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POTENTIATION AND STABILIZATION OF GLUTARALDEHYDE BIOCIDAL ACTIVITY UTILIZING SURFACTANT–DIVALENT CATION COMBINATIONS

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SUMMARY

Various surfactant-cation combinations were examined as possible potentiators of glutaraldehyde activity. The magnesium salt of sulphated lauryl alcohol was shown to potentiate and stabilize the biocidal activity of acid glutaraldehyde. Sporicidal activity of the formulation was maintained over a 12-month storage period and the effect of operating temperature and storage temperature on sporicidal activity was also examined. The formulation had excellent bactericidal and fungicidal activity. Glutaraldehyde concentration and polymerization in the stored formulation is discussed in terms of the state of the molecule.

INTRODUCTION

The wide antimicrobial spectrum of glutaraldehyde (1,5-pentanedial) has been described in a number of papers as reviewed by Russell and Hopwood (1976) and 2% (w/v) alkaline glutaraldehyde (CIDEX) has proved to be an effective sporicide (Pepper and Lieberman, 1962). Borick et al., (1964), however, demonstrated an alkaline solution to be inherently unstable and recommended that such solutions should be discarded after 2 weeks. In contrast, acid glutaraldehyde is stable but is a poor sporicide. Attempts have been made to overcome these difficulties by storing glutaraldehyde under acid conditions and then, just prior to use, activating the solution to alkaline pH with 0.3% (w/v) sodium bicarbonate.

The search for a biocidal synergistic additive which would stabilize glutaraldehyde while also increasing its antimicrobial activity has produced several formulations. Stonehill (1966) suggested the use of cationic surfactants in the pH range 5–8. The benefit of non-ionic surfactant addition has also been demonstrated (Sidwell et al., 1970; Boucher, 1971; Wilkoff et al., 1971).

Glutaraldehyde achieves its bactericidal effect via combination of a partial sealing of the outer membrane of the cell wall and inactivation of cell wall-associated or periplasmic-located enzymes (Gorman and Scott, 1977a). Study of the action of the alkalinating agent, sodium bicarbonate, has shown that it has a role other than solely changing the pH and state of the glutaraldehyde molecule. The sodium ions cause disruption of the loosely bound outer layer and outer membrane of the cell wall enabling increased uptake and penetration of the disinfectant to its optimum site of action (Gorman and Scott, 1977b). Further investigations showed that Na^+ in NaCl could replace $NaHCO_3$, thus retaining the stability of the acid glutaraldehyde solution but with somewhat reduced biocidal activity. The activity was enhanced by substituting divalent cations, in particular Mg^{2+}, for the monovalent Na^+ (Gorman and Scott, 1979).

It therefore appeared feasible that a further potentiation or synergistic effect might occur on combining divalent inorganic cations with certain types of surfactant and adding this combination to acid glutaraldehyde solution.

MATERIALS AND METHODS

Organisms and growth conditions

The non-sporing bacteria: *Escherichia coli* 15 TAU, NCIB 10430, *Pseudomonas aeruginosa* NCTC 10332 and *Staphylococcus aureus* NCTC 8532, were grown to exponential phase in nutrient broth (Oxoid). The sporing organism: *Bacillus subtilis* (NCTC 8236 and NCTC 10073) was grown on Sabouraud Dextrose agar (SDA) in Roux bottles at 32°C until total sporulation had occurred, as determined microscopically. Both vegetative cell and spore suspensions were washed twice with and suspended in sterile Ringer's solution. Spores were not further treated. To ensure measurement of sporicidal activity only, in studies with glutaraldehyde, zero time was taken after 5 min cell contact with disinfectant since 2% glutaraldehyde killed all vegetative cells in this time, as previously shown by Stonehill et al. (1963).

The fungi: *Aspergillus niger* IMI 25325 and *Trichophyton mentogrophytes var. interdigitale* (clinical isolate), were maintained and harvested for spores as described by Gorman and Scott (1977c).

Glutaraldehyde solutions

Aqueous solutions of glutaraldehyde were prepared from a 50% (w/v) solution (Kodak Ltd, Kirkby, Liverpool). The solution was made alkaline when required by the addition of 0.3% (w/v) sodium bicarbonate.

Chemicals

Dodecyl sodium sulphate and dodecyl benzene sodium sulphonate were obtained from Koch-Light Labs, Colnbrook, England. Empilan KA 880, an 80% solution of ethoxylated fatty alcohols in water, and Empicol ML 26A, a 26.5% (w/v) solution of magnesium dodecyl sulphate in water which also contains 1.5% magnesium sulphate and 0.2% magnesium chloride, were both obtained from the Marchon division of Albright and Wilson Ltd. Solan E is a solid, water soluble ethoxylated lanolin marketed by Croda Chemicals Ltd.

Antibacterial activity of the surfactants

A 5 ml volume of *E. coli* suspension (2×10^8 viable cells/ml) was added to 5 ml of each concentration of surfactant. At suitable intervals a 1 ml sample was withdrawn and serially diluted in sterile water before plating by the method of Miles and Misra (1938) onto nutrient agar. Incubation followed at 37°C for 48–72 h.

Sporicidal activity of glutaraldehyde combined with surfactants

A 5 ml volume of spore suspension (2×10^8 spores/ml) was added to 5 ml of glutaraldehyde–surfactant mixture giving a final glutaraldehyde concentration of 2.0% (w/v). Various surfactant concentrations were examined and time survival measurements made over a 2 h period as above. Serial dilutions were made in sterile water in which glycine (1% w/v) was incorporated as inactivator (Gorman and Scott, 1976). Viable counts were taken after incubation at 32°C for 48–72 h on nutrient agar.

The effect of addition of a divalent inorganic cation on the sporicidal activity of glutaraldehyde–surfactant mixtures was subsequently measured. $MgCl_2$ was added to give a final Mg^{2+} concentration of 0.2 M. The pH and physical appearance of the formulations were noted.

Potentiation by use of the divalent ionic salt of an anionic surfactant – Magnesium Dodecyl Sulphate (Empicol)

(1) Effect of surfactant on pH of glutaraldehyde solution. Equal volumes of the surfactant concentration under examination and acid glutaraldehyde were mixed and the pH measured on a Corning-EEL digital pH meter. Glutaraldehyde concentrations of 0.01% and 2.0% (w/v) were examined.

(2) Bactericidal activity. Empicol was examined for inherent bactericidal activity as described above. The same procedure was followed when examining solutions of Empicol–glutaraldehyde (0.01% w/v) for bactericidal activity. Glycine (1% w/v) was employed in the serial dilutions as inactivator.

(3) Fungicidal activity. The formulation was examined for stability in deactivating the spores of *A. niger* and *T. mentagrophytes* as described by Gorman and Scott (1977c). Initial spore count was 10^6/ml.

(4) Sporicidal activity. A 5 ml volume of *B. subtilis* spore suspension (2×10^8 spores/ml) was mixed with 5 ml of the formulation and time-survival measurements made over a 3 h period at room temperature as described above.

The procedure was repeated at temperatures of 37°C and 55°C, samples being removed at intervals for estimation of survivors.

Stability of solutions

(1) Biocidal activity. Glutaraldehyde solutions were formulated as indicated in Table 6, and examined for sporicidal activity as previously described. The solutions were subsequently stored at 18°C and 4°C and examined at suitable intervals for retention of activity.

(2) Chemical stability. The stored solutions were examined at suitable intervals for glutaraldehyde concentration and solution pH as described by Gorman and Scott (1977a). Glutaraldehyde polymerization rate was also determined in each of the solutions (Gorman and Scott, 1977b).

RESULTS

Examination of surfactant antimicrobial activity showed that Tego 103G (1% w/v) produced a drop in viable count of one log cycle over 30 min contact with *E. coli*. Other concentrations had little effect. All other surfactants had negligible antimicrobial activity at all concentrations investigated.

Potentiation of sporicidal activity was obtained with all the glutaraldehyde–surfactant formulations examined, with and without Mg^{2+} (Table 1). Considerable variation in the degree of potentiation was observed and the final pH of the formulation is of importance in this respect. Formulations in the neutral to alkaline range should essentially be avoided due to glutaraldehyde stability problems. The effect of surfactant and glutaraldehyde concentration on final pH is shown in Table 2. Considering this variation in pH, the data obtained from the bactericidal, fungicidal and sporicidal determinations on the glutaraldehyde–Empicol formulation may be evaluated comparatively with the acid and alkaline glutaraldehyde solutions (Tables 3–5). In each case, the glutaraldehyde–Empicol formulation is shown to have considerable activity which increases substantially at higher temperatures (Table 5).

TABLE 1

SPORICIDAL ACTIVITY OF GLUTARALDEHYDE (2% W/V) IN THE PRESENCE OF VARIOUS ADDITIVES (*B. subtilis* initial spore count : 1×10^8/ml)

Additive	(%) Concentration	Formulation pH	% viable spores after 2 h contact
Dodecyl benzene sodium sulphonate (DBSS)	0.25	4.18	1.0
DBSS	1.0	7.04	0.01
DBSS + Mg^{2+} [a]	0.25	3.82	0.08
DBSS + Mg^{2+}	1.0	6.15	0.001
Dodecyl sodium sulphate (DSS)	2.5	3.56	1.0
DSS	10.0	3.57	6.0
DSS + Mg^{2+}	2.5	2.76	0.1
DSS + Mg^{2+}	10.0	2.78	0.2
Cetrimide	0.1	6.5	0.0004
Cetrimide + Mg^{2+}	0.1	5.5	0.0001
Empilan KA880	1.0	5.4	0.5
Empilan + Mg^{2+}	1.0	5.2	0.1
Polysorbate 80	0.25	3.26	1.0
Polysorbate 80	1.0	3.38	1.0
Polysorbate 80 + Mg^{2+}	0.25	2.78	0.2
Polysorbate 80 + Mg^{2+}	1.0	2.92	0.2
Solan E	1.0	5.4	0.1
Solan E + Mg^{2+}	1.0	5.2	0.05
None	–	3.6	30.0
$NaHCO_3$	0.3	7.9	0.0005

[a] Mg^{2+}: $MgCl_2 \cdot 6H_2O$ (0.2 M).

TABLE 2

EFFECT OF ADDITION OF EMPICOL ML26A ON THE pH OF GLUTARALDEHYDE SOLUTIONS

Concentration (%) of Empicol	pH of surfactant + glutaraldehyde (0.01%)	pH of surfactant + glutaraldehyde (2.0%)
10.0	7.60	4.52
5.0	7.31	4.12
2.5	7.08	3.89
1.0	6.62	3.76
0.1	5.94	3.68

TABLE 3

BACTERICIDAL ACTIVITY OF GLUTARALDEHYDE FORMULATIONS (0.01% W/V) AT 18°C (initial viable count: 1×10^8/ml)

Additive	(%) Concentration	Formulation pH	Time (min) for 99.9% kill of:		
			E. coli	*Staph. aureus*	*Ps. aeruginosa*
Empicol	2.5	7.08	22	9	25
Empicol	10.0	7.60	15	7	20
None	–	4.6	120	100	65
$NaHCO_3$	0.3	7.9	20	12	35

TABLE 4

FUNGICIDAL ACTIVITY OF GLUTARALDEHYDE FORMULATIONS (0.5% W/V) AT 18°C (initial spore count: 1×10^6/ml)

Additive	(%) Concentration	Formulation pH	Time (min) for 99.9% kill of:	
			A. niger	*T. mentagrophytes*
Empicol	2.5	4.8	110	75
Empicol	10.0	4.9	105	65
None	–	4.3	>180	>180
$NaHCO_3$	0.3	7.9	80	45

TABLE 5

SPORICIDAL ACTIVITY OF GLUTARALDEHYDE FORMULATIONS (0.2% W/V) AT VARIOUS TEMPERATURES (initial *B. subtilis* spore count: 1×10^8/ml)

Additive	(%) Concentration	Formulation pH	Time (min) for 99.9% kill at °C		
			18°C	37°C	55°C
Empicol	2.5	4.8	95	15	4
Empicol	10.0	4.5	55	7	2
None	–	3.6	>300	25	19
$NaHCO_3$	0.3	7.9	40	17	13

TABLE 6

STABILITY OF GLUTARALDEHYDE FORMULATIONS (2% W/V) WITH REGARD TO SPORICIDAL ACTIVITY AT 18°C (*B. subtilis* spore count: 10^8/ml)

Additive	Concentration	Formulation pH	Time (min) for 99.9% kill with formulation stored for weeks:				
			0	2	4	24	52
Empicol	10.0%	4.5	55	55	55	55	60
$MgCl_2$	0.2 M	3.5	120	120	120	120	125
None	–	3.6	>300	>300	>300	>300	>300
$NaHCO_3$	0.3%	7.9	40	120–180	>300	>300	>300

TABLE 7

STABILITY OF GLUTARALDEHYDE FORMULATIONS (2% W/V) WITH REGARD TO GLUTARALDEHYDE CONCENTRATION

Formulation	Storage temperature	(%) concentration of glutaraldehyde on storage for weeks:				
		0	2	6	12	24
Acid glutaraldehyde	4	2.0	1.9	1.6	1.6	1.6
	18	2.0	1.9	1.5	1.5	1.5
Alkaline glutaraldehyde	4	2.0	1.7	1.3	1.1	0.6
	18	2.0	1.6	0.7	0.6	0.3
Acid glutaraldehyde–Empicol	4	2.0	2.0	1.8	1.8	1.8
	18	2.0	2.0	1.7	1.7	1.6

Investigations into the sporicidal activity of the glutaraldehyde–Empicol formulation on storage indicated that activity of this solution was retained for several months in contrast to the alkaline solution which lost its activity within two weeks of activation (Table 6). This retention and loss of activity in the two solutions correlates with data obtained from studies on glutaraldehyde concentration and polymerization in the solutions over the same period (Table 7, Fig. 1).

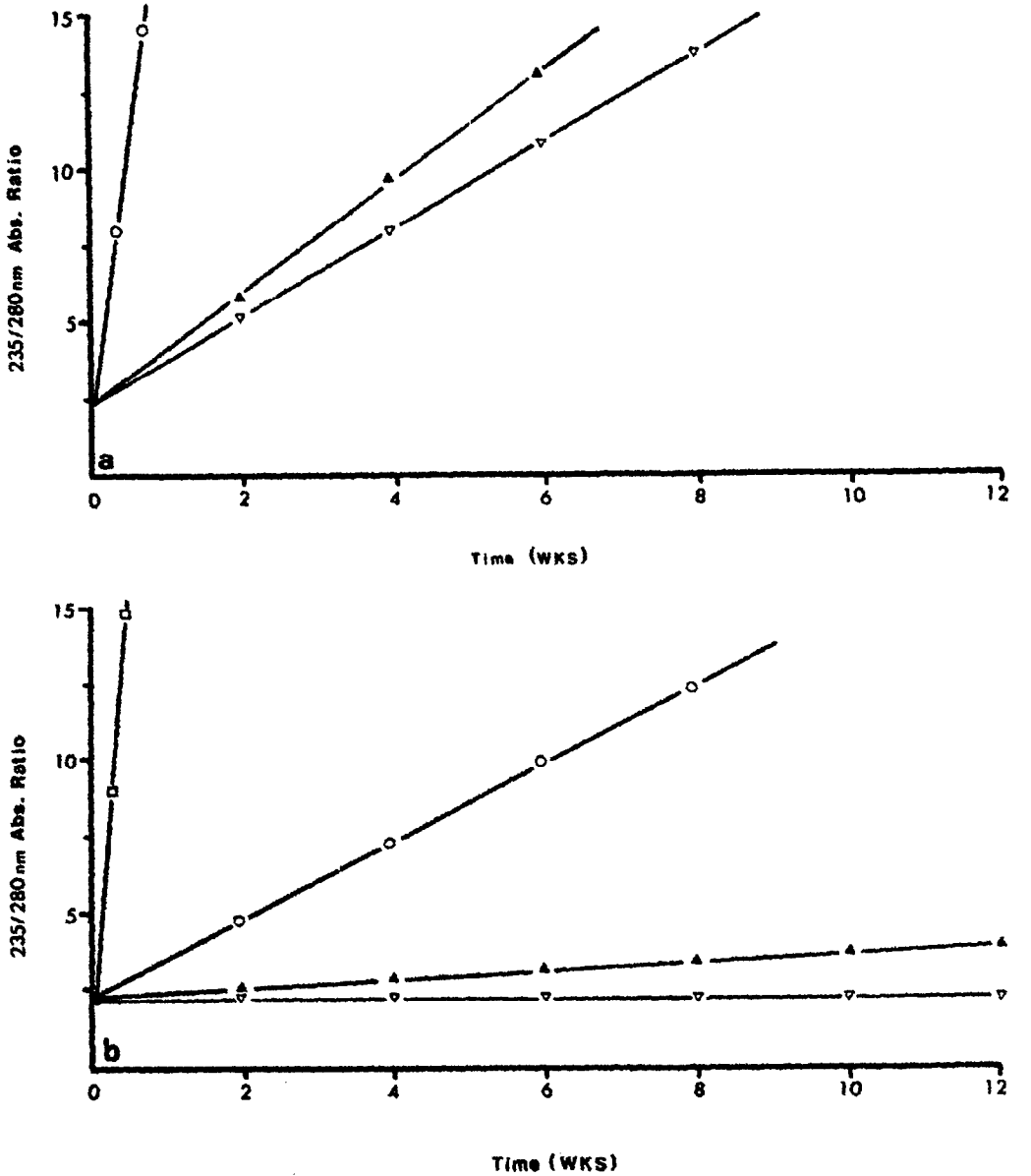

Fig. 1. Polymerization of glutaraldehyde in (a) alkaline (b) acid and acid–Empicol solution on storage at temperatures: ▽, 4°C; ▲, 18°C; ○, 37°C; □, 55°C.

DISCUSSION

Dodecyl benzene sodium sulphonate, suggested by Boucher (1971) as giving increased stability and activity to acid glutaraldehyde, was examined in the present study, together with another C_{12} anionic surfactant, sodium dodecyl sulphate. Both these compounds exhibited considerable activity in potentiating acid glutaraldehyde, though in the case of the former compound some of the increased activity may be attributed to an induced change in pH from acid to alkaline at higher concentrations of surfactant.

Voss (1963) in a study on the effect of inorganic cations on the bactericidal activity of anionic surfactants found that the effectiveness of two alkyl aryl sulphonates and alkyl sulphate against *Staph. aureus* was increased in the presence of low concentrations of divalent cations. Gorman and Scott (1979) showed that Mg^{2+} potentiated glutaraldehyde activity, so it became apparent that an acid glutaraldehyde solution could be potentiated by addition of both Mg^{2+} and an anionic surfactant, preferably an alkyl sulphate, in order to ensure retention of acid pH and thus stability in the formulation. To facilitate the preparation of such a formulation, the magnesium salt of sulphated lauryl alcohol (Empicol) was used. Empicol was shown to have no inherent antimicrobial activity and excellent potentiation was observed. The acid pH of the sporicidal glutaraldehyde (2% w/v)–Empicol formulation explains the slight decrease in comparative activity with the alkaline bactericidal glutaraldehyde (0.01% w/v)–Empicol formulation. This difference in activity was reduced as the operating temperature of the disinfectant was increased until at 55°C the glutaraldehyde (2%)–Empicol (2.5%) formulation surpassed alkaline glutaraldehyde (2%) in sporicidal efficiency.

The sporicidal activity of the potentiated formulation is shown to be stable on prolonged storage in contrast to the rapid fall in activity over 2 weeks of the alkaline solution. The glutaraldehyde concentration and the pH of the former formulation is also maintained over a long period.

The same pattern of polymerization was observed for acid glutaraldehyde solution and the glutaraldehyde–Empicol formulation showed little increase in the rate over a 3-month period at room temperature. In contrast, the rate, even at 4°C, was rapid in the alkaline glutaraldehyde solution. Cross-linking reactions in alkaline solution cause rapid polymerization to give the aldol-type polymer (Hardy et al., 1969; Monsan et al., 1975). Loss of free aldehyde groups occurs so that with time and temperature the degree of polymerization is increased with a corresponding loss of biocidal activity. This is apparent in the decrease in sporicidal activity of an alkaline glutaraldehyde solution after 2 weeks storage at room temperature. The potentiated formulation retains its excellent sporicidal activity due to the presence of discrete monomers and acetal-like polymers. Heating acid formulations raises the molecular energy level and also produces more active monomers from the acetal-like polymers. This explains the significant increase in sporicidal activity observed on heating the acid glutaraldehyde–Empicol formulation in contrast to that observed on heating the alkaline solution.

The presence of surfactant could conceivably potentiate the effect of the Mg^{2+} which was shown to produce optimum potentiation due to a combination of factors which include disruption of the loose layer and outer membrane of the cell wall thus increasing the surface area for uptake and entry of glutaraldehyde to its site of action (Gorman and Scott, 1977a).

Three basic requirements are therefore indicated for optimum biocidal activity of glutaraldehyde: (1) alkaline pH, (2) divalent cations and (3) surfactants. The shelf-life of solutions formulated at alkaline pH is extremely short, as shown in this study, and are consequently unsafe to use after several days storage. In contrast, solutions formulated on the basis of requirements (2) and (3) possess a shelf-life of months as acid pH is maintained. Gorman and Scott (1977d) have also shown that acid glutaraldehyde solutions have negligible reaction with organic matter in comparison to the marked interaction with alkaline solutions. Further potentiation of acid glutaraldehyde solutions may be attained by use of higher temperatures.

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