THE INTERFERENCE OF NONIONIC EMULSIFIERS WITH PRESERVATIVES. III*

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Nonionic surfactants have become widely adopted by many industries because of their stability toward acids, alkalis and salts, as well as the ease with which their hydrophile/lipophile balance can be adjusted to a given need. We have reported earlier (1, 2) on the fact that nonionic emulsifiers interfere with the preservative properties of ethyl gallate; DCMX; bithionol; hexachlorophene; dichlorophene; methyl *p*-hydroxybenzoate; benzoic, dehydroacetic and sorbic acids. Other preservatives since tried are *candicin*, *penicillin*, *Aureomycin*, ascosin, usnic acid and five laboratory chemicals supplied by two companies, which show possible preservative properties. Data on these are not included here, however.

In this paper we discuss some of the factors believed to influence this interference. We can now also state that practically all nonionics based on the addition of ethylene or propylene oxide to fatty acids, alcohols, esters or polyglycols, interfere with the preservative properties of compounds containing either a phenolic or carboxylic hydroxyl group in the molecule. This interference is apparently due to the formation of complexes by hydrogen bonding.

Most of the work reported in this paper deals with methyl *p*-hydroxybenzoate and G-3720 working at a pH of 5.6. The acid range was chosen because most nonionic emulsions are acid in reaction and, also, molds grow better in acid media. The problems in acid media are mainly concerned with yeasts and molds both of which make spoilage readily apparent.

A constant pH was chosen because it has been shown by Rahn and Conn (3) that the amount of benzoic acid needed at pH 5.8 to prevent growth of a yeast was almost thirty times greater than at pH 4.1. As a result, one must work under fixed test conditions to draw relevant conclusions. Furthermore, most usable preservatives are more effective in the acid range. Figure 1 shows the loss in effectiveness of several compounds as the pH changes.

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* Presented at the December 13, 1956, Meeting, New York City.

Based in part upon a thesis to be submitted by M. G. deNavarre to the Graduate School of Wayne State University in partial fulfillment of the requirements for the degree Master of Science.

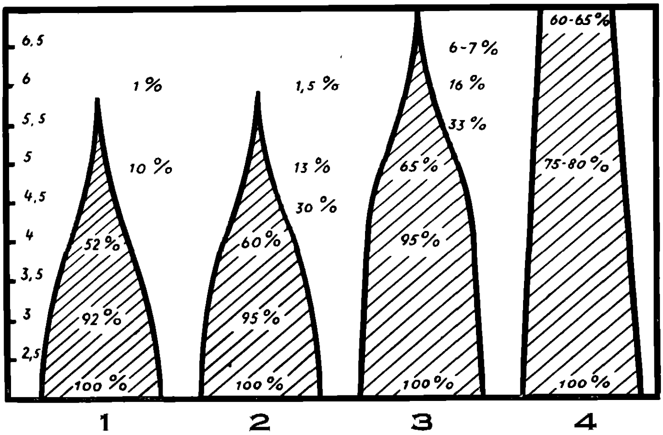

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Figure 1.—The Effect of pH on Preservative Activity.

1. *p*-Chlorobenzoic Acid
2. Benzoic Acid
3. Dehydroacetic Acid
4. Methyl *p*-Hydroxybenzoate

Experimental

Generally, Jaag liquid medium was used owing to its favorable effect on mycelium growth. To this was added 2 per cent of the nonionic and 0.1 per cent of preservative, usually adjusted to a pH of 5.6 unless otherwise stated.

Aspergillus niger was the principal microbe used in these tests because it is a common cause of spoilage, grows on liquid media, produces much visible mycelium and sporulates easily.

The *Aspergillus* cultures were grown in 25- × 200-mm. capped test tubes containing 15 cc. of solid Sabouraud medium, pH 5.6, slanted so the full length of the tube contained medium. After ten days' growth the spores were harvested, using 50 cc. of Jaag medium or distilled water, containing 1:100,000 of Aerosol OT. The suspension was strained through sterile J & J gauze filter discs to remove agar, mycelium and spore clumps somewhat after the procedure of Berry and Perkin (4). The resulting suspension was used in amounts of 1 cc. in all tests. Many tests were run in triplicate and the balance in duplicate. All tests were incubated at a temperature of 20 to 25°C. Results were usually recorded weekly as one plus or more depending on the growth. At the early stages, some tests were observed daily to determine earliest time of growth. Figure 2 shows the method of scoring.

Almost forty nonionics were used. Nevertheless, much of the present work was done with G-3720, a polyoxyethylene cetyl ether (20 ETO) for

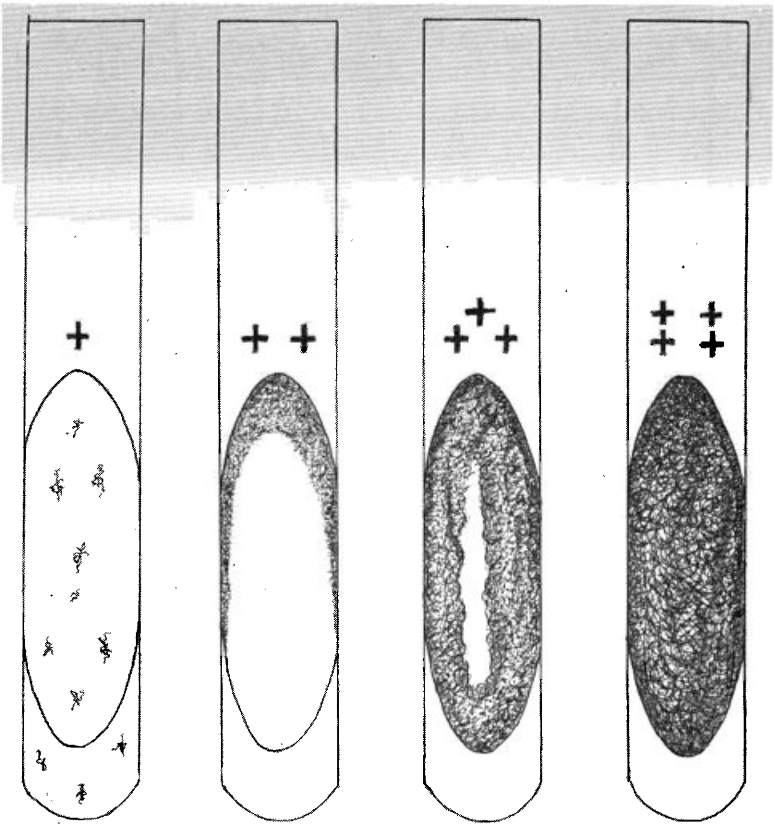

Figure 2.

reasons of convenience, after it was found that other ETO nonionics behaved similarly.

pH of Nonionics in Solution

The pH of 2 per cent solutions of the nonionics was determined before and after autoclaving, in deionized water and in Jaag medium. All gave pH readings in the acid range. In water the pH varied from 3.2 to 6 4 before autoclaving with most being in the 5.0 to 5.2 range while in Jaag medium they were from 3.2 to 5.7 with most showing a pH of 4.7. Deionized water had a pH of 6.5 while Jaag medium exhibits a pH of 5 1 After autoclaving, some became slightly more acid; others increased in pH a few decimals, but all were acid.

Effect of pH

The preservative added to a finished product acts in a physical or chemical way to prevent multiplication of organisms. This activity is influenced by the degree of dissociation of the preservative which in turn is influenced by pH. All other factors being constant, the greater the number of microbials in a given volume, the greater the possibility for some or all of the microorganisms to be uninhibited and to multiply.

Microbial contamination in good manufacturing practice is not believed to be as great as the proportionate size of inoculum used in laboratory work. However, in the baking industry, *Aspergillus*, *Mucor* and *Penicillium* spores are common, running as high as 11,000 per gram of flour (5). Since these comprise about 10 per cent of the flora, one can readily see the magnitude of the problem. While this is not directly translatable to cosmetics, airborne microbes must be exceedingly numerous even under ideal conditions of cosmetic manufacture.

A further complication is the tendency for poorly water soluble preservatives to go into the dispersed oil phase, leaving the water phase unprotected (6).

Jaag medium containing 2 per cent G-3720 and 0.1 per cent methyl *p*-hydroxybenzoate was adjusted with N/10 HCl or N/10 NaOH to the proper pH. The tubes were inoculated with a 1 cc. suspension of spores of *Aspergillus*. After two weeks' incubation the mycelium was removed, washed, dried to constant weight and the weight recorded. This procedure has variables. Results obtained with it are said to be difficult of interpretation according to Molho and Lacroix (7). Table 1 shows the comparison of visual observation denoted by plusses, with actual weights of mycelium. The figures are all proportionate to the sample with the lowest weight of mycelium.

Table 1—Effect of pH on Mycelium Growth

pH	Wt. Mycelium	Visual
7.5	1	+++
7.0	1.3	+++
6.5	1.6	++++
6.0	1.3	+++
5.5	2.0	++++
5.0	1.6	+++
4.5	1.2	+++
4.0	1.7	+++

Table 2—Effect of Size of Inoculum on Mycelium Produced

Inoculum	Visual	Wt. Mycelium
0.1	+++	1.0
0.2	+++	1.24
0.4	++++	1.07
0.6	++++	1.17
0.8	++++	1.32
1.0	++++	2.61

One sees a variation here but no consistent change unless we take the figures at pH 5.5 and those at pH 7.5 where there is a 100 per cent difference. This may be the point of optimum growth. But for all practical purposes, there is significant and damaging growth at all pH levels. Similar results were obtained with dehydroacetic acid.

Size of Inoculum

Using 0.1 per cent sorbic acid with 2 per cent G-3720 in Jaag medium adjusted to pH 5.6, varying amounts of a suspension of spores of *Aspergillus* were added. Tubes were shaken and incubated for two weeks. Table 2 shows the plusses noted by visual observation and the weight of dried mycelium alongside.

The weight of the mycelium in the tube with the smallest inoculum was taken as unity. All other weights recorded are proportionate to the first. Although there is a significant difference between the top and bottom figures of mycelium weight, they are not of the magnitude expected from the tenfold greater amount of inoculum used.

Later tests, repeating the entire experiment, made with 0.2 per cent sorbic acid gave a four plus reading for all.

Purity of Nonionic

It has been suggested that the unreacted fatty acid or free polyol in a nonionic is responsible for the interfering effect previously described. Accordingly, two series of tests were set up to check this possibility. In one set, increasing amounts of free sorbitol 5 to 15 per cent based on the weight of Tween 40, the nonionic used, were introduced into the test medium containing the corresponding nonionic and the preservative.

Table 3 shows the effect of the presence of free sorbitol. In the second set, excess oleic acid (the acid most often mentioned as the possible causative agent) was added to the nonionic incorporated in Jaag medium with the preservative.

Table 3—Effect of Excess Sorbitol on Growth of Aspergillus

5%	++	Poor sporulation
10%	++	Poor sporulation
15%	++	Poor sporulation
Control 1	++++	Poor sporulation
Control 2	++++	Heavy sporulation
Control 3	+++	Heavy sporulation

Control 1—Tween 40 and 10% XS Sorbitol and Jaag medium.
Control 2—Tween 40 and Jaag medium.
Control 3—Jaag medium.

Table 4—Jaag Medium with 2 Per Cent Nonionic and 0.1 Methyl *p*-Hydroxybenzoate, and 2% Nonionic

Carbowax 1540	+
Oleic Acid	—
25% Oleic in Tween 80	—
Tween 40	++++
Arlacel 83	—
Tween 80	+++
Myrj 59	+++
G-3720	++++
Span 20	+++
PEG 1000 Monolaurate	+++

Oleic Acid

The effect of excess fatty acid was determined using 2 per cent of fatty acid alone in test medium along with the preservative. Both stearic and oleic acids were previously used in solid Sabouraud medium. Similar concentrations of mineral oil, oleyl alcohol and sodium oleate were used as controls.

After months of observation we got no more than trace growth.

Later, using liquid Jaag medium, Arlacel 83, oleic acid alone, a 25 per cent mixture of oleic acid in Tween 80 and Tween 80 alone all in 2 per cent concentration with and without methyl *p*-hydroxybenzoate, we were unable to find any effect ascribable to excess of free oleic acid or the oleate radical not found with other nonoleate nonionic. Table 4 shows results with the above mentioned materials and 1:1000 of methyl *p*-hydroxybenzoate.

Effect of Inorganic Matter

At the outset we used liquid and solid Sabouraud medium adjusted to pH 5.6 after addition of all test materials. Bolle and Mirimanoff (8) however, used a liquid, synthetic, peptone-free Jaag medium described in an earlier paper by us. We then switched to the use of Jaag medium throughout most of the balance of this work. Since Jaag medium contains several inorganic salts in addition to sucrose, a series of experiments compared tap and deionized water, to Jaag medium as a potential source of nourishment.

It has been repeatedly observed that where distilled or deionized water was used as the control alongside of Jaag medium, growth in distilled water rarely showed more than two plus and more often one plus. No matter how long the test continued this did not increase appreciably. However, when Jaag medium was used, growth continued to three and four plus. As would be expected, traces of inorganic materials and polyols especially the sugars, can increase spoilage, all other factors being equal.

Effect of Fatty Radicals in Nonionic Molecule

The fungistatic properties of fatty acids are well established as existing at a maximum in the 8–12 saturated carbon acids. On the other hand, it has been claimed that oleic acid in particular is stimulating to microbial growth. So much so that oleic acid or sodium oleate and more recently Tween 80 (9) have been used in media for the cultivation of certain mycobacteria. However, Minami (10) has just reported that oleic acid and oleates were bactericidal to tubercle bacilli.

There is, therefore, the possibility that the fatty substituent if any, might influence the interference of nonionics with preservatives. A series of tests were made with Carbowax 1540 as a fat-free nonionic along with a diversified group using Jaag medium, pH 5.6, 2 per cent nonionic and 1:1000 of several preservatives. Table 5 shows results with sorbic acid. Other preservatives in double the concentration behaved similarly as shown in Table 6.

Water as the Liquid Medium

The effect of a wide variety of nonionics on the growth of *Aspergillus* when distilled water brought to pH 5.6, was the medium, was then de-

Table 5—Effect of Fatty Radical and 1:1000 Sorbic Acid on Growth Aspergillus

Carbowax 1540	++++
Pluronic L-64	++++
Myrj 59	++
G-3720	++++
G 3920	++++
Tween 20	++
Span 20	++

Table 6—Effect of 0.2 Per Cent Preservative with (2%) and (4%) G-3720 on Growth of Aspergillus

Sorbic Acid (2)*	++++
Sorbic Acid (4)	++++
DHA (2)†	++
DHA (4)	++
Methyl *p*-Hydroxybenzoate (2)	—
Methyl *p*-Hydroxybenzoate (4)	+++
Control Jaag only (2)	++++
Control Jaag only (4)	++++

* Figures in Table 6 in parentheses indicate per cent nonionic.
† Dehydroacetic acid.

termined. The tests were repeated using Jaag medium at the same pH. The nonionics included every type exemplified by Pluronic L-64, Carbowax 1540, ethoxylated propoxylated glycerin, Myrj 59, G-3720, ethoxylated castor oil, ethoxylated cholesterol, PEG 600 ricinoleate and stearate, PEG 1000 monolaurate, all the Spans and Tweens, a 25 per cent solution of oleic acid in Tween 80 and a 30 per cent mineral oil Span-Tween nonionic emulsion.

When distilled water was the medium, growth in the nonionic mixture and water was little or no greater than in distilled water without nonionic, which was usually a one plus growth. These results are to be expected.

When water was replaced with Jaag medium, a four plus growth in the Jaag control and the nonionic mixtures, including the mineral oil emulsion was obtained.

Effect of Other Surfactants

Since the advent of synthetic surfactants, many publications report synergistic effects with anionics or quaternary compounds when used together with various antiseptics.

We find that several anionics (Duponal C, Aerosol OT, Nacconol NRSF and MP 189) when present alone in water or in Jaag medium, in concentrations of 0.1 per cent without any preservative, fail to support growth of *Aspergillus*.

However, upon the introduction of a representative nonionic, such as G-3720, we immediately get growth of *Aspergillus*. Indeed, when a mixture of from 1 per cent to 10 per cent of these anionics in G-3720 were used in concentrations of 2 per cent in distilled water or in Jaag medium containing 1:1000 and 1:500 of methyl *p*-hydroxybenzoate, we got from two plus to four plus growths readily. Data are accumulating to indicate that higher concentrations of anionic in the nonionic neutralize the interference in some way.

Failing to completely overcome the interference being studied with anionics, attention was turned to the combined use of cationic germicides along with nonionics, G-3720 in particular, with and without methyl *p*-hydroxybenzoate. Among the compounds tried were Roccal, Hyamine 10X and Ethyl Cetab in varying combinations with G-3720. Roccal, in particular shows promise although Hyamine 10X is also being further studied.

Among the classes of surfactants to be tested are the ampholytes. These results are not yet complete, hence will be reported later.

Discussion

From the tests made so far several observations are possible.

(1) Preservative usefulness is still a problem specific to a given product formulation.

(2) It takes more than 0.1 per cent of methyl *p*-hydroxybenzoate, benzoic, sorbic and dehydroacetic acids to be effective in the absence of a nonionic emulsifier.

(3) In the presence of nonionics, even double this amount is not dependable.

(4) Practically all nonionics based on ethylene or propylene oxide condensates, with each other, fatty esters, alcohols or acids, inactivate all presently usable drug and cosmetic preservatives.

(5) Anionics sometimes used with nonionics may favorably prevent this interference if used at higher concentrations.

(6) Roccal and Hyamine 10X of a group of quaternary compounds showed possibilities in preventing preservatives from being inactivated by nonionics.

(7) Results with the ampholytes now being tested will be reported later.

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