THE INTERFERENCE OF NONIONIC EMULSIFIERS WITH PRESERVATIVES VIII

By JOHANNA POPRZAN and MAISON G. deNAVARRE*

Presented September 18, 1958, International Symposium, Gesellschaft Deutscher Kosmetik Chemiker, Bonn, Germany

IN THE COURSE of investigating the inactivation of preservatives by nonionic surfactants, a number of significant developments were noted but hitherto not published. In addition other progress has suggested new lines of research in this unsolved problem.

Indeed, the recent report (1) that 3 per cent hexylene glycol was used successfully with 0.2 per cent sorbic acid to preserve a nonionic product has led us to re-examine some earlier results (1955) and to further examine the effect of all commonly used glycols along with other polyols.

Propylene glycol had been used as a solvent for preservatives in one of our (M. G. deN.) early experiments. Checking the glycol controls led to the verification of the established inhibitory concentration for propylene glycol against *Aspergillus niger*. This was found to be at least 16 per cent, in Jaag medium, with or without the presence of the nonionic G-3720. This figure is somewhat higher than the published concentrations recommended. However, when the glycol, 0.1 or 0.2 per cent methyl *p*-hydroxybenzoate and 2 per cent G-3720 were added to Jaag medium, there were indications that somewhat less than 10 per cent propylene glycol would have either a potentiating effect on the preservative, or that it might interfere with the suspected complexing of the preservative with the nonionic.

With this background, one of us (J. P.) set up experiments to evaluate the effect of position and number of hydroxyl groups on a polyol in preventing inactivation of methyl *p*-hydroxybenzoate by nonionics.

Experimental. The microörganisms used in this series were *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Aspergillus niger*. Nutrient broth was the bacterial medium while Czapek Dox medium was used for the mold.

The test methods and other procedures were the same as one of us had previously published (2).

* Cosmetic Laboratories, Inc., Div. of Beauty Counselors, Inc., Detroit 7, Mich.

The commercial nonionic G-3720 was used throughout this work. It is a polyoxyethylene (20) stearyl alcohol. A 2 per cent concentration was used.

Tween 80 was used in a concentration of 2 per cent in some tests. It is a polyoxyethylene (20) sorbitan oleate.

Methyl *p*-hydroxybenzoate 0.2 per cent was the only preservative tried.

Varying amounts of glycol were used as the test required with due adjustment of water content to compensate for the glycol (1).

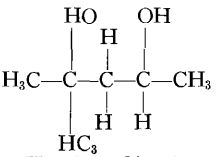
The polyols used were propylene glycol of commerce; 1,3-propanediol; dipropylene glycol; 1,4-butanediol; hexylene glycol; sorbitol and glycerol.

Discussion. In this work, results with molds cannot be considered on the same basis with usual bacteriological tests. Thus, it is found that Jaag medium preserved with 0.1 per cent methyl *p*-hydroxybenzoate showed no growth of *A. niger* for four months. At the end of seven months, a very heavy growth was obtained. Too often such tests are reported on a seven-, fourteen- or thirty-day basis. Our tests with molds are continued for at least a year.

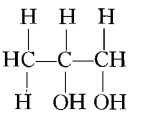
If the number and position of hydroxyl groups on a polyol have an effect on preservative inactivation, then it is desirable to examine the configuration of the polyols used.

The propylene glycol of commerce is largely 2,3-propanediol with a small amount, rarely over 5 per cent, of 1,3-propanediol.

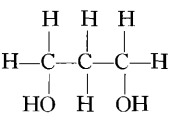
Hexylene glycol of commerce is 2-methyl 2,4-pentanediol having the following configuration:



Hexylene Glycol



2,3-Propanediol



1,3-Propanediol

In hexylene glycol, the two hydroxyl groups are not attached to adjacent carbons as they are in ordinary propylene glycol. This configuration could account for the difference in behavior. At the same time if this line of reasoning is valid, then such commercial compounds as 1,4-butanediol, 1,3-butanediol, hexanetriol-1,2,6 and others may have similar effects, but sorbitol and glycerol would be comparatively ineffective.

Results. We find that 8 per cent propylene glycol when added to the

medium containing G-3720 or Tween 80 and methyl *p*-hydroxybenzoate showed no growth after six months using the four bacteria and *A. niger*.

In view of the effect of hexylene glycol previously mentioned, a series of tubes was set up using various concentrations of the glycol with G-3720 and methyl *p*-hydroxybenzoate. The results showed that 4 per cent hexylene glycol is as effective as 8 per cent propylene glycol in preventing growth of the four bacteria and *A. niger* for four months.

Hexylene glycol as the sole preservative failed to prevent growth of both bacteria and *A. niger* in 2, 3 and 4 per cent concentrations. In a concentration of 5 per cent the *Pseudomonas* and *Proteus* bacteria were still able to grow as did the *Aspergillus*. These results are at variance with those mentioned earlier (1).

Concentrations of glycerol and sorbitol ranging from 2 to 12 per cent failed to prevent inactivation of methyl *p*-hydroxybenzoate by G-3720. While growth periods varied, all samples grew actively within two months.

In screening 1,4-butanediol, dipropylene glycol and 1,3-propanediol, arbitrary concentrations of 5 and 10 per cent were added to the appropriate medium containing methyl *p*-hydroxybenzoate and G-3720. In all three cases the 5 per cent concentration failed to prevent growth of only *Pseudomonas aeruginosa*, but a 10 per cent concentration of either glycol inhibited the growth of all the organisms. Further tests are necessary to find the more exact concentration of glycol that will prevent growth of these microörganisms in a nonionic medium containing 0.2 per cent methyl *p*-hydroxybenzoate.

G-3720, %	Growth	Months	Ratio G-3720:MP*
0.1	+	10	1/2:1
0.2	+	13	1:1
0.4	+	9	2:1
0.6	++++	2	3:1
0.8	++	2	4:1
1.0	++++	2	5:1
2.0	++++	4	10:1
4.0	+++	1 1/2	20:1
6.0	+++	1 1/2	30:1
10.0	++	1 1/2	50:1

* Methyl *p*-hydroxybenzoate.

Figure 1.—Growth *A. niger* in Jaag Medium with G-3720 and 0.2% MP

Effect of Ratio of Nonionic to Preservative

Pisano and Kostenbauder (3) have found that 5 per cent Tween 80 in water bound 78 per cent of the methyl and 90 per cent of propyl *p*-hydroxybenzoates, using a dialysis technique. Somewhat later it was found that these figures are increased by bacteriological media.

Our tests, using G-3720, in various ratios and a uniform 0.2 per cent concentration of methyl *p*-hydroxybenzoate in Jaag medium showed growth at all concentrations and ratios of the nonionic to the preservative; the heaviest growth occurring at 0.6, 1.0, 2, 4.0 and 6 per cent of the nonionic. Heavy growth is first noticeable when the ratio of nonionic to preservative is 3:1.

However, using 2 per cent Tween 80 and concentrations of methyl *p*-hydroxybenzoate from 0.2 per cent to 0.5 per cent, an oil (complex?) eventually separated from the medium when the concentration of methyl *p*-hydroxybenzoate reached 0.3 per cent.

In a similar experiment using Myrj 49, no oil or crystalline separation took place, but it required 0.5 per cent of methyl *p*-hydroxybenzoate to prevent growth of *A. niger* in Jaag medium.

In a similar series it required 0.4 per cent of methyl *p*-hydroxybenzoate to preserve 2 per cent G-3720 against *A. niger*. While at 1 per cent of methyl *p*-hydroxybenzoate and 2 per cent G-3720, a crystalline compound settled out.

Igepal Co	Growth	Moles ETO
210	++	1.5
430	++	4
630	++++	10
730	+	15
850	++++	20
880	++	30

Figure 2—Eleven Month Growth *A. niger* Jaag Medium, 0.2% MP* and 2% Igepal

* MP = Methyl *p*-hydroxybenzoate.

	Growth	Months
Carbopol 934	++	6
Cellosize	−	8
Tragacanth	+++	5
PVP K-30	++	5
Methocel 1500	++	2

Figure 3.—Growth of *A. niger* in Jaag Medium Plus 0.2% MP* Plus 2% Gum

* MP = Methyl *p*-hydroxybenzoate.

Enzymic Activity

We have found that polyoxyethylene ethers of the type of G-3720, particularly in the presence of dehydroacetic acid are apparently not only split by a possible "etherase" but that the product of hydrolysis is either selectively split from the nonionic liberating lower molecular weight alcohols, from the mixture comprising commercial stearyl alcohol or that the stearyl alcohol is somehow degraded. This splitting is also found occasionally in mixtures preserved with sorbic acid.

A similar splitting has been observed in mixtures of sodium alkyl sulfate with G-3720, Tween 60, Myrj 49 and ethoxylated glyceryl monostearate. Oddly enough, no mold growth has been found in any of these tests. It is obvious that in terms of a commercial emulsion, any splitting of the emulsifier by microbial esterases could lead to a separation of the emulsion phases.

Sorbic Acid Tests

It has been thought that the discrepancy between our results and those reported by others using sorbic acid, could be in the medium employed. Both magnesium and ferrous iron have been suggested as forming sorbates less soluble than the free acid. However, magnesium sorbate is far more water soluble than sorbic acid itself. In experiments made with Jaag medium with and without these two ions, no difference in growth was observed. When deionized water served as the medium, the addition of these ions in the concentration found in Jaag medium showed no effect on preservative action.

Though Lach (4) has found sorbic acid to be less complexed by polyglycols than the *p*-hydroxybenzoates, our work to date, using mainly *A. niger* as the test organism does not confirm this. Furthermore, one of us (M. G. deN.) has found that at a pH above 5.0, sorbic acid is a poor preservative even in the absence of nonionics in Jaag medium against *A. niger*.

	Roccal	Ethyl Cetab	Hyamine 10X
0.1% cationic only*	−	−	−
0.1% cationic plus 2% G-3720*	++++	++++	++++
0.1% MP†	+	++	+++
0.15% MP	−	−	++++
0.2% MP	−	−	+

Figure 4.—Twelve Month Growth of *A. niger* in 2% Mixture of Cationic (20%) in G-3720 with Methyl *p*-Hydroxybenzoate

* No other preservative.

† Methyl *p*-hydroxybenzoate.

	12 Months
Tween 80	+++
Myrj 49	+++
G-3720	++
G-3810	+++
G-1425	++

Figure 5.—Growth *A. niger*, in Jaag Medium, 2% Nonionic 0.2% MP*

* Methyl *p*-hydroxybenzoate.

Miscellaneous Observations

Of the many preservatives tested, we found benzoic, sorbic and dehydroacetic acids very sensitive to pH in their antimicrobial activity. Ethyl vanillate and the parabens had a fairly wide pH spectrum.

In general, we found that the greater the number of moles of ethylene oxide in the ether or ester chain, the greater the inactivation of preservative. These data are based on tests of a series of ethoxylated nonyl phenols, glyceryl monostearate, stearic acid and cetyl alcohol.

We found *Aspergillus* growing slightly in 0.2 per cent formaldehyde (100 per cent basis) in Jaag medium containing 2 per cent G-3720. Similar

tests with lower concentrations of formaldehyde showed, as would be expected, heavier growth, but in no case was it more than moderate.

At the end of one series of tests using Jaag medium, *Aspergillus* and methyl *p*-hydroxybenzoate in concentrations of 0.1, 0.15, and 0.2 per cent, we added 0.1 per cent of a commercial perfume compound dissolved by 2 per cent G-3720 to the group. In two months tubes preserved with 0.1 per cent methyl *p*-hydroxybenzoate showed a very heavy growth. At a concentration of 0.15 per cent methyl *p*-hydroxybenzoate, a heavy growth took place in one year. At a concentration of 0.2 per cent methyl *p*-hydroxybenzoate no growth was discernible at the end of one year.

It was of interest to note the profound effect of the nonionic G-3720 on the growth of *A. niger* in Jaag medium containing Duponol C. At 0.1 per cent concentration of Duponol C alone, tubes were free of growth at the end of one year. When 0.1 per cent G-3720 was added, moderate growth was observed in five months. At a concentration of 0.3 per cent G-3720, and 0.1 per cent Duponol C, heavy growth occurred in four days.

When the sodium alkyl sulfate was ethoxylated with $1^1/_2$ to 2 moles ethylene oxide, moderate growth was readily noticeable in three weeks.

Based on a suggestion made in Geneva in 1957, we used five materials because their main action was fungicidal. Jaag medium was made with 2 per cent G-3720 and variable amounts of the fungicides with the following results.

Vancide 89 (5) showed a very heavy growth in a 0.01 per cent concentration in thirty days. Isothan Q-15 (6) in 0.1 per cent concentration showed a heavy growth in twenty days. Salicylanilide 0.1 per cent took one hundred fifty days to give a very heavy growth. Rotax (7) 0.1 per cent gave a moderate growth in one hundred twenty days, while TMTD (8) 0.1 per cent has shown no growth for nine months.

We find that not only nonionics but other macromolecules inactivate preservatives when tested in Jaag medium. Thus, 0.5 per cent Carbopol 934 (9) containing 0.2 per cent methyl *p*-hydroxybenzoate shows moderate growth of *A. niger* in from two to four months depending on the alkali used in neutralizing the material. Cellosize (10) can be preserved with as little as 0.15 per cent methyl *p*-hydroxybenzoate against *A. niger*. However, 2 per cent tragacanth in Jaag medium shows slight growth of *A. niger* in five months in the presence of 0.2 per cent methyl *p*-hydroxybenzoate. Methocel 1500 (11) gives moderate to heavy growth of *A. niger* in concentrations of 0.1 to 0.2 methyl *p*-hydroxybenzoate. PVP, two types, shows moderate growth of *A. niger* in 0.2 per cent methyl *p*-hydroxybenzoate after five months.

We now have indications that the presence of around 8 per cent ethyl alcohol in the preserved nonionic medium, may retard inactivation of the preservative toward *A. niger*.

Another observation indicates that the incorporation of 20 per cent of a quaternary germicide, such as Hyamine 10 X, Roccal or Ethyl CETAB based on the weight of the nonionic, will prevent growth in the presence of 0.2 per cent methyl *p*-hydroxybenzoate but not in its absence. These tests have now been running continuously for eighteen months.

Summary

The prevention of inactivation of preservatives by alkoxylated nonionics appears a step closer to solution. The addition of 10 per cent and in some cases less, of ethyl alcohol; propylene glycol; 1,3-propanediol; 1,4-butanediol or 2, methyl 2,4-pentanediol is effective for this purpose against four bacteria and *A. niger*, using 0.2 per cent methyl *p*-hydroxybenzoate and 2 per cent Tween 80 or G-3720.

These results are to be expanded to include other preservatives, nonionics and microörganisms.

References

(1) Barr, M., and Tice, L. F., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 445 (1957).
(2) deNavarre, M. G., *J. Soc. Cosmetic Chemists*, **8**, 68 (1957).
(3) Pisano, F. E., and Kostenbauder, H. B., Am. Pharm. Assoc., Sci. Sect., Los Angeles Meeting, April (1958).
(4) Lach, J. L., Ravel, K., and Blaug, S. M., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 615 (1957).
(5) A brand of N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide. R. T. Vanderbilt Co., Inc., New York 17, N. Y.
(6) A brand of lauryl isoquinolinium bromide, Onyx Oil and Chemical Co., Jersey City, N. J.
(7) A brand of mercaptobenzothiazole, R. T. Vanderbilt Co., Inc., New York 17, N. Y.
(8) Tetramethyl thiuram disulfide.
(9) A brand of polymethylene carboxylate, B. F. Goodrich Chemical Co., Cleveland 15, Ohio.
(10) A brand of ethoxylated cellulose, Carbide and Carbon Chemicals Div., Union Carbide and Carbon Corp., New York 17, N. Y.
(11) A brand of methyl cellulose, The Dow Chemical Co., Midland, Mich.