Studies on the Stability of Kathon® CG/ICP Microbicide in Alpha Olefin Sulfonate Based Systems*

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Kathon® CG/ICP Microbicide (Rohm & Haas Co.) which contains 1.5% 5-chloro-2-methyl-4-isothiazolin-3-one and 0.35% 2-methyl-4-isothiazolin-3-one as active ingredients has been found to be a highly efficient antimicrobial preservative for formulated products. However, its use has been contra-indicated for formulations containing certain anionic surfactants, especially AOS, because of the suspected presence of residual bisulfite ion added to remove residual hypochlorite remaining at the end of the bleaching process.

It has been found that sulfite ion is not stable in the complex mixture of components in commercially produced AOS. AOS samples tested, whether bleached or not, contained residual reducing capacity, but of a sufficiently low redox potential as to not affect Kathon® CG/ICP Microbicide stability.

Alternative analytical methods for analysis for bisulfite in AOS and other surfactant systems are discussed.

A new HPLC method for determination of Kathon® CG/ICP Microbicide level in formulated products was developed.

In the formulation of products for the household and personal-care products industry, it is essential that a preservative be included to protect the formulation from antimicrobial attack. A number of different preservative systems are available for this purpose. One of these, a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, offered as Kathon® CG/ICP Microbicide by Rohm and Haas Company, has been receiving increasing attention for this use because of its effectiveness at very low (5-10 ppm) active levels (1-3). Kathon® CG/ICP Microbicide is available as a water solution of 5-chloro-2-methyl-4-isothiazolin-3-one (1.05%-1.25%) and 2-methyl-4-isothiazolin-3-one (0.25%-0.45%), which also contains magnesium chloride and magnesium nitrate, as shown in Table 1. However, there has been some concern about its use in formulations made with surfactants such as alpha-olefin sulfonates which have been subjected to an oxidative bleaching process in manufacture. This is because sodium sulfite is added in a step subsequent to the bleaching step to neutralize residual oxidizing agent, and Kathon® CG/ICP is known to be degraded by sulfite or bisulfite (4). In fact, the use of bisulfite solution is recommended by the Rohm and Haas Company for destruction of Kathon® CG/ICP prior to disposal. Recommended decontaminant solutions contain about 10% sodium bisulfite and are used at a pH of 4 to 5.5 (5). This degradation is reported to occur by nucleophilic attack of the bisulfite ion to give the ring-opening reaction shown in Scheme 1 (4). The possibility of resulting concentrations of sulfite in the surfactant sufficient to inactivate the Kathon® CG/ICP Microbicide has raised questions regarding the advisability of its use in products formulated with bleached surfactants. Surfactant manufacturers who have been contacted do not have sulfite specifications for their products nor do they routinely analyze for sulfite. Therefore, good data on actual sulfite levels in surfactants were not available. Since the use of Kathon® CG/ICP Microbicide provides certain benefits to the formulator, and specifically since there is significant interest in the use of Kathon® CG/ICP Microbicide as a preservative in AOS-based systems, it was decided to investigate the scope of this problem.

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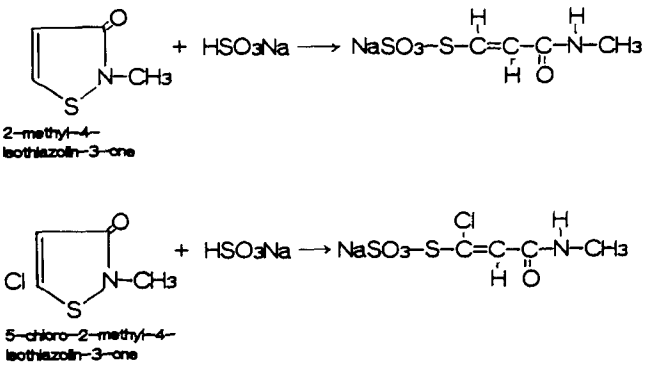
TABLE 1

Kathon® CG/ICP Microbicide

Typical Composition	% wt.
Active ingredients	
5-chloro-2-methyl-4-isothiazolin-3-one	1.05-1.25
2-methyl-4-isothiazolin-3-one	0.25-0.45
Inert ingredients	
Magnesium chloride	0.5-1.0
Magnesium nitrate	21-23
Water	74-77



Scheme 1 - Degradation Reactions of Kathon® CG/ICP

EXPERIMENTAL

In this work, sulfite analysis was by a conventional iodometric method in which a sample acidified with acetic acid is titrated with standard 0.05 N potassium iodate in the presence of potassium iodide and a starch indicator. The endpoint is taken as the first appearance of a blue color (in water solutions) or brown color (in surfactant solutions) which is stable for 30 seconds. For titration of aqueous solutions of sodium sulfite, aliquots of the solution to be analyzed were placed into 100 ml of 5% acetic acid, KI and starch indicator added, and the sample titrated. For analysis of surfactants, 25 ml of acetic acid was added to the surfactant sample (usually 50 grams), followed by 75 ml water, KI and starch, and the titration was completed. Lowest level of sodium sulfite which could be measured using this procedure was 25 ppm.

Determination of total reducing agent in AOS samples was done by addition of an excess of standard 0.1 N iodine solution to a 1 gram sample in 100 ml of deionized water, acidified by the addition of 5 ml of 50% H2SO4. After an appropriate time period, during which the solution was stored away from light, the excess iodine was titrated with a standard 0.02 N sodium thiosulfate solution, with the addition of starch indicator near the endpoint.

For potentiometric titrations, 50 gram samples of surfactant were acidified by addition of 5 ml of 50% H2SO4. Redox potential was read by a Corning Redox Combination Electrode (Cat. No. 476064), used with a Beckman Model 4500 digital pH meter set to display output in millivolts. Titrant was delivered by a Manostat Cassette Pump, Junior Model. Titration curves were recorded on a Linear Instruments Corp. Model 281 strip chart recorder. Titrants and flow rates used are given in the discussion which follows.

The HPLC system used for analysis of active components of Kathon® CG/ICP Microbicide consisted of two Waters M-45 solvent pumps, a Waters U6K injector, an Alltec/Applied Science Econosil C18, 10u, 4mm × 25cm column, a Waters Model 450 variable wavelength detector, a Waters Model 720 Data Module and a Waters Model 730 System Controller. Chromatographic conditions are shown in Table 2. The separation of compounds of interest is done in an isocratic mode, with the gradient used to clear the column of interferring materials prior to the next injection. Kathon® CG/ICP standards were prepared by dilution of 1.5% active material (as provided by Rohm and Haas) in deionized, HPLC grade water to give 1.5 ppm solutions. Surfactant and product samples were prepared by dilution of 5 gram samples to 25 grams with deionized, HPLC grade water. Standard and sample solutions were filtered through a 0.45 u filter prior to injection into the chromatograph. Accuracy of the quantitative determination of Kathon® CG/ICP is estimated to be ± 5%.

TABLE 2

Chromatographic Conditions

Analysis of Kathon® CG/ICP Microbicide

- C18 reverse phase column
- UV detector, 275 nm
- Chart speed, 0.5 cm/min
- Solvent:
  A. 25/75 MeOH/H_2O w/0.4% dibasic ammonium phasphate
  B. 100% methanol
- Solvent gradient

Time (min)	Flow (ml/min)	% A	% B
Initial	1	100	0
14	1	100	0
14.5	1	0	100
16	1	0	100
16.5	2	0	100
20	2	0	100
21.5	2	100	0
24	2	100	0
25	1	100	0
30	1	100	0

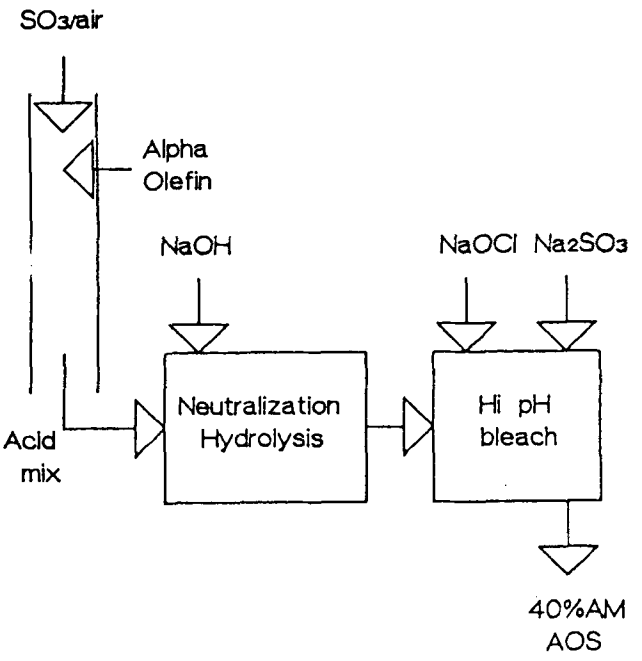


FIG. 1. AOS manufacture.

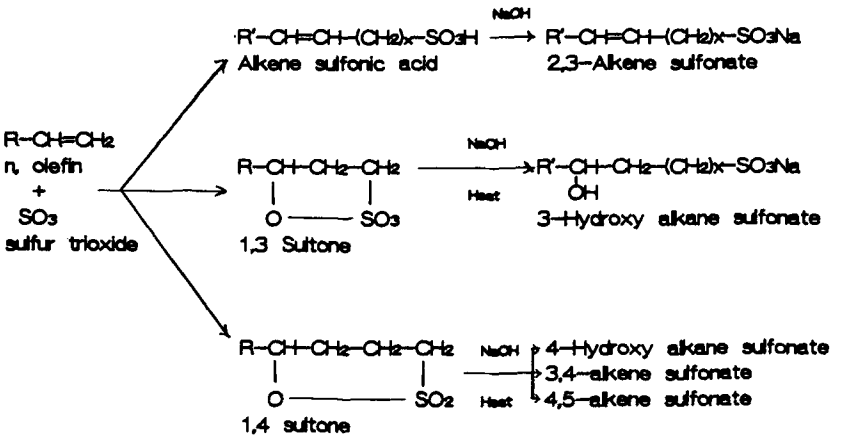
DISCUSSION

In Figure 1 is a simplified flow-diagram for a continuous process for manufacture of alpha-olefin sulfonate (6,7). In the process, alpha-olefins are reacted with sulfur trioxide in a thin film reactor under controlled temperature conditions. The reaction mixture is neutralized to yield a complex mixture of ingredients (6,8) as shown in Scheme 2. The neutralization is done under strongly alkaline (pH > 12) and elevated temperature conditions to not only neutralize alpha-olefin sulfonic acids but also to hydrolyze the cyclic inner esters or sultones to alkene sulfonates and hydroxy alkane sulfonates and disulfonates. In addition, there are several side reactions which may occur when the reactor is run under less than optimum conditions (6,8). These are shown in Scheme 3. The primary result of these reactions is the formation of disulfonates.

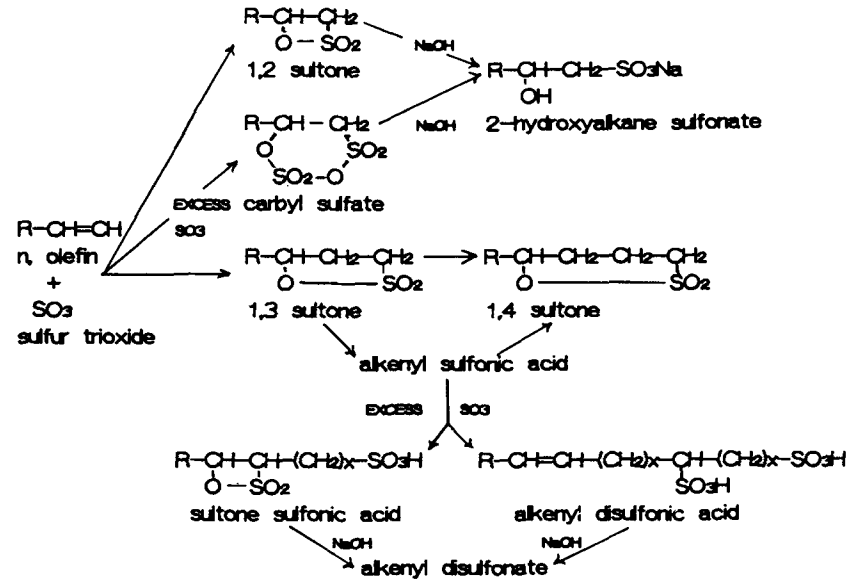
Following the neutralization and hydrolysis step and prior to pH adjustment (still at pH > 12), the reaction mixture may be bleached. Bleaching by hydrogen peroxide is reported in the literature (9) but is most commonly done by addition of sodium hypochlorite. Following the bleaching step and while the slurry is still at the high pH, sodium sulfite is added to neutralize the residual hypochlorite remaining at the end of the bleaching process. This sulfite addition must be done before pH reduction because sultones can be reformed if the pH is reduced before the hypochlorite is destroyed. After sulfite treatment, the final product is neutralized to a pH which is typically around 8.0 to 8.5.

The final product will usually contain about 40% surfactant actives of which alkene sulfonates, hydroxy alkane sulfonates and disulfonates are present in the ratio of about 70:30:10 along with minor amounts of other reaction products (6) as shown in Table 3.

In the course of this work, one of the initial requirements was to find a reproducible, accurate method for the determination of sulfite in the surfactant raw mate-



Scheme 2 - Primary Reactions in the Sulfonation of AOS



Scheme 3 - Secondary Reactions in the Sulfonation of AOS

rials of interest. Iodimetric methods are commonly used for the determination of sulfite or bisulfite in aqueous solutions. A direct titration with iodine has been used, but it has been reported that this method can give low results as a result of air oxidation of the sulfurous acid or its salts during the titration. Better results are reported to be obtained with a method where an excess of iodine is added and back-titrated with a reducing agent such as sodium thiosulfate (10). However, it was found that neither of these methods was suitable for the determination of sulfite in AOS samples due to interference from components of the surfactant present in the titration solution. In the titration of AOS, the direct titration with iodine suffered from a lack of reproducibility due to a changing endpoint which was dependent on the speed with which the titration was carried out, and the method utilizing an excess of iodine and back titration with standard thiosulfite gave very large blank values, even in AOS samples which had not been bleached during manufacture and to which no sulfite had been added. These findings were confirmed by the laboratory of an AOS manufacturer. This method was, in fact, used to estimate total reducing agent in AOS samples.

TABLE 3

AOS Composition

	% W
Active matter	
Alkene sulfonates	
Alkane hydroxysulfonates	35-40
Disulfonates	
Neutrals	
Alpha olefin	
Internal olefin	
Sec-alcohols	0.5-1
Paraffins	
1.4-Sultones < 50 ppm	
Salts	
Sodium sulfate	1-2
Sodium chloride	0.5-1
Sodium hydroxide	0.1
Water	balance
	100

TABLE 4

Effect of pH on Stability of Sodium Sulfite

	% Activity				
Time	pH 2.0	pH 4.6	pH 7.0	pH 8.8	pH 11
0	100.0	100.0	100.0	100.0	100.0
24 hr	90.0	92.6	60.6	51.1	48.9
48 hr	53.8	91.1	51.0	11.2	11.5
Final pH	1.9	3.8	3.9	7.2	10.7

TABLE 5

Effect of Dissolved Oxygen on Recovery of Sodium Sulfite

Order of Addition in KIO_3 Titration	% Recovery
1. Water 2. Na_2SO_3 3. Acid	87
1. Water 2. Na_2SO_3 (wait 6 minutes) 3. Acid	83
1. Water 2. Acid 3. Na_2SO_3	98
1. Water (deaerated) 2. Na_2SO_3 3. Acid	98

It was found that a method involving the titration of an acidified sample with standard potassium iodate solution in the presence of potassium iodide and starch indicator was most suitable for the determination of sulfite in surfactant samples. In the acid environment of the titration, any sulfite present will be converted to bisulfite which is oxidized by the iodate titrant. At the endpoint, excess iodate oxidizes the iodide to iodine which is detected by a starch indicator solution. The endpoint is a blue or brown color, depending on the sample being titrated. Acetic acid is preferred for the acidification step because of improved stability, in solution, of sulfite/bisulfite at a pH around 4, as will be discussed below.

Since it is the residual sulfite ion in the surfactant raw material resulting from the neutralization of excess bleaching agent which is reported to be the problem with respect to Kathon® CG/ICP Microbicide stability, it is important to briefly consider the chemistry of sulfite and bisulfite, which exist in equilibrium: $HSO_3 + OH <-----> SO_3 = + H_2O$. At the alkaline pH of the finished surfactant, the bisulfite-sulfite equilibrium would be strongly on the sulfite side (11).

Also of importance is the fact that oxygen in the air can oxidize sulfite to sulfate by the reaction $2\ SO_3 + O2 -----> 2SO_4 =$. The ease of air oxidation of sodium sulfite under alkaline conditions is supported by work of Urone and Boggs who investigated the stability of sodium sulfite in water and in dilute sodium hydroxide (12). They found about 54% loss of activity within 4 days in water and about 90% loss of activity within 2 days in 0.1N NaOH. The rate of this reaction is dependent on pH, as shown in Table 4, where stability was tested at pHs of 2.0, 4.6 (normal pH of $NaHSO_3$), 7.0, 8.8 (normal pH of Na_2SO_3) and 11.0. Initial sulfite concentrations were 0.1% and solutions were unbuffered. As can be seen, stability is greatest at pH 4.6 with decreased stability at pHs either above or below this value. As can be seen, the pH of all solutions changed over the storage period, some very significantly, becoming more acid in every case.

It was found that the oxygen which is normally dissolved in the water used in sample dilution can be a factor in analysis of low levels of sodium sulfite. This was studied by changing the point of addition of sodium sulfite during the titration procedure. In these experiments, about 0.05 grams of solid sodium sulfite, which is equivalent to 1000 ppm in a 50 gram sample, were dissolved in about 100 ml of water. As shown in Table 5, when solid sodium sulfite samples were dissolved in water, then acidified and titrated as usual, low results (about 87% recovery) were obtained, even if the acid was added immediately following the addition of solid sodium sulfite. In an experiment where exactly six minutes were allowed to elapse between addition of sodium sulfite and acid, recovery dropped to about 83%. However, when the water was acidified prior to addition of solid sodium sulfite, recovery was better than 97%. Further, when water was deoxygenated by saturation with nitrogen, recovery was better than 97% even when the sample was added to water prior to acidification. This suggests that in the analysis of low levels of sulfite, it is important to acidify the sample prior to dilution with water, or to acidify the water used to make the dilution. Because of improved stability of sulfite (bisulfite) at pH 4-5, acetic acid is preferred.

When AOS samples were received from a manufacturer who bleaches with hypochlorite and neutralizes with sodium sulfite, analysis by the iodate method showed no residual sulfite. As a result of this, a series of experiments was done to attempt to put the perceived problem of residual sulfite into perspective. In these experiments, AOS samples were spiked with known quantities (ca 1000 ppm) of sodium sulfite. It was found with both bleached and nonbleached AOS that titratable levels of sulfite, by the iodate method, dropped very rapidly and were at negligible levels within about 10 minutes. Similar tests were done using an alcohol ether sulfate (AES) surfactant. It was found that this surfactant reacts much differently than does AOS. When samples of AES are spiked with sulfite, sulfite stability is comparable to that in an aqueous solution. Results are shown in Table 6. This difference is attributed to reactive components in the complex reaction mixture obtained in the sulfonation of AOS.

In titrations of AOS samples it was found that endpoints faded rather quickly, suggesting that there were

TABLE 6

Stability of Sodium Sulfite in AOS or AES

	ppm Sodium sulfite* remaining	
Initial	AOS 1000	AES 1000
10 min	32	n/a
30 min	32	455
60 min	25	436
120 min	25	n/a
6 hr	25	357

*25 ppm is lowest level measurable under analytical conditions used.

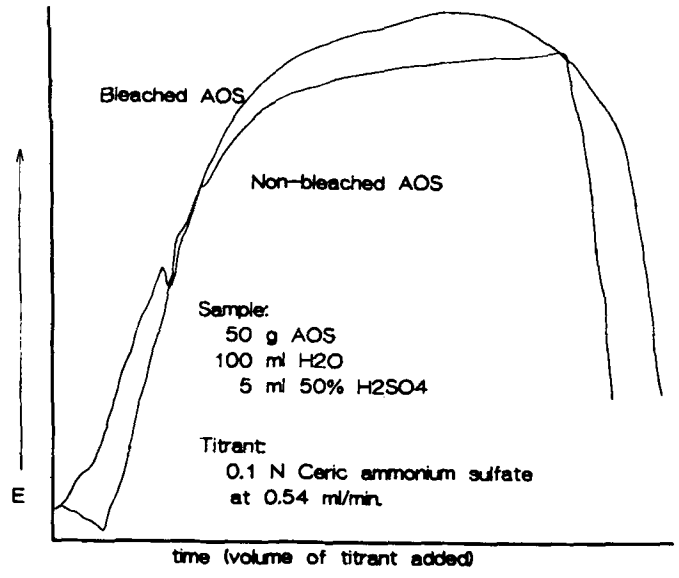


FIG. 2. Potentiometric titration of AOS.

other oxidizable reducing) species in AOS. To investigate this, titrations of AOS samples were done using a ceric ammonium sulfate solution with ferroin (1,10-phenanthroline ferrous sulfate solution) as the indicator. Titrations with this somewhat stronger oxidizing agent (oxidation potential +1.443 as compared to +0.535 for iodine) gave nonstable endpoints which were very dependent upon the rate of titration, and permanent endpoints could not be obtained even at relatively large titration values. This reducing property of AOS was studied in another set of experiments in which the potentiometric titrations of acidified AOS solutions were done using the ceric ammonium sulfate solution. In these potentiometric titrations it was found that bleached AOS showed a definite level of immediately oxidizable material before the potential began to increase with continued titrant addition as shown in Figure 2. When cerate addition was ceased, the measured potential dropped toward the original level, indicating that the cerate ion was being reduced. The unbleached AOS showed no indication of easily oxidizable material. With continued titrant additon, the potential increased but began to level off at a potential lower than that attained with the bleached sample. When titrant addition was ceased, the oxidation potential fell very rapidly as the cerate ion was reduced, as is shown in Figure 2.

Total reducing agent in the AOS samples was determined by the addition of an excess of standard iodine solution to an acidified water solution. Samples were stored in the dark overnight and titrated for residual iodine with standard sodium thiosulfate. Results showed the nonbleached AOS to have a total reducing capacity of 0.365 meq/gram; the bleached AOS had a reducing capacity of 0.313 meq/gram. To put this into perspective, this is equivalent to 4.62% and 3.94% sodium sulfite in the two samples, respectively.

High-performance liquid chromatographic (HPLC) methods have been found to be extremely useful in the determination of Kathon® CG/ICP levels in surfactants

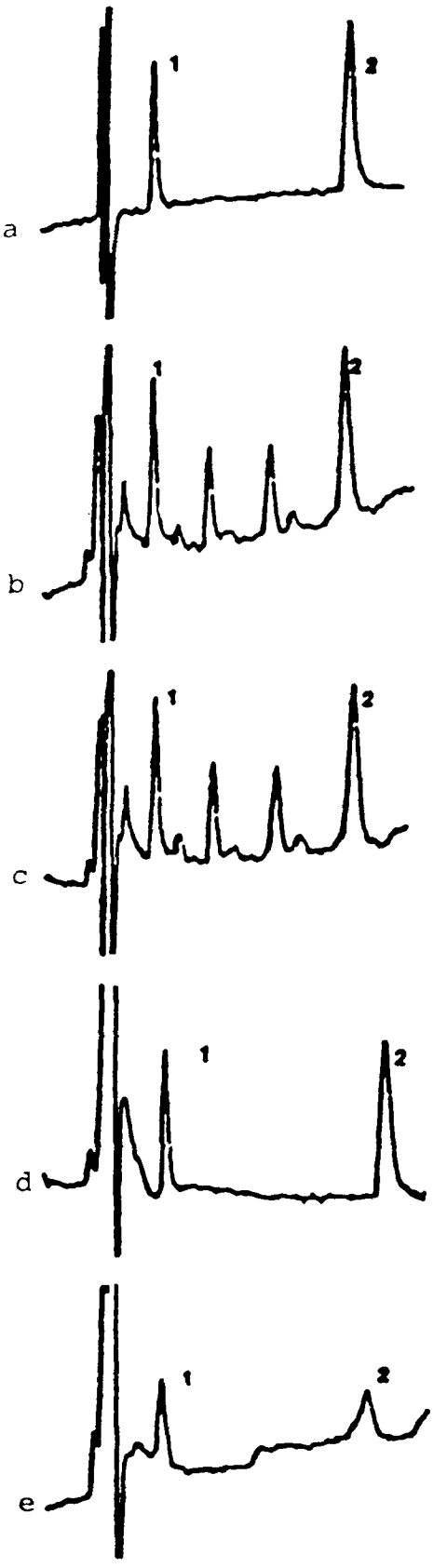


FIG. 3. HPLC chromatogram of Kathon® CG/ICP Microbicide. 1, 2-methyl-4-isothiazolin-3-one: 2, 5-chloro-2-methyl-4-isothiazolin-3-one: a, Standard; b, In AOS; c, In AOS after 24 hr; d. In AES; e, In AES after 24 hr.

TABLE 7

Efficacy of 10 ppm Kathon® CG/ICP Microbicide in AOS Containing Sodium Sulfite[1]

Kathon® CG ppm Al added	Sodium sulfite ppm Al added	Bacteria (CFU/ml) remaining			
		2 wks	4 wks	6 wks	8 wks
0	0	1.7×10^6	3.8×10^5	1.5×10^5	6.6×10^6
10	0	<10	<10	<10	<10
10	100	<10	<10	<10	<10
10	500	<10	<10	<10	<10
10	1000	<10	<10	<10	<10
0	500	720	6.0×10^5	2.2×10^6	—
0	1000	320	280	2.6×10^6	—

Note: — = not plated

[1]Rohm & Haas Co.—used by permission.

TABLE 8

Kathon® CG/ICP Microbicide Active Ingredient Level in Sodium Lauryl Ether Sulfate[1]

	Unbleached	Bleached 0 residual bisulfite	Bleached 25 ppm residual bisulfite	Bleached 50 ppm residual bisulfite
Initial	10.6	9.6	9.9	10.6
2 weeks	10.5	9.5	9.8	10.5
4 weeks	10.4	9.6	9.5	10.5
6 weeks	10.4	9.3	10.0	10.3
8 weeks	10.3	9.1	9.7	10.5
12 weeks	10.2	8.2	9.7	10.1
22 weeks	9.8	7.3	9.2	9.8
pH (10%) Initial	7.3	7.2	6.7	7.0
22 weeks	7.1	7.1	6.3	6.6

[1]Rohm & Haas Co.—used by permission.

and formulated products (13). The analytical conditions suggested by Rohm and Haas Co. (14) were initially tried, but it was found that there were interferences with components of a hand cleanser formulated with AOS, as well as with AOS itself. Therefore, all of the results reported here were run using a modification of that procedure, as was detailed in the discussion of experimental methods.

A chromatogam of an aqueous solution of Kathon® CG/ICP Microbicide obtained using the procedure indicated is shown in Figure 3a. Peaks for the two active components are seen at about 5.2 minutes for the nonchlorinated material and 14.4 mintues for the chlorinated material, respectively.

In Figure 3b is a chromatogram of an AOS sample containing 0.05% Kathon® CG/ICP Microbicide (7.5 ppm active) and no sulfite. A number of peaks attributed to the components present in AOS can be seen. However, the two Kathon® CG/ICP peaks are apparent and fairly well-separated from the AOS components. Figure 3c shows a chromatogram of an AOS sample spiked with 1000 ppm sodium sulfite, to which Kathon® CG/ICP was added at 0.05%, after the sample had aged for 24 hours. Quantitation of the two Kathon® CG/ICP peaks shows essentially 100% recovery. Chromatograms of this same sample after about 10 days show similar results. Results of experiments done by Rohm and Haas Co. to determine the effect of sulfite on Kathon® CG/ICP levels on antimicrobial efficacy in AOS are shown in Table 7. These show that the addition of up to 1000 ppm sodium sulfite has no effect on the preservative efficacy of Kathon® CG/ICP in AOS, as would be expected based on our findings.

Shown in Figure 3d is a chromatogram of AES containing Kathon® CG/ICP Microbicide at 0.05%, without added sulfite. The chromatogram of AES is much cleaner than is seen with AOS. Figure 3e shows the chromatogram of a similar AES sample, to which sodium sulfite has been added at 1000 ppm, obtained after 24 hours. As can be seen, sodium sulfite at this level has a very significant effect on the active Kathon® CG/ICP level with significant reductions in both peaks. In fact, we found a complete disappearance of the chlorinated component of Kathon® CG/ICP within 10 days. However, experiments done by Rohm and Haas Co. with AES, as shown in Table 8, indicate that up to 50 ppm residual bisulfite has no effect on the level of active Kathon® CG/ICP Microbicide in the sample.

A prototype hand cleaner formulation which contained 20% of a 40% active nonbleached AOS, 2% of a 35% active coco-amidopropylbetaine, 3% of a 100% active coco-diethanolamide, 1.5% ethylene glycol monostearate and about 0.03% (4.5 ppm active) Kathon® CG/ICP Microbicide was found to have 4.9 ppm active Kathon® CG/ICP remaining after more than 11 months storage. The initial level reported is based on the nominal concentration of Kathon® CG/ICP added. The product was not analyzed at the time of manufacture.

Based on these findings, it is concluded that the perceived problem related to residual sulfite levels in bleached AOS is not of significant importance because of the inherent instability of the sulfite ion in the complex mixture of components making up that surfactant. It also appears that, although both bleached and nonbleached AOS have a significant reducing capacity, the redox potential is low enough that this is not a significant factor in Kathon® CG/ICP Microbicide stability. The effect of residual sulfite may, however, be a factor in other bleached surfactants. In any given formulation, it is the sulfite level of the final mixture after all dilutions have taken place which is probably most significant.

It must be recognized that the effect of sulfite or bisulfite ion on Kathon® CG/ICP Microbicide stability and its effectiveness as a preservative is only one facet of a much larger and more complicated picture. As with the use of any preservative chemical, the formulator should perform appropriate chemical and antibacterial challenge tests with each formulation, using all approved raw materials, to determine that the product formulated is adequately preserved at the time of manufacture and that the preservative has adequate stability in the formulation.

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