MICROBIOLOGICAL CONTAMINATION AND PRESERVATION OF PHARMACEUTICAL PRODUCTS

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INTRODUCTION

CONTAMINATION AND SPOILAGE

Contamination is the introduction of microorganisms into a product. Contaminating organisms may arise from many sources during the course of manufacture and subsequent use of the product.

A spoiled product may be described as one that is unfit for use.

Microbial spoilage can be caused by bacteria, yeasts or fungi which are all extremely versatile in their metabolic activities since they can adapt to very broad range of environmental conditions.

As a result, all classes of natural organic compounds are susceptible to degradation and synthetic compounds are also attacked, although often less readily.

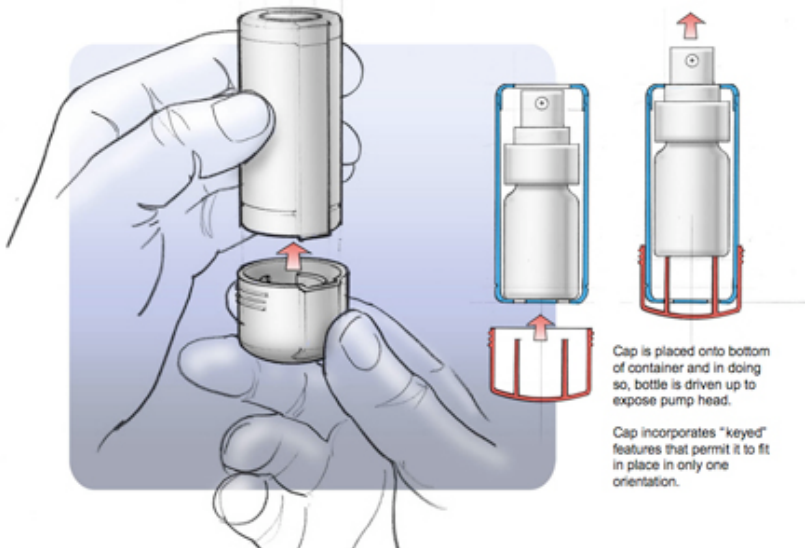
CONTAMINATION AND SPOILAGE

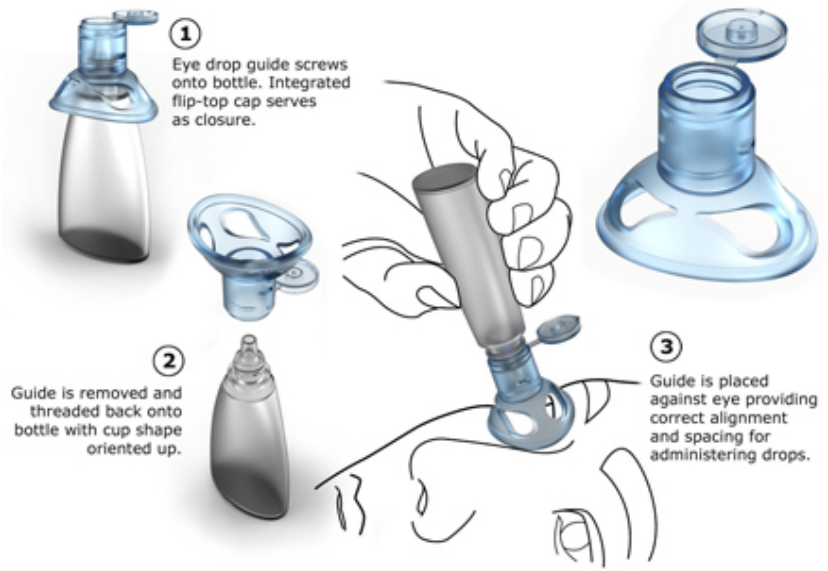
- The processes of Good Manufacturing Practice (GMP) are used to reduce contamination during manufacture but contamination arising from patient is largely out of control of the manufacturer except by container design and labeling.

- Spoilage is followed by contamination and describes the processes and consequences of the microbial growth in the product.

- Taking appropriate steps for reducing the steps to minimize the risk of spoilage is the responsibility of the formulation scientist and the manufacturer.

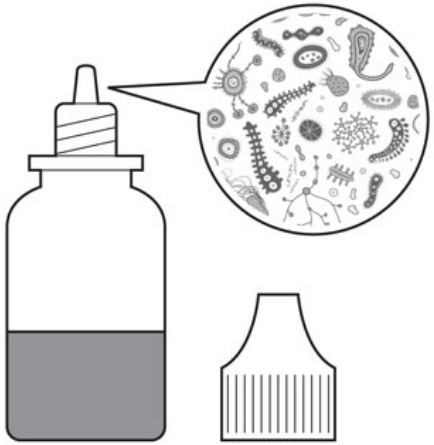
Packaging design





Current product drawback:

Germ sticking cannot be avoided once opened for most eyedrop products. This may lead to infection especially to patients with eye disease.



Reasons for exclusion

The three reasons why microorganisms should be excluded from medicines are:

1. Products or raw materials contaminated with pathogenic organisms may be an infection hazard.
2. Microorganisms may cause chemical or physical changes in the product that causes it to be less potent or effective.
3. Microbial growth is likely to make the product unacceptable to the patient or consumer even if there are no significant infection risks or loss of efficacy.

Manifestations and Mechanisms of Microbial Spoilage

- Before spoilage can occur organisms which are capable of altering the components of a product must first be introduced via raw materials, the processing plant, packaging materials, operatives or elsewhere in the environment.

- Although spoilage does not necessarily depend upon the growth of these contaminants it is generally facilitated if the formulation and the ambient conditions of temperature and humidity encourage their multiplication.

- When these criteria are satisfied, changes in the product will occur and may ultimately manifest themselves to the user in one or more of the following ways:

- **Toxic effects:** Microbial toxins produced by microorganisms can produce toxic effects. E.g. Endotoxins, produced by Gram- negative bacteria such as *Escherichia coli* are very important in connection with injectable products.

- **Metabolic products:** Simpler catabolic products such as organic acids and amines, which can be toxic to human, may be produced.

- As these compounds are considerably less toxic than are the classic bacterial toxins, relatively high concentrations have to be attained before a spoiled product causes illness.

- **Change of activity:** The inactivation of biologically active molecules by organisms contaminating a formulation have been observed to occur in practice. A classic example is the destruction of penicillins by penicillinases enzymes produced by a broad range of micro-organisms.

- A loss of atropine of up to 20% in eye drops could be caused by *Corynebacterium* and Pseudomonas spp. Isolated from the eye drops and atropine itself. Another organism, *Corynebacterium hoffnaii*, which was isolated from laboratory dust, metabolized the analgesics aspirin, phenacetin and paracetamol.

- **Visible effects:** In liquid formulations contaminants may be seen as a sediment, turbidity or a pellicle while on more solid preparations colonies, often colored, of bacteria, yeasts or moulds may form.

- **Color changes:** Color changes due to alterations in the components of a product may result from pH, redox or other changes caused by the metabolic activities of an organism, or to pigment production by the contaminants themselves. Members of the Pseudomonas genus are often implicated in spoilage of this type.

- These organisms metabolize a very broad range of compounds and can also produce soluble pigments ranging in color from blue-green to brown. Similarly, in an acidic product, oxidative yeasts can cause arise in pH by utilizing organic acids and this w ill encourage bacterial growth.

- **Gas production:** If microbial metabolism produces gas in a sufficient amount to exceed its solubility in a product visible bubbles, frothing and other manifestations of an increase in pressure occur. Products containing carbohydrates or other fermentable substrates are particularly susceptible to this type of spoilage.

Other Changes:

- **Irritancy:** The eye is particularly susceptibel to infection from contaminated products and it is also at risk from the direct effect of irritant metabolites left in a product even after the organisms producing them have been eradicated.

- **Olfactory effects:** production of characteristic odor e.g. sulphur-containing metabolites such as hydrogen sulphide, fishy odor of amines and the astringency of ammonia.

- **Texture:** Creams can become lumpy or 'gritty' and changes in viscosity of liquid preparations, which can be detected when applied to the skin.

Sources of Contamination

- Microbial contamination occurs from three principal sources:
- The raw materials, including water, from which the product is manufatured.
- The manufacturing environment, including the atmosphere, equipment and work surfaces.
- Manufacturing personnel.

- **Water:** Distilled water can readily pick up organisms from pipes and tubing and ion-exchange columns may actually serve as a reservoir of organisms because nutrient organic residues are not removed by the process.

- Without effective treatment to minimize contamination, water can, within a few days, contain large numbers of initially Gram-negative and Gram-positive bacteria and subsequently a wide variety of bacteria, moulds and yeasts.

- At this stage visible and olfactory spoilage occurs and a foul taste may develop. Often the responsible organisms are pseudomonas which are highly resistant to preservative

- **Simple aqueous solutions:** Some moulds will grow on such unlikely media as strong solutions of copper sulfate or sulfuric acid and simple solutions of inorganic compounds will support the growth of many sorts of microbe.

- Detection of turbidity due to algae, moulds, bacteria or yeasts in a multiplicity of different solutions including ammonium carbonate, neutral ammonium tartrate, calcium digluconate and potassium citrate have been reported.

- **Suspensions:** Aqueous suspensions of inorganic material for pharmaceutical use frequently support microbial growth, particularly as added preservatives tend to be absorbed and inactivated by the suspended matter.

- Unless growth is at the surface, as with mould contaminants, it is not easily detected visually because of the opacity of these products.

- When the lid is removed spoilage is sometimes manifested by an offensive odor or an unpleasant taste. Thus, a medicament for the treatment of an intestinal disorder may exacerbate, rather than alleviate, the condition.

- However, a part from visible growth, a variety of other changes in appearance may be seen and preparations of this type can thin, separate, decolorize, or change color.

- **Emulsions:** O/W emulsions are particularly susceptible to spoilage as the water in the continuous phase allows contaminants to spread throughout the product.

- Spoilage in emulsions can be manifest by changes in rheological properties, including separation or 'breaking down'. Decolorization, changes in odor and taste and signs of visible growth also occur.

- **Creams and lotions:** Mould growth is one of the most common causes of spoilage of creams of all types and can occur in products as varied as antifungal, calamine, baby and hair creams and a number of other cosmetic formulations including moisture and cleansing creams.

- **Syrups:** The sugar content of syrups (67%) inhibits the growth of many micro-organisms by virtue of its high osmotic pressure but osmo tolerant moulds and yeasts are a source of trouble.

- Fermentation of the sugar by these organisms causes foul flavors due to the production of alcohol, lactic acid and other organic acids.

- Fluctuating storage temperatures then cause sufficient condensation of water vapor to dilute the syrup at its surface so that growth can occur.

- **Raw Materials:** Solid raw materials may serve as a source of contaminants which will later spoil a formulated product.

- Solids can be contaminated with *E. coli* and *Staphylococci*.

- Spoilage of the solid raw material itself is largely due to mould growth on the surface due to improper storage with inadequate coverings in a damp environment or under conditions of fluctuating temperature.

- Tablets: Visible spoilage of tablets generally manifested as surface discoloration may be caused by the growth of moulds.

- Spores from the environment, container or tablet itself may find sufficient moisture to initiate growth on the tablet surface even under apparently dry conditions.

- For instance, fluctuations in temperature or variations between those in different parts of the container can cause corresponding changes in relative humidity.

Packaging Materials: Containers for pharmaceuticals are becoming increasingly elegant and are now made from a large variety of materials, particularly plastics.

- This should result in a reduction in microbial spoilage because plastics are not biodegradable like the cellulose materials, paper and card.

- In addition cellulose materials soak up liquids thus providing a substrate for moulds.

- In any case, liners of paper and card and cork closures often contain many micro-organisms and therefore frequently are a source of mould contamination where as plastic closures are free from this defect.

Plastics suffer from some disadvantages:

- They are porous to varying degrees and some allow the diffusion of oxygen and carbon-dioxide and may thus facilitate microbial growth in the packed product. They also encourage condensation of water and, if mould spores are present, can facilitate spoilage by these organisms.

Factors Influencing Growth of Microorganisms

In addition to water, the growth of a contaminant within a raw material or manufactured medicine include:

- **Nutrient availability:** Products containing glycerol, sugars, amino acids or proteins can represent ideal media for microbial growth even in the presence of preservatives.
- **Temperature:** Bacterial growth in manufactured medicine can be insignificant at temperatures between 15ºC to 20ºC within a two year shelf life.
- **pH:** Most bacteria have optimum growth pH values of 5-6.
- **Redox potential:** it represents whether oxidizing or reducing conditions exist in a liquid which may be required for the metabolism of different types of microorganisms.
- **The presence and concentration of antimicrobial chemicals:** Different preservatives posses different antimicrobial activity and thus need to be carefully selected. E.g. propylene glycol is a preservative whose activity is mostly limited to topical products.

Control of Contamination and Spoilage during Manufacture

- Standards for atmospheric cleanliness in the manufacturing areas for the production of both sterile and non-sterile products can be followed.
- Air supply can be invariably HEPA filtered and the filters need to be checked and cleaned according to protocols.
- Cleaning and dis infection procedures should be followed properly as mentioned in the Orange Guide.
- Health, hygiene, clothing and training of operators should be ensured.

- Raw materials may be exposed to radiation, ethylene dioxide to reduce high level of contamination.
- Filtration units and UV-light can be used to remove contamination in water.
- If the water is to be used as an ingredient for injectable product, filtration is preferred over UV-light.
- Water can be maintained at 8^{o}C whenever possible during manufacturing process in order to prevent microbial growth.
- Preservatives should be chosen carefully during formulation of the medicinal products.

- Parabens are used in topical and oral products.

- Eye drops contain benzalkonium chloride as preservative.

- Preservatives can be used in combinations to offer synergistic effect. Synergy is most likely to be exhibited when two agents have dissimilar modes of action.

- Examples of preservatives: ethanol, isopropanol. Propylene glycol and glycerol.

Conclusion

- In practice, regular monitoring during development and manufacture establishes the type and minimum number of organisms which are achievable for each specific product.

- Providing, of course, that this level is compatible with microbiological stability it forms the best approximate guide-line for a product.

HAPPY TO ANSWER IF U HAVE ANY QUESTION

THANK YOU