblasts to normoblasts.. It is given on a daily dose of 5 - 20 mg in megaloblastic anaemia associated with iron deficiency, and 200 - 500 ug daily in the prophylaxis of megaloblastic anaemia of pregnancy. It is readily absorbed from the gastrointestinal tract and well tolerated.

3.1.2 REASONS FOR THE CHOICE OF FOLIC ACID IN THE INVESTIGATION OF MICROBIAL CONTAMINATION OF TABLETS DURING MANUFACTURE

Commercial folic acid tablets are being produced by both wet granulation and direct compression, methods. The quantity of Folic acid in each tablet is very small, hence necessitating incorporation of vehicles

and other tablet excipients before granules/powder mixture can be compressed into tablets. The possibility of incorporation of these excipients enables one to study their effects on the overall microbial contamination level of the final tablets.

Bacteria do not utilise already made folic acid in its biochemical reactions. They synthesize their own internally within the cytoplasm from folic acid precursors. Folic acid beside, is not a nutrient for bacteria, and has no antibacterial effect. These enumerated properties prompted the choice of folic acid tablet, as the prototype, in the investigation of microbial contamination of tablets during manufacturing.

3.2 MATERIALS AND EQUIPMENT

3.2.1 MATERIALS

Most of the raw materials used were obtained from bulk packages in the Department of Industrial Pharmacy and Quality Control and few others from the Department of Pharmaceutics and Pharmaceutical Microbiology, both of Ahmadu Bello University, Zaria, Nigeria. The materials and their original sources were as stated below.

Dicalcium Phosphate Powder

obtained from A. C. Christlieb (Nig.) Ltd.,

Lagos, Nigeria;

Folic acid powder Lot No 642651

F. Hoffmann-La Roche Co, Ltd, Basle, Switzerland;

Gelatin B.P. 1973.

Tennaxt Ltd, U.K.;

Maize Starch (Powder) Lot No 70680

May and Baker Ltd, U.K.;

Microcrystalline Cellulose (Avicel) PH101, size 50μ

Batch No 881, Fluka AG;

Lactose (Anhydrous) B.P.

obtained from A. C. Christilied (Nig.) Ltd,

Lagos, Nigeria;

Lactose (Hydrous) U.S.P. Batch No 7259

Whey Products, U.K;

Polyethylene glycol 6000. Prod. 29577

British Drug Houses Chemical Ltd, U.K;;

Talc (fine Powder), Art No 8070

E. Merch, Darmstadt, U.K.

3.2.2 EQUIPMENTS

Erweka Disintegration Apparatus G.M.B.H

Type ZT3 No 41728

Made in Western Germany

Erweka Granulator G.M.B.H.

Type FG3, No 40536

Erweka Tabletting Machine

Type EKO, Single Station Punch

Made in Western Germany

Gallenkamp Hot Oven Box, size 3

Mettler Instruments AG, Switzerland

Mettler Loading Balance, Type P163

Mettler Instruments AG, Switzerland

Roche Friabilator, Erweka Type TA3R

Made in Western Germany

Manesty Granulator with Shaft

Manesty Machines Ltd

Liverpool, England

Manesty Tablet Press, 23 - Station rotary set

Manesty Machines Ltd

Liverpool, England

Pressier Vernier Callipers

Monsanto Hardness Tester

Monsanto Co, St Lious, United States of America

Mortar and Pestle

Manesty Drum Mixer.

Manesty Machines Ltd, Liverpool, England.

3.3 EXPERIMENTAL

3.3.1 WET GRANULATION METHOD

Table 3.1 WET GRANULATION METHOD WORKING FORMULA*

	COMPOSITION	QUANTITY	
1.	Folic acid	2.5 kg	Initial Powder mix
	Maize Starch	50.0 kg	
2.	Gelatin	1.4 kg	Binder solution
	Distilled water	26.6 kg	
3.	Folic acid	2.5 kg	
	Talc	0.932 kg	
	Magnesium stearate	0.286 kg	

*This is the working formula being used in a local Pharmaceutical Manufacturing firm in Kaduna State.

The weighed amount of Folic acid in Stage I was mixed with the calculated weighed amount of maize starch powder in a Manesty Drum mixer for twenty to thirty (20 - 30 minutes). This was the approach for large batch size (about 500,000 to 1,000,000 tablets batch size). In the case of small batch size of about five thousand tablets, the mixing was done using the spatula technique.

The gelatin binder solution was prepared as follows:- The weighed amount of gelatin powder was hydrated for about 10 - 15 minutes. It was then heated on hot plate until a homogenous clear solution was obtained. Sufficient quantity of hot water was added until the required amount, was obtained and it was allowed to cool to 35°C before being used.

The prepared binder solution in case of a large batch size, was added to the initial powder mix (in stage 1), in a Manesty Granulator, granulated for about 30 minutes. In case of small batch size, the binder solution was added in portions to the powder mix and kneaded thoroughly, using pestle in a mortar, granulation being done at a constant speed until a wet mass was obtained. The wet mass was subjected to wet screening using sieve mesh size of 1.25 mm in an Oscilliating granulator (Erweka Type FGS). The wet screening granulations were dried at 50°C for at least one hour. Another batch was similarly prepared but dried at the room temperature (28 - 30°C) overright.

The dried granules were then dry screened using either a 1.0 mm or 0.8 mm mesh screens in an oscillating granulator. The obtained granules were

then mixed with the remaining quantity of Folic acid, the lubricants and glidants (Talc and magnesium stearate), in a drum mixer.

3.3.2 DIRECT COMPRESSION

In each formulation, the calculated amount of each ingredient was weighed and mixed thoroughly together in a drum mixer and taken to the Tablet Press for compression.

Table 3.2 DIRECT COMPRESSION WORKING FORMULA

COMPOSITION	FORMULATION			
	I	II	III	IV
Folic acid	500 g	500 g	500 g	500 g
Microcrystalline Cellulose (Avicel PH 101)	2.2 kg	3.5 kg	2.0 kg	2.5 kg
Dicalcium Phosphate Powder	-	2.0 kg	-	-
Anhydrous Lactose	3.0 kg	-	1.2 kg	-
Polyethylene glycol 6000	-	-	2.0 kg	-
Maize starch granules	-	-	-	2.8 kg
Magnesium stearate	30 g	60 g	30 g	100 g
Talc	300 g	-	300 g	100 g

3.3.3 TABLET COMPRESSION

Whichever method was used for the preparation of the granules (for compression), the compression process was the same. The compression into tablets were done on a power driven single Punch (eccentric) Tablet Press (Erweka Type Eku) using a single set of 5.00 mm bevelled edge punches and die. The compression pressure and die-fill settings were held constant for each batch production.

3.4 QUALITY CONTROL TESTS

3.4.1 THICKNESS TESTS

The thickness (in mm) of at least ten tablets of each batch was determined using a Pressier Verinier Calliper. Three sets of readings were obtained. The variation from the average is an indication of the uniformity or otherwise of the weights of the tablets, provided the same compressional force was used throughout in a batch.

3.4.2 WEIGHT VARIATION TEST

The mean weight of at least ten tablets were taken and the weight of individual tablets also determined using a Laboratory Mettler balance P163. The percentage deviations from the mean weight were

also calculated and compared to the BP 1980 specifications for allowable weight variations (see table below).

Table 3.3 BP. SPECIFICATIONS FOR ALLOWABLE WEIGHT VARIATIONS

No. of Tablets in sample	Average weights of Tablets in sample	% Deviations form average	% Deviations form weight
20	80 mg or less	± 10	± 15
	Greater than 80 mg, but less than 250 mg	± 7.5	± 12.5
	250 mg or more	± 5	± 10.0
	No. of tablets in the sample that may deviate by more than the above percentage	Not more than two	None
10	80 mg or less	± 10	± 15
	Greater than, 80 mg, but less than 250 mg	± 7.5	± 12.5
	250 mg or more	± 5.0	± 10.0
	No. of tablets in the sample that may deviate by more than the above percentage	Not more than one	None

A perfect manufacturing procedure would yield a batch of tablets having identical weights and medical content; hence the determination of weight variation gives an indication of the variation of tablet active ingredients.

3.4.3 FRIABILITY TEST

For each formulation, ten tablets were weighed and put in a Roche Friabilator, Operated at 25 rpm. (rotations per minute) for four minutes. The loss in weight (friability) is expressed as a percentage of the original weight. For a four minute test, the percentage loss should not be greater than 0.8 % w/w while for a 10 minute test, the loss should be in the range of 1 - 5 % w/w.

3.4.4 CRUSHING STRENGTH TEST

This is another parameter for the determination of hardness test of tablets. The crushing strength of ten tablets of each formulation were determined using a Monsanto Hardness Tester. The mean values of these were recorded. There is no official limit for the crushing strength of tablets but nevertheless, they must be hard enough to withstand handling without breaking, chipping or crumbling but not so hard as to prolong the disintegration time.

3.4.5 DISINTEGRATION TEST

Taking six tablets at a time (B.P. method), the disintegration times were determined on a Manesty Disintegration Test Unit, (ZT3, No. 41728). Distilled

water thermostated at 37° ± 1°C was used as the disintegration medium. Test was repeated to obtain three sets of reading for each batch. No disc was used throughout.

The B.P. 1980 Specified disintegration time for uncoated and compression coated tablets is within 15 minutes.

3.5 RESULTS

The results for friability, disintegration times, hardness (crushing strength) and thickness are as shown in Table 3.4 and weight variation percentages shown in Table 3.5(a) and 3.5(b) Tablets of C_2 (see Table 3.4) was produced by wet granulation method by a Pharmaceutical firm and was therefore subjected to Quality Control tests, to serve as a standard for comparison for those produced by the same experimental procedure in this research work. Tablets of C_1 which were produced by direct compression was used to serve as a standard for those folic acid tablets produced by same procedure in this research work. In the wet granulation, tablets produced by drying the wet granules at room temperature were more colour intense and have greater tendency to cap than those produced from granules dried at 50°C. The percentage weight variations of all the tablets are within official limits.

(Table 3.4: Quality Control Test Values of the various Tablets

	Wet Granulation Method		Direct Compression Method B					
	*A_1	*A_2	I	II	III	IV	C_1	C_2
Thickness (mm)	2.95	2.65	2.25	2.75	2.55	2.40	2.00	2.90
Friability % w/w	0.7 - 1.0	0.4	0.32	0.32	0.62	0.3	0.26	0.33
Hardness (kgf)	1.0 - 1.2	2.8	5.0	3.0	3.8	4.0	3.30	1.5
Disintegration Time (Secs)	30 - 50 sec.	5 mins (300 secs)	2 mm 25sec (145 sec)	2 min (120 secs)	20 mins (1200 secs)	35 secs	1 min 15 sec (75 secs)	1 min (60 secs)

*A_1 - Granulation by wet method dried at room temperature

*A_2 - Granulation by wet method dried at 50°C

A_1 & A_2 has same composition.

C_1 - Folic acid tablets produced by Pharbita C●., Holland.

C_2 - Folic acid tablets produced by Industrial Pharmacy and Quality Control department, A.B.U., Zaria.

Table 3.5(a): Percentage Weight variations from Average Weights of Tablets

Sample No.	Average	A_1 Weight = 58.65 mg		Average	A_2 weight = 59.8 mg	
	Weight (mg)	% Weight	% Deviation from Average	Weight (mg)	% Weight	% Deviation from Average
1	59	100.60	0.60	60	100.33	0.33
2	59	100.60	0.60	60	100.33	0.33
3	58	98.89	1.11	61	102.01	2.01
4	58	98.89	1.11	60	100.33	0.33
5	57	97.19	2.81	58	96.99	3.01
6	59	100.60	0.60	60	100.33	0.33
7	56	95.45	4.52	58	96.99	3.01
8	59	100.60	0.60	60	100.33	0.33
9	60	102.30	2.30	61	102.01	2.01
10	59	100.60	0.60	59	98.66	1.34
11	59	100.60	0.60	62	103.68	3.68
12	59	100.60	0.60	58	96.99	3.01
13	57	97.19	2.81	60	100.33	0.33
14	59	100.60	0.60	62	103.68	3.68
15	60	102.30	2.30	60	100.33	0.33
16	59	100.60	0.60	61	102.01	2.01
17	61	104.01	4.01	61	102.01	2.01
18	58	98.89	1.11	59	95.32	4.68
19	58	98.89	1.11	60	100.33	0.33
20	59	100.60	0.60	60	100.33	0.33

3.6 DISCUSSION

The two most important or commonly applied methods of tablet manufacturing, wet granulation and direct compression methods, have been used in the production of folic acid tablets.

The results depicted in table 3.4, shows two important observations vis-a-viz effect of temperature of drying wet granules, and the type of granulation employed. The first prominent observation indicates that tablets of wet granulation dried at 50°C (A2) have superior quality with respect to the various quality control tests especially with reference to hardness, friability and disintegration patterns, when compared to those produced from granules dried at room temperature (A1). Tablets of A1 beside having greater thickness, lower crushing strength and very low disintegration, time, have friability values greater than the officially accepted limit of 0.8 % w/w. Drying at room temperature resulted in granules of higher moisture content (moisture content at room temperature is invariably higher than at 50°C). Rees and Hearsey (1972) showed that moisture exerts some additional effects (beside its surface tension effect) which cause a reduction in compact strength. The predominant effect of

moisture is to reduce the strength of actual bonds formed during compaction. On application of compressional force, an appreciable hydrodynamic pressure within the interstitial fluid will resist compaction and prevent intimate contact between the particles of the compressed material. This is probably the basis behind the higher values of thickness of tablets produced by drying granulation at room temperature. Invariably, where the compact strength of tablets is low, the disintegration is very fast since the interparticulate bond in such cases is weak.

It is not quite clear why the tablets produced from granules dried at room temperature is more colour intense than those produced from granules dried at 50°C. In A1, the slow rate of drying enables the sparingly soluble microcrystalline folic acid to penetrate the particles of the filler the more. Also, the wetter surfaces of granules of A1 (because of its higher moisture content) allows it to affix more of the second portion of folic acid which was added before compression.

The type of granulation employed greatly affects the quality of the resulting tablet. Tablets produced by direct compression showed good qualities such as low friability, relatively higher hardness values and good disintegration pattern except formulation B III. The relative high friability value of formulation B III is due to the plastic nature of one of its component - polyethylene glycol. It is this polyethylene glycol, because of its hydrophobic nature that is responsible for the high disintegration time (20 minutes). In direct compression, there is no wetting stage involved. Some of the vehicles like Avicel, Dicalcium phosphate and Lactose have inherent property of absorbing moisture from the atmosphere. The little amount of water they contain and or that absorbed is adequate in most cases to act as a lubricant by reducing interparticulate friction which had to be overcome before a compact can be made. The lower disintegration in direct compression tablets (2 minutes compared to 5 minutes for A2) is due to the fact that in direct compression, during disintegration, the ingredients break-up directly into its individual particles whereas the break-up in wet granulation tablets is first into granules and later into individual particles.

One of the major problems confronting direct compression is its *flowability*. In this work, forced feeding of the die cavity had to be resorted to because of poor flow from the hopper to the die. Overcoming this great drawback, tablet production will be more economically produced and resulting tablets are assured of better quality.

The tablets produced by the two methods of tablet manufacturing compared very favourably with the commercial folic acid tablets as judged on the B.P. Quality Control tests. Therefore, observations on microbial contamination during various stages could be taken as generally typical of occurences during tablet production.

CHAPTER FOUR

DETERMINATION OF MICROBIAL CONTAMINATION OF RAW MATERIALS OF FOLIC ACID TABLETS

GENERAL METHODS

Microbial limit tests are used for the estimation of the number of viable aerobic bacteria and fungi present, and to ensure the absence of some designated microbial pathogens from Pharmaceutical products. In routine tests, four organisms: Ps. aeruginosa, Staph. aureus, E. coli and Salmonella species are the objects of focus.

4.1.0 METHODS OF DETECTION OF MICROBIAL CONTAMINANTS

The methodology should take into account the properties of the product to be examined.

For example:-

(a) The solubility of the product and the nature of the components. The sample can be diluted, dissolved, suspended or emulsified as the case may be, in a suitable sterile liquid. Polysorbate 20 or 80 can be used for emulsification.

(b) The presence of any antimicrobial properties; which, can be eliminated either by dilution,

In the first stage, the sample to be tested is suspended and diluted in nutrient broth, and later incubated. Nutrient broth serves as a general purpose medium and a non-toxic diluent. Growth from the nutrient broth is sub-cultured (about 1 ml) into a selective liquid medium, to selectively cultivates the desired species. Typical examples of such media include Salt meat media for Staph. aureus, Selenite and tetrathionate broth for Salmonella species.

The growth obtained from above are sub-cultured into differential media, which are usually both selective and diagnostic. They are not only designed to allow for growth of specific type(s) of organisms, but in addition contain indicator substances which are affected by the growth of particular organisms to produce characteristic reactions. For example, indicators of acid and gas production, usually with a change of colour. Examples of these type of media include: MacConkey Agar, [illegible] Mannitol Salt Agar, Trip le Sugar Iron Agar, Vogel Johnson Agars among many others.

From the diagnostic media, colonial characteristics, cell morphology and biochemical tests are performed as shown in Table 4.1. The tests can only

give presumptive identifications while complete identification require more considerable informations.

Table 4.1: CHARACTERISTICS OF THE COMMONLY DETECTED CONTAMINANTS

Organisms	Selective media	Differential media	Biochemical tests
Staph. aureus	Salt meat media	Vogel Johnson Agar, Mannitol Salt Agar, Baird Parker Agar	Gram stain coagulase phosphatase
Salmonella, spp	Selenite and tetrathionate broth	Xylose lysine deoxy-cholate (XLD), Deoxycholate citrate Agar, Bismuth sulfite Agar	Sugar fermentation, glucose, lactose sucrose, dulcitol Mannitol. Gram stain
E. coli	Minerals modified glutamate	MacConkey Agar, Eosin Methylene blue Agar	Indole (44°C), MRVP citrate, lactose ricinoleate broth (44°C) Gram stain
Ps. aeruginosa	Lilly-Lowbury broth (containing nalidixic acid)	Cetrimide (0.03 %) Agar.	Oxidase Diffusible pigment production, growth at 42°C Gram stain.

4.1.1 TESTS FOR STAPHYLOCOCCUS AUREUS

A 10 % homogenate of the sample is made. 10 ml of the homogenate is inoculated into 90 ml of Casein-soyabean digest medium or nutrient broth. If

there is growth after incubation for 24 - 48 hours at 37°C, streaks are made from the medium onto Agar surfaces of either Vogel Johnson, Baird-Parker, or Mannitol Salt medium. This is then incubated at 37°C for 2 - 3 days, after which the resulting colonies are compared in their characteristic appearance to those shown in Table 4.2. If there is any correspondence, some biochemical tests are carried out to further ascertain presence of Staph. aureus. It is however preferable to carry out a cortrol test at the same time with a Standard Staph. aureus cells, subjected to the same treatment as with the test sample.

Table 4.2 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF Staph. aureus ON DIAGNOSTIC/SELECTIVE MEDIA

Differential medium	Vogel-Johhson Agar	Mannitol Salt Agar	Baird-Parker Agar
Characteristic colonial morphology	Black surrounded by yellow zones.	Yellow colonies with yellow zones.	Black shinning colonies surrounded by clear zones (2 - 5 mm).
Gram stain	Gr +ve Cocci in clusters	+ve Cocci in clusters	+ve Cocci in clusters
Coagulase test	+ve	+ve	+ve
Phosphatase test	+ve	+ve	+ve

It is known that some species of bacteria are however able to grow on some of these media e.g. Bacillus and Staph. epidermidis. It is therefore important that the coagulase and phosphatase tests are done which are both negative for Staph. epidermidis.

The coagulase test which is commonly done routinely is carried out as follows:- With a loop, cultures from the selective media are transferred to tubes containing 0.5 ml of rabbit or horse plasma, with or without suitable additives and kept in a water bath regulated at 37°C for a period of 24 hours. The tubes are examined at 3 hourly intervals for coagulation. Absence of coagulation indicates absence of Staph. aureus hence the sample can be considered to pass the test. For a sufcessful coagulase test, it is necessary that an 18 - 24 hours culture of the organism is used. This is done by sub-culturing from the differential media into nutrient broth, then incubated at 37°C for 18 - 24 hours before performing the test.

4.1.2 TEST FOR PSEUDOMONAS AERUGINOSA

A 10 ml of the homogenate is inoculated into Lilly Lowbury broth, where this is available or into either Casein-soyabean digest broth or nutrient broth

and incubated at 37°C for 24 — 48 hours. From this, streaking is done on cetrimide agar (0.03 %) and incubated at 37°C for 2 - 3 days. The presence of well developed colonies of gram negative bacilli with an intense greenish diffusible zones around the colonies is a conclusive evidence of the presence of Ps. aeruginosa. Development of weak pigment or none indicates the need for a confirmatory test.

From the cetrimide agar surfaces, cultures are transferred to surfaces of other agar media for detection of pyocyanin and flourescin, incubating for three days at 37°C. Results confirming to those in Table 4.3, and also if it tallies with that of control, gives a presumptive indication for the presence of Ps. aeruginosa.

For confirmation, oxidase test is done. This involves the transfer of cultures of the suspected organism onto a strip of filter paper which had been previously treated with N,N-dimethyl-p-phenylene diamine dihydrochloride. Failure of the development of pink colour within seconds which changes to purple, shows the absence of Ps. aeruginosa.

Table 4.3: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS FOR Ps aeruginosa ON DIAGNOSTIC AGAR MEDIA

Media	Cetrimide Agar	Ps. aeruginosa Agar medium for detection of flourescin	Ps aeruginosa Agar medium for detection of pyocyanin
Characteristic colonial morphology	Generally greenish	Generally colourless to yellowish	Greenish yellow to yellow.
Flouresce U.V. light	Greenish	Yellowish	Blue
Oxidase test	+ve	+ve	+ve
Gram Stain	gr -ve rods	gr -ve rods	gr -ve rods
Growth at 42°C	+ve	+ve	+ve

It is known that pigment production by Pseudomonas is very variable and depends markedly on environmental conditions. The two main pigments are pyocyanin (blue-green, diffusible) and flourescin (Diffusible, green under U.V.), but there are some strains that produce brown pigment and some melanin. Further, up to 10 percent of strains may be non-pigment. Early examination of cultures is essential or the characteristic pigments may be masked. Well isolated colonies are essential as pigment may diffuse and be taken up by non-pigmented, non-pseudomonads, thereby giving a false impression. Also, addition of nalidixic acid at a concentration of 15 ug/ml further ensures the

growth of Ps. aeruginosa only on cetrimide Agar medium. Addition of peptone and magnesium salts to the medium is also known to enhances the production of flourescent pigments, which are characteristic to Ps.aeruginosa.

4.1.3 TESTS FOR ESCHERICHIA COLI

A 10 % homogenate solution of the sample is made, and incubated at 37°C for 24 - 48 hours. Ten mililitres of this is inoculated into 100 ml of MacConkey broth, and incubated at 37°C for 18 - 24 hours. One millitre of the medium is subcultured into 5 ml of MacConkey broth and incubated at 44°C for 24 hours. From this medium, cultures are plated out onto the agar surfaces of MacConkey medium, incubated once again at 37°C for 24 - 48 hours. Emergence of red colonies, non-mucoid, of gram negative bacilli and surrounded by a reddish precipitation zone, gives a presumption of the presence of E. coli. Biochemical test such as Mackenzie tests or alternatively, tests for methyl red (MR), citrate, Indole and Voges - Proskauer (VP) are performed. Control test should be carried out at same time. Simulation to the characteristics given in Table 4.4 confirms the presence of E. coli.

Additional test that can be performed include:-

(i) Innoculation of a tube of lactose ricinoleate→

Table 4.4: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTIC OF E. COLI ON DIFFERENTIAL MEDIA

Media	Characteristic colonial Morphology	Biochemical	Reactions
MacConkey Agar	Brick-red, may have surrounding zones of precipitated bile.	Indole	+
		MR	+
		VP	-
		Citrate	-
		LRNB ($44^{o}C$) 24 hrs	Gas
		Gram Stain	-ve cocco-bacilli

→ broth (LRNB) with the suspected culture and incubated at $44^{o}C$. Production of gas within 24 hours indicate the presence of E. coli;

(ii) Streaking from suspected colonies to agar surfaces of Levine Eosin-Methylene-Blue medium and incubating at $37^{o}C$ for 18 - 24 hours. Upon examination, if colonies exhibit a characteristic metallic sheen under reflected light and a blue-black appearance under transmitted light, then E. coli presence is confirmed.

4.1.4 TESTS FOR SALMONELLA SPECIES -

A 10 % homogenate of the sample in nutrient broth or fluid soya-bean Casein digest medium is incubated at 37°C for 2 days. 1 ml of the portion is transferred into about 10 ml of either Selenite cystine broth or fluid tetrathionate media, incubating at 37°C for 12 - 24 hours. Streaks from this medium are made onto agar surfaces of Brilliant Green, Bismuth Sulfite or xylose lysine deoxycholate medium, and incubated for 1 - 2 days at 37°C. Characteristics of the resulting colonies are compared to those shown in Table 4.5

Table 4.5: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF SALMONELLA SPECIES ON DIFFERENTIAL MEDIA

Medium	Bismuth sulfite Agar	Xylose-lysine Deoxycholate Agar	Brilliant Green Agar
Characteristic Colonial Morphology	Black or green	Red with or without black centres	Small, transparent, colourless or pink to white, opague, Surrounded by Pink
Gram Stain	Gr -ve bacilli	Gr -ve baccili	Gr -ve bacilli
Sucrose	-ve	-ve	-ve
Lactose	-ve	-ve	-ve
Dulcitol	AG (Variable*)	AG (variable)*	AG (variable)*
Glucose	AG (A)*	AG (A)*	AG (A)*
Mannitol	AG	AG	AG
Urea	-ve	-ve	-ve

AG - Acid and gas

A - Acid only

* result for Sal. typhi

Characteristic colonies are inoculated into a slant butt tube of Tripple sugar Iron Agar medium, and also into urea broth, The formation of gas and acid in the butt (typified by yellow colouration) and the absence of acidity from the surface (shown by red or no colour change) together with a concomittant absence of red colour in the urea broth indicates presence of Salmonella. If acid but no gas is produced in the butt, further confirmatory test have to be done. Typical biochemical results are as shown in Tables4.5 and 4.6 for most Salmonella species and Sal. typhi. Complete identification can only be based on a detailed serological studies.

Table 4.6: PERFORMANCE OF SOME SALMONELLA SPECIES ON TRIPPLE SUGAR IRON AGAR MEDIUM

Organisms	Butt	Slope	H_2S
Salmonella typhosa	A	NC or ALK	-
Sal. paratyphi	AG	NC or ALK	-
Sal. Schottmuelleri	AG	NC or ALK	-
Sal. Choleraesuis	AG	NC or ALK	-
Sal. enteritidis	AG	NC or ALK	+
Sal. typhimurium	AG	NC or ALK	+

A = Acid (yellow)
AG = Acid (yellow) and gas formation
NC = No change in colour
ALK = Alkaline (red)
\+ = H_2S (black)
\- = no H_2S (no black colour)

4.1.5 QUANTITATIVE EVALUATION OF ENTEROBACTERIA

This test has now become official as recommended by the committee of official Laboratories and Drug Control Services, and the section of Industrial Pharmacists, FIP, 1975, which limits the number of other enterobacteria besides E. coli and Salmonella species in non-sterile pharmaceutical preparations. This test involves inoculating decreasing quantities of the homogenate (corresponding to 1.0, 0.1 and 0.01 gram) into suitable quantities of selective media such as Entereobacteriace Enrichment broth (Mossel) and incubating at 37°C for 24 - 48 hours. From this, cultures are streaked onto agar surfaces of crystal violet, neutral red, bile Salt Agar with dextrose medium and incubated at 37°C for 18 - 24 hours. Growth of well-developed colonies, generally red or reddish, of gram negative bacilli constitute a positive result. The data in Table 4.7 indicates the acceptable level provided that the tests for E. coli and Salmonella are negative.

Table 4.7: ACCEPTABLE LIMITS OF ENTEROBACTERIA IN NON-STERILE PREPARATIONS

Results with quantities			Interpretation
1.0 g	0.1 g	0.01g	
+	+	+	Rejected
+	+	-	Accepted
+	-	-	Accepted
-	-	-	Accepted

4.1.5 METHODS OF ENUMERATION OF VIABLE ORGANISMS

The most common methods of determining level of microbial counts are plating methods, membrane filtration and the tube dilution techniques. Normally, plating methods are preferred but other techniques can be employed depending on the nature of the product. For filterable liquid products, membrane filtration is the method of choice especially if microbial contamination level is low. For similar products that are water insoluble, the tube dilution technique is utilized.

(a) PLATING METHODS

Plating methods generally involve placing a known volume of a suitable dilution of the sample in or on a solid medium and incubating. The resultin

colonies are then counted. A dilution of the sample that would yield between 30 and 300 colonies per plate is preferred. If the count is low, a high margin of error may occur. High numbers of colonues would lead to overcrowding of colonies. The more common plating methods include the following:-

(i) Pour Plate Method

This can be of a single agar layer or double agar layer. In the single agar layer method, 1 ml of a suitable dilution of the sample is added to a 20 ml melted nutrient agar that has been cooled to 45°C, gently mixed and poured into a sterile plate. It is allowed to set, cooled and then incubated at inverted position at 37°C for 1 - 2 days. In case of fungi and moulds, Sabouraud dextrose agar is used in place of Nutrient agar. Some antibiotics such as tetracyclines, chloramphenicol and Penicillin G in calculated amount can be incorporated in the media to prevent the growth of bacteria. Incubation is done at 22 - 25°C for five days.

In the double agar layer procedure,.15 ml of nutrient agar is allowed to set on the petri-dishes. 1 ml of the solution of the sample is then mixed with 7 ml of sufficiently cooled (to 45°C) nutrient agar and poured over the first layer, allowed to set and incubated at 37°C for 1 - 2 days in an inverted position. In both cases, the number of colonies growing within and on the surface of the agar layer represents the number of viable cells in the 1 ml of the sample solution used.

(ii) Surface Viable Method

In this case, a standard dropping pipette is used to drop five drops of the solution of the sample on the surface of an over-dried nutrient agar plate. The pipette tip is held 2 - 3 cm from the agar surface. The drops are allowed to dry, care taken not to allow the drops to run into each other. The plates are inverted and incubated at 37°C for 1 - 2 days. The number of colonies represent the number of viable cells in the volume of 5 drops of the sample solution. The volume of each drop should have been previously calculated. The surface viable method may yield nigher count to damaged cells.

(iii) Roll - Tube Method

In this method, plugged test tubes containing about 2 ml nutrient agar are melted and cooled to 45°C. With a standard dropping pipette, 5 drops of the solution of the sample are dropped to the molten agar test tubes directly in such a way that the drops fall straight to the bottom of the tube and do not run down the sides of the tubes. The test tube is then shaken slightly without introducing air bubbles and is rolled under a jet of cold water from the tap so that a thin film of solidified agar uniformly covers the test tube. The test tube is then inverted and incubated at 37°C for 24 - 48 hours. The number of colonies is recorded and it represents the amount of viable cells in the 5 drops of the sample solution, from which by extrapolation, the number present in 1 ml or 1 gm of sample can be obtained.

(b) TUBE DILUTION METHODS

These are based on the premise that counts are distributed in accordance with the Poisson Theory which predicts that at any given level of mean count, there is a known probability of getting no organism in the sample i.e. no growth

is obtained when sample is introduced into broth and incubated. All other samples will have one or more organisms and so will show growth on inoculation into broth.

The techniques involve the preparation of a series of dilutions and inoculating from each dilution into serveral tubes of nutrient broth. After incubation, the pattern of sterile and fertile tubes is recorded and the count determined by reference to prepared tables. This is the "Most Probable Number" MPN. Tube dilution methods are generally used when very low counts particularly of swarming organisms are expected or when the presence of insoluble solids would interfere with other methods. The errors involved here are however large.

(i) Tube Dilution Method (U.S.P.) 1980

In this methods fourteen 20 x 150 mm test tubes containing 9 ml of sterile nutrient broth are used. Twelve of the tubes are arranged in four sets of three tubes each. One set of three tubes is set aside to serves as negative control to prove the sterility of the medium. The other two test tubes are labelled as A and B respectively. 1 ml of the dilution

of sample is pipetted into each of a second set of three test-tubes and test tube A. The contents are shakened thoroughly to effect mixing. The set of three tubes is labelled 1:10 dilution. From tube A, 1 ml is pipetted into another set of three test tubes and as well into tube B. The contents are again mixed. This new set of three tubes is labelled 1:100 dilution. From tube B, 1 ml is again pipetted to the last set of three test tubes, labelled 1:1000 dilutions and mixed. All the four sets of three tubes together with tubes A and B are incubated at 37°C for 48 hours. The number and pattern of tube showing growth is recorded and from prepared tables, the probable number of organisms in the dilution of the sample used is calculated (Table 4.8).

Table 4:8 MOST PROBABLE NUMBER (MULTIPLE) U.S.P.

Observed Combinations of no. of tubes showing growth in each set. No of mg or ml of sample per litre (Dilution levels)			MPN of organisms per gm/ml
1:10	1:100	1:1000	
3	3	3	1100
3	3	2	1100
3	3	1	460
3	3	0	240
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	93
3	1	3	160
3	1	2	120
3	1	1	75
3	1	0	43
3	0	3	95
3	0	2	64
3	0	1	39
3	0	0	23

(ii) FISHER AND YATES METHOD

This is a mathematical approach to calculating the most probable number of viable organisms in a product. The process of dilution series is same as employed for the U.S.P. tube dilution method. In this, the following notations are given:-

S = number of dilution levels

a = dilution factor

n = number of tubes at each dilution level.

The most accurate estimate of the number of viable bacteria per tube at any given level is obtained by solving the equation of maximum likelihood. By statistical data, 87 .7 % of the information is contained in the total number X of fertile or Y of sterile tubes counted without regard to level. If X is the number of organisms per tube at the highest concentration, the value of X for which the expected number of sterile tubes is equal to the observed number is given by the question below:

$$Y = n(e^{-\lambda} + e^{-\lambda/a} + e^{-\lambda/a^2} + \ldots\ldots e^{-\lambda/a^{s-1}})$$

The solution is easily obtained for a 10-fold dilution from Fisher and Yates table. All that is required to be done is to calculate the mean fertile level, $x = \frac{X}{n}$ and the mean sterile level $y = s - x = \frac{Y}{n}$ and to find the tabular value K corresponding to the observed x or y for the appropriate number of levels. Then

$$\log X = x \log a - K.$$

For an accurate determination of X, more dilution levels than are used in the U.S.P. method is necessary.

(c) MEMBRANE FILTRATION METHOD

A known volume of sample solution is passed through a sterile bacteria proof membrane filter. The membrane containing the cells is aseptically transferred to freshly prepared nutrient agar plate or a sterile filter paper pad soaked in nutrient broth. After incubation at 37°C for 24 - 48 hours, the resulting colonies are counted. The number of colonies should not be more than 200 per membrane. This number represents the sample filtered. Usually, a membrane filter of pore size of 0.45 u is made use of. This method is very suitable for relatively large volumes of solution containing small numbers of bacteria. For yeast and mould counts, the same procedure should be followed and the membrane filter placed on a fungal medium containing antibiotics.

Among the three major methods for the enumeration of bacteria in a sample, the plate method is the best because it permits a precise count within a large range of concentrations. Filteration method is least suitable because only 20-50 colonies are allowed per membrane while the precision of Most Probable Number (MPN) is very poor. Differences in the latter between workers can be statistically highly significant.

4.2.0 MATERIALS

The raw materials used were same in source and quality as described in Chapter Three, Page 51 - 52

CULTURE MEDIA

Nutrient broth, Nutrient Agar, Sabouraud Dextrose Agar, Cetrimide Agar, Mannitol Salt Agar, Bismuth Sulphite Agar, MacConkey Agar and Tripple Sugar Iron Agar. They were all dehydrated oxoid Products produced by Oxoid Ltd, London

4.3.0 EXPERIMENTAL

4.3.1 Preparation of Homogenate Solution

Nutrient broth was used throughout as the dilution medium. 10 gm of the raw material was weighed into sterile small beakers, and later transferred aseptically to the 90 ml nutrient broth in the conical flasks. The contents were uniformity mixed either by slight agitation or by shaking vigorously for a minute or two depending on the solubility of the raw materials. Such substances like lactose and maize starch were readily soluble at the 10 % w/v homogenate solution.
Others like talc, Avicel, Folic acid and Dicalcium

phosphate were easily dispersed in suspension forms. However, for magnesium Stearate powder, 2 % v/v Polysorbate 80 (Tween 80) was added to the nutrient broth, before it could be uniformly dispersed. For gelatin granules, a lower percentage of 2·5% w/v of the homogenate solution was made as a 10 % w/v one easily gelled on standing. A preliminary investigation was also carried out, especially to ensure that appropriate dilution levels were used which would give a desirable microbial level range that can easily be counted.

4.3.2 Pour Plate (Viable Counting) Method

The prepared sterile Nutrient Agar and Sabouraud Dextrose Agar were melted in a water-bath at 98° - 100°C. Using sterile pipettes, 1 ml of the prepared homogenate solution were drawn into sterile plates. The melted agar was allowed to cool to 45°C and slightly below, poured on the plate containing 1 ml of the homogenate solution. Replacing the cover of the plate, the content were mixed uniformly by tilting gently and rotating the plate. After, the content was allowed to solidify and set. Ten plates were used for each dilution, at each time, in case of Nutrient Agar while three plates, in case of Sabouraud Dextrose Agar. The whole process was

carried out under the Laminar Air Flow Cabinet. The set plates were then incubated in the inverted position at the appropriate temperature and time conditions - nutrient agar plates at $37^{\circ} \pm 2^{\circ}C$ for 24 - 48 hours and the Sabouraud Dextrose Agar plates at 22° - $25^{\circ}C$ for 2 - 3 days. The number of colonies were counted using a Gallenkamp Colony Counter, and the total number present per gram of the raw material was determined. In cases where the homogenate solutions had suspended particles, for example, Avicel, the suspension was allowed to settle and portions from the supernatant layer were used for the analysis.

4.3.3 EXAMINATION OF CONTAMINANTS

The common contaminants were examined based on their colonial morphology with simple staining and Gram stain.

4.3.4 Examination For Selective Organisms

Since nutrient broth was used in preparing the homogenate solution, it acted as a pre-enrichment medium. About 10 ml of the homogenate solutions were transferred to sterile test-tubes and incubated at $37^{\circ}C$ for 24 - 48 hours, to allow for sufficient growth. Subsequently, cultures from this were streaked (plated)

on appropirate dehydration agar. Cultures were taken from the 48 hours incubated homogenate solution and streaked on the agar surfaces of the differential/ diagnostic media.

From standard cultures of Staph. aureus (NCTC 6571), Salmonella, Escherichia coli (NCTC 10418) and Ps. aeruginosa (NCTC 10662), streaks were made on appropriate diagnostic agars such as Mannitol Salt, Bismuth Salphite, MacConkey and Cetrimide Agars, They (Standard Organisms) served as controls. Both test and control plates were incubated at 37°C for 18 - 24 hours. The colonial morphology of the test and Control plates were compared. Where there were similarities, microscopical examinations were done and where necessary, other differential media and or biochemical test were employed. (see Tables 4.2, 4.3, 4.4, 4.5 and 4.6).

4.4 RESULTS

The raw materials were tested for their level of contamination and types of contaminants. The result for the number of organisms are shown in Table 4.9.

The various types of contaminants were differentiated based on their morphological characters, and Gram stain results. Tests showed the absence of the designated specific pathogens

Ps. aeruginosa, Staph. aureus, E. coli and Salmonella species. The different bacterial contaminants are as shown in Table 4.10.

Table 4:9 MICROBIAL LEVELS IN THE RAW MATERIALS

Raw materials	Bacterial Counts *cfu per gram	Fungal Count cfu per gram
Folic Acid	5	3
Microcrystalline Cellulose PH 101 (Avicel)	3	2
Dicalcium phosphate (Powder)	5	6
Magnesium Stearate	35	3
Lactose USP	60	1
Starch Granules	391	17
Maize Starch	420	2
Talc	930	5
Gelatin	1200	0
Polyethylene glycol 6000 (Powdered)	10	20

*Cfu - Colony forming Units

Table 4.10: Various Morphological Forms of Bacterial Contaminants in Raw Materials for Folic Acid Tablets

Substance	Colonial Morphology	Microscopical characters	Pathogens Identified
A. Folic Acid Powder	1. Light yellowish circular, flat, entire, Opaque, Smooth and shinning colony having a halo around it.	Gr +ve bacilli, in long chains 0.5 u x 3.0 u	
	2. Off-white, flat, Opaque, circular, slightly granuler, radially striated and crenated edged colony.	Gr +ve cocci, in chains and clusters. $(0.7 - 0.8\ u)^2$	None
	3. Orange, circular, flat, transluent, lobate edged, smooth and shining colony with wide halo.	Gr -ve rods, singly and diplobacillus. 0.4 u x 0.8 - 1.0 u	
B. Magnesium Stearate	Light yellow, circular, raised, entire, opaque and shinning colony.	Gr +ve cocci in chains and clusters. 0.4 u x 0.4 u	
	2. Red, circular, raised, lobate, Opaque, dull and striated colony.	Gr +ve cocci, mostly single. 0.6 u x 0.6 u	None
	3. White to dirty white, irregular, flat, Opaque, crenated, and smooth colony.	Gr +ve bacilli, singly with spores. 0.5 u x 3.0 - 4.0 u	
	4. White, spreading, flat, Opaque, crenated, smooth and shinning colony.	Gr +ve bacillus, singly, with terminal spores. 0.5 u x 1.5 - 2.0 u	

Substance	Colonial Morphology	Microscopical characters	Pathogens Identified
C. Lactose powder B.P/ U.S.P.	1. Light Orange, ciruclar, flat, Opaque entire, dull and striated colony.	Gr -ve cocco-bacilli, singly and in short chains 0.5 u x 0.8 u.	
	2. Milky white, irregular, raised, opaque, undulate and shinning colony.	Gr +ve single rods with terminal spores. 0.4 - 0.5 u x 1.0 u.	
	3. Cream, irregular, opaque, slightly raised, lobate, granular with papillae.	Gr +ve single bacillus with spores. 0.4 u x 1.0 - 2.0 ug	None
	4. Dirty white, irregular, flat, opaque with fimbriate edge, smooth and dull colony.	Gr +ve single and diplobacillus. 0.5 u x 1.0 - 1.5 u.	
D. Talc	1. Pink to Pinkish red, circular, raised, opaque entire, smooth and shinning colony.	Gr +ve cocci, in clusters, few in chains. $(0.5 - 0.7 \text{ u})^2$	
	2. Orange, circular, raised, opaque, entire, radially striated, and granular colony.	Gr -ve rods, singly. 0.5 u x 0.8 - 1.5 u.	
	3. Yellow, circular, raised, entire, opaque and smooth colony.	Gr +ve cocci, in groups, singly and diplo. 0.4 u x 0.4 u.	
	4. Dirty white, irregular, flat, translucent dull, lobate and smooth colony	Gr +ve rods, diplo, in chains of 4 cells, with terminal spores. 0.8 u x 1.0 - 2.0 u.	

Substance	Colonial Morphology	Microscopical characters	Pathogens Identified
	5. Colourless, circular, raised, transparent, slightly lobate and shinning colony.	Gr +ve cocci, in groups and chains of 2 - 3 cells.	None
	6. White, circular, raised, opaque, almost entire, shinning colony.	Gr +ve rods, singly with polar spores. 0.5 u + 2.0 u.	
	7. Grey brown, circular, entire, raised, translucent, smooth colony.	Gr +ve cocci, mostly diplococcus. $(0.4 - 0.5\ u)^2$	
	8. Colourless, irregular, crenated, flat, transparent and smooth colony.	Gr -ve bacillus, mostly singly, few diplobacillus 0.5 u x 0.8 u.	
	9. Ash, circular, entire, dome, opaque dull and granular colony.	Gr +ve cocci, chains mostly. 0.7 u x 0.7 u.	
	10. Milky white, circular, dome, entire, opaque, smooth and shinning colony of diameter size 1 - 2 mm.	Gr +ve cocci, singly, some in in groups. $(0.5 - 0.6\ u)^2$	
E. Micro-crystalline cellulose (Avicel)	1. Milky white circular raised, entire, opaque, smooth and shinning colony.	Gr +ve cocci, mostly in clusters. $(0.4 - 0.5\ ug)^2$	None
	2. Yellow, circular, flat, entire, opaque, shinning colony with pronounced papillae.	Gr +ve cocci, mainly in chains. 0.7 u x 0.7 u.	

Substance	Colonial Morphology	Microscopical characters	Pathogens Identified
F. Maize Starch Powder	1. Yellow, circular, raised, entire, Opaque, smooth and shinning colony.	Gr +ve cocci, in groups, and singly. $(0.7 - 0.8 ug)^2$	
	2. Off-white, irregular to spindle shaped, opaque, dull, undulate to entire, slightly raised, smooth colony.	Gr +ve cocci, singly and in groups. $(0.5 - 0.7 u)^2$.	
	3. Pinkish-brown, circular, raised, opaque, entire, and granular colony.	Gr +ve bacillus, mostly single and diplobacillus, with spores. 0.5 u x 1.0 - 2.5 u.	None
	4. Cream, irregular, rhizoid, opaque, umbonate, coarse granular colony	Gr +ve bacillus, mostly diplobacillus. 0.5 u x 2.0 u.	
	5. Pinkish red, dome, entire, circular, opaque, smooth and shinning colony of diameter size 4 mm.	Gr +ve cocci, in short chains of 3 - 6 cells. $(0.4 - 0.6 u)^2$	
G. Starch Granules (Wet granulated)	1. Buff to colourless, spreading, flat, almost entire, translucent, slightly granular colony of diameter size greater than 30 mm.	Gr +ve, small rods, few diplo and s short chains, with central, oval spores, 0.5 u x 1.0 - 1.5 u.	

Substance	Colonial Morphology	Microscopical characters	Pathogens Identified
	2. White, serrated, almost flat, translucent, coarse colony of diameter size 10 - 12 mm.	Gr -ve rods, singly and few diplob- acillus. 0.5 u x 0.8 - 1.0 u.	None
	3. Yellowish orange, circular, flat, entire, almost opaque, smooth and shinning colony of diameter size 5 - 6 mm.	Weakly gr +ve rods, singly and few diplobacillus with granules. 0.8 - 1.2 u x 1.5 - 2.5 u.	
	4. White, circular, raised, entire, opaque smooth colony of diameter size 2 - 3 mm.	Gr +ve rods, mostly single, with central, subterminal, oval spores. 0.4 u x 2.0 u.	
H. Gelatin	1. Greenish yellow, circular, entire, dome, opaque, smooth and shinning colony.	Gr +ve cocci, mostly in chains, few clusters. $(0.5 - 0.6 u)^2$	
	2. Red, circular, entire, dome, opaque, smooth and shinning colony.	Gr +ve cocci, singly and diplocci. 0.6 u x 0.6 u.	
	3. Yellowish red, circular, flat, undulate, some raised, transl- ucent smooth colony.	Gr +ve diplobacillus, some in chains. 0.4 - 0.5 u x 1.5 u.	None

Substance	Colonial Morphology	Microbcopical characters	Pathogens Identified
H. Gelatin	4. Colourless, irregular, lobate, flat, transparent, granular and dull colony.	Gr -ve, mostly single and few diplobacilus. 0.5 - 0.7 u x 0.8 - 2.0 u.	
	5. Light to grey brown, circular, crenated, raised, granular and transparent colony.	Gr +ve cocci, mostly single, $(0.4 - 0.5\ u)^2$.	None
	6. Buff to grey, circular, flat, crenated, transparent and granular colony.	Gr -ve cocco-bacillus, mostly single. 0.4 - 0.5 u x 0.8 u	

4.5 DISCUSSION

The results obtained especially with respect to microbial levels of the raw materials conform to the observations that had been made by an earlier investigator (Westwood 1971). Synthesized chemicals such as folic acid, microcrystalline cellulose (Avicel), Dicalcium phosphate and Magnesium stearate have very low microbial counts. Lactose which is also produced by chemical process is relatively not much contaminated. The contaminants in the above raw materials are likely to have come from the air, personnel and the environment where they were made, since the manufacturing processes for these chemicals are normally not condussive for microbial growth.

Maize starch was found to be relatively quite contaminated. Starch, a product from plant, is a carbohydrate, and can serve as a source of nutrient for bacterial growth. The common contaminants as found showed both Gram positive cocci and bacilli, and Gram negative bacillus aerobic organisms, which is typical of substances obtained from botanical sources. In the case of the contaminants from microcrystalline cellulose and magnesium stearate, the predominant contaminants are Gram +ve cocci (normal residents of the skin and respiratory tract) and Gram +ve rods.

Usually, substances of natural earth are heavily contaminated. This was the case with Talc. It had been explained (Hugo and Wilson 1971, and Wilson, 1972) that the high level of contaminants associated with talc is due to nutrients leached from it, which aids bacterial proliferation. The contaminants are mostly gram positve rods and cocci. Some of the gram positive cocci are likely to be of the genus Staphylococcus. The gram positive spore bearing bacilli are also a representative of soil bacteria. The source of talc had been shown to influence its level of microbial contaminants.

Gelatin was found to be heavily contaminated, the common contaminants being gram positive and gram negative bacilli. The high level of contaminants could be due to the nutritive value of gelatin. Moreover, gelatin has the ability of absorbing large quantity of moisture from the atmosphere without much altering its physical appearance. The amount of moisture taken could be such that the minimum water activity (Aw_i) especially in damp areas, for most bacteria is reached, thus allowing for bacterial proliferation.

The level of moulds was found to be generally low in all of the raw materials, the highest being that of starch granules and polyethylene glycol due to their exposure during preliminary processing into granules. The presence of a few fungal spores in a raw material stored in a damp environment, can lead to quality deterioration of the material due to rapid growth of moulds that easily spread and biochemically act on it.

While some pharmacopoieas do not impose any limit on the level of microbial contaminants except the requirement for absence of pathogenic organisms, others like the Swedish Pharmacopoiea and European Pharmacopoiea did state that raw material should not contain microbes of more than than 100 gm. If this limit is accepted, it would mean that all synthesized products used in this investigation meet this requirement. Though this limit can not be applied to raw materials of natural and inorganic origins, it is desirable nevertheless, that raw materials of low microbial counts should be used as a prelude to obtaining finished products of low microbial counts.

CHAPTER FIVE

DETERMINATION OF MICROBIAL CONTAMINATION OF FOLIC ACID TABLETS PRODUCED BY WET GRANULATION METHOD

The wet granulation method of tablet production involved at least six various stages, Starting from the stage of raw materials, milling, through mixing stages to the stage of tablet compression. This multistage process highly enhances the likelihood of microbial contamination of the final product, much more than in a two or three-stage process.

The wetting stage involves use of water which had been shown to be of a high microbial contaminant. (1971 Pharmaceutical Society Working Party Report). The main objective of the work reported in this chapter was to determine the microbial contamination potential of the production environment, as well as the various stages of this method of tablet manufacturing.

5.1 MICROBIAL FLORA OF AIR

Air contamination can be measured in two ways:-

(i) by direct exposure of agar plates to air in the production environment, and the result expressed in terms of the number of colonies per minute of exposure;

(ii) by the use of a Slit Sampler using a vacuum pump. The latter method was employed as it ensures a better sampling within a shorter time.

5.2 MICROBIAL FLORA OF SURFACES

Microbial contamination of the working surfaces including those of the major machines used had to be considered. Microbial level of surfaces could be determined by several methods including Swabbing and rinsing which break up bacteria clones; and contact plating or direct surface agar plating, which replicate surface bacterial clones. The Swab method was utilized, as this was both convenient and reliable.

5.3 MATERIALS AND METHODS

5.3.1 MATERIALS

Maize Starch Powder
Magnesium Stearate Powder
Talc Powder
Gelatin and
Folic acid Powder.

The raw materials as mentioned above, were the same in source and quality as expressed in Chapter three, page 51.

CULTURE MEDIA

The culture media used were Nutrient Agar, Nutrient Broth, Sabouraud Dextrose Agar, Bismuth Sulfite Agar, Mannitol Salt Agar, MacConkey Agar, Cetrimide Agar and Tripple Sugar Iron Agar, all dehydrated Oxoid Products.

5.3.2 PRODUCTION OF FOLIC ACID TABLETS BY WET GRANULATION METHOD

The weighed amount of folic acid powder was mixed with the required amount of Maize Starch powder in a Manesty drum mixer in environment A. The gelatin binder solution which was prepared by adding a certain quantity of an almost boiling distilled water, was added to the above mixture and granulated until a wet mass was obtained. It was then wet-screened through an appropriate mesh screen, in an Oscillating granulator, after which the wet granules were dried at 50°C or at room temperature as desired. The resulting dried granules were dry-screened through an appropriate mesh screen. The lubricant and glidant (magnesium Stearate and talc), together with the rest amount of the folic acid powder were mixed with the screened dry granules, and then taken for compression in a tablet press. (Detailed method and working formula have

Table 5.1 Microbial Contamination Level ofAAir in Two Tablet Production Environments A and B

	Environment	Bacterial count cfu/m^3	Fungal count cfu/m^3
	Tabletting Room	2300	
*A	General Packaging section	3000	2700
	Production Room (People present)	3300	
	Production Room (No personnel present)	2000	
+B	Student Laboratory	5000	1000

*A - A Pharmaceutical Manufacturing Firm in Kaduna State.

+B - Process Laboratory of the Department of Pharmaceutics and Pharmaceutical Microbiology, A.B.U., Zaria, NIGERIA.

Table 5.2 Various Morphological Types of Bacterial Contaminants of Air in Environment.

Sample analysed	Colonial Morphology (size is that of diameter expressed in mm)	Microscopical character	Pathogens Identified
Air in Environment A	1. Bright yellow, circular, convex, entire, slightly translucent, smooth and shinning colony of size 1 - 2 mm.	Gr +ve cocci, in clusters, 0.8 u x 0.8 u.	
	2. Dark orange, circular, raised, entire, opaque, smooth and shinning colony of size 2 - 3 mm.	Gr +ve cocci, in scattered groups. $(0.6 - 0.7 \text{ u})^2$	
	3. Creamy white, circular, convex, entire, opaque, smooth and shinning colony of 2 - 3 mm.	Gr +ve cocci, mostly in clusters, few in chains of 3 - 5 cells. 0.5 u x 0.5 u	Staph. aureus.
	4. Dirty white, almost circular, entire, flat, dull and granular, opaque colony of size 8 - 10 mm.	Gr +ve rods in chains with central spores, 2 - 8 cells per chain. 0.4 u x 1.5 - 6.0 u.	
	5. White, circular, slightly raised, entire, smooth and opaque colony of size 3 - 4 mm.	Gr +ve rods, singly with central, oval spores larger than vegetative cell. 0.5 u x 2.0 u.	
	6. Red, circular, almost convex, entire, opaque, smooth and shinning colony of size 2 - 2.5 mm.	Gr +ve cocci, mostly in chains of 4-7 cells, few tetrads. $(0.3 - 0.4 \text{ u})^2$	

Sample analysed	Colonial Morphology (size is that of diameter expressed in mm)	Microscopical character	Pathogens Identified
Air in Environment A	7. Yellowish, orange, circular, raised, entire, opaque, dull and slightly granular colony of size 4 mm.	Gr +ve rods in chains of 2 - 4 cells, with central spores. 0.8 u x 1.5 u.	
	8. Faint Pink, circular, raised, entire, opaque, smooth and shinning colony of size 2.0 - 2.5 mm.	Gr +ve cocci, mostly singly and in small groups. $(0.4 - 0.5 u)^2$	
	9. Milky white, circular, convex, entire, smooth, and shinning, opaque colony of size 1 mm.	Gr +ve cocci, in diplo and in small groups. 0.8 u x 0.8 u.	

Table 5.3: Various Morphological Forms of Bacterial Contaminants of Air in Environment B

Item analysed	Colonial Morphology (size is that of diameter, expressed in mm)	Microscopical character	Pathogens Identified
Air in Environment B	1. Yellow, circular, raised with wide papillae, entire, opaque, smooth and shinning colony of size of 3 - 4 mm.	Gr -ve cocco-bacilli, mostly diplobacillus. 0.4 u x 0.6 - 0.8 u.	
	2. Cream, circular, flat, with papillae, entire, opaque and granular colony of size 4 mm.	Gr +ve cocci, singly and some diplococcus. $(0.6 - 0.8\ u)^2$	
	3. Milky white, circular, raised, entire, opaque, smooth and shinning colony of size 1 - 2 mm.	Gr +ve cocci, in clusters. $(0.5 - 0.6\ u)^2$	
	4. Pink, circular, raised, entire, opaque, smooth and shinning colony of size 4 - 5 mm.	Gr +ve cocci, singly, few in groups. 0.5 u x 0.5 u.	Ps. aeruginosa.
	5. Light yellow, circular, raised, entire, smooth and shinning, slightly translucent colony of size 4 - 5 mm.	Gr -ve motile, single rods, few are diplobacillus. 0.4 x 0.5 u x 0.8 - 1.0 u.	
	6. Light orange, circular, raised, entire, opaque, smooth and shinning with papillae, colony of size	Gr +ve cocci, mostly diplococcus and few in chains of 3 cells. $(0.5 - 0.7\ u)^2$	

Item analysed	Colonial Morphology (size as that of diameter expressed in mm)	Microscopical character	Pathogens Identified
Air in Environment B	7. Slightly dirty white, flat, spreading, lobate, opaque, smooth and shinning colony of size 10 - 15 mm.	Gr +ve rods, in long chains, some with sub-terminal spores. 0.5 u x 1.5 - 2.5 u.	
	8. Off-white, circular, flat, entire, opaque, smooth, shinning colony with concentric circles, of size 3 - 6 mm.	Gr +ve rods, mostly single, with sub-terminal spores, larger than vegetative cells. The cells with spores look like tennis racquet. 0.4 - 0.5 u x 1.5 - 2.0 u.	
	9. Golden yellow, circular, raised, entire, smooth and shinning, slightly translucent colony of size 1.5 - 2.0 mm.	Gr +ve cocci, mostly diplococcus and short chains of 3 cells. $(0.4 - 0.6\ u)^2$	

Table 5.4: Microbial contamination level of Distilled water used for wet granulation

Type of Water		Bacterial count cfu/ml	Fungal count cfu/ml
Distilled water	Freshly distilled water (collected aspetically)	0	0
	Distilled water in a sterile container, uncovered during collection	5	0
	Freshly distilled water in an unsterile containers	30	10
	Distilled water, stored for a day	1070	-
	Distilled water stored for four days	67000	160
Tap, undistilled water		24500	100

Table 5.5: Various Morphological Forms of Bacterial Contaminants of Distilled Water

Colonial Morphology (Size is that of diameter expressed in mm).	Microscopical character	Pathogens Identified
1. Light golden yellow, raised, circular, entire, translucent, smooth and shinning colony of size 1.0 - 1.5 mm.	Gr -ve rods, mostly singly. 0.4 u x 0.8 u.	
2. Faint greenish-yellow, circular, entire, raised, translucent, smooth and shinning colony of size 1.0 - 1.5 mm.	Gr -ve rods, singly and few diploba-cillus. 0.4 u x 0.5 - 0.8 u.	Ps. aeruginosa
3. Light Orange, circular, raised, entire, Opaque, smooth colony of size 0.5 - 1.0 mm.	Gr +ve cocci, in diplococci and chains of 3 cells, $(0.5 - 0.6\ u)^2$	
4. Pink, circular, raised, entire, opaque smooth and shinning colony of size 4 - 5 mm.	Gr +ve cocci, singly, few in groups. 0.5 u x 0.5 u.	

Table 5.6: Various Morphological Forms of Bacterial Contaminants of Tap Water.

	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical character	Pathogens Identified
1.	Dark Orange to slightly reddish Orange, circular, convex, entire, opaque, smooth and shinning colony of size 2 - 3 mm.	Gr +ve cocci, in diplo but mostly in chains of 3 - 4 cells. 0.4 u x 0.4 u.	
2.	Yellowish Orange, circular, entire, convex, Opaque and smooth colony of size 1 - 2 mm.	Gr +ve rods, diplobacilli only. 0.4 u x 0.8u.	Ps. aerugi-nosa.
3.	Creamy white, circular, convex, entire Opaque, smooth and shinning colony of size 3 - 4 mm.	Gr -ve motile, rods, mostly single. 0.4 u x 0.8 u.	
4.	Faint greenish yellow, circular, entire raised, translucent, smooth and shinning colony of size 1 mm.	Gr -ve rods, singly and diplobacillus. 0.4 u x 0.8 u.	

Table 5.7: Microbial Contamination Level of Oven and Tablet Press

Equipment Sampled	Environment A		Environment B	
	Bacterial count cfu/cm^2	Fungal count cfu/cm^2	Bacterial count cfu/cm^2	Fungal count cfu/cm^2
Oven	6.0	8	8.5	0.7
Tableting machine	8.5	6.5	10.3	4

Table 5.8: Various Morphological Forms of Bacterial Contaminants of Tabletting Machine in Environment A

Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical character	Pathogens Identified
1. White, circular, flat, almost entire, slightly translucent, smooth and shinning colony of size 8 - 10 mm.	Gr +ve, rods, with oval, central and subterminal spores 0.8 u x 2.5 u	
2. Light yellowish white, circular, convex, Opaque, smooth and shinning colony of size 1.0 - 1.5 mm.	Gr -ve rods, mostly single and motile. 0.4 u x 0.7 u.	
3. Milky white, circular, convex, entire, opaque, smooth and shinning colony of size 1 - 2 mm.	Gr +ve cocci, mostly in clusters. $(0.6 - 0.8\ u)^2$	
4. Yellow, circular, convex, entire, slightly translucent, colony of size 0.5 - 1.0 mm.	Gr +ve cocci, diplo, tetrads and in groups. $(0.5 - 0.6\ u)^2$	None
5. Colourless, circular, raised, entire, transparent, smooth and shinning colony of size 3 - 4 mm.	Gr +ve slender rods, singly a few with subterminal spores. 0.3 - 0.4 u x 1.0 - 3.5 u.	
6. Off-white, raised, entire, opaque, slightly circular, granular and dull with papillae colony of size 3 - 5 mm.	Gr +ve (weak) rods, mostly in long chains with granules, cells wound mound each other 1.0 u x 2.0 - 4.0 u.	

Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical character	Pathogens Identified
7. Slightly off-white, flat, lobate, translucent, granular, dull, slightly irregular colony of size 10 - 14 mm.	Gr +ve rods, single, diplo, and short chains, with Oval, Central and subterminal spores. 0.6 - 0.7 u x 1.5 - 2.0 u.	
8. Light Orange, flat, lobate, dull, smooth, trarslucent, slightly irregular colony of size 8 - 12 mm.	Gr +ve rods, in small chains with liberated spores. 0.7 u x 2.0 - 2.5 u.	

Table 5.9: Various Morphological Forms of Bacterial Contaminants of Tabletting Machine in Environment B

	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical character	Pathogens Identified
1.	Light yellow, circular, raised, entire, opaque, granular with papillae. Colony size of 3 - 4 mm.	Gr +ve rods, in long chains of at least 8 cells, wound in a mass. 0.8 x 1.0 u x 1.5 - 2.0 u.	
2.	Milky white, convex, circular, opaque, entire, smooth and shinning colony of size 1.0 - 1.5 mm.	Gr +ve cocci, in small clusters. $(0.6 - 0.8\ u)^2$	
3.	Creamy Orange, circular, raised, entire, opaque, smooth and shinning colony of size 2.0 - 2.5 mm.	Gr +ve cocci, singly, diplococcus, and in small groups. 0.5 u x 0.5 u.	
4.	White, circular, entire, Opaque, flat, smooth colony with concentric circles on surface. Size is 4 mm.	Weakly gr +ve rods, in long chains, with granules, wound round each other. 0.8 x 1.0 u x 2.5 - 4.0 u	None.
5.	Light Pink, circular, raised, entire opaque, smooth colony of size 0.5 mm.	Gr +ve cocci, mostly single. $(0.5 - 0.7\ u)^2$	
6.	Colourless, flat, smooth, dull, serrated edged transparent, spreading colony of size greater than 50 mm.	Gr +ve rods, in long chains with sub-terminal spores. 0.5 u x 1.5 - 2.5 u	

Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical character	Pathogens Identified
7. Light to golden Orange, circular, entire, smooth, raised, translucent colony of size 1.0 mm.	Gr +ve cocci, few diipl but most ar short chains and small groups. 0.5 u x 0.5 u.	
8. Dirty white, irregular, lobate, flat, Opaque. smooth colony of size 8 - 10 mm.	Gr -ve rods, singly and 2 - 3 cells per chain. 0.4 - 0.5 u x 1.0 u.	
9. Buff to almost colourless, lobate, spreading, slightly raised, slightly granular, translucent colony.	Gr +ve rods, mostly single, few in chains. 0,5 u x 1.0 - 1.5 u.	

Table 5.10: Various Morphological Forms of Bacterial Contaminants of Oven in Environment A and B

Item analysed		Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical character	Pathogens Identified
Oven in Environment A	1.	Creamy Orange, circular, entire, convex, Opaque, smooth and shinning colony of size 1.0 - 2.0 mm.	Gr +ve cocci, in groups. 0.5 u x 0.5 u.	
	2.	Light yellow, circular, convex, entire, Opaque, smooth and shinning colony of size 1 mm.	Gr +ve cocci, tetrads and in groups. 0.5 u x 0.5 u.	
	3.	Milky white, raised, circular, entire, Opaque, smooth and shinning colony of size 1 - 1.5 mm.	Gr +ve cocci, mostly in clusters. $(0.6 - 0.8 \text{ u})^2$	
	4.	Almost colourless, circular, entire, raised, transparent and shinning colony of size 4 - 6 mm.	Gr +ve rods, slender, singly with subterminal spores larger than vegetative cell 0.4 u x 2 - 4.0 u.	None.
	5.	Dirty white, irregular, flat, lobate, granular, translucent colony of size 7 - 10 mm.	Gr +ve rods, diplo and in chains with central and subterminal spores. 0.7 u x 2.0 u.	
	6.	Cream, circular, raised, entire, Opaque, smooth and shinning colony of size 2 - 3 mm.	Gr +ve cocci, tetrads and in small clusters. 0.4 u x 0.4 u.	

Item analysed	Colonial Morphology (Size is that of diameter in mm)	Microscopical character	Pathogens Identified
Oven in Environment A	7. Light orange, circular, flat, translucent, smooth, almost entire colony of size 8 - 10 mm.	Gr +ve rods, with oval, terminal spores, diplobacillus and in chains 0.8 - 1.0 u x 2.0 u.	
	8. Slightly colourless, flat, fimbriate, dull, smooth, spreading, transparent colony of size greater than 40 mm.	Gr +ve rods, singly and diplobacillus, with oval, central spores, 0.5 u x 1.0 - 2.0 u.	
Oven in Environment B	1. Buff to almost colourless, circular, flat, entire, translucent, slightly granular colony of size 5 - 7 mm.	Gr +ve rods, singly, diplo and short chains, with oval subterminal spores. 0.6 u x 1.5 - 2.0 u.	None.

Table 5.11: Microbial Contamination Levels at Various Stages of Wet Granulation method in Environment A

Stages	Batch I		Batch II		Batch III	
	Bacterial count cfu/gm	Fungal count cfu/gm	Bacterial count cfu/gm	Fungal count cfu/gm	Bacterial count cfu/gm	Fungal count cfu/gm
A Initial mixture	437	6	378	10	400	7
B Wet mixture	27000	20	10380	70	15000	30
C Dried Granules	900	10	145	30	200	10
D Final Mixture	400	20	83	40	133	24
E Produced Tablets	50	5	43	10	38	5

	Bacterial count, cfu/ml	Mould count cfu/ml
Distilled water in second Batch	6.7×10^3	40
Binder solution in second Batch	1.5×10^4	50

FIG 5.1: MICROBIAL CONTAMINATION LEVELS AT VARIOUS STAGES OF WET METHOD

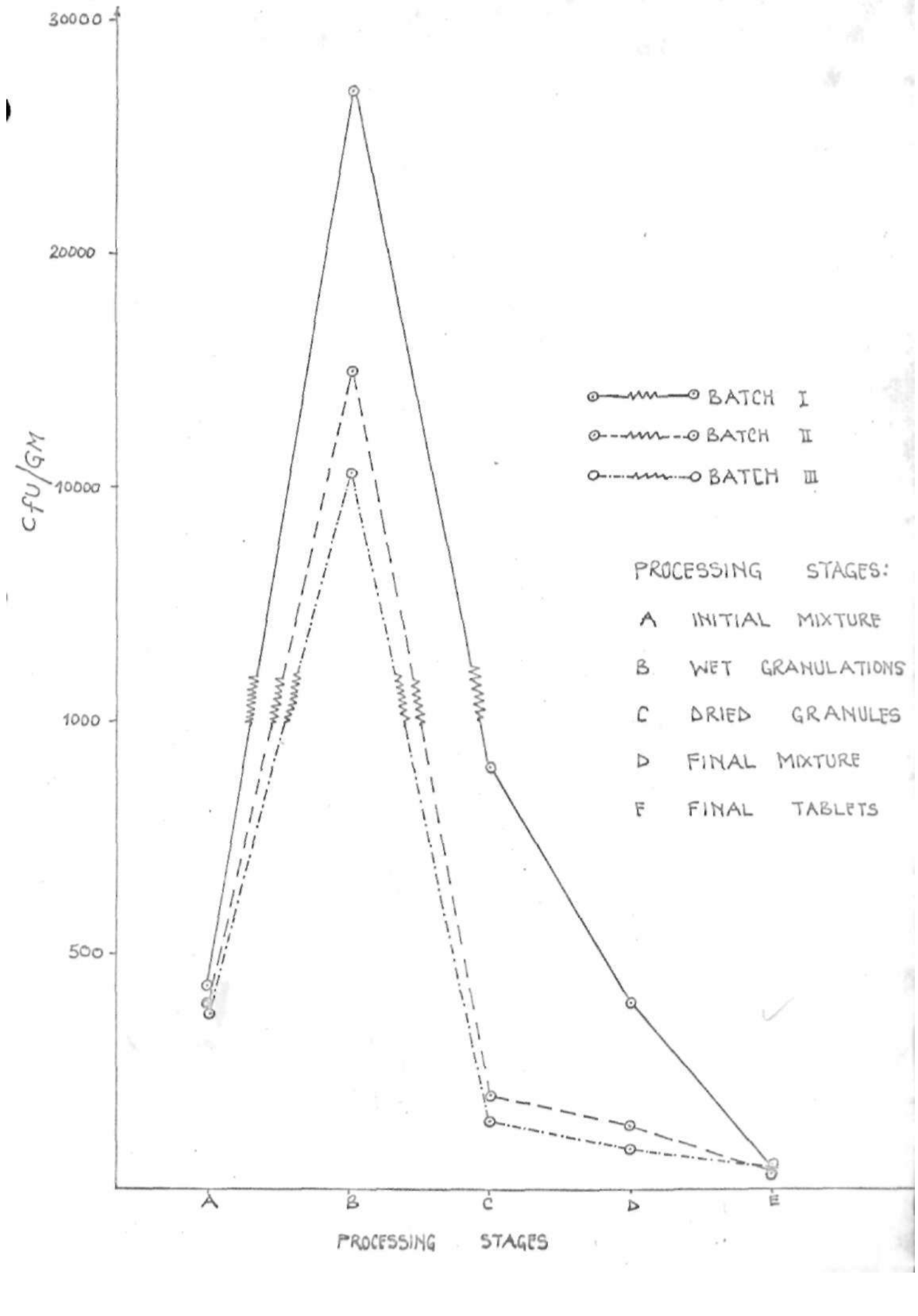


Table 5.12: Various Morphological Forms of Bacterial Contaminants at various Production Stages of Folic acid Tablets

Wet Granulation Method Stages	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical characters	Pathogens Identified
A (Initial mixture)	1. Milky white, circular, raised Opaque, entire, smooth and shining colony of size 2 - 5 mm.	Gr +ve cocci, in clusters. $(0.6 - 0.8\ u)^2$	
	2. White, flat, translucent, coarse and dull, fimbriate, spreading colony of size greater than 40 mm.	Gr -ve motile rods. 0.5 u x 1.0 u.	
	3. Red, circular, convex, Opaque, entire, smooth and glistening colony of size 3 mm.	Gr +ve cocci, mostly in groups. $(0.6 - 0.7\ u)^2$	None.
	4. Faint yellow, irregular, flat, lobate, smooth transparent colony of size 15 - 18 mm.	Gr -ve rods, mostly single. 0.5 u x 0.8 u.	
	5. Off-white, flat, irregular, lobate, Opaque, slightly granular colony of size 10 - 14 mm.	Gr +ve rods in chains with spores. 0.5 u x 2.0 - 2.5 u.	
	6. Buff, circular, flat, Opaque, entire, smooth and shinning colony of size 4 mm.	Gr +ve rods, mostly sirgle with terminal spores. 0.8 u x 1.5 - 2.5 u.	

Wet Granulation method stages	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical characters	Pathogens Identified
B Wet mixture (After Addition of Binder Solution)	1. Golden yellow, circular, convex, slightly translucent, smooth and shinning, entire colony of size 1 - 3 mm.	Gr +ve cocci, in diplo and chains of 3 - 4 cells. $(0.4 - 0.5 u)^2$	
	2. White, flat, circular, entire, Opaque, smooth and shinning colony of size 4 - 5 mm.	Gr -ve rods, singly, some massed together. 0.6 - 0.8 u x 2.0 u.	
	3. Milky white, circular, convex, entire, Opaque, smooth and shinning colony of size 2 - 3 mm.	Gr +ve cocci, in clusters. 0.8 u x 0.8 u.	*Staph. aureus.
	4. Off-white to buff, flat, irregular, lobate, granular, translucent colony of size 8 - 10 mm.	Gr +ve rods, singly, some in chains of 3 - 4 cells, with central spores. 0.5 u x 2.0 - 2.5 u.	*Ps. aeruginosa
	5. Cream, circular, raised, entire, Opaque, smooth and shinning colony of size 2 mm.	Gr +ve cocci, mostly in clusters. 0.5 u x 0.5 u.	
	6. Almost colourless, circular, flat, translucent, smooth with granular inner portion, colony of size 2 - 3 mm.	Weakly gr +ve, single and diplobacillus, with spores. 0.4 u x 1.5 - 2.0 u.	
	7. Faint yellow, flat, irregular, lobate, smooth, transparent colony of size 15 - 18 mm.	Gr -ve rods, mostly single 0.5 u x 0.8 u.	

Jet Granulation method stages	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical characters	Pathogens Identified
	8. Light yellow, flat, entire, Opaque, almost circular, slightly granular colony of size 3 - 4 mm.	Weakly gr +ve rods in chains of 4 - 8 cells, wound round each other. 0.8 - 1.(u x 2.5 u.	

*Diagnostic and biochemical tests showed the presence of Staph. aureus and Ps aeruginosa in one batch produced at Environment A. In the subsequent stages of that Batch, Ps.aeruginosa were also detected.

Table 5.12: Various Morphological Forms of Bacterial Contaminants at various Production Stages of Folic acid Tablets

Wet Granulation Method Stages	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical characters	Pathogens Identified
C (After Drying at 50°C for 60 - 90 minutes)	1. Milky white, circular, raised, Opaque, entire, smooth and shinning colony of size 2 - 3 mm.	Gr +ve cocci, mostly in clusters. (0.6 - 0.7 u)^2	
	2. Off-white to almost colourless, irregular, flat, spreading, fimbriate, dull, translucent colony of size 30 - 40 mm.	Gr +ve rods, singly with central spores. 0.5 - 0.6 u x 1.0 - 1.5 u.	
	3. Off-white, circular, raised, entire, smooth, and translucent colony of size 2 mm.	Gr -ve rods, mostly single. 0.4 u x 0.6 - 0.8 u.	None.
	4. Light yellow, circular, convex, entire, smooth, shinning, translucent colony of size 1 - 2 mm.	Gr +ve rods, mostly diplobacillus with central spores. 0.5 u x 1.5 - 2.5 u.	
D Final Mixing	1. Light yellow, circular, convex, entire, smooth, shinning, translucent colony of size 1 mm.	Gr +ve rods, singly and diplobacillus 0.5 u x 1.5 - 2.5 u.	
	2. Dirty white, circular, flat, granular, almost entire, slightly translucent colony of size 6 - 8 mm.	Gr +ve rods, in chains with central spores. 0.5 - 0.6 u x 2.0 - 3.0 u.	

Wet Granulation method stages	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical characters	Pathogens Identified
	3. Colourless, irregular, flat, lobate, granular and slightly translucent colony of size 10 - 12 mm.	Gr +ve rods, in long chains with central and terminal spores.	None.
	4. Colourless, circular, raised, entire, granular and slightly translucent of size 1 - 2 mm.	Gr +ve rods, in long chains. 1.0 u x 5.0 - 5.0 u.	
	5. Milky white, circular, raised, entire, smooth, shinning, Opaque colony of size 2 - 3 mm.	Gr +ve cocci, in groups 0.5 u x 0.5 u.	
E The freshly produced tablets	1. Colourless, Spreading, irregular, flat, fimbriate, granular, dull, translucent colony of size 40 - 50 mm.	Gr +ve rods, singly and diplobacillus with central spores. 0.5 u x 1.0 -	
	2. Milky white, circular, raised, entire, Opaque, smooth and shinning colony of size 1 mm.	Gr +ve cocci, singly and in clusters. 0.5 u x 0.5 u.	
	3. Off-white, slightly irregular, raised, Opaque, smooth and shinning, colony of size 3 - 5 mm.	Gr +ve bacillus, diplobacillus with terminal spores. 0.5 - 0.6 u x 2.0 - 3.0 u.	None.
	4. Light yellow, circular, entire, flat, slightly granular, translucent colony of size 6 - 8 mm.	Weakly gr +ve rods, mostly in chains with granules on cell surfaces. 0.8 - 1.0 u x 2.5 - 3.0 u.	

Table 5.13: Comparison of Microbial Contamination Levels At Various Processing Stages in Two Different Environments

Stages	Environment A		Environment B	
	Bacteria count cfu/Gm	Fungal count cfu/Gm	Bacterial count cfu/Gm	Mound count cfu/Gm
A Initial Mixture	378	10	148	4
B Wet mixture	10380	70	1048	50
C Dried Granules	145	30	138	10
D Final Mixture	83	50	210	10
E Final Tablets	43	10	118	4

FIG.5·2 : MICROBIAL CONTAMINATION LEVELS AT VARIOUS STAGES OF WET GRANULATION METHOD IN ENVIRONMENTS 'A' AND 'B'

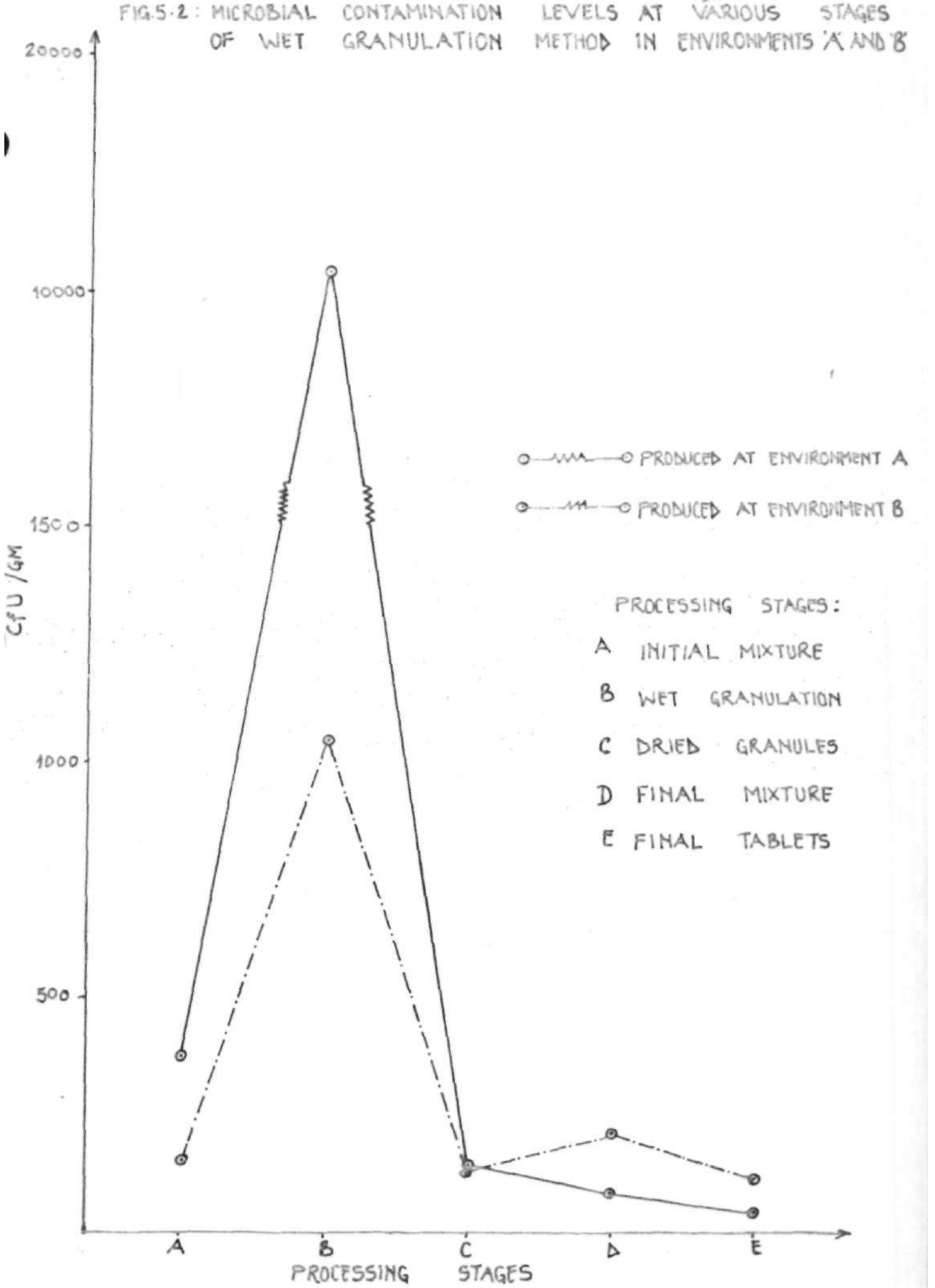


Table 5:14: Effect Of Drying Temperatures on the Microbial Contamination of Tablet Granules.

Stages	Drying of wet granules at 50 - 52°C		Drying of wet granules at room tempe.(28 - 30°C)	
	Bacterial count cfu/Gm	Fungal count cfu/Gm	Bacterial count cfu/Gm	Fungal count cfu/Gm
A Initial mixture	143	4	148	4
B Wet mixture	1048	50	1048	50
C Dried granules	138	10	1814	210*
D Final mixtures	210	10	1948	267*
E Final Tablets	118	4	1500	110*

*Most of the Nutrient agar plates and the Sabouraud Dextrose agar plates on incubation were fully coverred with mucor by 24 hours.

FIG. 5.3: MICROBIAL CONTAMINATION LEVELS AT VARIOUS PROCESSING STAGES OF GRANULATIONS DRIED AT 50-52°C AND ROOM TEMPERATURE (28 - 30°C)

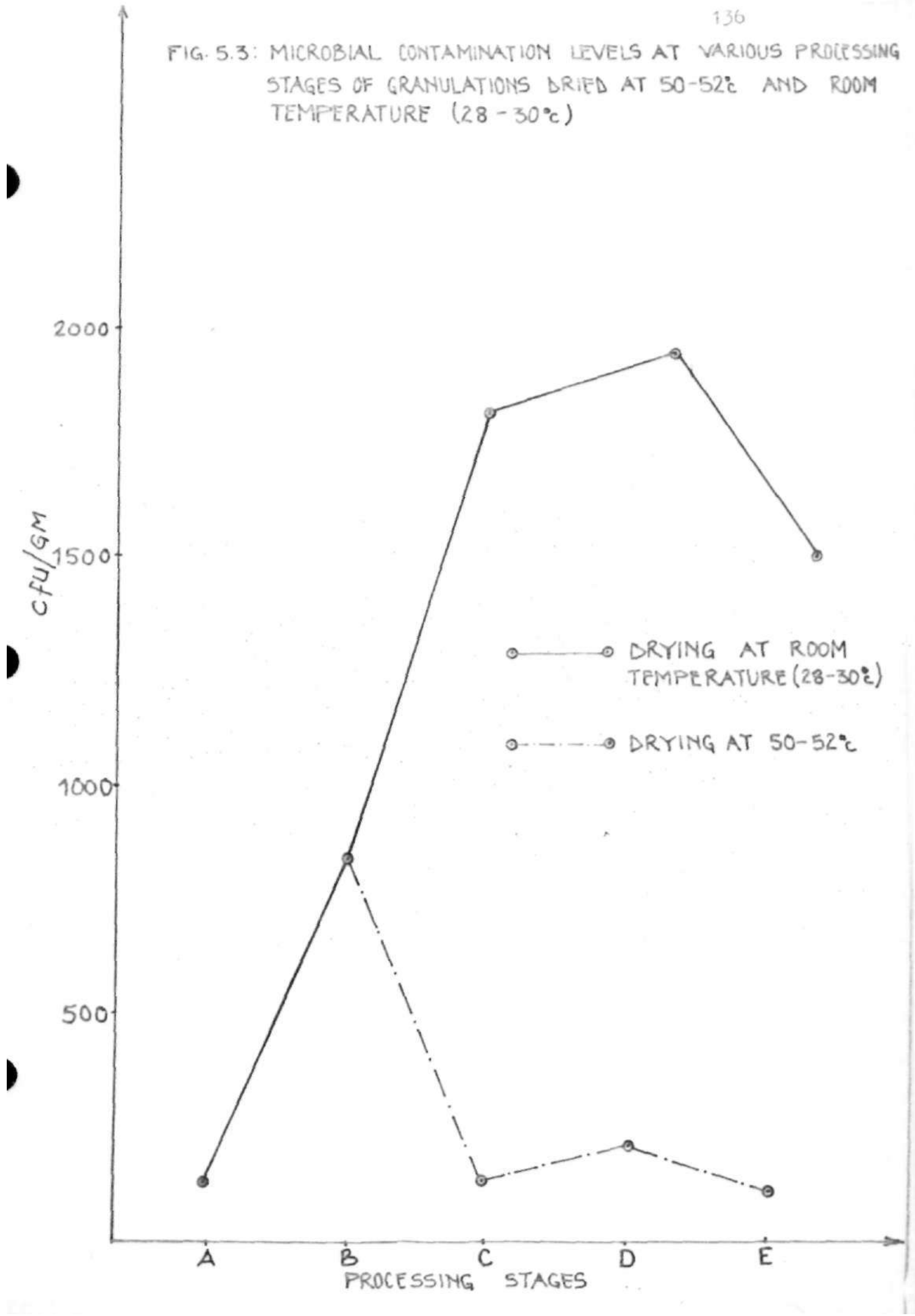


Table 5:15: Various Morphological Forms of Bacterial Contaminants of Tablets produced from Granules Dried at Different Temperatures

	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical characters	Pathogens Identified
Tablets from granules dried at 50 - 52°C	1. Colourless, flat, fimbriate, translucent, granular, dull, spreading colony, covering whole plate.	Gr +ve, motile rods with central spores, mostly single. 0.4 - 0.5 u x 1.0 - 1.2 u.	
	2. Light yellow, circular, raised, with papillae, entire, Opaque, granular colony of size 2 - 3 mm.	Weakly gr +ve rods, singly and in chains, with granules, wound round each other. 0.8 u x 2.5 - 5.0 u.	
	3. Milky white, circular, raised, entire, Opaque, smooth and shinning colony of size 1 mm.	Gr +ve cocci, singly and in clusters. 0.5 u x 0.5 u.	None.
	4. Off-white, raised, Opaque, entire, circular, smooth and shinning colony of size 3 - 5 mm.	Gr +ve rods, mostly diplobacillus, few singly with terminal spores. 0.5 - 0.6 u x 2- 3 u.	

	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical characters	Pathogens Identified
Tablets from granules Dried at room temperature (26 - 28°C)	1. Milky white, circular, raised, entire, Opaque, smooth and shinning colony of size 1 - 1.5 mm.	Gr +ve cocci, in chains and clusters. $(0.6 - 0.3\ u)^2$	
	2. Cream, circular, raised, entire, Opaque, smooth and shinning colony of size 1 - 2 mm.	Gr +ve cocci, singly and in groups. $(0.7 - 0.8\ u)^2$	
	3. Light yellow, circular, flat, Opaque, entire, smooth and glossy colony of size	Gr +ve rods, spores forming, mostly single. 0.8 - 1.0 u x 3.0 - 6.0 u.	Staph. aureus Ps. aeruginosa
	4. Colourless, spreading, flat, fimbriated, dull, slightly granular, translucent colony of size greater than 40 mm.	Gr -ve rods, mostly single, some diplobacillus. 0.5 u x 0.8 - 1.2 u.	
	5. Dirty white, regular, flat, lobate, Opaque dull and granular colony of size 1 - 2 mm.	Gr +ve rods, singly and diplobacillus, spore forming. 0.5 u x 1.0 - 1.5 u.	
	6. Faint yellow, to off-white, circular, raised, smooth, Opaque, entire colony of size 1 - 2 mm.	Gr +ve cocci, in bunches $(0.8 - 1.0\ u)^2$.	
	7. Light Orange, cir circular, raised, entire, Opaque, smooth and shinning colony of size 2 - 3 mm.	Gr +ve cocci, in small groups. 0.4 u x 0.4 u.	

5.4 RESULTS AND DISCUSSION

The results depicted in Table 5.1 shows that the bacterial contamination level of air in environment A ($3300 cfu/m^3$) is much lower than that in environment B ($5000 cfu/m^3$), whereas the reverse is the case with respect to mould count ($2700 cfu/m^3$ in A compared to $1000 cfu/m^3$ in B). The lower bacterial contamination level in environment A might be due to the general cleanliness maintained therein, which included frequent floor and wall washings. The windows of the Production area were usually locked and the entrance door was so designed that it always remained closed. On the other hand, floor and wall washings was less thorough, windows had to be opened during practical classes, and the number of operators were a crowd (greater than 30), in environment B, compared to those in environment A. The bacterialflora of the two production environments also differred greatly (Tables 5.2 and 5.3). While Staph. aureus was found in enviornment A, a Pseudoemonas species was detected in enviornment B, which could be due to the proximity of the latter environment to a Microbiology laboratory. Pseudomonads especially Ps. aeruginosa have been found to occur in some environments and absent in others. Whitby and Rampling (1972), and Baird et al (1976)

showed that while Ps.aeruginosa was present in high numbers in the hospital pharmacies and wards, they were absent in corresponding areas in domestic homes. Result of Table 5.1 also depicts the variation in the microbial contamination levels of the different compartments in environment A. The significant difference in the microbial flora and level between the two environments, and also between the different compartments in environment A could be due to the following factors:

(a) the number of people in the environment;

(b) the sort of activities being carried out in the environment;

(c) the amount of movement of the people;

(d) the direction and strength of wind, and

(e) the humidity of the atmosphere. The works of Hooper et al. (1972) confirmed some of the above enumerated factors. The relative high value of mould count in environment A could be due to large bulk of carbohydrate raw materisl that are frequently handled in this area. Carbohydrates in a moist environment provide a good menstrum for the growth of moulds.

Freshly distilled water is supposedly free from micro-organisms since in the distillation process, the bacterial cells and spores can not accompany the steam into which water is first turned, before condensation to water. As Table 5.4 shows, microbial contamination of distilled water can occur either through the use of unsterile containers, or through air due to inadequate closure of the vessel during the time of collection. It is at the storage point that the microbial contamination level greatly increases. Favero et al (1971), reported a thousand fold increase in the level of Ps. aeruginosa in distilled water stored at 37°C for 24 hours and even a higher value at 25°C.

The microbial flora of distilled water is usually a reflection of the microbial flora of the environment and the collecting vessel. Most of the contaminants observed in this study belong to the gram negative rods, along with few gram positive cocci and spore forming gram positive bacilli (Table 5.5). The presence of Ps. aeruginosa in the distilled water could be due to the tap water used in the cleaning and rinsing of the collecting vessel for the distilled water. Another possible source from which Ps. aeruginosa could have arisen is the

Microbiology Laboratory which is in close proximity to the room where distilled water was prepared. The growth of Ps. aeruginosa in distilled water despite its lack of nutrients is, according to Favero et al (1971) due to the ability of the organism to utilize organic compounds that had been absorbed in the distilled water during storage. Morecver Ps aeruginosa is non exacting in its nutritional requirement. The relative few types of organisms detected in tap water examined, could be due to the pretreatment of the water by chlorination at the reservoir before distribution (Table 5.6).

The microbial contamination level of the Tabletting machine (Table 5.7) shows that the bacterial count per unit surface area in environment A (8.5 cfu/cm^2) was lower than that in environment B (10.3 cfu/cm^2). The mould count of the tabletting machines are a reversal with respect to their environmental areas. This result is in agreement with the order of the air microbial levels obtained for the two production environments. The microbial flora and level of an equipment is a reflection of the air microbial flora and level of that environment in which it is placed and the sort of treatment the equipment has and is being subjected to.

The microbial contamination flora and level of Oven (Table 5.7 and 5.10) indicates a higher value for that in environment A (6.0 cfu/cm^2 for bacteria and 8.0 cfu/cm^2 for moulds) compared with the values for the oven in environment B (0.5 cfu/cm^2 for bacteria and 0.7 cfu/cm^2 for moulds). The high level of microbial contaminants associated with the Oven in environment A is due to the low temperatures of 50 - 52^oC at which the oven is usually used in drying the wet granules. This temperature level can hardly kills bacteria except those belonging to the psychrophillic group. The spores which are heat resistant are also less adversely affected at the temperature range. Moreover, the oven in environment A is hardly subjected to chemical disinfection. The oven in environment B was, beside being used for drying wet granules at 50 - 52^oC, frequently employed in the drying of glasswares at elevated temperatures of 130^oC, a temperature at which virtually no vegetative cells and few bacteria spores survive.

Table 5.11 shows the microbial contamination level at various stages of the wet granulation method of tablet production. It clearly indicates that the highest level of contamination occurred during the

wet mixing stage and that this is substantially decreased when dried at 50 - 52°C. The high level of microbial contamination of wet mix is due to the addition of binder solution which in itself is heavily contaminated as a result of the use of highly contaminated water, its major component and vehicle. The bacterial flora at this stage revealed the presence of many gram negative rods, gram positive cocci and gram positive spore forming rods. During drying at 50°C, most bacterial vegetative cells of psychrophiles and some mesophiles are either killed or irreversibly inactivated, due to the destruction of protein moieties of enzymes involved in the biochemical reactions of bacteria, which are necessary for bacterial growth. The spores and thermophillic micro-organisms require much higher temperature to cause their death. Though the thermophillic organisms can grow easily at this temperature (50 - 52°C), they are not normally as abundant as the former two groups

There is a further decrease from the dried granules to the final mixtures before compression (Fig. 5.1). The decrease (55 % in Batch I, 43 % in Batch II and 37°C in Batch III) is more than the theoretical calculated decrease value of 5 %. The microbial contamination level of the three different batches produced at same temperature under

environment A is not very different (Table 5.11). The mould count in all the three batches generally conform to the trends of the bacterial count at various stages. In one batch, Staph. aureus and Ps. aeruginosa were detected at the wet mixture stage. While the Ps. aeruginosa was traced to the distilled water used in preparing the binder solution, the Staph aureus was probably from the environment (see Table 5.12). In this batch, Ps. aeruginosa was detected in the subsequent stages, up to the final produced tablets.

Table 5.13 and Fig. 5.2 show the comparative microbial levels at various production stages up to the final tablets of folic acid at the two production enviornments. Tablets produced in environment A had lower bacterial count (43 cfu/gm) than those produced in environment B (118 cfu/gm). The reverse was the case with respect to the mould counts. This result revealed that the microbial level of the environment at which production of pharmaceuticals take place greatly affects the microbial contamination level of the finished product. Investigations carried out by the Swedish National Board of Health, in 1955, traced the high microbial contamination levels of some non-sterile pharmaceuticals to their manufacturing environments.

Table 5.14 and Fig. 5.3 show the effect of the temperature at which the wet tablet granules are dried on their microbial contamination level. The results indicate that the granules dried at 50 - 52°C were substantially decontaminated, compared with those dried at 28°C. In fact, drying at lower temperature encouraged bacterial proliferation. Drying at a low temperature of 28°C, coupled with the high moisture content of the wet granules, and aided by the presence of gelatin and starch, favours bacterial proliferation of the psychrophiles and some mesophiles. A similar 'favour' applied to the moulds. The microbial flora showed that tablets produced by drying the wet granules at room temperature consisted of greater varieties of bacteria than those produced by drying the wet granules at 50 - 52°C. The bacteria flora consisted mostly of Gram positive cocci and Gram positive sporulating rods (Table 5.15).

The results of this investigation have shown clearly that the microbiological quality of tablets produced by wet granulation method, is influenced by such factors as:-

(a) the microbiological quality of the environment;

(b) the microbial quality of the binder solution used in the preparation of the wet granules; and

(c) the temperature at which the resulting wet granulations are dried.

The microbial contamination levels of the equipment used in the production are a direct reflection of the microbial quality of the environment and the sort of cleanliness they had been subjected to.

CHAPTER SIX

DETERMINATION OF MICROBIAL CONTAMINATION OF FOLIC ACID TABLETS PRODUCED BY DIRECT COMPRESSION METHOD

The direct compression process of tablet manufacture is a two-stage operation, involving the blending of all the required excipients with the active ingredients and the compression of the mixed ingredients in a tablet press to form a compact. The critical Stages of wetting and drying, involved in wet granulation method, are not encountered in this method. Becuase of the few steps involved, it is less exposed to the environment, hence contamination by personnel and from the environment should be minimal. The microbiological analysis of the direct compression stages in the tablet manufacture is undertaken to determine the contamination potential of this method compared with that of the wet granulation.

6.1 MATERIALS AND EQUIPMENT

6.1.1 MATERIALS

The raw materials used include the following:-

1. Dicalcium phosphate (Powder)
2. Folic acid Powder

3. Magnesium Stearate Powder
4. Microcrystalline cellulose (Avicel) pH 101, size 50 u
5. Lactose Powder (Hydreous)
6. Polyethylene Glycol 6000
7. Starch (granulated)
8. Talc (fine powder)

They were the same in source and quality as previously described. (Chapter three page52)

CULTURE MEDIA

Nutrient Broth, Nutrient Agar, Sabouraud Dextrose Agar, MacConkey Agar, Mannitol Salt Agar, Cetrimide Agar, Bismuth Sulfide Agar, and Tripple Sugar Iron Agar. They were all oxoid dehydrated media.

6.1.2 EQUIPMENT

1. Tumbler mixer
2. Tabletting Machine (Single-Station Punch), Korsch Western Germany,

6.2 EXPERIMENTAL

6.2.1 MICROBIAL FLORA OF AIR

Using a MacIntosh and Fildes Anaerobic jar, along with a vacuum pump, the experiment was repeaced as described in chapter five. From the number of resulting colonies, the number of microorganisms per unit volume of air was calculated.

6.2.2 MICROBIAL FLORA OF TABLETTING MACHINE

The swabbing method was followed as described previously in chapter five.

6.2.3 MICROBIAL FLORA DURING DIRECT COMPRESSION PROCESSES

In each formulation, all the ingredients which were already in powders were blended together in a tumber mixer, and taken to the hopper of a tablet press for compression. Since the formulations were not flowing uniformly and adequately into the die cavity, forced feeding was resorted to. Reproduced below are the composition of the different formulations used. (Table 6.1). The two stages which were subjected to microbiological analysis for microbial contamination were:-

(a) Powder mixture of all the ingredients;

(b) Final produced tablets.

Microbial Counts were carried out, and tests for the presence of some specific pathogens were also performed. Where there were presumptive positive results, further biochemical and diagnostic tests were carried out. The production of tablets was carried out in the Process Laboratory of the Department of Pharmaceutics and Pharmaceutical Microbiology, which had earlier been designated as Environment B in Chapter 5.

Table 6.1: Composition of Four Formulations of Tablets Produced by Direct Compression

	FORMULATIONS			
Composition	I	II	III	IV
Folic Acid	500 gm	500 gm	500 gm	500 gm
Lactose (Hydrous)	3.0 kg	-	1.2 kg	-
Microcrystalline Cellulose (Avicel)	2.2 kg	3.5 kg	2.0 kg	2.5 kg
Polyethylene Glycol 6000	-	-	2.0 kg	-
Starch granules	-	-	-	2.8 kg
Dicalcium Phosphate	-	2.0 kg	-	-
Talc	300 gm	-	300 gm	100 gm
Magnesium Stearate	30 gm	60 gm	30 gm	100 gm

Table 6.2: Microbial Contamination Levels of Air And Tabletting Machine

Item Examined	Microbial Levels	
	Bacterial count	Mould count
Air	4545 Cfu/m^3	705 cfu/m^3
Tablet Press	8 cfu/cm^2	2 cfu/cm^2

Table 6.3: Microbial Contamination Level Of The Two Stages In Direct Compression Method

Formulations	Mixture of All Ingredients		Final Tablets	
	Bacterial count cfu/gm	Mould count cfu/gm	Bacterial count cfu/gm	Mould count cfu/gm
I	114	12	53	5
II	40	5	15	3
III	50	20	21	6
IV	230	26	53	8

Table 6.4: Various Morphological Forms of Bacterial Contaminants of Formulation I

Direct Compression Formulation	Colonial Morphology (size is that of diameter expressed in mm)	Microbial character	Pathogens identified
Stage A: Powder mixture	1. Light Yellow, circular, raised, entire, Opaque granular, wrinkled and striated colony of diameter size 4 - 6 mm.	Gr +ve rods, mostly in chains with granules within the cells, many wound round to form mass. 1.0 u x 1.5 - 4.0 u.	
	2. Colourless, circular, raised, entire, almost transparent, smooth and shinning colony of diameter size 1 - 4 mm.	Gr +ve long rods, mostly singly and diplobacillus. 0.4 - 0.9 u x 2.0 - 5.0 u.	None
	3. Dirty white, circular, raised (slightly), entire, smooth and shinning, translucent colony of size 2 - 3 mm.	Gr +ve rods, mostly single with subterminal large spores giving some tennis racquet shape. 0.5 u x 1.0 - 3.0 u	
	4. Ash, irregular, flat, serrated, dull, coarse with visible branches (finger-like) and translucent colony of size 8 - 10 mm.	Gr +ve single rods, with central, oval spores. 0.4 u x 1.0 - 1.3 u	

Direct Compression Formulation I	Colonial Morphology (Size is that of diamnter expressed in mm)	Microscopical character	Pathogens Identified
Stage B: Produced Tablets.	1. Light vellow, circular, raised, Opaque and granular colony of size 3 - 5 mm	Gr +ve rods, in long strands of 6 - 12 cells wound round to form a mass. 0.8 - 1.0 u x 3.0 - 4.0 u.	
	2. Colourless, circular, raised, entire, smooth and shinning transparent colony	Gr +ve rods, mostly diplobacillus. 0.4 u x 1.5 - 3.0 u.	
	3. Intense pink, circular, raised, entire, Opaque, smooth and shinning colony of size 3 - 4 mm.	Gr +ve cocci, relatively large, singly and in small groups. $(0.8 - 1.0\ u)^2$	
	4. Milky white, slightly raised, entire, Opaque, smooth and shinning colony of size 2 - 3 mm	Gr +ve cocci, mostly in groups (clusters) $(0.8 - 1.0\ u)^2$	None.
	5. Golden yellow, circular, convex, entire, translucent, smooth and shinning colony of size 1 mm.	Gr +ve cocci, diplo and short chains and small groups $(0.3 - 0.5\ u)^2$	
	6. Dirty white, circular, slightly raised, entire, smooth and shinning, translucent colony of size 2 mm.	Gr +ve rod, mostly single with sub-terminal, spores. giving a tennis racquet shape. 0.5 u x 1.0 - 2.5 u.	

Table 6.5: Various Morphological Forms of Bacterial Contaminants of Formulation IV

Direct Compression Formulation IV	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical character	Pathogens Identified
Stage A: Powder Mixture	1. Faint Pink, circular, raised, entire, Opaque, smooth and shinning colony of size 1,2 mm.	Gr +ve cocci, singly and in small groups. $(0.4 - 0.5\ u)^2$	
	2. Orangish red, circular, raised, entire, Opaque, smooth and shinning colony of size 1 - 2 mm.	Gr +ve, slender rods, singly with central and oval spores. 0.4 u x 2.6 - 4.6 u	
	3. Slightly off white, irregular, flat, Opaque, dull and granular, fimbriate to serrated colony of size 10 - 13 mm.	Gr +ve rods, singly, diplo with sub-terminal spores, 0.5 u x 1.5 - 2.5 u.	None
	4. Yellow, circular, convex, entire, Opaque, smooth and shinning colony of size 1 - 2 mm.	Gr +ve cocci, single, short chains and clusters. 0.5 u x 0.5 u.	
	5. Buff to colourless, circular, raised, entire, smooth and slightly transparent colony of size 1 mm.	Gr +ve cocci, mostly single, few diploccus. $(0.7 - 0.8\ u)^2$	

Direct Compression Formulation IV	Colonial Morphology (Size is that of diameter expressed in mm)	Microscopical character	Pathogens Identified
Stage B: Produced Tablets	1. Faint Pink, circular, convex, entire, Opaque, smooth and shinning colony of size 1 mm.	Gr +ve cocci, mostly single, few in clusters. (0.5 - 0.7 u,^{2}	
	2. Yellow Orange, circular, flat, entire, slightly translucent, smooth with pronounced concentric ridge, colony of size 5 - 6 mm.	Weakly gr +ve rods some curved, with granules Some are straight rods. 1.0 u x 2.0 - 7.0 u.	
	3. Yellow, circular, convex, entire, smooth and shinning, Opaque colonies of size 1 - 2 mm.	Gr +ve cocci, singly and few clusters. 0.5 u x 0.5 u.	None.
	4. White, irregular, almost flat, lobate granular and dull, Opaque colony of size 8 - 11 mm.	Gr +ve rods, singly and diplo with central, oval spores. 0.4 - 0.5 u x 1.0 - 2.0 u.	

Table 6.6: Comparison of the theoretical and obtained Microbial Contamination Levels in the first stage of Direct Compression.

Formulations	Theoretical Values		Practical obtained Values	
	Bacterial count cfu/gm	Fungal count cfu/gm	Bacterial count cfu/gm	Fungal count cfu/gm
I	78	2	114	13
II	3	3	40	5
III	65	8	50	20
IV	197	9	230	26

6.3 RESULTS AND DISCUSSION

Table 6.2 depicts the microbial contamination levels of the environmental air and the tablet press surface. The microbial flora of the two had earlier been treated in Chapter 5, under Environment B. The microbial contamination levels obtained in these cases were fairly similar to those obtained from those of the Wet granulation method.

Three significant observations were made from this investigation. (1), the microbial contamination level of the produced tablets were much lower than that obtained for the mixture of all the ingredients prior to compression (Table 6.3). In most cases, the reduction in the microbial level were more than

fifty percent (50 %); the same trend was also observed in the wet granulation method, between the final mixtures prior to compression and the produced tablets. This decrease in microbial contamination level after compaction may be attributed to

(i) the high temperatures attained within the tablet matrix which may lead to localized melting and subsequent killing of contaminating micro-organisms;

(ii) death of some bacterial cells due to compaction force, and

(iii) death of some aerobic bacteria due to oxygen starvation in the tablet core. The works of Bowden and Tabor (1964) and York and Pilpel (1972) supported the theory of localized melting.

Starting with granules of low microbial contamination levels, results in final tablets of low microbial contamination levels, as seen in Table 6.3, formulations II and III compared with I and IV. In all cases, there was a proportional decrease of at least 50 % from the initial microbial contamination levels.

The third significant observation is depicted in Table 6.6, which shows that the obtained experimental values for microbial contamination level for the formulations were higher than the calculated theoretical values (except formulation III). The theoretical values were based on the percentage composition of the ingredients in each formulation and the initial contamination lvels of the raw materials. Since the direct compression method involves just two operations, it can be reasonably inferred that the environmental contamination including the equipment (mixer), in part accounted for the increment in the microbial contamination level. Bacteria are continually multiplying, though some are dying as well. The increment in microbial contamination level may be due to the fact that those bacteria proliferating are more in number than those dying.

The results obtained from this study have indicated that tablets produced by the direct compression method are less liable to microbial contamination than with the wet granulation method.

CHPATER SEVEN

GENERAL DISCUSSION AND CONCLUSION

7.1 COMPARISON OF THE TABLETS PRODUCED BY DIFFERENT METHODS

For a valid comparison of the microbial contamination levels of the tablets produced by both the wet granulation and direct compression methods, the results obtained by their production under the same environmental conditions were used. The highest microbial contamination count obtained from the direct compression tablets, for example 53 cfu/gm from formulation IV was much lower than those from tablets produced by the wet granulation method, for example 118 cfu/gm.

The significant difference in the microbial count of the tablets produced by the two methods can be attributed to the relative few numbers of production stages (two) in direct compression compared with those of the wet granulation in which six to nine production stages are involved. Moreover, the high microbial levels obtained during the wet granulation stage is due in part to the high microbial level of the distilled water used in preparing the binder solution. The microbiological quality of distilled water, the binder used, and the

temperature at which the resulting wet granules are dried greatly determines to a very large extent the microbiological quality of the resulting tablets. These factors are inconsequential in direct compression because they are not involved. The general quality of the tablets produced by the direct compression method compared very favourably, and in some cases better, than those produced by the wet granulation. In terms of economic consideration, direct compression method of tablet production, where applicable, is preferred.

7.2 COMPARISON OF RESULTS WITH COMPENDIAL STANDARDS

The reports of infections associated with the use of contaminated Pharmaceutical products led to various standards being set for such products in different countries. While in some articles, absence of specific micro-organisms is set forth in their monographs, in others specific limit is placed on their aerobic count of viable organisms. In the second joint report of the Committee of Official Laboratories and Drug Control Services, and the section of Industrial Pharmacist, FIP (1976), the following recommendations were made.

Table 7.1: Limit of Microbial Contamination to Various Pharmaceutical Preparations

Product	Requirements
1. Injectable preparations	Sterility - Under Conditions of Pharmacopoiea
2. Ophthalmic Products	absence of viable organism in 1 gm or 1 ml
3. Topical Preparations (for skin abrassives, ear throat and nose)	Microbial limits of viable organisms: - 10^2 in 1 gm or 1 ml; - no enterobacteria; - no Pseudomonas aeruginos; - no Staphylococcus aureus.
4. Other Preparations	Limiting level of viable organisms: - 10^3 to 10^4 aerobic bacteria per gm or ml; - 10^2 yeast and moulds per gm or ml; Limits for specified micro-organisms: - absence of E. coli in 1 gm or 1 ml in certain cases; - absence of Salmorella in 1 gm or 1 ml - other enterobacteria (maximum 10^2/gm or ml). - absence of Ps.aeruginosa in 1 gm or 1 ml; - absence of Staph. aureus in 1 gm or 1 ml.

The following are the general recommendations of the USP XX and NF XV 1980:

(a) All natural plant and animal products to be free from Salmonella species;

(b) Oral solutions and suspensions be free from E. coli;

(c) Topical preparations be free from Staph. aureus and Ps.aeruginosa;

(d) Preparations for rectal, urethral, or vaginal administration should be checked for their total microbial counts.

In the individual monographs, all aluminium and magnesium oral liquid preparations are to be free from E. coli, with maximum aerobic count of 100/ml, while cocoa apart from being free from E. coli, should have total aerobic count maximal of 5000/gm.

Items required to be free from E. coli and Salmonella at same time are - gelatin (with aerobic count maximal of 1000/gm), Pancrelipase, thyroid, thyroglobulin, Starch, Pregelatinised Starch, tragacanth, xanthum, Sodium alginate (with total aerobic count of 100/gm) and Sugar (compressed and confectioner's). Chymotrypsin is required to be free

from Salmonella and Pseudomonads. The following articles are specified to be free from Salmonella - agar, Pectin, acacia, pancreatin, powdered digitalis, crystallized trypsin and rauwolfia serpentina.

The B.P. (1980) on the other hand recommends freedom of Pseudomonads in dried aluminium preparations. Articles such as Starch, pregelatinised Starch, and powdered acacia are required to be free from E. coli in 1.0gm. while Cochineal, prepared digitalis, gelatin and pancreatin are required to be free from E. coli in 1 gm and Salmonella in 10 gm. The Swedish Pharmacopoiea requires that all corticosteroid preparations be sterile and recommends absence of some specific organisms in many products and aerobic total limit count of 100 per gm or ml. Recognising the fact that natural products would not easily comply with this limit, the Swedish Medical Board therefore allowed higher microbial limits greater than 100/gm or ml for such products.

From the foregoing, only the Swedish Pharmacopoeia and the FIP (1976) specify a limit on the aerobic microbial level of non-sterile pharmaceutical products, with a limit of 100/gm or ml.

Based on this, the tablets produced by direct compression and those produced by wet granulation methods in environment A were within the limit. The value of 118 cfu/gm obtained for those tablets produced by wet granulation method in environment B was slightly outside the limit. However, the fact that there were absence of designated pathogens such as Staph. aureus, Ps. aeruginosa, E. coli and Salmonella species, makes it acceptable, especially by the USP (1980) and B.P. (1980) recommendations.

As stated earlier, standards imposed on the microbial limits for non-sterile pharmaceuticals have followed two approaches - namely tests for the complete absence of specific bacteria which are considered particularly harmful, and also restrictions on the total number of viable aerobic organisms permitted to be present. It is relatively simple to draw up standards for the former, but more difficult to define standards for counts. A major problem is that the numbers present in a product are rarely constant for very long. The bacteria are usually multiplying, resulting in ar increase in number with time or dying with a subsequent decrease in count, depending on the period and condition of storage before commencement of the microbial test.

For this reason, the B.P. (1980) and European Pharmacopoiea (E.P. 1971) do not include such a Standard. Further difficulties arise because the contaminants present is normally a mixture of different species, each with its own optimal growth requirements. The presence of one particular organism may interfere with the growth of another. Another problem is the fact that acute pathogens may occur in extremely low numbers. Markedly spoiled pharmaceuticals can often yield surprisingly low numbers of spoilage organisms. Although isolated in large quantities, a micro-organism may have little spoilage role in the product (e.g. ungerminated spores) or be secondary contaminant that has totally outgrown the primary spoilage organisms. Micro-organisms may be unevenly distributed within a pharmaceutical presenting sampling complications. Non-lethal, but inhibitory materials present in the formulation may require inactivation before assessment of the microbial population.

Another major drawback in the official standard for microbial limit test is the fact that tests are prescribed for aerobic organisms only. Some of the reported infections due to ingestion of oral non-sterile pharmaceuticals had been attributed to some anaerobes especially Clostridium species.

The low redox potential of some pharmaceuticals enable the growth of some facultative anaerobes and micro-aerophillic organisms. No one of the several methods of enumerating the number of bacteria in a product can give 100 % yield. The percentage yield or accuracy very much depends on the methodology used. There is extreme difficulty of performing accurate and precise microbial counts on materials other than simple aqueous solutions.

7.3 MEASURES TO REDUCE MICROBIAL CONTAMINATION

For effective reduction in the number of microbial contaminants and absence of others, contamination control criteria must be established which should include:-

(a) The types of microbial contaminants concerned, their biochemical properties and their mechanism of contamination i.e. source of contamination.

(b) A description of the contamination control system.

7.3.1 RAW MATERIALS

It is essential that raw materials are stored under conditions designed to deter microbial proliferation. Selection of materials of a good microbiological quality aids in the control of contamination levels in both product and the environment. All raw materials for use in manufacturing pharmaceuticals should have a stated degree of acceptable microbial purity, particularly emphasis on raw materials of plant and animal origins. Raw materials having high microbial count should be subjected to treatment such as using ethylene oxide if it is dry solid and compatible, irradiation and dry heat, so as to reduce the initial count. For hygroscopic raw materials, they should be stored under conditions that is below the minimum water activity (Aw) of bacteria, yeast and fungi. For liquid raw materials, pretreatment withfilteration can be employed. For some liquids and syrups which base their ability to inhibit osmophiles upon their osmotic pressure, they should be held under constant temperature since any variation may result in evaporation of the water content, followed by condensation and dilution of the surface layers to give Aw value

which may permit the growth of osmophiles and spoil syrups. Generally storage of raw materials in a clean dry air environment is recommended.

7.3.2 WATER

Stored distilled water can be irradiated by ultra violet light of wavelength 2400 - 2800 Å, but this is not very effective as the depth of penetration is low and this is further reduced by the presence of dissolved and suspended mineral and organic matters. The flow rate, contact time and hours of lamp operation are critical in U.V. irradiation, and these make U.V. irradiation inconsistent and unreliable. In case of de-ionized water which is easily contaminated due to the resin bed, the de-ionizing system is treated with 2 % formaldehyde, after having exhausted the resin beds with brine. The system can also be treated with live steam.

The sterilization of water by heat requires a temperature of 121°C and a pressure of 15 psi. For large quantities of water, large pressure vessels are necessary which are rare in most production units of firms producing non-sterile products. Filteration of especially large volumes of water

with low microbial counts can be employed. Usually a membrane of pore size of 0.45 u is adequate with application of negative pressure. The amount of water that is filtered at each time is however low for commercial significance. However, an effective filtration system by Ladish Co, Kenosha, Winsconsin, which has a flow rate of 115 gallons/min, at filtration cost of $2.70 per 1000 gallons is available (Dawson 1973). For use during the manufacturing process, freshly distilled water should be used. Supply of clean water can be maintained during storage in tanks by the use of U.V. lights, with provision for constantly recirculating the water, or the water kept at a constant temperature of 80°C, with a periodic monitoring system to ensure that it is in a good working condition. Periodic examination of the water for microbial contamination count is recommended.

7.3.3 EQUIPMENT

The equipment used for manufacturing must be thoroughly cleansed and sanitized. Use of Sanitizing solution is very effective in reducing incidences of microbial contamination. Traces of Sanitizing solution is removed by rinsing with steam

and hot water. The following points are common to many pieces of plant and serve as a general guide to reduce the risks of microbial contamination:-

(a) All equipment should be easy to dismantle and clean.

(b) All surfaces which are in contact with the product should be smooth, continuous and free from pits with rounded corners and junctions. All internal welding should be polished out smooth.

(c) There should be no inside screw threads while the outside ones should be accessible for cleaning.

(d) Coupling nuts on pipework and valves should be such that it can easily be taken apart for cleaning.

(e) Agitator blades should preferrably be of one piece and easy to clean.

(f) The product should be protected from any lubricant used in moving parts.

(g) Valves should be of the plug type instead of the diaphragm type where possible. Where the

latter is essential, then the valve must be routinely inspected and worn diaphragm replaced.

(h) All pipelines should slope away from the product source and all process and storage vessels should be self cleaning.

(i) If a vacuum exhaust system is used to remove steam or air from a preparation vessel, it is necessary to clean and disinfect all fittings regularly to prevent nutrients which may be drawn into them from supporting microbial growth, which may in the form of condensate contaminate subsequent batches of the product.

(j) Adequate covers should be provided for the equipment to prevent contamination between the intervals of cleaning and use. For this, polyethylene covers are suitable.

7.3.4 CONTAINERS AND CLOSURES

They should be well cleaned. Packaging serves both to contain the product and to prevent the entry of micro-organisms or moisture which may result in spoilage. Packaging materials which have a smooth impervious surface, free from crevices or interstices, made of cellophane, polyethylene, and

similar materials with low microbial counts are preferrably used where compartible. Multidose containers are prone to contamination in use and their use should be avoided, using unit dose containers especially for ophtahlmic and some topical preparations, while collapsible tube can be used for semi-solid topical preparations. Cardboard and cork liners should be replaced with metallic, glass and plastic ontainers and closures. Closures for products which are not adequately preserved may be sterilized by either formalin or ethylene oxide gas.

7.3.5 PERSONNEL

Human contamination can be minimized by placing the work between the clean air source and the operator. The use of protective garments e.g. use of Freostat nylon in areas where explosive gases are encountered and Polyester yarn in mechanical operations and areas where static electricity is not a problem, can reduce contamination from personnel. In selecting technicians for clean room work, temperament, appearance, psychological and physical factors must be considered. The technicians and other workers involved in production must be

educated on the essence of sanitization, cleanliness and the problems encountered if proper attitudes and procedures are not followed. Workers suffering from cold and other Communicable diseases and those having wounds or abscesses should report to the supervisor, so that theycan be exempted from production unit, during the period. Seminars should not only be frequently held for supervisors and trained researchers, but also for those that are actually involved in production, packaging and storage Hand gloves and face masks especially the type that covers the nose and mouth can be worn along with other protective garments.

7.3.6 ENVIRONMENTAL CONDITIONS

Another contact area concerned is, the Plant cleanliness and Sanitization. According to the regulation of Good Manufacturing Practice (GMP) for drugs, the building "Shall be maintained in a clean orderly manner and shall be of suitable size, Construction and location in relation to its Surrounding to facilitate adequate cleaning, maintenance and proper operation for their intended purpose - manufacturing, processing, packaging, labelling or holding for a drug",

(Runkle and Phillips 1969). With regards to microbial considerations, the regulations state that among others, control to minimize dissemination of microbes from one area to another is necessary.

The facility design features should include:-

(a) Use of ventilated cabinets, chambers, etc. to achieve an absolute or partial barrier to contain microbes at their point of use or exclude them from specific work area.

(b) Use of laminar air flow devices to exclude microbes from a particular environment. The dust free air brought into the room would eliminate virtually all air-borne bacteria.

(c) Use of differential air pressures within a facility or sub-facility so that air moves from a biological clean area towards an area of higher microbial contamination.

(d) Use of appropriately effective filtration for air supplied to and/or exhausted from rooms, cabinets, etc.

(e) Changing rooms, lockers, water or air shower rooms for personnel.

(f) Use of U.V. air locks and door barrier to separate areas of unequal microbial loading risks.

(g) Room arrangement or layout to achieve traffic control within the facility along a clean contaminated axis.

(h) Use of an effective intercommunication system in the facility to avoid unnecessary movement of personnel from one area to another.

The walls and ceilings should be smooth, impervious and washable, and this is done by cladding with laminated plastic. All floors should be easy to clean, impervious to water and laid on a flat surface. Where necessary, floors should slope towards a drain. Overhead pipes in all manufacturing area should be sited away from equipment to prevent condensation and possible contamination from falling into the product.

As the environmental contamination level is very much affected by the air microbial level, it is necessary to reduce air microbial count. Filtration is the most effective means of reduction of air microbial counts. Filters of cellulose, glass, wool or glass fibres are used. For many work areas, a standard of less than 100 particles per cubic feet of 0.5 um larger and the absence of particles larger than 5.0 um is adequate. Fine filters are normally proceded by coarse filters. Chemical disinfection which can also be used, has its limits because of its irritant effect when sprayed. Atomized propylene glycol at a concentration of 50 ug/litre and quarternary ammonium compounds at a concentration of 0.75 % may be used. Formaldehyde gas, fumigated at a concentration of 1 - 2 mg per litre of air and at a relative humidity of 80 - 90 % is effective. The use of ultra-violet irradiation to reduce air microbial count is limited because its effective depth is low.

7.3.7 STORAGE

Prevervatives are sometimes added to products to prevent or arrest growth and proliferation during the period of storage before use. A spoilage test is usually done to determine whether the product need

preservatives. Packaged drugs should be stored in dry, clean and well maintained environmental conditions, and at temperatures where microbial spoilage and proliferation is minimal.

7.4 CONCLUSION

The results of this investigation indicate that the microbial count of tablet products are influenced by the following factors:-

(1) the microbial flora and level of the starting raw materials especially in direct compression method;

(2) the tablet production environment;

(3) the quality of water used in making binder solution for wet granulation; and

(4) the temperature of drying the resulting granules.

Generally, the higher the microbial contamination level of these items or areas, the greater is the risk of contamination level of the final tablet product, and the greater the potential to health hazards. It is therefore expedient for manufacturers to be mindful of the quality of the

starting raw materials, not onlyiin terms of purity of contents, but to ensure minimum microbial contamination. Extra care of the production environment, equipment and even personnel cannot be over stressed. When wet granulation method is involved, the high microbial contamination potential of water should be minded, and adequate steps taken to minimize this risk. As much as possible, wet granules should be dried at above 50°C or by means of other processes that do not aid bacterial proliferation.

The manufacturing operations from the beginning to the end should be supervised by an expert. The microbiological control data at each stage of production should be kept for future reference in such cases as reviewing of procedures and in the determination of critical points of microbial control systems. From such datas, measures to adopt in limiting microbial contaminations can be formulated. Beside conformity of articles to their individual monographs, pharmaceutical manufacturers should ensure that their products as it gets to the consumers are microbiologically safe.

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