THE INTERFERENCE OF NONIONIC EMULSIFIERS WITH PRESERVATIVES WITH SPECIAL REFERENCES TO COSMETICS*,†

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Nonionic emulsifiers have become widely used in cosmetics because of their over-all usefulness. With a trend toward the formulation of emulsions having a pH similar to the skin, nonionics became the emulsifiers of choice. Furthermore, they find use in high electrolyte content formulations, solutions and emulsions of perfume oils and as both water-in-oil and oil-in-water emulsifiers for all purposes. They are comparatively inert and nontoxic.

This paper attempts to be a practical approach to the study of the interference of nonionics with preservatives, since in commerce any apparent microbial growth with or without other manifestations of spoilage is a financial loss.

In 1948 the spoilage of a commercial nonionic emulsion preserved with 0.15 per cent methyl *p*-hydroxybenzoate led to the present study. The solution to this problem seemed rather simple at first hand. But it was not. Indeed, it is not certain that a solution exists even now. For in 1950 the experimental work took a new twist with the publication of the Bolle and Mirimanoff paper entitled, "Antagonism Between Nonionic Detergents and Antiseptics."

Preservatives are merely antiseptics, and while the publication just mentioned did report a very limited number of tests with Nipagin, a well-known *p*-hydroxybenzoate preservative, it raised a number of questions with far reaching consequences. Basically, it had to be determined if antagonism between nonionics and all common preservatives did exist in fact. If so, to what degree did nonionics retard preservative action? Was the conflict limited to a particular class of preservative? Which types of nonionics were involved? If the inactivation were widespread, how could it be overcome?

The literature since 1950 contains a number of mentions of antiseptic

* Based on paper given at Symposium on Antimicrobial Preservatives, Society of American Bacteriologists, April 29, 1957, Detroit, Mich, by permission.

† Presented in Geneva, Switzerland, August 5, 1957.

inactivation by or incompatibility with certain nonionics, but there is no adequate study of the present problem.

At the outset of this work, a number of variables were apparent. The following were considered for their effect on results:

1. Type of nonionic.
2. Purity of nonionic.
3. Presence of mineral salts.
4. Other types of surfactants.
5. Use of sodium salts.
6. pH.
7. Solid versus liquid medium.
8. Ratio of nonionic to preservative.
9. Degree of ethoxylation.

Experimental

Seven microörganisms taken from the Wayne State University College of Pharmacy collection were used in the first tests. They consisted of the following: *Penicillium chrysogenum*, *Aspergillus niger*, *Rhizopus nigricans*, *Candida albicans*, *Alternaria solani*, *Oidium lactis* and *Mucor racemosa*. A *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli* and *Torula roseus* were also used in some of the tests but not in all of them, so the results are only summarized here. In addition two unknowns isolated from spoiled nonionic emulsions were also used in some tests. These organisms were later identified as *Paecilomyces varioti* and *Candida guillermondii*, respectively.

The first series of tests were made in test tube slants of Sabouraud dextrose agar at pH 5.6. The nonionic and preservative were dissolved in the medium, the pH adjusted, sterilized by autoclaving, slanted and, when cool, streaked with ten-day-old cultures of the molds under test or forty-eight hour cultures of the bacteria. While the concentration of nonionics commonly used in industry is usually between 1 and 25 per cent, they are rarely used in quantities less than 1 per cent. Accordingly, tests were made with 1 to 4 per cent concentrations of nonionic, mainly, although some tests were made with 0.1 to 10 per cent concentrations. The preservatives were used in amounts from 0.1 to 0.4 per cent of preservative with most tests at 0.2 per cent. Thus, a ratio of nonionic to preservative was generally 10 : 1.

In the greater number of experiments, *Aspergillus niger* became the sole test organism. It was chosen because it is easily seen when germinated and because it is a common contaminant in commercial products. Jaag liquid medium replaced Sabouraud dextrose agar. The main difference between Czapek-Dox medium and Jaag medium is that the latter contains more sucrose and nitrate. Figure 1 shows the method of scoring the results.

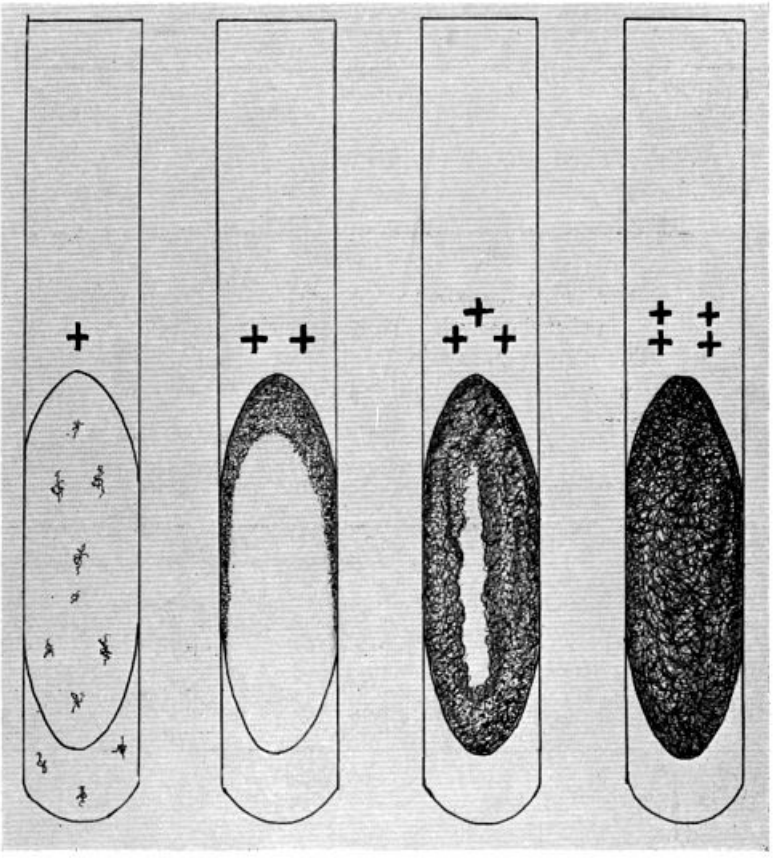

Figure 1.—Method of Scoring the Results Key to Figure:

\+ = Slight Growth
++ = Medium Growth
+++ = Heavy Growth
++++ = Very Heavy Growth

Effect of Nonionic Type

A representative group of nonionics was dissolved in 2 per cent concentration in Jaag medium, seeded with *A. niger* spores and observed for speed and degree of growth. Table 1 shows the results obtained in nine days.

It is apparent that ethoxylated nonionics do not *suppress* growth of *Aspergillus niger* in Jaag medium. Indeed, they seem to favor growth rather significantly. A nonethoxylated Span seems to retard growth in this test period.

Three different types of nonionics, Tween 80, Myrj 49 and G-3720 were dissolved in Jaag medium, in both 2 and 4 per cent concentrations along with 0.2 per cent of four different common cosmetic preservatives. It was

TABLE 1—GROWTH OF *A. Niger* IN 2% NONIONIC IN JAAG MEDIUM

	2 Days	9 Days
Span 40	–	–
Tween 80	++	++++
Carbowax 4000	+	++
G-3720	++	+++
Myrj 59	+++	++++
PEG 1000 Monostearate	++	++++
Jaag Control	+	++

found that methyl *p*-hydroxybenzoate, sorbic acid and ethyl vanillate showed slight to medium growth within three days at both concentrations of nonionic. The results shown in Table 2 were noted at the end of two months. Similar results were obtained with some 40 other ethoxylated nonionics of all types.

TABLE 2—GROWTH OF *A. Niger* IN JAAG MEDIUM, WITH 2 AND 4% NONIONIC AND 0.2% PRESERVATIVE

	DHA*	MP†	EV‡	SOR§
2%				
Tween 80	+	+++	++	++++
Myrj 49	++	+++	+++	++++
G-3720	++	++++	+++	++++
4%				
Tween 80	+	++++	+++	++++
Myrj 49	++	++++	++++	++++
G-3720	++	+++	+++	++

* Dehydroacetic Acid.
† Methyl *p*-hydroxybenzoate.
‡ Ethyl Vanillate.
§ Sorbic Acid.

PURITY OF NONIONIC

Nonionic surfactants as they are used in industry are commercially pure materials with a somewhat variable composition. The starting fatty materials are usually mixtures, though largely a given compound. Thus, Tween 80, an ethoxylated sorbitol oleate contains traces of free oleic acid and some unreacted hexitol or its anhydrides have been suggested as being responsible for the inactivation or of stimulating microbial growth in the presence of preservatives. The microbiological literature has a number of references to the stimulating action of oleic acid or oleates on the growth of microörganisms, although there are also references to the contrary. Numerous tests were made to prove these claims against *Aspergillus niger*. Table 3 shows the effect of 2 per cent oleate and nonoleate based nonionics, some with an excess of free oleic acid in Jaag medium with 0.1 per cent methyl *p*-hydroxybenzoate. One can hardly point to any influence of oleic acid or oleate based nonionic in these data.

TABLE 3—GROWTH OF *A. Niger*, JAAG MEDIUM, 2% NONIONIC AND 0.1% METHYL *p*-HYDROXYBENZOATE

		Days
Carbowax 1540	+	90
Tween 20	++++	21
Span 20	++	21
Oleic Acid	+	21
25% Oleic Acid*	+	65
Pluronic L 64	++++	60
Control	++++	21

* In Tween 80.

Limiting the number of nonionics but using different types with a higher concentration of preservative, Table 4 shows us what we get with 0.2 per cent methyl *p*-hydroxybenzoate.

TABLE 4—GROWTH OF *A. Niger*, IN JAAG MEDIUM, 2% NONIONIC 0.2% MP*

	10 Days
Tween 80	+++
Myrj 49	++
G-3720	++
G-3810	++
G-1425	–

* Methyl *p*-hydroxybenzoate.

Now checking the possible effect of free unreacted sorbitol or sorbide in nonionics based on sorbitol, Jaag medium was made up in the conventional way and also by replacing the sucrose with sorbitol and sorbide, respectively. No nonionic or preservative was added. *Aspergillus niger* spores were introduced and growth observed.

Both sucrose and sorbitol type media produced heavy growth at approximately the same rate in about twenty days. However, the sorbide modified Jaag medium still showed no growth at the end of three months and only slight growth in seven months. Such results hardly support any stimulating effect of either sorbitol or sorbide that is greater than that of sucrose. As a matter of fact, other tests made with sucrose and sorbitol in aqueous solution with and without preservative show no particular effect specifically attributable to the hexitol when *Aspergillus niger* was the test organism. A similar test made with 0.1 per cent methyl *p*-hydroxybenzoate, 2 per cent Tween 40 in Jaag medium with an excess of sorbitol gave medium growth as compared to a parallel series without the added sorbitol.

EFFECT OF MINERAL SALTS

The need for minerals and nitrogen in the metabolism of microörganisms is well known. It can be said that the data presented here are on an enriched medium, which does not always exist in commercial products.

Many of these tests were made using aqueous solutions of the nonionics with and without preservative. As would be expected, aqueous solutions produced slight to medium growth, while parallel tests in Jaag medium produced heavy to very heavy growth.

However, most commercial cosmetics will contain trace minerals from the materials used, whether natural, synthetic or derived as well as from containers and equipment. True, the amount of metal thus obtained is small but sufficient to be a factor in spoilage. Nitrogen can come from the alkanolamines, urea or allantoin, common cosmetic ingredients. Sorbitol syrup and glycerin are carbohydrate sources.

As a result, commercially made cosmetics may not show as rapid and heavy growth as can be had in Jaag medium, but growth will be greater than that obtained in fresh deionized or distilled water.

Results with Other Microörganisms

Summing up the work with bacteria, yeast, other molds and yeast-like organisms, mentioned earlier in this paper, it was found that, in general, all grew readily on solid or in liquid media in the presence of 0.2 per cent preservative and 2 per cent of G-3720 nonionic.

The Effect of Sodium Salts of Preservatives

At this stage of the work it became apparent that the suspected hydrogen bonding between the hydrogen of the preservative hydroxyl group and the ether oxygen of the nonionic was indeed taking place. In an attempt to offset this effect, the sodium salts of dehydroacetic and benzoic acids, *o*-phenylphenol and methyl *p*-hydroxybenzoate were suggested and tried. Concentrations of 0.1 and 0.2 per cent of preservative, calculated as the sodium free material, with 2 per cent G-3720 were run in a parallel series containing the straight sodium-free preservative at a pH of 7.5 using *Aspergillus niger*. Heavy to very heavy growth appeared in all cases; slightly less growth occurred in tubes containing sodium *o*-phenylphenate.

Effect of pH

While pH is an important factor in microbiological tests, it was less an influence here than expected.

In a series using 2 per cent G-3720, 0.2 per cent methyl *p*-hydroxybenzoate and *Aspergillus niger* in Jaag medium, pH ranging from 4.0 to 7.5, by half units, medium growth was obtained at all pH levels with growth starting on the fourteenth day. There was a slightly greater growth at pH 6.0, 7.0 and 7.5.

Similar results were obtained with sorbic and dehydroacetic acids. One thing is certain that neither of the preservatives were effective at any pH at a concentration of 0.1 per cent against *Aspergillus niger*. At a con-

centration of 0.1 per cent sorbic acid failed to protect at pH 3 to 8, with or without a nonionic present, although there was somewhat less growth at pH 3 to 5.0. At a concentration of 0.2 per cent sorbic acid failed without nonionic at a pH above 5.0. With dehydroacetic acid, maximum growth occurred at pH 6.0.

The pH of most nonionics is in the acid range whether in aqueous solution or Jaag medium. A summary of pH values of a group of 22 nonionics in 2 per cent concentration tested both in aqueous solution and Jaag medium, before and after autoclaving shows a pH variation of 2.9 to 6.7 all in the acid range with an average of 2.7 to 3.5 with the higher figure in Jaag medium.

Effect of Other Surfactants

In the course of this work it was decided to test the effect of the other three remaining types of surfactants, namely, anionic, cationic and amholytic.

Earlier tests had shown that 0.1 per cent of anionic surfactants in Jaag medium failed to show growth of *Aspergillus niger* for a year. When 0.1 per cent methyl *p*-hydroxybenzoate was added to the anionics in Jaag medium, tubes over a year old were still free from growth. This suggested the possibility of preventing inactivation of the preservative by using an anionic in conjunction with the nonionic. Such combinations are sometimes used for other reasons in cosmetic formulations.

Mixtures of 1, $2^1/_2$, 5 and 10 per cent Duponol C, Nacconol NRSF and Aerosol OT were made in G-3720. Two per cent of the mixture was used in Jaag medium to which was added 0.1 per cent methyl *p*-hydroxybenzoate. All concentrations of the anionic in the mixture failed to prevent the interference of the nonionic with the preservative resulting in heavy growth of *Aspergillus niger*.

Four representative nonionics in 2 per cent concentration were used with 0.2 per cent Duponol C and 0.2 per cent of preservative all in Jaag medium using *Aspergillus niger* as the test organism. The control contained every-

Table 5—Five Month Growth of *A. Niger* in Jaag Medium Containing 2% Nonionic, 0.2% Duponol and 0.2% Preservative

	MP*	SORBIC†	DHA‡	CONTROL
G-3720	+	++	++	++++
Myrj 49	++	++	++	++++
Tween 60	++	++	++	++++
Carbowax 1540	−	−	−	−
Pluronic L-64	+	+	−	++++
GMS 20 ETO	++	++	++	++++
0.2% Duponol	−	−	−	−

* Methyl *p*-hydroxybenzoate.
† Sorbic Acid.
‡ Dehydroacetic Acid. GMS = Glyceryl monostearate ethoxylated (20).

thing but the preservative. Table 5 shows the results. A concentration of Duponol C equivalent to the amount of preservative appears to reduce or prevent interference by the nonionic in this short test period.

The tests were repeated using three representative cationics with the nonionic G-3720. A 10 and 20 per cent mixture of the cationic in the nonionic was made, but results made with the latter are reported only. Two per cent of this mixture was dissolved in Jaag medium, preservative added and seeded with *Aspergillus niger* spores. Table 6 gives the results after eight months' growth.

TABLE 6—GROWTH OF *A. Niger* IN JAAG MEDIUM CONTAINING 2% OF A G-3720-CATIONIC MIXTURE AND METHYL *p*-HYDROXYBENZOATE

	Roccal	Days	Ethyl Cetab	Days	Hyamine 10 X	Days
0.1% Cationic	−	210	−	210	−	320
0.1% Cationic + 2% G-3720	++++	25	++++	40	++++	20
" " 0.1% MP*	+	250	−	150	+++	40
" " 0.15% MP	−	150	−	150	+	40
" " 0.2% MP	−	150	−	150	−	160

* Methyl *p*-Hydroxybenzoate.

These results show possibilities for further testing if they do not change over a longer period of time.

A similar test was made with one ampholyte, Deriphat XD-150 A, using it as a 20 per cent mixture in nonionic G-3720. This was added in a 2 per cent concentration to Jaag medium containing varying amounts of methyl *p*-hydroxybenzoate and seeded with *Aspergillus niger* spores. Table 7 shows the results after nine months.

TABLE 7—GROWTH OF *A. Niger* IN JAAG MEDIUM CONTAINING 2% OF A MIXTURE OF G-3720 AND DERIPHAT XD-150 A AND METHYL *p*-HYDROXYBENZOATE

Methyl *p*-Hydroxybenzoate	pH	Results in Months 1	3	7	9
0.1	7.8	−	−	−	++
0.1	5.6	−	−	−	+
0.15	7.8	−	+	+	+
0.15	5.6	−	−	−	−
0.2	7.8	−	−	−	−
0.2	5.6	−	−	−	−
Control	5.6	−	+	++++	

Ampholytes, being Zwitterion types of compounds can be anionic or cationic surfactants depending on the pH of the medium. At a pH below 7 they are cation-active while above a pH of 7, they are anion active. Here again are possibilities for future research if the results do not change in a longer period of time.

Effect of Ratio of Nonionic to Preservative

Test were made on ratios of nonionic to preservative ranging from 1 to 1 all the way to 50 to 1 using concentrations of 0.1 and 0.2 per cent methyl *p*-hydroxybenzoate.

It was found that all ratios of nonionic to preservative showed growth although lesser amounts of nonionic gave slower growth.

Effect of Degree of Ethoxylation

In general it has been found that when there are above six moles of ethylene oxide in the ethoxylated chain, the nonionic becomes water soluble or dispersible. Almost in a parallel way, the greater the degree of ethoxylation, the more rapid the inactivation, excepting in the case of the Carbowax series. It seems that an alkyl or aryl group at one end of the nonionic is necessary to get maximum inactivation of the preservative. In a narrow series, Myrj 49 inactivates preservative more slowly than Myrj 59 and G-3810 is slower than G-3816 and it in turn slower than G-3820.

When 0.1 per cent sodium lauryl sulfate was compared to 0.1 per cent ethoxylated sodium lauryl (ether) sulfate in Jaag medium, *Aspergillus niger* grew in the ethoxylated medium within three weeks showing heavy growth.

Solid *Vs.* Liquid Media

A liquid medium was preferred for this work although over a thousand tests were made on solid Sabouraud dextrose agar slants. It was easier to quantitate results. Because nonionics are surfactants, it was sometimes difficult to get clear, smooth surfaces due to foaming after autoclaving a solid medium. Some nonionics are precipitated out of solution during sterilization remaining as a glob in the bottom of the tube. Using a liquid medium, the tubes were placed on a slow shaking machine until dissolved. The foam formed did not interfere with seeding of spores, since on standing the foam collapsed. A solid medium could not be used this way.

Summary

Over the last several years over forty nonionics and twenty preservatives have been tested in varying concentrations to determine if all types of nonionic surfactants inactivate all usuable cosmetic preservatives. From this group of preservatives, four were used, namely, methyl *p*-hydroxybenzoate, for the main part, dehydroacetic acid, ethyl vanillate and sorbic acid. The principal nonionic used was G-3720 for its ease of handling and for producing rapid results. *Aspergillus niger* was the test organism most often used.

Factors possibly influencing results were examined.

Conclusion

In general any water soluble ethoxylated nonionic surfactant will inactivate the commonly used cosmetic preservatives when employed in their usual concentrations if tested on Sabouraud dextrose agar or Jaag medium against the organisms mentioned.

SOME USES OF PAPER CHROMATOGRAPHY FOR THE ANALYSIS OF COSMETICS*

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In Switzerland, cosmetics are subject to control based on the Federal decree of May 26, 1936, regulating trade in foodstuffs and various common substances, with particular reference to Article 467 of the decree. Medicinal cosmetics and those on the market with indications claiming curative properties are controlled by the Inter-cantonal Office for the Control of Medicaments (O.I.C.M.).

Article 467 states that preparations used for the care of the mouth, skin and hair, hair dyes and make-up must not contain metalloidal or toxic metallic (arsenic, antimony, lead, mercury, thallium) substances or harmful organic components.

Paragraph 2 of article 467 mentions the following organic components considered to be noxious: para-phenylene, diamine, formaldehyde, paraformaldehyde, pilocarpine and nitrobenzine, but this list is not exhaustive, for it ends with "etc." The Federal Public Health Service may at any time declare other organic components to be noxious and may forbid their use. Thus the use of thioglycerin in permanent wave liquids has been prohibited since 1956.

As regards coloring matters, cosmetics may in principle be colored only with foodstuff colorings listed in Article 441 of the above decree. Paragraph 6 of Article 467, however, allows the use of harmless coloring matters other than those used in foodstuffs, provided that their chemical composition is communicated to the Federal Public Health Service.

It will be agreed that Swiss legislation on cosmetics is very liberal.

The great variety of substances used for the composition of cosmetics makes their analysis exceedingly difficult. The "Swiss Foodstuffs Manual," 4th edition, 1939, containing official methods for analysis of foodstuffs

* Presented at the August 2, 1957, Meeting, Geneva, Switzerland.