

# Influence of delmopinol on bacterial $\zeta$ -potentials and on the colloidal stability of bacterial suspensions

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Delmopinol is a low molecular weight surface-active compound that has been shown to be effective against dental plaque both *in vitro* and *in vivo* and against gingivitis *in vivo*. To study the mode of action of delmopinol, the influence of the compound on the stability of bacterial suspensions, both with and without saliva, and on the  $\zeta$ -potentials of oral streptococci was studied. The results showed that delmopinol reduced the magnitude of the  $\zeta$ -potentials, but, in contrast, the colloidal stability of the bacterial suspensions without saliva was increased. The explanation of these observations could be that non-DLVO interaction components, such as repulsive hydration/steric forces, have come into effect at very close distances between two approaching bacterial cells. To judge from the present results, it is possible that delmopinol forms films on bacterial cells in a plaque, thereby facilitating mechanical removal. □ *Bacteria, oral; dental plaque; electrophoretic mobility; flocculation; gingivitis; streptococci*

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Delmopinol is a low molecular weight surface-active compound that has been shown to be effective against dental plaque both *in vitro* and *in vivo* and against gingivitis *in vivo* (1, 2). It is predominantly cationic under experimental conditions. The antimicrobial effect, measured as minimum inhibitory concentrations in a tube-diluting system, is comparatively low as compared with chlorhexidine (2), but the mode of action of the compound is not yet known in detail.

It has previously been shown that oral bacteria might be regarded as being components of a colloidal biologic system and that plaque formation, at least partly, may be governed by general, physicochemical laws (3, 4). The same series of studies also showed that the properties of saliva as a colloidal suspension medium for oral bacteria differed among individuals in such a manner that saliva from 'light' plaque formers had a greater capacity to flocculate suspensions of a plaque-forming bacterium than saliva from 'heavy' plaque formers (5). Those results are in line with the finding of Magnusson et al. (6) that saliva-induced bulk aggregation of oral bacteria could be a protective mechanism against plaque formation.

The tendency of particles to aggregate is a most important physical property of a colloidal system. Aggregation is caused by attractive van der Waals forces, whereas stability against aggregation is caused by repellent electrostatic forces due to the net surface charge, the  $\zeta$ -potential, of the particles (7, 8). One possible mechanism of action of delmopinol could be that it has an influence on the physicochemical phenomena that govern plaque formation, and it was therefore thought worthwhile to investigate the influence of the compound on the colloidal stability of bacterial suspensions and on the bacterial  $\zeta$ -potentials, both in the absence and in the presence of saliva.

## Materials and methods

### *Test substance*

The substance used was delmopinol hydrochloride (batch 002; chemical purity, 99.7%; pKa, 7.1). The compound was originally synthesized at the Chemistry Department of Biosurface AB, Malmö, Sweden. The structural formula is given in Fig. 1.

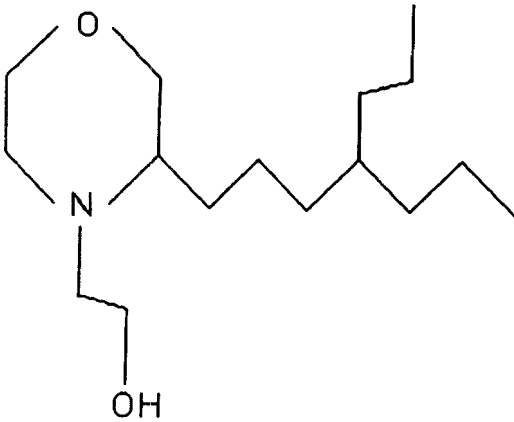


Fig. 1. The structural formula of delmopinol.

### Bacteria

The bacterial strains used were *Streptococcus mutans* I-B (9) and *Strep. sanguis* KRF3 (3). The strains were stored at  $-80^{\circ}\text{C}$  and subcultured at most five times before each experiment. Subculturing was done on blood agar (Oxoid Blood Agar Base 2) containing 6% citrate-treated horse blood (SVA, Håttunaholm, Sweden). The blood agar plates were incubated in 95%  $\text{H}_2$  and 5%  $\text{CO}_2$  (GasPac<sup>®</sup>, BBL Microbiology Systems) for 18 h at  $37^{\circ}\text{C}$ . The cells were washed twice in their respective test media and thereafter suspended to a density of  $10^8$ – $10^9$  cells/ml.

### Test solutions

The test solutions used were 0.01 mol/l sodium acetate buffer at pH 4, 5, and 6,

containing 0, 0.01, 0.02, 0.05, and 0.1% (w/v) delmopinol hydrochloride (0, 0.32, 0.64, 1.60, and 3.25 mmol/l, respectively). pH was measured with a PHM 83 pH-meter (Radiometer, Copenhagen, Denmark), and conductivity with a conductometer type 644 (Metrohm, Herisau, Switzerland).

### Saliva

The saliva used in the experiments consisted of parotid saliva, which was collected from one person on one occasion by the aid of Lashley cups (10). Measurements of colloidal stability in the presence of saliva were performed at pH 5 and 6 at 0%, 0.01%, and 0.1% delmopinol, and  $\zeta$ -potentials in the presence of 10% saliva in buffer were calculated at pH 6 and 0%, 0.01%, and 0.1% delmopinol.

### Measurements of colloidal stability

The colloidal stability was measured turbidimetrically according to Simonsson et al. (3) in a spectrophotometer (Hitachi, type 100-40) at 700 nm, 1-cm path length. The temperature was kept at  $25 \pm 1^{\circ}\text{C}$ . Curves of absorbance versus time were recorded for 1 h on a recorder type SE 120 (Goerz, Metrawatt, Vienna, Austria). Each experiment was repeated three times for all test suspensions. The colloidal stability was calculated planimetrically by the area fraction program of an AMS Image Analyser (Shirehill Industrial Estate, Shirehill, U.K.) from the curves of absorbance versus time. The measured area reached from an optical den-

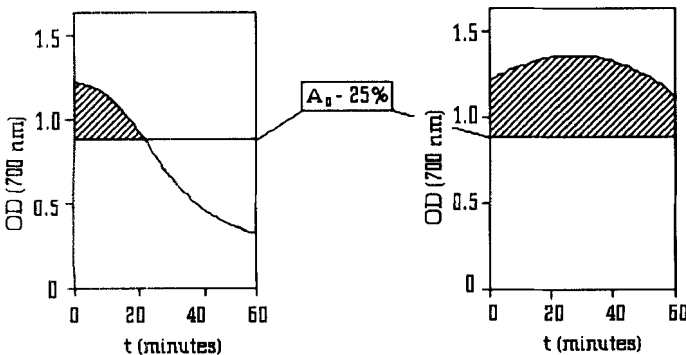


Fig. 2. Planimetric measurements of colloidal stability (shaded areas).

sity (OD) of 1.300 at time 0 until the absorbance had decreased by 25% (OD 0.975) at time  $t$  (Fig. 2, left), and if  $t > 60$  min, the area between line OD = 0.975 and the curve (Fig. 2, right).

*Calculations of  $\zeta$ -potentials*

The bacterial  $\zeta$ -potentials were calculated from particle-microelectrophoretic measurements, as previously described elsewhere (3). The apparatus used was a particle-microelectrophoresis apparatus type Rank Mark II (Rank Brothers, Cambridge, U.K.). Platinum electrodes and the flat type of glass cell were used. Migration of the bacteria was observed in the stationary levels of the cell, and adequate current was applied for 5–35 sec. The electrophoretic mean velocity was estimated by timing 10 individual bacterial cells over a distance of 62  $\mu$ m. To minimize a possible influence of the shape of the microbial cells, measurements were made only for cells found not to rotate or flip over at the change of electrode polarity. All measurements were made by the same person.

Under the given experimental conditions, the  $\zeta$ -potentials were calculated in accordance with Olsson & Glantz (11):

$$\zeta = 12.9u,$$

where  $u$  is the electrophoretic mobility ( $\mu$ m/V/cm) (V/cm).

**Results**

The present study showed that increasing concentrations of delmopinol in bacterial suspensions without saliva gave a successive increase of the  $\zeta$ -potentials at all the studied pH levels, as shown in Figs. 3 and 4. At certain pH levels the colloidal stability was also increased with increasing concentrations of delmopinol in the suspensions (Figs. 3 and 4).

The influence of delmopinol, both on the  $\zeta$ -potentials and on the colloidal stability, was much less pronounced in the presence of saliva (Figs. 5 and 6). It was, however, observed that the  $\zeta$ -potentials decreased with increasing concentrations of delmopinol, whereas the colloidal stability was increased at both the investigated pH levels.

The values of the colloidal stability in Figs. 3–6 are means and standard deviations from three tests for each suspension, and the corresponding values for the  $\zeta$ -potentials are means and standard deviations of 10 different bacterial cells run in each suspension.

**Discussion**

Flocculation of particles is an important physical property of a colloidal system, and it has been suggested that flocculation of bacteria in saliva in vivo is a protective mechanism against plaque formation, since fewer

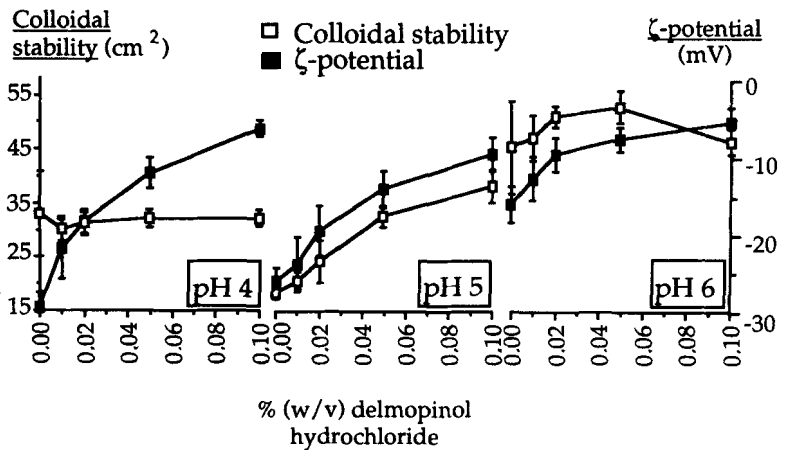


Fig. 3. Colloidal stability and  $\zeta$ -potential of *Streptococcus mutans* I-B at pH 4, 5, and 6 and at various concentrations of delmopinol in pure buffer solutions.

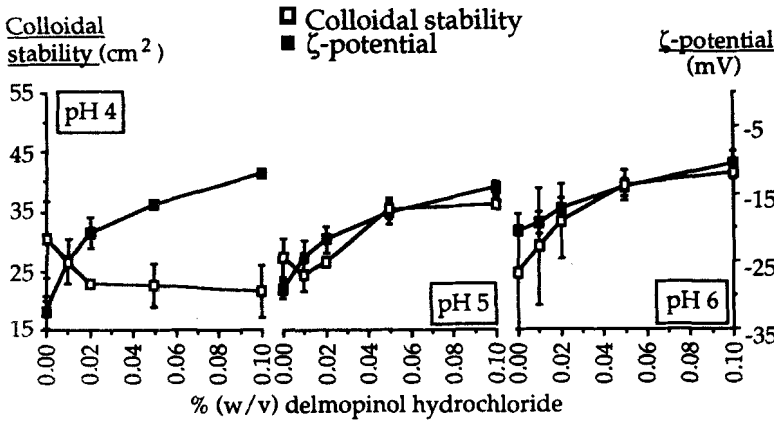


Fig. 4. Colloidal stability and  $\zeta$ -potential of *Streptococcus sanguis* KRF3 at pH 4, 5, and 6 and at various concentrations of delmopinol in pure buffer solutions.

single bacterial cells will be available for attachment to tooth surfaces (5, 6).

When two particles, in this case bacteria, in a liquid approach each other, they will experience a long-range attraction, which is due to van der Waals forces. This attraction is initially weak but increases strongly with distance between the particles. At close distances an electrostatic repulsive force will come into effect due to interactions between the electric double layers around each particle. In the case of bacteria the electrostatic repulsive force arises because almost all biologic surfaces and cell surfaces are negatively charged in aqueous media around physiologic pH. Provided that the

magnitude of the electrostatic repulsion is sufficiently high, an energy barrier preventing aggregation will be present. The size of this barrier depends on, among other things, the surface potentials of the particles. Interactions between particles that can be described by the sum of the energy due to the electric double layer overlap and van der Waals energy are usually referred to as DLVO interactions. In practical terms this means that addition of counterions to a suspension of particles may cause aggregation due to a reduction of the net surface charge of the particles by incorporation of the added ions into the electric double layer. Flocculation will occur when the range of the

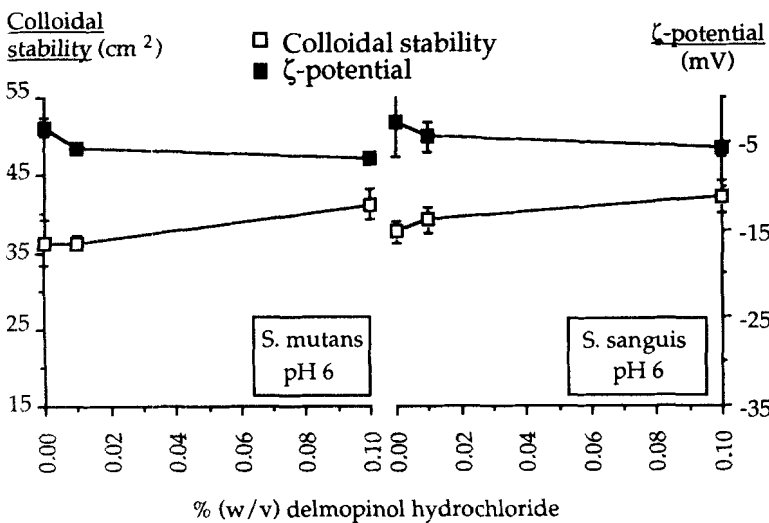
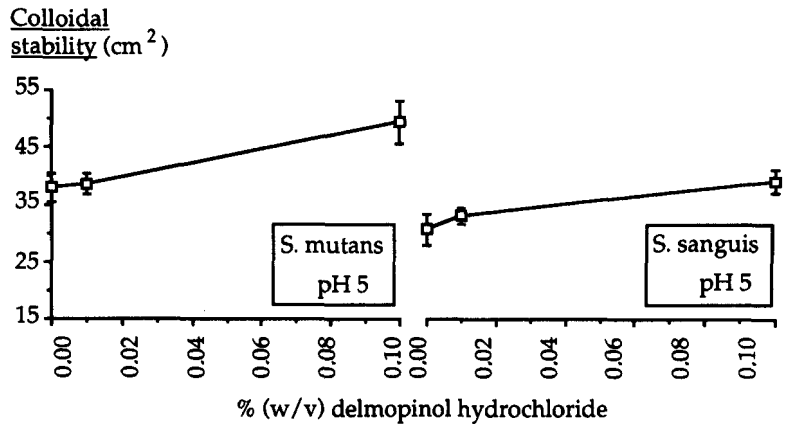


Fig. 5. Colloidal stability and  $\zeta$ -potential of *Streptococcus mutans* I-B and *Streptococcus sanguis* KRF3 and pH 6 and at various concentrations of delmopinol in buffer solutions containing 10% saliva.

Fig. 6. Colloidal stability of *Streptococcus mutans* I-B and *Streptococcus sanguis* KRF3 at pH 5 and at various concentrations of delmopinol in buffer solutions containing 10% saliva.



double-layer interaction is reduced sufficiently to permit particles to overcome the energy barrier and van der Waals attractive forces to predominate again at short distances (for reviews, see Refs. 7, 8, 12–14).

The present study has shown that delmopinol, which is a cationic surfactant, has an influence on bacterial  $\zeta$ -potentials as well as on the colloidal stability of bacterial suspensions. It was found that the magnitude of the  $\zeta$ -potentials decreased with increasing concentrations of delmopinol in the suspension media, which can be explained by adsorption of delmopinol molecules to the negatively charged bacterial cell surfaces. This means that the electrostatic repulsion barrier between two bacteria is reduced, and a flocculation would be expected to be facilitated. In contrast, it was observed that the colloidal stability of the bacterial suspensions without saliva increased with increasing concentrations of delmopinol.

The explanation of an increased colloidal stability of the bacterial suspensions in the presence of delmopinol in spite of a reduced magnitude of the  $\zeta$ -potentials could be that non-DLVO components, such as short-range repulsive hydration forces, have come into effect at very close distances between two approaching bacterial cells. Another explanation could be a steric hindrance of flocculation due to a changed surface structure of the cells after the delmopinol adsorption.

In the presence of saliva the effect of delmopinol was less pronounced. The difference in behavior might depend on the responsibility of saliva for the colloid-chemical behavior of the system. The possibility cannot, however, be ruled out that presence of saliva could have caused some gassing at the electrodes during the electrophoretic measurements. Gas development indicates that chemical processes take place around both electrodes, and thus the standardized experimental conditions are no longer relevant. The electrophoretic measurements in the presence of saliva could therefore to some extent be suspected of uncertainty. Even so, the tendency for the colloidal stability was that the suspensions became more and more stable with increasing concentrations of delmopinol.

It has previously been suggested that one clinical function of delmopinol is to penetrate into established plaque and to make it less cohesive, thereby making it more accessible for mechanical removal (2, 15). The findings of the present study point in the same direction. It is possible that delmopinol forms films after adsorption in vivo, and those films could be of clinical importance by facilitating mechanical removal of dental plaques, and it cannot be excluded that the possible presence of hydration/steric forces could contribute to this effect. Hydration/steric forces could also be of importance for prevention of bacterial cell adhesion to tooth surfaces.

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