

COMMUNICATIONS

The state of the glutaraldehyde molecule in relation to its biocidal activity

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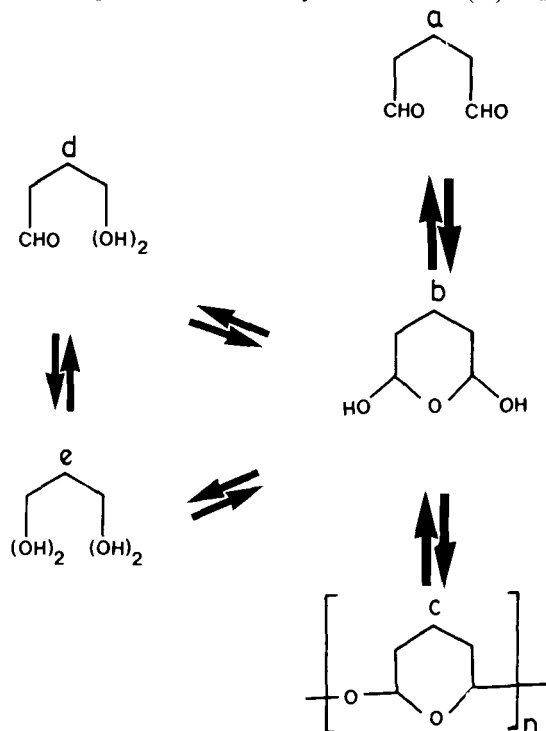
Glutaraldehyde (1, 5-pentanedial) is a compound having excellent chemosterilizing properties at alkaline pH but poor activity at acid pH in which form it is commercially available. Biocidal activity of the molecule at acid pH is however relatively constant in contrast to the gradual decrease in activity observed in alkaline solutions of glutaraldehyde (Russell & Hopwood 1976). The immediate effect of alkalization on bactericidal activity has been shown to be on the bacterial cell rather than on the glutaraldehyde molecule (Gorman & Scott 1977). Retention and degree of biocidal activity, however, appear to depend on the state of the molecule which varies with pH, time and temperature. The effect of increasing temperature on the acid solution is to increase biocidal activity and on the alkaline solution to decrease activity (Sierra & Boucher 1971). Aldehyde concentration in freshly prepared alkaline solutions has been shown by several authors to fall by approximately 45% in 14 days (Borick 1968; Miner et al 1977; Gorman & Scott 1979).

The state of the glutaraldehyde molecule at different pH values, i.e. monomer to polymer ratio and type of polymer present, has been the subject of numerous publications in recent years. Despite some controversy a consensus can be identified. Rubbo et al (1967) suggested that the aldehyde existed as a monomer (Ia, 25%) in equilibrium with cyclic hemiacetal (Ib). They thought that the presence of free aldehyde groups was necessary for biocidal activity. Richards & Knowles (1968) indicated that protein cross-linking reactions in commercial acid glutaraldehyde were possible because of the presence of α , β -unsaturated aldehydes (II a, b). Subsequently, Hardy et al (1969) in a p.m.r. study on commercial acid glutaraldehyde showed the α , β -unsaturated material to be a minor component essentially an impurity. Hardy et al found that pure, acid glutaraldehyde on dissolution in water undergoes rapid hydration to give three hydrates in equilibrium (Ib,d,e). An acetal-like polymer (Ic) was also shown to exist in acid solution and this is similar to that suggested by Aso & Aito (1962). Hardy et al (1969) found polymerization to be slow in acid solution and they concluded that cross-linking using such solutions must

involve the monomer or one of its hydrates and not an unsaturated polymer. They did find polymerization occurred in alkaline solution to give unsaturated polymers (aldol-type), such as II (a, b), or intermediates, and they suggested that these could be responsible for cross-linking reactions in alkaline solution.

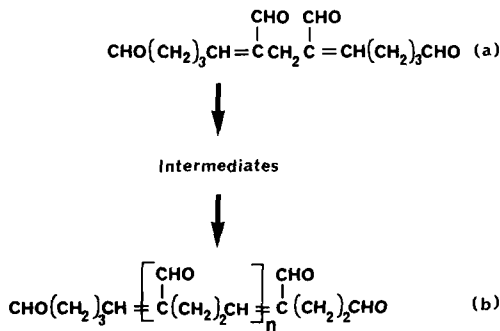
Progression to the higher polymeric form (IIb) could be possible with time and rise in pH since it has been shown that there is an extensive loss of aldehyde groups from polymerization in alkaline solution (Fein et al 1959).

Korn et al (1972), also using p.m.r. on a commercial acid solution, stated that monomeric glutaraldehyde (Ia) was in equilibrium with the cyclic hemiacetal (Ib) and



SCHEME 1. Scheme for glutaraldehyde polymerization in aqueous acid media. monomer (Ia), cyclic hemiacetal (Ib), acetal-like polymer (Ic), monohydrate (Id), dihydrate (Ie).

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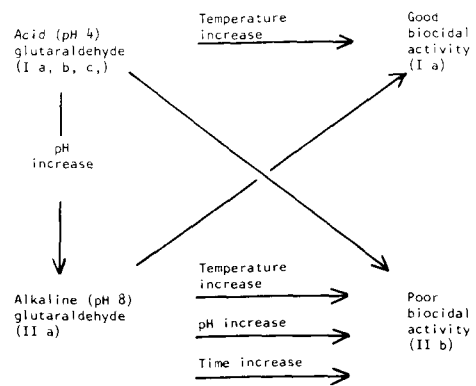


SCHEME 2. Scheme for glutaraldehyde polymerization in aqueous alkaline media showing progression of the aldol-type polymer (IIa) to the higher polymeric form (IIb).

the acetal-like polymer (Ic) but that a small quantity of Id and Ie possibly also existed, though only at 25°C. At temperatures of 50°C and higher the equilibrium tended to proceed from Ic → Ib → Ia giving 15% monomer at 25°C and 60% at 75°C. Two further studies on commercial acid glutaraldehyde using [¹³C] NMR produced results similar to those from the previous studies but differing slightly in the ratio of the various forms (Whipple & Ruta 1974; Holloway & Dean 1975). These studies showed 4% monomer (Ia) present at 23°C increasing with temperature by reversion of Ib (71% at 23°C). The low percentage of Id and Ie remained constant even with increasing temperature. Monsan et al (1975) confirmed these results for acid solutions and also showed aldol-like polymers (IIa, b) formed at alkaline pH. These authors proposed it is as an aldol-type polymer that glutaraldehyde reacts readily with protein, not in its free form, reaction in alkaline solution with amino groups giving an imino bond stabilized by resonance with the ethylenic double bond.

Reviews by Boucher et al (1973) and Boucher (1975) are misleading in so much as it was stated that at alkaline pH the hydrate equilibrium (Ia, b, d, e) operates and is irreversible on heating whilst at acid pH it was maintained that the acetal-like polymer (Ic) was of prime importance. The significance of the aldol-type polymer (II a, b) at alkaline pH was disregarded.

Our interpretation on consideration of the information about the polymerization shown by glutaraldehyde at acid and alkaline pH, and the relationship with biocidal activity is as outlined in the schemes. The loss of the excellent biocidal activity in alkaline solution is apparent in the decrease in free aldehyde groups necessary for optimum reaction with amino groups. It would appear from the preceding information that the degree of polymerization in alkaline solution could dictate the extent of biocidal activity, optimum activity and preferential protein interaction occurring when acid solutions are immediately made alkaline.



SCHEME 3. Influence of time, temperature and pH on the biocidal activity of acid and alkaline glutaraldehyde solutions. Iabc and IIab relate to state of the molecule as described.

It has previously been stated that biocidal activity in acid solution is dependent upon the presence of free aldehyde groups. Thus the percentage of dialdehyde monomer in acid solution is increased by increasing temperature resulting in enhanced biocidal activity.

October 3, 1979

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