Principles of Preservation

What is preservation? When we speak as formulators about preservation, we are referring to the protection of our products from contamination by bacteria, yeast, or mold from the time the product is manufactured up until the time the product is completely used up by the consumer. In order to understand this problem and its solutions it is necessary to first define a number of terms.

A preservative is defined as a material that will prevent the growth of or react with and destroy microorganisms that might damage the product or create a health hazard by growing on or in the product. An **antiseptic** is a material that prevents the growth of and/or destroys microorganisms when applied to living tissue. An example of an antiseptic would be hydrogen peroxide. **Disinfectants** are materials that destroy disease-producing microorganisms on inanimate objects. **Germicide** is a general term used to refer to products that kill microorganisms. Two common suffixes used in microbiology are the terms **cidal** meaning kill and the term **stasis** meaning inhibition of growth.

Unlike disinfectants and many antiseptics that must act quickly and powerfully, often against specific organisms, preservatives must act steadily and effectively against a wide range of microorganisms over a long period of time. Preservatives however are not a replacement for basic good housekeeping procedures. This does not mean that the types of products that we are concerned about here must all be manufactured under sterile conditions, but a reasonable level of cleanliness is dictated by "GMP's" (Good Manufacturing Procedures). In other words, the preservative is intended to keep a clean product free of incidental contamination, not kill the overwhelming number of microorganisms that can be introduced during manufacturing in an unclean plant.

Now that we have defined preservation, it is important to understand why products need to be preserved. Microbial contamination of cosmetic products is of concern to industry, to regulatory agencies and most importantly to the consumer. Contamination of finished products may result in visible changes which may include off odor, color changes, viscosity and texture changes, gas production, degradation of active ingredients, or possibly, the most important and often invisible concern, potential health hazards. Regulatory agencies are particularly concerned about inadequately preserved topicals which may come into contact with the eye or which may be used on infants, the sick or the elderly.
Although we live in a world filled with microorganisms, these groups are particularly vulnerable to microbial attack. For example, infections caused by Pseudomonas aeruginosa, a Gram-negative bacterium, can be fatal to burn victims.

General Microbiology

The organisms that are capable of compromising both product integrity and safety are divided into 3 broad categories: bacteria, yeast and mold.

For optimum growth, bacteria in general prefer neutral or slightly alkaline pH and warm temperatures of 30-37'C. Bacteria can be divided into two classes based on a differential staining procedure, which distinguishes the chemistry in the bacterial cell wall. Gram-positive bacteria retain the primary stain while Gram-negative bacteria do not retain the stain material. This distinction is very important because many of the Gram-negative bacteria are considered pathogenic or disease producing while only a very few of the Gram-positive bacteria are considered pathogenic. Gram-negative bacteria are extremely difficult to control because of the complexity of their multilayered cell walls. The genus of Gram-negative bacterium that is of greatest concern to formulators is Pseudomonas.

Pseudomonas species are widely distributed in nature and can be isolated from soil, tap water, marine water, and even from the skin. Many Pseudomonas species are noted for their nutritional versatility and adaptability. Pseudomonas can be found which can degrade a wide variety of organic compounds such as starch, cellulose, hydrocarbons, and resins. They are resistant to most antimicrobials and are often severe health hazards. Pseudomonas aeruginosa is often responsible for bum and wound infections, urinary tract infections, and severe eye infections, which can result in conjunctivitis or loss of sight.

Yeast usually prefer an acidic pH and room temperature for optimal growth. They are of concern due more to their effects on the aesthetics of the formulation than to health hazards. Candida albicans is the most common representative of this group.

Mold, like yeast, also prefer an acidic pH and room temperature. They reproduce by spore formation and the spores can continue to survive indefinitely under favorable conditions. Spores are difficult to control as they can remain dormant in a hostile environment and can then become activated when the circumstances become conducive to their growth. A typical member of the group is Aspergillus niger, a widely distributed mold capable of product spoilage.

Microorganisms, like most other living things, normally have three basic requirements that are essential for growth: water, air (for aerobes) and nutrients.

Water is required in order to secure food and to eliminate waste products.
However, even anhydrous or non water-based products should be protected by a preservative system. During use conditions, for example in the bathroom, a film of water can form on an anhydrous product and in the high local concentration of water, microorganisms will grow.

Most microorganisms require air for survival and growth-, they are called aerobes. Air is required for the conversion of nutrients into energy.

Nutrients or food are required for the synthesis of cell-building materials or as a source of energy. Practically any carbon compound can nourish one microbe or another. The list ranges from the usual growth substances such as proteins and carbohydrates to unusual nutrients such as rubber, oil or paints.

Cosmetic formulations in particular are an excellent source of nutrients for microorganisms. Examples of ingredients that are especially good sources of food include: alcohols such as glycerol, sorbitol, mannitol and fatty alcohols, fatty acids and their esters, sterols including lanolin and its derivatives, proteins, vitamins, and botanical extracts.

There are many variables that influence the effectiveness of a preservative system. These include concentration of the preservative, contact time, the number of microorganisms present, PK inactivation or enhancement by other ingredients in the formulation, and packaging. We will explore each of these in greater detail.

In general, the higher the concentration of the preservative, the more effective it will be. Often preservatives are cidal, that is they kill, at high concentrations and exhibit stasis, that is prevent growth, at low concentrations. There is sometimes a tendency to want to "over preserve" products. This is not advised because high levels of preservative activity are often associated with toxic or irritant properties on animal tissues. One must remember that preservatives are specifically designed to kill living cells. Therefore, at some concentration they will undoubtedly begin to effect the skin. On the other hand, too low a concentration of preservative may be ineffective or may even stimulate microbial growth.

A second factor is contact time between the preservative and the formulation. This is important since the longer the contact time the greater the number of organisms killed. Theoretically, microorganisms are killed at a logarithmic rate. Under a specific set of conditions, the same percentage of a microbial population should be killed with each unit of time.

The third factor regulating preservative activity is the number of microorganisms challenging the system. Obviously, the greater the number the longer it takes for the preservative system to drop the count to some acceptable level. Too many organisms can overwhelm the preservative system.

The pH of the formulation is a fourth factor, as some preservatives, such as benzoic acid or parabens are only active in their acidic form.

One must also consider the interaction between the preservative and other ingredients in the formulation. This interaction can result in either inactivation or enhancement of the preservative depending on the chemical reaction taking place. For example, anionic surfactants usually inactivate preservatives that are cationics. Proteins inactivate quats, parabens and phenolics. Alternatively, enhancement of the preservative can occur when using raw materials such as alcohols, aldehydes, and acids because they often have some antimicrobial activity of their own. Another example of preservative enhancement is the use of EDTA (ethylenediaminetetraacetic acid) in a formulation. EDTA can be used to increase the permeability of the cell wall by chelating the metal ions that are part of its composition, thus increasing the organism's sensitivity to a wide variety of preservatives. In addition, EDTA's chelating ability allows it to tie up metal ions in the organism's surroundings, depriving it of essential mineral nutrients.

A related problem is the potential interaction with packaging. The finished product packaging should be designed to prevent access of contaminants into the container, but also must be made of materials that will not cause inactivation of the preservative due to adsorption or complexation. Low density polyethylene, for example, may adsorb parabens from a product.

Preservative Testing

Preservative efficacy testing is an essential part of substantiating the safety of a product. Most large personal care manufacturers have a microbiology staff that performs preservative testing. Smaller companies may use the services of an outside micro lab for testing. The goal of efficacy testing is to determine, not only which preservative system to use against the strains of microorganisms to which the product may be exposed, but also that concentration of preservative that will preserve the product during manufacturing and under use conditions.

The microbiologist's most important procedure for testing if a sample is contaminated is the Aerobic Plate Count or APC. The APC is used to determine the number of viable organisms present in a sample. This is carried out on agar plates that contain materials that support microbial growth. Each colony is assumed to represent growth from a single organism.

Preservative testing is often lengthy and time consuming. Therefore, there are a number of fairly rapid screening methods that are used by microbiologists. The most common of these is called the Minimum Inhibitory Concentration or MIC test. This test determines the lowest concentration of the preservative system that will retard microbial growth. It uses inoculations of standard organisms that are representative of both Gram-positive and Gram-negative bacteria, yeast and mold.

In light of the previous discussion of all of the factors that may influence the activity of a preservative, it is essential to test the preservative system in the actual finished formulation. This is done by means of an Adequacy of
Preservation Challenge Test or Challenge Test. For this test, the preservative is generally incorporated into a product base and "challenged" or inoculated with a large number of standard organisms along with various "house" organisms.
"House" organisms are organisms that have adapted to a particular product or environment and whose metabolic activity is varied from the norm of its particular strain. They are often unique to a manufacturing plant. Assays are performed over a predetermined period of time, typically 4 to 8 weeks, sometimes with a rechallenge at 3 or 4 weeks.

The "Ideal" Preservative

Before we can discuss the chemistry and function of preservatives, it is important to review the proper-ties of the so-called "ideal" preservative system. Please keep in mind that the "ideal" preservative does not exist and probably cannot exist in a single chemical entity.

An ideal preservative would have broad spectrum activity, which is the ability of the preservative to kill a wide range of microorganisms. Such a product would be effective against both Gram-negative and Gram-positive bacteria, yeast and mold. Usually, a multiple preservative system is needed to accomplish this goal.

A preservative should be effective at low concentrations in order to reduce costs, minimize toxicity effects and not adversely affect the physical properties of the finished product.

The preservative should be stable under whatever conditions it may encounter in the manufacture of the finished product (i.e. temperature, pH, etc.).

It should not affect either the color or the odor of the product and it should be compatible with the wide range of ingredients that may be found in the formulation.

The ideal preservative should offer protection during manufacturing and should maintain activity throughout the intended life of the product in the hands of the consumer.

It should be easy to analyze in the finished product. This is more difficult than it sounds since determining the concentration present does not necessarily indicate that it is all available to preserve the product. For example, some of the preservative may be bound to other chemicals or even the packaging, as mentioned previously, and therefore not be active.
The material should be easy to handle and safe to both the environment and to humans. This is also not easy since a chemical that will kill microorganisms is biologically toxic, and therefore has the potential to adversely affect the environment and mammalian tissue.