Adverse Effect of Ammonium Salts on the Antibacterial Activity of Paraformaldehyde Solutions

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ABSTRACT

MYERS, G. E. (University of Alberta, Edmonton, Alberta, Canada) AND R. G. L. McCREADY. Adverse effect of ammonium salts on the antibacterial activity of paraformaldehyde solutions. Appl. Microbiol. **11**:357–361. 1963.—The antibacterial activity of aqueous solutions of paraformaldehyde in concentrations from 0.1 to 0.4% (w/v) is bacteriostatic rather than bactericidal in the presence or absence of ammonium chloride. The presence of ammonium chloride significantly lengthened the time of exposure to paraformaldehyde necessary for inhibition of growth of the test organism (*Staphylococcus aureus* FDA 209) when unbuffered solutions were used. Elevation of the pH of the reacting mixture of paraformaldehyde and ammonium chloride by partial buffering lengthened the time of exposure necessary for inhibition of growth of the test organism. Decrease of antibacterial activity was concomitant with the disappearance of paraformaldehyde from the reacting mixture. The reaction of paraformaldehyde with ammonium chloride was rapid at room temperature (25 C) and at pH levels near neutrality. The fate of the reacting paraformaldehyde, including the possibility of the formation of hexamethylenetetramine or methylenimine, is discussed with particular reference to loss of antibacterial activity.

In the petroleum industry, control of fermentation of the starch in so-called starch-base drilling mud is commonly attempted by adding a preservative, usually an antiseptic such as paraformaldehyde (Farrow, 1950). Operators of drilling rigs occasionally report difficulty in maintaining the desired concentration of paraformaldehyde in the mud during drilling. Investigating one such occurrence, Myers (1962) demonstrated the disappearance of paraformaldehyde from a sample of starch-base drilling mud in the laboratory. He suggested that the paraformaldehyde may have reacted chemically with ammonium chloride, present in large amounts in produced water which mixed with the mud during the drilling operation.

The present paper relates the results of experiments done to determine the effects of various concentrations of ammonium chloride and variations in pH on the antibacterial activity of paraformaldehyde.

MATERIALS AND METHODS

The test organism used in all experiments was *Staphylococcus aureus* FDA 209. The paraformaldehyde used in all our experiments contained 86% available formaldehyde when assayed according to the method of the *British Pharmaceutical Codex* (Pharmaceutical Society of Great Britain, 1959). The remaining 14% is believed to be water of crystallization. A stock solution was prepared by dissolving 4.00 g of paraformaldehyde in sufficient distilled water to make 1 liter. Complete solution at room temperature (25 C) required 12 days. The solution was diluted in distilled water so that a series of nine 1-ml volumes was obtained, containing 0.4, 0.33, 0.25, 0.2, 0.16, 0.14, 0.125, 0.11, and 0.1% (w/v) paraformaldehyde. These solutions were used to determine the antibacterial activity of the paraformaldehyde. To determine the effect of ammonium chloride on the antibacterial activity of paraformaldehyde, a 20% (w/v) stock solution of ammonium chloride was prepared in distilled water. Sufficient of this solution was added to the paraformaldehyde stock solution so that three series of dilutions of paraformaldehyde were prepared containing, respectively, 1, 2, and 3% (w/v) ammonium chloride. The mixtures of paraformaldehyde and ammonium chloride were allowed to stand at room temperature (25 C) for 18 hr before testing, to allow for equilibration of any chemical reaction. To each dilution of paraformaldehyde or paraformaldehyde plus ammonium chloride was added 1 ml of a 24-hr broth culture of the test organism. At 30-min intervals during 6 hr thereafter, a standard loopful (diameter, 2 mm) of the mixture in each tube was transferred to 10 ml of sterile nutrient broth. Broth cultures were incubated at 37.5 C and examined for visible growth after 24, 48, 72, and 96 hr of incubation. All broth cultures were subcultured on nutrient agar plates at each examination. Agar plate cultures were incubated for 24 hr and then checked for the presence of growth typical of the test organism.

The experiments were repeated with stock solutions of paraformaldehyde and ammonium chloride prepared in Gifford's (1935) buffer (pH 7.2).

The reaction rates of paraformaldehyde with various concentrations of ammonium chloride at room temperature (25 C) were studied by use of a modification of the sodium sulfite method originally described by Lemme (1903).

The amount of paraformaldehyde in solution was determined at 30-min intervals over a period of 6 hr after the preparation of the paraformaldehyde-ammonium chloride mixtures. The pH levels of the aqueous solutions of paraformaldehyde and of the aqueous ammonium chloride solutions were determined before mixing the two, and the pH of the reacting mixtures was determined at each 30-min interval thereafter. Identical experiments were performed with solutions of paraformaldehyde and ammonium chloride prepared in Gifford's buffer (pH 7.2).

RESULTS

The antibacterial activity of paraformaldehyde in concentrations from 0.1 to 0.4% (w/v) is bacteriostatic rather than bactericidal, in that prolongation of incubation time for subcultures increases the exposure time required to inhibit growth (Table 1). The same is true when ammonium chloride is present and when the solutions are buffered (see Table 2).

Examination of the results shown in Table 1 (see 96 hr of incubation) reveals that the exposure time required for

TABLE 1. *Effect of ammonium chloride on the bacteriostatic activity of unbuffered solutions of paraformaldehyde (test organism: Staphylococcus aureus FDA 209)*

Paraformaldehyde concn (w/v)	Ammonium chloride concn (w/v)	pH of mixture	Exposure (min) required to inhibit growth when subcultures incubated for			
			24 hr	48 hr	72 hr	96 hr
%	%					
0.4	0	5.9	75	120	120	120
	1	4.3	75	135	165	165
	2	3.7	75	135	180	195
	3	3.6	75	165	210	210
0.33	0	5.8	90	120	120	120
	1	4.3	105	150	180	180
	2	3.8	105	165	195	210
	3	3.7	105	195	240	240
0.25	0	5.8	90	135	165	165
	1	4.4	120	180	210	225
	2	3.9	120	195	210	240
	3	4.0	120	255	300	300
0.2	0	5.9	105	150	165	165
	1	4.5	150	210	240	255
	2	4.2	150	225	240	270
	3	4.2	150	300	345	345
0.16	0	6.0	120	165	180	180
	1	4.7	165	225	255	270
	2	4.4	165	240	270	285
	3	4.3	165	315	—*	—
0.14	0	5.9	135	180	195	195
	1	4.8	180	240	270	285
	2	4.5	180	255	285	330
	3	4.4	180	315	—	—
0.125	0	5.9	150	180	225	225
	1	4.9	210	255	300	330
	2	4.6	210	270	330	360
	3	4.5	210	—	—	—
0.11	0	6.0	150	195	225	225
	1	5.1	210	270	330	330
	2	4.7	240	270	360	—
	3	4.6	255	—	—	—
0.1	0	6.0	150	195	225	225
	1	5.1	210	270	330	360
	2	4.8	240	270	—	—
	3	4.6	255	—	—	—

* Indicates no bacteriostasis despite 6 hr of continuous exposure.

TABLE 2. *Effect of ammonium chloride on the bacteriostatic activity of partially buffered solutions of paraformaldehyde (test organism: Staphylococcus aureus FDA 209)*

Paraformadehyde concn (w/v)	Ammonium chloride concn (w/v)	pH of mixture	Exposure (min) required to inhibit growth when subcultures incubated for			
			24 hr	48 hr	72 hr	96 hr
%	%					
0.4	0	6.8	60	60	90	90
	1	4.1	60	90	105	105
	2	3.7	60	90	120	165
	3	3.6	60	90	120	165
0.33	0	7.3	60	60	90	90
	1	4.4	60	90	120	135
	2	3.9	75	105	150	195
	3	3.8	90	105	150	195
0.25	0	7.5	60	90	120	120
	1	4.7	60	120	150	195
	2	4.3	90	135	195	210
	3	4.2	105	135	210	210
0.2	0	7.4	60	90	120	150
	1	4.9	90	135	185	225
	2	4.6	105	165	210	210
	3	4.5	120	165	240	300
0.16	0	7.5	90	120	150	180
	1	5.1	105	150	225	255
	2	4.8	120	180	270	330
	3	4.7	150	180	285	330
0.14	0	7.4	90	120	150	180
	1	5.4	135	225	265	265
	2	5.0	150	240	345	360
	3	4.9	180	240	360	360
0.125	0	7.5	120	150	180	210
	1	5.6	165	315	330	345
	2	5.2	180	330	360	—*
	3	5.1	195	360	—	—
0.11	0	7.5	180	180	210	210
	1	5.7	180	330	360	360
	2	5.3	195	360	—	—
	3	5.3	210	—	—	—
0.1	0	7.5	180	180	210	210
	1	5.9	180	360	—	—
	2	5.4	240	—	—	—
	3	5.4	240	—	—	—

* Indicates no bacteriostasis despite 6 hr of continuous exposure.

inhibition of the growth of the test organism by unbuffered solutions of paraformaldehyde significantly increases when ammonium chloride is present; e.g., the exposure time required for inhibition of the growth of the test organism by an aqueous solution containing 0.33% (w/v) paraformaldehyde increases 50% in the presence of 1% (w/v) ammonium chloride. The effect becomes more pronounced when the amount of ammonium chloride is increased; e.g., with the aqueous solution containing 0.33% (w/v) paraformaldehyde, the exposure time necessary to inhibit growth of the test organism increases 75% in the presence of 2% (w/v) ammonium chloride and 100% in the presence of 3% (w/v) ammonium chloride. Results obtained with partially buffered solutions of paraformaldehyde plus ammonium chloride (see Table 2, 96 hr of incubation) show that the increase in exposure time required to inhibit the test organism is generally greater than when unbuffered solutions are used; e.g., the exposure time required for inhibition of growth of the test organism by a partially buffered aqueous solution containing 0.33% (w/v) paraformaldehyde increases 116% in the presence of 2 to 3% (w/v) ammonium chloride.

The loss of paraformaldehyde due to reaction with ammonium chloride is very rapid at first (Tables 3 and 4), gradually slowing until equilibrium is reached. These results agree with the observation that the amount of paraformaldehyde present in the drilling mud investigated by Myers (1962) significantly decreased within 30 min after preparation. The results also agree with those obtained by Boyd and Winkler (1947). The reaction goes further as the amount of ammonium chloride present is increased. In tests using buffered solutions of slightly higher pH than the unbuffered solutions (see Table 4), the decrease in pH that occurred during the reaction was not as pronounced, probably owing to the conversion to a weaker acid of a portion of the hydrochloric acid produced.

For comparison of rates of reaction, a series of tests was done with ammonium hydroxide used in place of ammonium chloride, so that water would be formed rather than hydrochloric acid. The solutions were not buffered. Reaction rates increased with increased concentrations of ammonium hydroxide and, in fact, paraformaldehyde could not be detected almost immediately after the addition of 3% ammonium hydroxide to a solution containing 0.4% (w/v) paraformaldehyde. The pH levels of the ammonium hydroxide solutions were as follows: 1% ammonium hydroxide, pH 10.95; 2% ammonium hydroxide, pH 11.20; and 3% ammonium hydroxide, pH 11.40, as compared with pH 5.45, 5.10, and 4.80, respectively, for solutions containing the same concentration of ammonium chloride. It is obvious that the rate of reaction is very rapid at a high pH and a relatively low temperature (room temperature, 25.8 C). These results agree with those of Polley, Winkler, and Nicholls (1947).

Discussion

Loew (1888) discovered the antibacterial activity of formaldehyde and indicated that formaldehyde is more active in this regard than any of its condensation products or any of the other aldehydes. The antibacterial activity of paraformaldehyde is attributed to depolymerization yielding formaldehyde (Noller, 1951). Dissociation of paraformaldehyde occurs to a greater extent in basic solutions, and this can account for the slightly greater antibacterial activity of our partially buffered solutions (pH range, 6.8 to 7.5) as compared with unbuffered solutions (pH range, 5.8 to 6.0).

Butlerov (1859, 1860) first prepared formaldehyde in 1859, and in 1860 observed the almost quantitative reaction with ammonia which results in the formation of hexamethylenetetramine (methenamine, Hexamine, or Urotropin). Polley et al. (1947) demonstrated that the

TABLE 3. *Reaction rate of paraformaldehyde and ammonium chloride in unbuffered solution at 25.8 C*

Reaction time	Initial concn and pH of ammonium chloride solution					
	1% (w/v); pH 5.45		2% (w/v); pH 5.10		3% (w/v); pH 4.80	
	Paraformaldehyde concn (w/v)	pH of mixture	Paraformaldehyde concn (w/v)	pH of mixture	Paraformaldehyde concn (w/v)	pH of mixture
min	%		%		%	
0	0.151	5.20	0.151	4.80	0.151	4.50
30	0.130	4.30	0.130	4.00	0.103	4.20
60	0.110	4.10	0.103	3.90	0.089	3.50
90	0.103	3.95	0.097	3.80	0.082	3.35
120	0.097	3.80	0.089	3.70	0.082	3.35
150	0.097	3.80	0.089	3.60	0.075	3.30
180	0.097	3.80	0.082	3.55	0.075	3.30
210	0.097	3.80	0.082	3.55	0.075	3.30
240	0.097	3.80	0.082	3.50	0.075	3.30
270	0.097	3.80	0.082	3.50	0.075	3.30
300	0.097	3.80	0.082	3.50	0.075	3.30
330	0.097	3.80	0.082	3.50	0.075	3.30
360	0.097	3.80	0.082	3.50	0.075	3.30

TABLE 4. *Reaction rate of paraformaldehyde and ammonium chloride in partially buffered solution at 25.8 C*

Reaction time	Initial concn and pH of ammonium chloride solution					
	1% (w/v); pH 7.0		2% (w/v); pH 7.0		3% (w/v); pH 7.0	
	Paraformaldehyde concn (w/v)	pH of mixture	Paraformaldehyde concn (w/v)	pH of mixture	Paraformaldehyde concn (w/v)	pH of mixture
min	%		%		%	
0	0.158	6.50	0.158	6.45	0.158	5.80
30	0.130	5.05	0.130	4.65	0.137	4.10
60	0.123	4.90	0.117	4.40	0.123	3.95
90	0.117	4.80	0.110	4.30	0.117	3.75
120	0.110	4.70	0.103	4.20	0.103	3.65
150	0.103	4.60	0.097	4.10	0.089	3.65
180	0.097	4.50	0.089	4.10	0.075	3.65
210	0.082	4.40	0.075	4.00	0.068	3.65
240	0.082	4.40	0.075	4.00	0.059	3.65
270	0.082	4.40	0.075	4.00	0.059	3.65
300	0.082	4.40	0.075	4.00	0.059	3.65
330	0.082	4.40	0.075	4.00	0.059	3.65
360	0.082	4.40	0.075	4.00	0.059	3.65

reaction will occur in the pH range 4.0 to 8.0 but is enhanced by alkaline conditions and low temperature (0 to 30 C). Werner (1917) noted that when formaldehyde and ammonium chloride are mixed in solution the liquid quickly becomes strongly acid (compare with our results, Tables 1 to 4), and hexamethylenetetramine will be formed only if the acid is neutralized or removed. Hexamethylenetetramine owes its antiseptic activity to the formaldehyde released when it hydrolyzes (Nicolaier, 1894, 1899) and is a much weaker antiseptic than formaldehyde for this reason. According to Hinman (1913), Jordan (1911), and Hanzlik and Collins (1913), hydrolysis of hexamethylenetetramine occurs only under acid conditions. Shohl and Deming (1920) demonstrated that hydrolysis of hexamethylenetetramine in urine increases as the pH is lowered, and liberation of formaldehyde is as follows: pH 7.6, 0%; pH 6.4, 3%; pH 5.8, 8%; pH 5.4, 13%; and pH 5.0, 20% of the theoretical amount. Walker (1913) demonstrated that the presence of ammonia prevents the splitting-off of formaldehyde from hexamethylenetetramine and that under this condition no antiseptic activity can be demonstrated.

Myers (1962) demonstrated the disappearance of paraformaldehyde from a sample of starch-base drilling mud in the laboratory. When the mud was used for drilling, the pH was continually adjusted to or near neutrality by the operator in an effort to prevent the development of acidic conditions and consequent drill-stem corrosion. The degree of acidity which develops when paraformaldehyde and ammonium chloride react (see Tables 3 and 4) will tend to increase the rate of corrosion (Updegraff, 1955; von Wolzogen Kuhr, 1937). Doig and Wachter (1951) noted that corrosion decreases when a high pH is maintained, and for this reason it has become common practice to adjust the pH of drilling mud toward the alkaline side. However, as we have seen, adjustment toward alkalinity will facilitate the reaction of formaldehyde with ammonia to produce hexamethylenetetramine and at the same time prevent hydrolysis of the hexamethylenetetramine to release formaldehyde. In addition, the presence of ammonia will tend to prevent the hydrolysis of the hexamethylenetetramine. The total result will be the loss of antibacterial activity, which our results demonstrate.

In our experiments in the laboratory, the pH of the reacting mixture could not be maintained at neutrality despite the use of buffer, and the development of an acidic condition was only partially arrested (see Tables 2 and 4). Under these circumstances, it is questionable whether hexamethylenetetramine was produced. However, Duden and Scharff (1895), Werner (1917), and Baur and Rüetschi (1941) suggested that, under acidic conditions, the initial step in the reaction of formaldehyde with ammonia is the formation of methylenimine, which is stable under acidic conditions. Werner (1917) noted that hexamethylenetetramine is not found under acidic conditions. Thus, it is possible that methylenimine was produced in the reaction that occurred in our experiments.

If, during the drilling operation, the pH of the mud is maintained at neutrality or slightly on the alkaline side of neutrality, paraformaldehyde will depolymerize to yield formaldehyde. If ammonia is present and the pH is maintained at neutrality or slightly alkaline, the formaldehyde will react with the ammonia to form hexamethylenetetramine. Hexamethylenetetramine will not hydrolyze to release formaldehyde and thus exert antibacterial activity unless an acidic condition develops. However, if an acidic condition does develop and ammonia is present, no antibacterial activity will be evident, for the formaldehyde and ammonia react to form methylenimine, which is stable under acidic conditions, and the reaction goes no further. In either case, the net result is loss of free formaldehyde and antibacterial activity and, in the latter situation, the development of the acidic condition will also increase the possibility of corrosion.

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