UNIT -V

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Microbial Spoilage

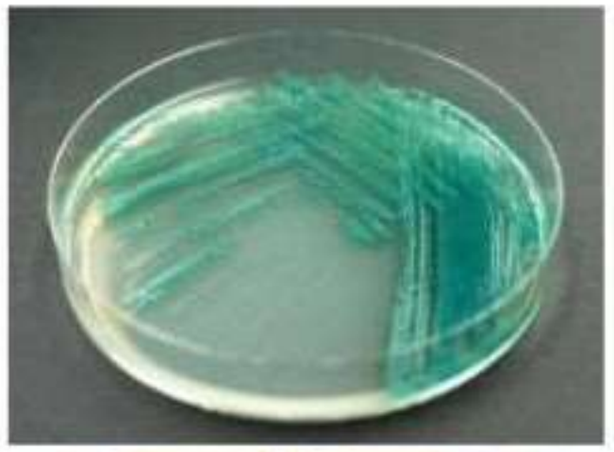
Microbial spoilage is defined as “deterioration of pharmaceutical products by the contaminant microbe.

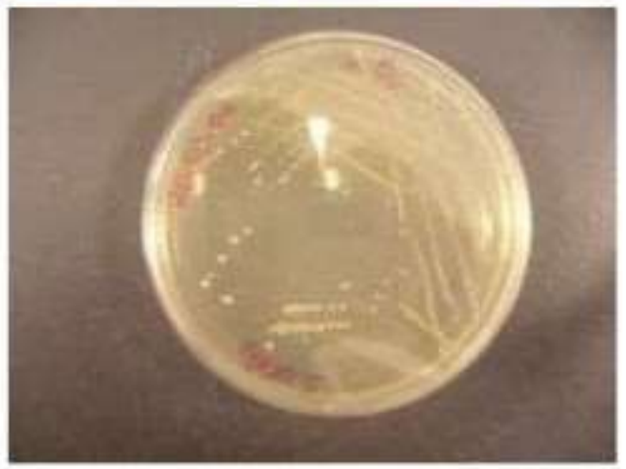
Preservation

Inhibiting or minimizing preferably during the storage and multi-dose application the risk of microbial contamination of pharmaceutical products

MICRO-ORGANISMS INVOLVED IN THE SPOILAGE OF PHARMACEUTICALS

1.Pseudomonas aeruginosa
2.Salmonella cavaban
3.Escherichia coli
4.Staphylcoccus aureus
5.Clostridium botulinum
6.Clostridim perfingens
7.Streptococcus faecalis
8.Proteus sp
9.Pseudomonas sp
10.Gram negatives can be isolated in many pharmaceutical products as contaminants(e.g.Pseudomonas, Serratia, Klebsiella)





SOURCE OF CONTAMINATION

In manufacture

raw materials (water and materials with natural origin) microbiological quality

pharmaceutical industry environment-

wet sites, cleaning equipment

packaging e.g. cardboard,corks,papers are unsuitable packaging materials

containers that are frequently re-used

contamination of disinfectants due to re-used containers

repackaging of products purchased in bulk into smaller containers

processing

storage

transportation

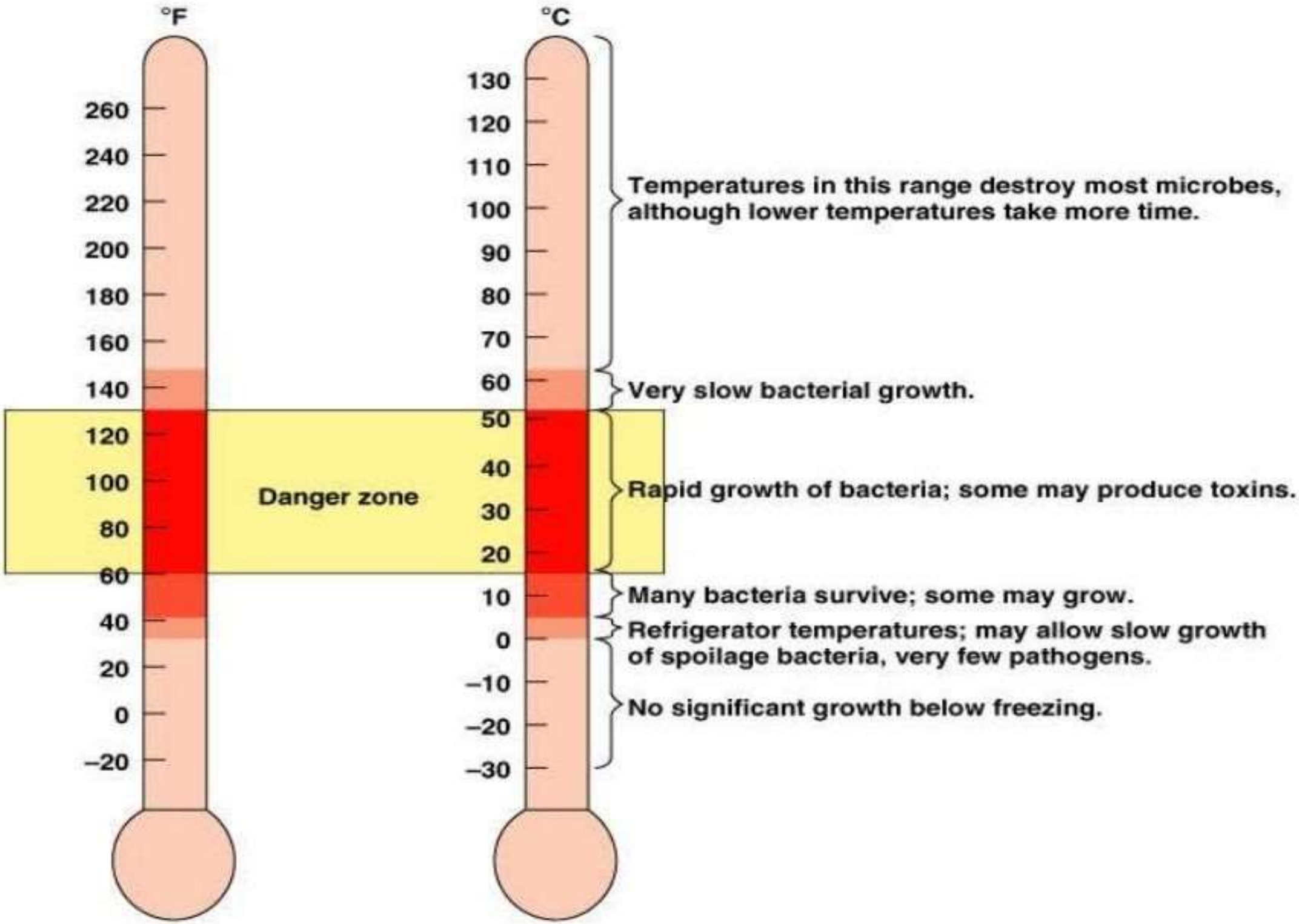
Microbial growth in food is dependent on

- **Intrinsic Factors**: physical and chemical properties of the food
- **Extrinsic Factors**: Storage conditions
- **Implicit Factors**: Physiological properties of microorganisms
- **Process Factors**: heating, cutting,..

Intrinsic factors

- pH
- Water activity (a_w)
- Redox potential (Eh)
- Nutrient content
- Antimicrobial constituents
- Biological (antimicrobial) structures

PH



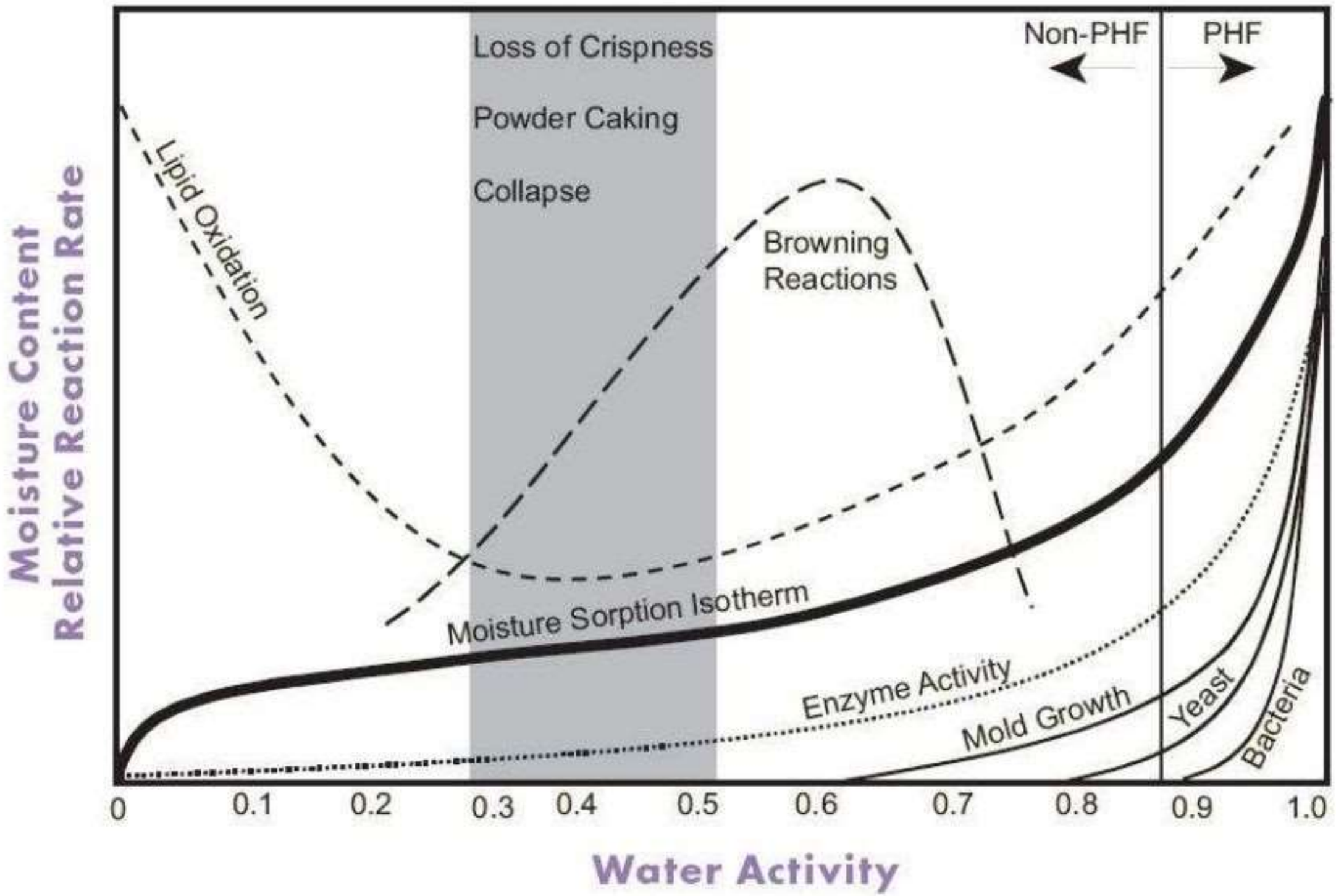
The intensity of acidity of a food is expressed by its pH value. The pH of a food is one of several important factors that determine the survival and growth of microorganisms during processing, storage and distribution. Consequently, food processors are interested in determining the pH of foods and in maintaining pH at certain levels to control microbial growth and prevent product deterioration and spoilage. Every microorganism has a minimum, an optimum and a maximum pH for growth. Most microorganisms grow best at pH values around 7.0 while only a few grow below pH 4.0. Yeasts and molds are generally more acid tolerant than bacteria and can grow at lower pH values. Foods with pH values below 4.5 are usually not easily spoiled by bacteria but are more susceptible to spoilage by yeasts and molds. Microorganisms can grow in wide pH ranges and these ranges are probably the difference between different bacterial strains, types of food or growth medium and the type of acid or base used to adjust pH.

Water Activity

Microbial growth is the reason water activity began to be used in the food industry. Before the discovery that water activity controls microbial growth, food producers never knew why some batches molded and others didn't. They suspected it had something to do with water, but everyone knows that high-moisture salt pork doesn't mold, while low moisture nuts still do. Water activity better predicts the growth of microorganisms because microorganisms can only use "available" water, which differs considerably depending on the solute. On average, ions bind the most water, whereas polymers bind the least water; sugars and peptides fall into an intermediate position. At the same molecular concentration, salt lowers the water activity more than sugar.

When a substance is added to lower water activity, the result can be complicated. Ideally, an inert material could be added which would decrease water activity without any other effects such as increased ionic strength and decreased surface tension. In reality, the choice of substance can have a profound effect. For instance, salt could be added to one reaction mixture and sugar to another. The amounts can be controlled so that the resulting compounds have identical water activity. But the results of the reaction will differ because of the differing influences of salt and sugar on biological reactions. Salt and sugar form different additional hurdles.

Water Activity - Stability Diagram



Redox potential

This is the ratio of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of a substance.

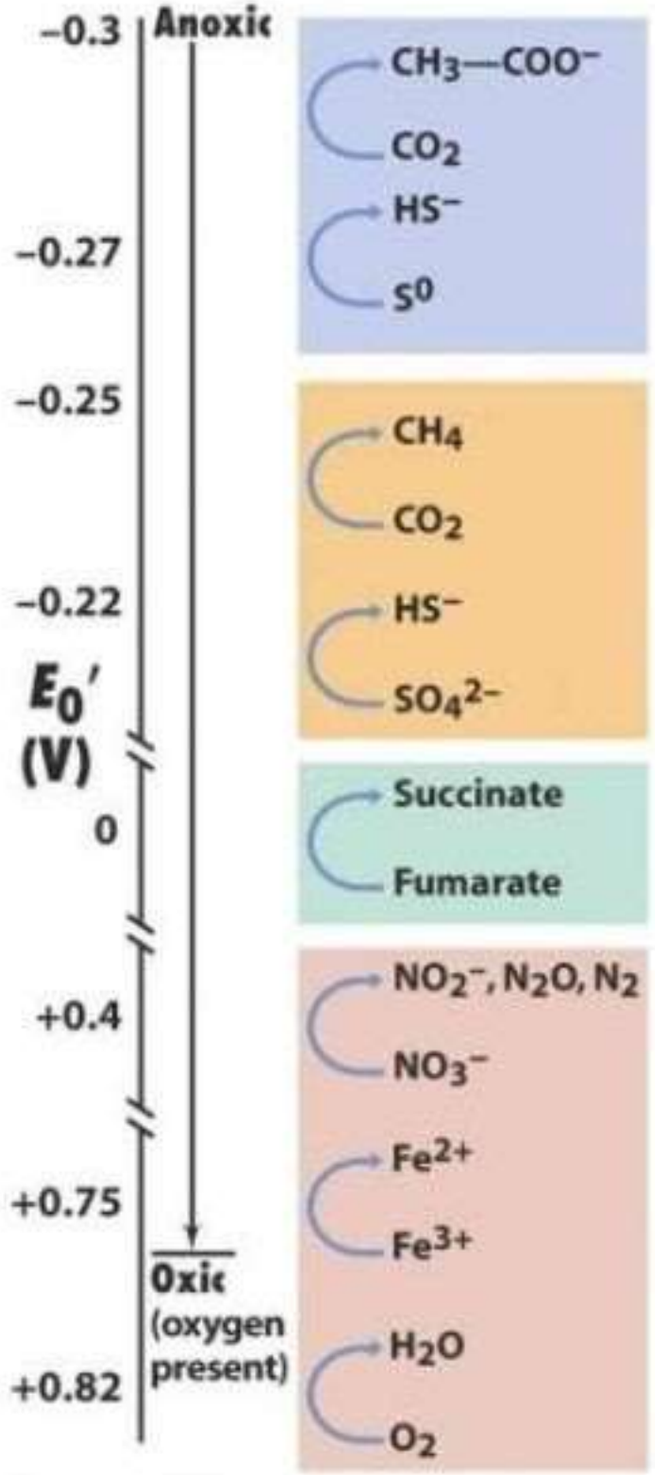
Eh is a measurement of the ease by which a substance gains or losses electrons.

Eh is measured in millivolts (mV)

The more oxidized substances, the higher the Eh; the more reduced substances, the lower the Eh.

Microorganisms that grow at:

- ✓ high Eh or +ve Eh (require oxygen) – Aerobes
- ✓ low Eh or –ve Eh (oxygen is toxic)- Anaerobes
- ✓ high and low Eh (+ve /-ve Eh) – Facultative anaerobes
- ✓ relative low Eh values – Micro-aerophilic



Nutrient Content:

- In order to grow and function normally the microorganisms of importance in foods require the following:
  - Water
  - Source of energy
  - Sources of nitrogen
  - Vitamins and related growth factors
  - Minerals

Nutrient Content

Microorganisms require

A. Energy source such as carbohydrates, amino acids, proteins, organic acids and alcohol.
B. Nitrogen source such as amino acids,peptides,nucleotides,urea, proteins and ammonia.
C. Carbon source
D. Minerals such as phosphorus, iron, manganese,magnesium,calcium and potassium.
E. Vitamins and other growth factors

Anti-microbial

Antimicrobials prevent microbial spoilage.Some products cannot be easily attacked by micro organisms due to presence of naturally occurring substances having anti-microbial activity.

Examples:

1.Eggs have lysozymes along with conalbumin which provides fresh eggs with a efficient anti microbial system.

2.Fruits,vegetables,tea contain hydroxycinnamic acid which shows an anti-microbial activity.

3.Natural constituents of foods which affect microbial growth are:

- ✓ Lysozyme e.g. Eggs
- ✓ Lactoferrin e.g. Milk
- ✓ Lactoperoxidase e.g. Cow's milk
- ✓ Conglutinin e.g. Cow's milk
- ✓ Essential oils e.g. Spices and vegetables

Preservatives such as benzoic acid, sorbic acid.

Biological Structures

Some foods are naturally covered and these covering provide excellent protection against the entry and subsequent damage by spoilage organisms.

Testa of seeds, the **outer covering of fruits** the **shell of nuts**, the hide of animals and the **egg shells**.

EXTRINSIC PARAMETERS

These are the properties of the storage environment that affect both the foods and their microorganisms. These are:

- Temperature of storage
- Relative humidity of the environment
- Presence and concentration of gases
- Presence and activities of other microorganisms

Temprature

- Individual groups of micro organisms grow over a wide range of temperature requirements for growth, microorganisms can be placed in three different groups.
- Psychrotrouphs: Are those organisms that grow well at or below 7 degree celsius and their optimum temperature between 20 to 70 degree celsius.
- Mesophiles: Those organisms that grow well between 20 -45 degree celsius with optimum temperature between 30-40 degree celsius.
- Thermophiles: Those micro organisms that grow well at and above 45 degree celsius with optima between 55 degree celsius and 65 degree celsius.

Temperature dependent growth

Start amount
1.000 bacteria per gram food

16.000
bact.

2.000
bacteria

20 °C

4.000
bacteria

7 °C

260.000
bact.

4 Million
bact.

8.000
bact

Related humidity of environment

When foods with low Aw values are placed in environments of high R.H the foods pick up moisture until equilibrium has been established. Foods that undergo surface spoilage from moulds,yeasts and certain bacteria should be stored under condition of low R.H. Improperly meats such as whole chickens and beef cuts tend to suffer much spoilage in the refrigerator before deep spoilage occurs. This due to high R.H of the refrigerator and the fact that meat spoilage microorganisms are aerobic in nature.

Presence of Concentration of Gases in The Environment

Controlled atmosphere (modified atmosphere storage)

- This is the atmosphere containing increased amounts of CO_2 up to about 10% CO_2 is applied from mechanical sources or by use of solid CO_2
- Modified atmosphere is employed in storage of fruits e.g. apples and pears. CO_2 retard fungal rotting of fruits
- CO_2 atmospheres extend the storage life of meat carcasses. In general the inhibitory effects of CO_2 at lower temperatures, and the pH of meats stored in high- CO_2 environments tends to be slightly lower than that of air – stored due to carbonic acid formation.

- Gram-negative bacteria are more sensitive to CO_2 than gram-positive.

- Ozone (O_3); when added to food storage environment has a preservative effect on certain foods. At levels of several parts per million (ppm) O_3 has been found to be effective against a variety of microorganisms O_3 should not be used on high-lipid content foods because it would cause an increase in rancidity as it is a strong oxidizing agent

Susceptibility of pharmaceutical Ingredients to microbial degradation .

- **Organic polymers**
- pectin, cellulose, dextran
- microbial depolymerisation by a number of extracellular enzymes
- agar-agar
- inert polymer
- PEG
- easily degraded
- synthetic polymers (nylon, polystyrene, polyester)
- extremely resistant

- **Preservatives And Disinfectants**
- Many preservative & disinfectants can be metabolized by a wide variety of gram negative bacteria but at concentration below their effective use levels.
- Pseudomonas has outstanding degradative capacity
- **Surface active agents**

VISIBLE SIGNS OF MICROBIALDEGRADATION

- loss of viscosity and sedimentation due to depolymerisation of
- suspending agents
- pH change
- gas production
- Unpleasant smelling & tasting metabolites such as “sour” fattyacids, “fishy” amines, “bad eggs” bitter, earth or sticky taste or
- smell indicates the spoilage.
- Products may become unappealingly discolored by microbial pigments of various shades.
- Microbial polymerization of sugars & surfactant molecules can produce shiny, viscous masses in syrups, shampoos & creams & fungal growth in creams has produced gritty textures.
- Metabolism of surfactant in o/w emulsions reduce stability & accelerate creaming of the oil globules. Release of fatty acids lower
- pH & encourage coalescence of oil globules & cracking of emulsion

FACTORS AFFECTING MICROBIAL SPOILAGEOF PHARMACEUTICAL PRODUCTS

- **1. Types and size of contaminant innoculum**
- **2. Nutritional factors**
- **3. Moisture content**
- **(water activity)**
- **4. Redox potential**
- **5. Storage temperature**
- **6. pH**
- **7. Packaging design**
- **8. Protection of microorganisms within**
- **pharmaceutical products**

HAZARD OF MICROBIALCONTAMINATION TO HEALTH

- since the early part of the 20th century -increasing number of documented medicament-related infections; significant decrease in only 1960s and 70s
- In 1989, poorly contaminated Chloroquine syrup killed several children in U.N.T.H,
- Enugu in the early „80s of which there is no statistics, partly because many of the
- deaths were not even reported.
- In 2003 fake cardiac stimulant (Adrenalin) contributed to the death of three children during open-heart surgery at UNTH, Enugu. Further investigations by NAFDAC revealed that even the muscle relaxant used was substandard and the infusion was not sterile.
- CJD; human growth hormone derived from human pituitary glands. These particles are considered responsible for such diseases as scrapie, bovine spongiform encephalopathy, kuru, and Creutzfeldt-Jakob disease.
- microbial toxins
- bacterial endotoxin (LPS), bacterial exotoxins (food poisoning),aflatoxin.
- In 1990, the "Paracetamol syrup disaster" occurred when 109 children died in Ibadan
- and Jos, after taking paracetamol syrup produced with the toxic ethylene glycol solvent instead of propylene glycol. This tragedy occurred more than fifty years after that of the U.S.A.
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Preservatives

- inhibit or minimize preferably during the storage and multi-dose application the risk of microbial contamination of pharmaceutical products.
- The inclusion of preservatives may be unnecessary in case of tablets, powders and capsules.
- Preservatives should never be added to mask poor manufacturing process

The ideal preservative:

- **The Ideal Preservative**
- **Is free of toxic or irritant effects at the concentrations used.**
- **Is effective in preventing the growth of micro-organisms most likelyto contaminate the preparation.**
- **Is sufficiently soluble in water to achieve adequate concentrations inthe aqueous phase of a system of two or more phases.**
- **Has adequate stability to heat and prolonged storage, with no chemical decomposition or volatilisation during the desired shelf-life.**
- **Is chemically compatibility with all other formulation components andretains the undissociated form at the pH of the preparation.**
- **Is not adversely affected by the product‟s container or closure.**
- **Has an acceptable odour and colour.**
- **Is cheap!**

Methods of Preservation of Pharmaceutical Products

- The five basic methods of preservation are as follows:
- physical protection
- preservative coating only
- waterproof protection
- Watervapor proof protection
- Watervapor proof protection with desiccant

SAFETY PRECAUTIONS

- QA = quality assurance; encompasses a scheme of management which embraces all the procedures necessary to provide a high probability that pharmaceutical product will conform consistently to a specified description of quality
- R&D = formulation design and development
- GPMP = Good Pharmaceutical Manufacturing Practice
- QC = quality control; controlling the totalmanufacturing process in each step
- post-marketing surveillance
- contamination risk assessment

GPMP = Good Pharmaceutical Manufacturing Practices

- GMP is a part of a quality system covering the manufacture and testing of active pharmaceutical ingredients,diagnostics, pharmaceutical products, and medical devices. It includes the following,
- 1.Precautions must be taken to prevent unauthorized persons from entering storage areas.
- 2. Storage areas should be designed or adapted to ensure good storage conditions. In particular, they should be clean and dry and maintained within acceptable temperature limits. Where special storage conditions are required on the label (e.g. temperature, relative humidity), these should be provided.
- 3. Storage areas should be clean, and free from accumulated waste and vermin.

Thankyou

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