J. Soc. Cosmetic Chemists, **18**, 191–198 (Mar. 4, 1967)

The Application of Microbiology to Cosmetic Testing

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Presented September 20–21, 1966, Seminar, New York City

Synopsis—Sanitation and preservation hold the key to the control of microbial contamination in cosmetic products. Ultraviolet radiation can be used industrially to control the build-up of microflora in the stored deionized water that is utilized in the manufacture of cosmetics. A titration technique has been developed for measuring the relative antimicrobial activity of test preservative systems in products. The technique employs varying dosages of selected test microbes which are inoculated into the test systems. The method has been found predictive in that preservative systems inactivating high dosages of test microorganisms are effective under practical conditions.

Introduction

Cosmetics need not be completely free from nonpathogenic bacteria and fungi, but the residual organisms present in any product at the time produced must be prevented from multiplying during the product's shelf and use life by an effective preservative (1).

While the desired objective of a microbiological program in the production of cosmetics is to develop "sterile" products, the desired objective is not always readily attainable. "Sterile," as used in this context, means free from living microflora which can be detected by routine sterility tests. Ideally, cosmetics should be self-sterilizing against all microbes encountered during production, packaging, and usage. When complete sterility is not feasible, the cosmetics must be free of viable human pathogens and inhibitory against residual nonpathogens.

Actively viable microorganisms can be deleterious to both the esthetics and to the functional characteristics of cosmetic products.

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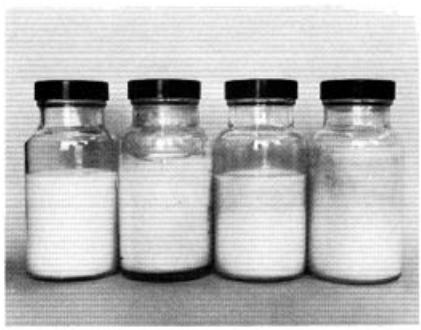

Figure 1. Hair conditioning lotion. The sample on the left is sterile. Pseudomonads are propagating in the other three samples

Figure 3. Hair styling gel. The sample on the left is sterile. Aspergillus mold is propagating in the sample on the right

Figure 2. Shampoo. Pseudomonads have attacked the shampoo in both bottles

Effects on color, odor, emulsion stability, foaming, and clarity can be demonstrated. Some samples for illustration are as follows:

Figure 1 shows four samples of an experimental hair conditioning lotion. The sample on the left is a sterile control. Pseudomonads have been allowed to propagate in the other three samples. The first contaminated sample shows an emulsion separation due to microbial attack on the nonionic emulsifiers. The last two contaminated samples illustrate discoloration due to Pseudomonad pigmentation.

Figure 2 shows two samples of a grossly contaminated sodium lauryl

sulfate type shampoo. Pseudomonads have attacked the detergent, causing the product to discolor and separate badly.

Figure 3 illustrates what can happen to an inadequately preserved hair styling gel. The control sample on the left represents a clear gel. Mold growth in the sample on the right has caused the gel to become turbid. Aspergillus mold was isolated from the turbid sample.

To avoid problems of this type, sanitation techniques and preservative methods need to be selected and employed carefully. They need to be monitored continuously to seek improvements in the systems chosen as they are required.

Sanitation

During production, common sources of microbial contamination in cosmetic products are raw materials, equipment, and air.

Since water for batch-making can be the major threat to product sterility, control over the sanitary quality of this water will be emphasized in this discussion.

Under summer temperature storage conditions, demineralized or deionized water can easily support bacterial populations as large as 10^5 bacteria/ml. In a few cases as many as 10^6 bacteria/ml have been observed. To prevent gross pollution of the batch water supply, the propagation of microflora coming from the undeionized water, the deionizer units, and the storage tanks must be controlled.

Although radiation treatment of stored deionized water is not widely practiced in the cosmetic industry, it is potentially a valuable means for controlling water quality. This paper will stress the application of radiation to water sanitation and specifically the uses of ultraviolet (UV) radiation.

Effective forms of ionizing radiation include ultraviolet light, X-rays, cathode rays, and gamma rays. The target theory, hypothesizing that electron rays hitting a microbial cell cause vital cell atoms to ionize, has been used to explain the microbiocidal effect of ionizing radiations (2). In this connection, Hollaender (3) has reported that, when germicidal effectiveness of ultraviolet is plotted against wavelength, the resulting curve resembled the absorption curve for nucleic acids.

Mercury vapor sources of ultraviolet are classified (3) as either high-pressure (400–60,000 mm Hg) or low-pressure (0.004–0.02 mm Hg) lamps. The peak effectiveness of ultraviolet for microbiocidal activity has been shown by Luckiesh (4) to be at a wavelength around 2600 A, falling virtually to zero at 3200 A. Since low pressure mercury vapor

lamps exhibit a high output of radiation at 2537 A, this type of lamp is very efficient and is most commonly used industrially; about 90% of the emittance from these lamps is microbiocidal.

Studies conducted by Koller (5) showed that the killing power of UV is virtually unaffected by temperature in the 5–37°C range. While the shape of the ultraviolet effectiveness curve is generally independent of the type of bacteria, the tendency to spore formation does greatly influence the responses in specific cases. Thus the spore-forming *B. subtilis* is about 5–10 times as resistant to UV as *E. coli*. Molds and yeasts are usually 100–1000 times more resistant than bacteria. For example, to obtain a 0.0001 survival ratio in water, a UV exposure of 24,000 μw-sec/cm^2 would be required for bacterial spores and 192,000 μw-sec/cm^2 for fungi. "Survival ratio" is the fraction of the number initially present which survives UV radiation.

Koller (5) also notes that, in order to sterilize water effectively, the water must have a high transmission for UV. In other words, the water must be free from suspended matter which might shield microbes from radiation. The UV lamps may be installed in reflectors mounted over the water surface. The tank should be deep enough to absorb practically all the UV, since radiation absorbed by the walls is wasted. Arrangement of the water inlet and outlet should assure thorough mixing. The degree of disinfection, the survival ratio, depends upon the intensity of the source, the transmission depth, and the rate of water flow.

An interesting point, also noted by Koller (5), is helpful to the cosmetic chemist: Those bacteria surviving irradiation are more susceptible to subsequent cidal treatment, being more easily killed by mild disinfectants and exhibiting increased sensitivity to heat.

It may be useful now to describe a typical water sanitizing system employing ultraviolet radiation. Our plant employs such a process that has been in successful operation for a number of years. The deionized city water is continuously recirculated from two 5000 gallon storage tanks through an 85 gallon stainless steel UV exposure tank at the rate of 180 gallons/min. The water bed in the exposure tank is 25 cm deep, 90 cm wide, and 91 cm long. Baffles are installed in the tank to decrease the velocity of water flow at the bottom of the tank. This increases UV exposure time at the bottom. Mounted about 30 cm above the exposure tanks are seven General Electric (90 cm long) 30-watt UV lamps. These mlaps are spaced 13 cm apart and have specular aluminum reflectors. The lamps are low-pressure mercury lamps having a rated 4000 hour life. Based on six lamps being operative, the calculated UV

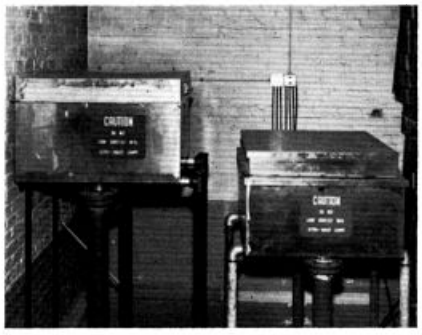

Figure 4. UV exposure tanks. Stored deionized water is recirculated continuously through these tanks

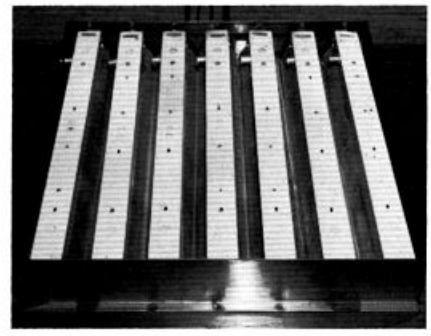

Figure 5. A UV exposure tank with cover removed. Seven specular aluminum reflectors are shown mounted over seven UV lamps on top of the tank

exposure at the water's surface is 26,000 μw-sec/cm^2, at the water's middle it is 18,000, and at the water's bottom it is 17,000. A single pass of the water through the UV exposure tank is calculated to result in a survival ratio of 0.0016.

Figure 4 shows the UV exposure tanks under actual operation. The tank on the left treats stored deionized city water. An identical exposure tank on the right is used to treat stored deionized well water. A view of the UV lamps mounted over the recirculating water is shown in Fig. 5.

When the UV treatment system for deionized city water was first placed in operation, the microbial count at zero time was 38,000 microbes/ml. After ½ hour operation the count dropped to 3300; after 1½ hours to 390; after 2 hours to 120, and after 18 hours to 81. The system has been in almost continuous operation since that time.

With periodic cleaning and sterilizing of the deionized water storage tanks, the counts have been kept at a relatively low level. Employing tight controls, the counts can be held to under 100 microbes/ml. Build-up of UV resistant organisms in the stored deionized water has not been a problem.

Periodic sterilization of the physical equipment—tanks, pipes and pumps—is required and desirable. Keeping a 2% solution of hydrogen peroxide in contact with the equipment for a two-hour period has been effective. Deionizer beds are treated with formalin as the need arises. Cruickshank *et al.*, (6) found that irrigation of ion exchange beds with 0.25% formalin (0.1% formaldehyde) served as an effective disinfectant.

Preservation

Even with good sanitation practices, cosmetics must be preserved to cope with contamination encountered during production, packaging, and normal usage. As other workers in the field of cosmetic preservation agree, test preservative systems must be evaluated in finished product formulations before final selection of a preferred system is made.

A useful technique for measuring the relative antimicrobial activity of various preservative system candidates is the inoculation of varying concentrations of selected test organisms into the test systems. This technique is predicated on the hypothesis that the higher the concentration of selected microbes a test system can inactivate the greater the efficacy of the system.

This titration technique for evaluating the efficacy of preservation systems is illustrated in an application to a hair-conditioning lotion. The product was an o/w emulsion having a pH between 3.25 and 4.25; nonionic emulsifiers were utilized in the formula at a concentration of approximately 4%.

Illustrative of a few of the preservatives evaluated in this product were: (a) 0.1% methyl *p*-hydroxybenzoate (MP), (b) 0.2% MP, and (c) 0.2% benzoic acid. It should be understood that these compounds are but a small sample of those normally employed in screening preservatives. Among the test organisms inoculated into the test systems was a Pseudomonad that had been isolated from a contaminated experimental batch of the product preserved with 0.1% MP.

A pure culture of the test Pseudomonad was taken from a nutrient agar slant and allowed to propagate for 24 hours at 30–32°C in nutrient broth. The resulting suspended culture was centrifuged and washed three times with buffered distilled water (pH 7.2). Serial dilutions of the washed culture were prepared in buffered distilled water (pH 7.2). One ml aliquot of each dilution were inoculated into 9 ml aliquots of each preserved product.

Sterility testing of the inoculated product samples showed that 0.1% MP inactivated levels of the test Pseudomonad up to about 10^6/ml of product. The product with 0.2% MP inactivated dosages up to about 10^7. The system containing 0.2% benzoic acid appeared bactericidal against the highest inoculum tested, *i.e.*, 10^9/ml of product.

Under practice conditions, Pseudomonad contamination was a recurring problem with the product containing only 0.1% MP. The product containing 0.2% benzoic acid has consistently been produced in a sterile condition.

The basic titration technique employed successfully on the hair conditioning lotion has been applied successfully to the development of preservative systems for a wide variety of cosmetic products. Included in these studies have been a variety of both o/w and w/o emulsions as well as dispersions and solutions. A broad spectrum of bacteria and fungi has been utilized in these evaluations of preservative systems in test products.

In addition to *Ps. aeruginosa,* initial screening evaluations of test preservative systems utilize representatives of the gram positive cocci, gram positive rods, and molds. If contamination has been a problem previously with a product similar to the one under study, pure cultures of these contaminants are also employed in the screening program. Thus, a typical screening study might include a Pseudomonad (*Ps. aeruginosa*), an Aspergillus mold (*Aspergillus niger*), a Bacillus (*Bacillus circulans*), a Micrococcus (*Micrococcus pyogenes v. aureus*), and any organisms isolated from products similar to the one under test.

The three most effective preservative systems, judged by the results of the screening tests, are then subjected to more extensive inoculation studies. During the extensive testing phase, organisms such as *Candida albicans, Cephalosporium sp., Corynebacterium pseudodiphthericum, Escherichia coli, Fusarium oxysporum, Penicillium sp.,* and *Streptococcus faecalis* are utilized.

The preferred preservative system is selected not only on the basis of its antimicrobial efficacy but on its product compatibility and medical acceptability as well.

Summary

As a protection to both the cosmetic products and the cosmetic users, adequate microbiological controls are an important part of a cosmetic testing program.

Contamination of the deionized water utilized in production can be minimized by ultraviolet radiation. UV treatment of the water accompanied by periodic chemical decontamination of the equipment can hold counts on stored water to under 100 microbes/ml.

Residual microorganisms finding their way into the finished cosmetic products are controlled through the development of effective preservative systems. A titration technique has been developed to measure the antimicrobial activity of preservative systems in products. This technique employs varying dosages of test microbes. The results indicate

that preservative systems inactivating high dosages of test microbes are adequate under production conditions.

Acknowledgment

The author gratefully acknowledges the assistance of the following co-workers in the preparation of this paper: Mr. Aaron Efron, Chemical Engineer; Mr. Carl Fraction, Microbiologist; and Miss Barbara Warnecke, Secretary.

(Received September 20, 1966)

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