

Adsorption of aminefluorides on human enamel

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ABSTRACT

Changes in surface characteristics of ground and polished human enamel after adsorption of two types of aminefluorides (AmF 297 and AmF 335) have been studied. After adsorption of aminefluorides from solutions with concentrations up to 10 mM for 2 min followed by rinsing of the surface with distilled water, contact angle measurements were carried out to yield surface free energies and ellipsometry was performed to yield the adsorbed layer thickness. In a separate experiment on powdered enamel, set up in an analogous way, zeta potential changes after adsorption of aminefluorides were determined in a 10 mM potassium phosphate buffer at pH 7.0. Surface free energies decreased from 88 erg·cm⁻² to 52 erg·cm⁻² and 35 erg·cm⁻² after adsorption of AmF 297 and AmF 335 respectively at $c = 1$ mM. Increasing the aminefluoride concentration in solution did not affect the values obtained. Zeta potentials, originally -36 mV, became positive after adsorption, while ellipsometry indicated the build-up of adsorbed layers with a thickness between 3 nm and 12 nm. All three types of experiments indicated that both AmF 297 and AmF 335 form an adsorbed monolayer on ground and polished enamel at a concentration of 1 mM. Negligible additional adsorption takes place at higher concentrations under the present experimental circumstances. *In vivo*, adsorbed aminefluoride layers will be rapidly covered by adsorbed protein layers, shielding both the adsorbed aminefluoride layer as well as its physicochemical characteristics. This effect has been studied *in vivo* by measuring surface free energy changes of ground and polished enamel, with AmF 297 and AmF 335 adsorbed at $c = 2.5$ mM as a function of the time, these samples were carried by test persons in partial dentures. On both types of AmF-coated enamel the surface free energies increased within 30 min to values approaching the one obtained previously for pellicle-coated ground and polished enamel (110 ± 9 erg·cm⁻²).

INTRODUCTION

Aminefluorides have been studied extensively with respect to their antiplaque adherent properties in clinical situations.

Since Balmelli et al. (1973) showed that applications of aminefluoride gave a reduction in the deposition of oral streptococci on rat molars, the addition of aminefluorides to toothpastes and mouthrinses has gained popularity (Mühlemann, 1983).

There are several possibilities to explain the working mechanism of aminefluorides as an antiplaque agent.

Most types of aminefluorides employed are considered to possess bacteriocidal properties and to reduce the growth of oral microorganisms (Gehring, 1983).

Application of aminefluorides to human enamel changes the physicochemical characteristics of the surface, thereby influencing the adhesion of microorganisms (Glantz, 1971). Using contact angle measurements, de Jong et al. (1984 b) showed that aminefluorides (Elmex Fluid; GABA, Basel, Switzerland) applied to ground and polished human enamel decreased the surface free energy from 85 to 56 erg cm⁻² ($1 \text{ erg}\cdot\text{cm}^{-2} = 1 \text{ mJ m}^{-2}$), which might explain their working mechanism on a thermo-

dynamic basis (Busscher et al., 1984), if the influence of electrical charge interactions can be neglected. Because the zeta potential of most oral microorganisms is negative (Olsson et al., 1976), while the zeta potential of enamel is also negative (Arends and Jongebloed, 1977; Arends, 1979), electrical charge interactions are expected to obstruct the adhesion of microorganisms (Rutter and Vincent, 1980; Reynolds and Wong, 1983). No information exists, however, on zeta potentials of enamel after aminefluoride adsorption.

In order to understand the working mechanism of aminefluorides as an antiplaque agent, based both upon bacteriocidal as well as on physicochemical effects, data on the accumulation of aminefluorides at the enamel surface are essential. In the present paper surface free energies, zeta potentials and adsorbed layer thicknesses of ground and polished human enamel after adsorption of two different types of aminefluorides are determined *in vitro*.

In order to study possible intermolecular interactions between the AmF molecules in the solutions, the surface tensions of the solutions were also measured.

In addition an *in vivo* experiment was carried out in which 10 test persons wore partial dentures containing blocks of ground and polished enamel after aminefluoride adsorption. Surface free energies were measured on these samples as a function of time to monitor the influence of protein adsorption.

MATERIALS AND METHODS

Enamel preparation

The outer 50–100 μm of the surface of freshly extracted human incisors was removed by grinding and polishing, thus removing not only the pellicle from the surface but also the outer mineral layer, which in general shows the greatest variation in chemical constituents.

Aminefluorides (GABA, Basel, Switzerland) AmF 297 and AmF 335 (*Fig. 1*) were dissolved in distilled water. After adsorption of aminefluorides on the enamel for 2 min at a volume to area ratio of 25 cm^3/cm^2 , the enamel surfaces were rinsed with distilled water and allowed to dry for several hours at room temperature in an enclosed chamber. Subsequently adsorbed layer

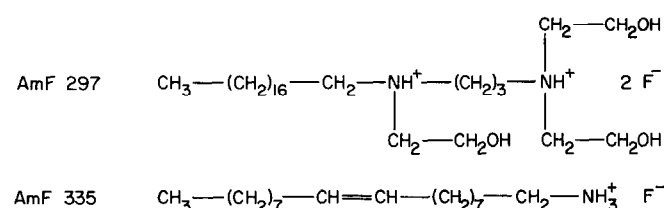


Fig. 1. Structural formulas of dihydroaminefluoride (AmF 297) and oleylaminefluoride (AmF 335).

thicknesses were determined by ellipsometry, and surface free energies were calculated from contact angle data.

In order to study the effect of protein adsorption on the surface free energy, samples of enamel (2 mm \times 2 mm) with AmF 297 and AmF 335 adsorbed from solutions with a concentration of 2.5 mM were put in partial dentures and carried by 10 test persons for 30 min, 1 day, and 6 days. During these experimental periods, the test persons were allowed to continue their habitual oral hygiene regimens, but were requested not to use any mouthrinses or to brush the inserts of enamel. After removal of the samples from the dentures, the surfaces were rinsed with distilled water to remove loosely bound material such as debris, and allowed to dry for 1 h in an enclosed chamber at 25°C before making contact angle measurements. The experiments were set up in such a way that each of the eight samples per test person were wetted only once.

Enamel particles with an average size diameter of 5 μm were obtained from human incisors. The particles were washed four times in distilled water and then suspended and gently agitated in the various solutions for 2 min. Finally, the particles were dissolved in a 10 mM potassium phosphate buffer at pH = 7.0 for zeta potential measurements.

Contact angle measurements and calculation of surface free energies

Advancing type contact angles (Darvell et al., 1987) were measured with water, six water/*n*-propanol (Merck, Darmstadt, FRG) mixtures and α -bromonaphthalene (Merck), at 25°C employing the sessile drop technique described by de Jong et al. (1982). As it is known that contact angles on proteinaceous surfaces are slightly time dependent, angles on pellicle-coated surfaces were measured as a function of time and extrapolated to $t = 0$. Subsequently data were least-square fitted to the geometric mean equation accounting for spreading pressures yielding the solid surface free energy γ_s (Busscher et al., 1983).

Zeta potential measurements

Zeta potentials were determined at 25°C in a potassium phosphate buffer (10 mM, pH 7.0, density 10⁸ particles cm^{-3}) with a Laser Zee Meter (Penkem Inc., Bedford Hills, NY, USA) using a potential difference between the electrodes of 150 V. Zeta potentials were calculated from the particle velocity employing the Helmholtz-Smoluchowski equation (Adamson, 1976).

Determination of adsorbed layer thicknesses

Adsorbed layer thicknesses were determined with the so-called null-type ellipsometer in the PCSA configuration (Polarisator, Compensator, Sample, Analyser) at a

wavelength of 632.8 nm and an angle of incidence of 50°. In principle, the ellipsometer determines two parameters Δ and Ψ defined as (Azzam and Bashara, 1977; Ericson et al., 1982):

$$\Delta = (\delta'' - \delta)_p - (\delta'' - \delta)_s \quad (1)$$

in which $(\delta'' - \delta)_{p,s}$ denotes the phase difference after reflection by the sample in the direction parallel (p) and perpendicular (s) to the plane of incidence respectively.

$$\tan \Psi = \frac{|E_p''|}{|E_p|} \bigg/ \frac{|E_s''|}{|E_s|} \quad (2)$$

in which $E_{p,s}$ and $E_{p,s}''$ denote the amplitude of polarized light prior to and after reflection in the p and s direction respectively.

From the values of Δ and Ψ for the clean enamel surface $\bar{\Delta}$ and $\bar{\Psi}$, the complex refractive index of enamel can be calculated, while Δ values measured on enamel with an adsorbed layer can be related to the layer thickness s by

$$\delta \Delta = \bar{\Delta} - \Delta = -\xi \cdot s \quad (3)$$

in which ξ is a complicated function of the complex refractive indices of the enamel substratum and the adsorbed layer, the wavelength of the incident light and the angle of incidence. Because the complex refractive index of the adsorbed AmF layer can only be estimated, it was decided to express the adsorbed layer thickness in relative units, i.e. $\delta \Delta$ values which are directly proportional to the adsorbed layer thickness s according to eq. (3)

Surface tension measurements of the aminefluoride solutions

The surface tension of the aminefluoride solutions were measured at 37°C using an Autotensiomat (Lauda, Königshofen, FRG). Measurements were carried out with a Du Nouy ring (circumference 6 cm) correcting for the Harkins-Jordan factor (Adamson, 1976). The accuracy of the reported surface tensions is $\pm 0.5 \text{ erg cm}^{-2}$.

RESULTS

Tables I and II show a selection of measured contact angles on enamel after adsorption of aminefluorides at various concentrations (Table I), and after adsorption of aminefluorides and being worn in a partial denture for various time intervals (Table II). Calculated surface-free energies are presented in Figs 2 and 3. Adsorption of aminefluorides gives a rapid initial decrease in surface free energies, which stabilizes at a low value for concentrations above 1 mM. After only 30 min *in vivo*, however, these low values have increased to values

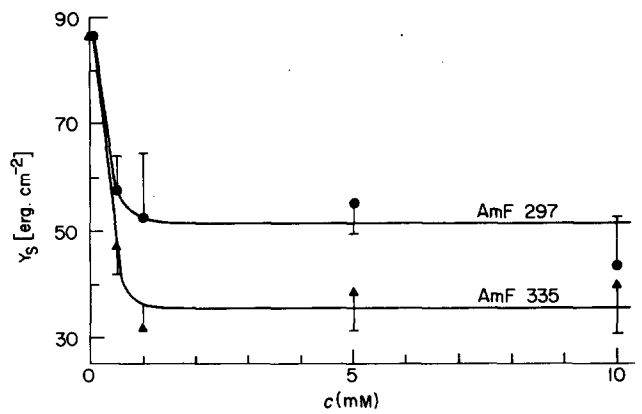


Fig. 2. Surface free energies of ground and polished human enamel after adsorption of aminefluorides from solutions with various concentrations (c). The bars denote standard deviation ($n = 10$).

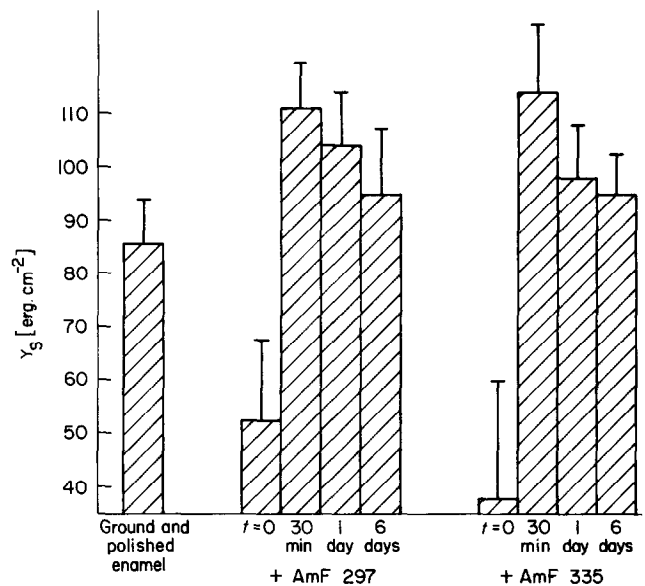


Fig. 3. Diagram of the surface free energy of: ground and polished human enamel (untreated); ground and polished human enamel after adsorption of AmF 297 and AmF 335 carried in a partial denture for various time intervals. The bars denote standard deviation ($n = 10$).

obtained previously by de Jong et al. (1984 a) for pellicle-coated enamel without adsorbed aminefluorides ($110 \pm 9 \text{ erg cm}^{-2}$).

The zeta potentials of enamel particles after adsorption of aminefluorides are given in Fig. 4. Initially the zeta potential is highly negative (-36 mV), after adsorption, however, positive values as high as $+20 \text{ mV}$ are obtained.

Adsorbed layer thickness (in relative units) as a function of the AmF concentration in solution is illustrated in Fig. 5. The clean surface values $\bar{\Delta}$ and $\bar{\Psi}$,

Table I. Selection of measured contact angles (degrees) \pm s.d. ($n = 10$) on enamel surfaces after adsorption of aminefluorides at various concentrations

Enamel surfaces	Water contact angle	α -Bromonaphthalene contact angle
Ground and polished	56 \pm 8	20 \pm 5
+ AmF 297		
<i>c</i> = 0.5 mM	72 \pm 7	34 \pm 9
<i>c</i> = 1 mM	75 \pm 10	42 \pm 10
<i>c</i> = 5 mM	76 \pm 9	23 \pm 6
<i>c</i> = 10 mM	77 \pm 17	48 \pm 9
+ AmF 335		
<i>c</i> = 0.5 mM	76 \pm 6	43 \pm 5
<i>c</i> = 1 mM	95 \pm 6	45 \pm 7
<i>c</i> = 5 mM	93 \pm 8	38 \pm 10
<i>c</i> = 10 mM	89 \pm 10	41 \pm 8

Table II. Selection of measured contact angles (degrees) \pm s.d. ($n = 10$) on enamel after adsorption of aminefluorides at $c = 2.5$ mM and after being carried for various time intervals in a partial denture

Enamel surfaces	Water contact angle	α -Bromonaphthalene contact angle
+ AmF 297		
<i>t</i> = 0	80 \pm 8	35 \pm 8
<i>t</i> = 30 min	34 \pm 6	31 \pm 7
<i>t</i> = 1 day	42 \pm 7	31 \pm 10
<i>t</i> = 6 days	44 \pm 7	38 \pm 10
+ AmF 335		
<i>t</i> = 0	88 \pm 9	38 \pm 7
<i>t</i> = 30 min	31 \pm 9	29 \pm 11
<i>t</i> = 1 day	44 \pm 10	28 \pm 8
<i>t</i> = 6 days	47 \pm 8	31 \pm 11

178.3° \pm 0.8° and 12.3° \pm 0.3° respectively result in a complex refractive index $\tilde{n} = 1.61 - i \times 0.02$.

Absolute values for the adsorbed layer thickness can be obtained by assuming a refractive index for the adsorbed film. Refractive indices of 1.3 or 1.45 yield ξ values (see eq. (3)) of 0.5 and 0.4°·nm⁻¹ respectively from which an absolute value for the adsorbed layer thickness may be calculated.

Surface tensions of the solutions, presented in Fig. 6, show a tendency of the aminefluorides to form micelles at concentrations above 1 mM.

DISCUSSION

The contact angle data as well as the surface free energy data of ground and polished enamel correspond well with previously published findings (Glantz, 1971; van Pelt et al., 1983). De Jong et al. (1984a, b) obtained similarly low values for enamel after application of a mixture of aminefluorides from a commercially available solution

(Elmex Fluid; GABA) as obtained in this study after adsorption of pure aminefluorides. De Jong et al. (1984 a) also observed increased values for the surface free energy of various materials after salivary protein adsorption *in vivo*.

Available data show a negative zeta potential for ground and polished enamel (Arends and Jongbloed, 1977; Arends, 1979; Reynolds and Wong, 1983). However, no data are available for enamel after aminefluoride adsorption, neither have adsorbed layer thicknesses been measured before.

The refractive index of the ground and polished enamel, as measured ellipsometrically, is in perfect agreement with data published by Spitzer and Ten Bosch (1975), while adsorbed layer thicknesses (3–12 nm), estimated from $\delta\Delta$ and assumed values for the refractive index of the adsorbed AmF layers, are in reasonable agreement with expectations based on molecular dimensions.

It is interesting to notice the differences between AmF 297 and AmF 335 with respect to the properties of its adsorbed layers on enamel:

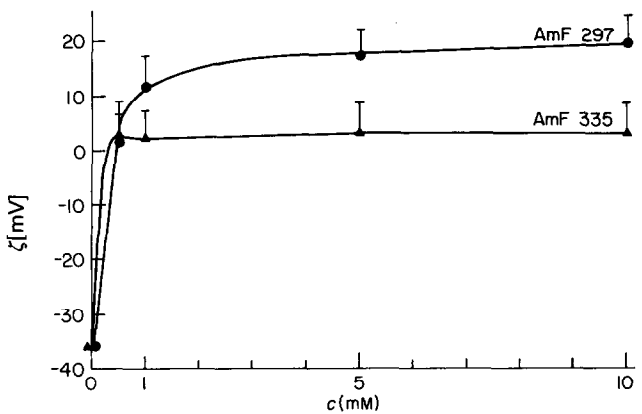


Fig. 4. Zeta potentials of human enamel particles after adsorption of aminefluorides from solutions with various concentrations (*c*). The bars denote standard deviations over six independent observations.

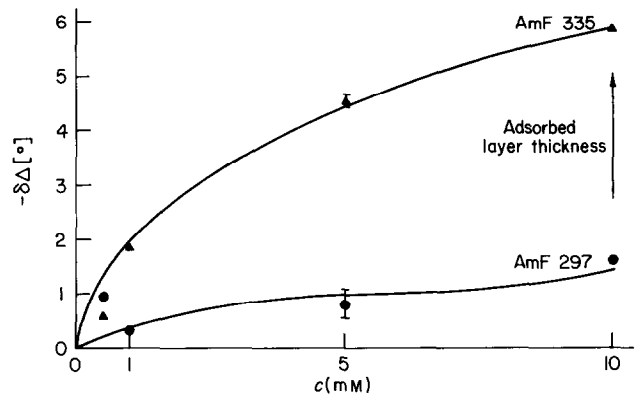


Fig. 5. Adsorbed layer thicknesses of aminefluorides adsorbed on ground and polished human enamel as a function of the concentration in solution (*c*). The bars denote standard deviation over five samples.

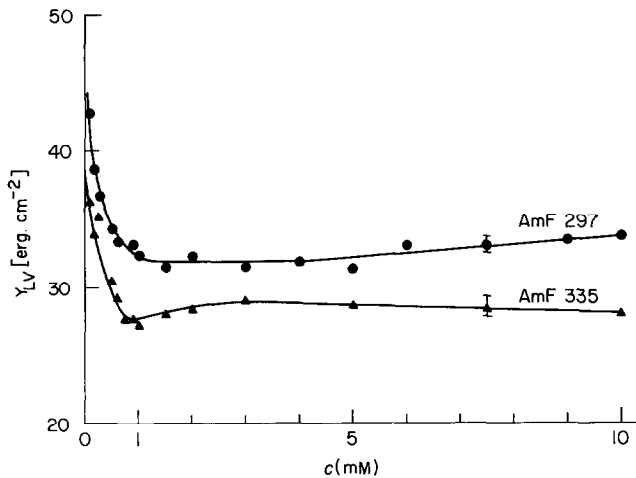


Fig. 6. Surface tensions of aminefluoride solutions as a function of aminefluoride concentration (*c*) measured at 37°C. The bars denote the experimental accuracy.

AmF 335 gives a lower surface free energy than AmF 297 (Fig. 2).

AmF 335 gives a less positive zeta potential than AmF 297 (Fig. 4)

and AmF 335 gives a thicker adsorbed layer than AmF 297 (Fig. 4).

It is suggested that these differences can be explained by assuming a bonding of AmF 297 with both NH⁺ groups to negatively charged phosphate groups at the enamel surface, thereby effectively decreasing the hydrocarbon chain lengths perpendicular to the enamel surface and the density of adsorbed molecules. Furthermore, the polar headgroup of AmF 297 is much larger than of AmF 335, which decreases the density at the surface.

The results of this study permit speculation on the structure and conformation of adsorbed AmF layers on enamel. In a similar study on aminefluoride adsorption to glass (Busscher et al., 1987), it was concluded that monolayer coverage occurs at *c* = 1 mM (approximately the same concentration at which micelles start to form in the solutions both at 25°C (Busscher et al., 1987) and, as shown in this study, at 37°C). Double layer formation at concentrations above 5 mM, as observed with AmF 297 on glass (Busscher et al., 1987), was not observed in this study, because the samples were rinsed before making the contact angle measurements. By contrast, de Jong et al. (1984 b) observed double layers of AmF on enamel but only after long application times (>5 min).

In a number of previous studies (Uyen et al., 1985; van Pelt et al., 1985; Busscher et al., 1986), it has been demonstrated that adhesion of most types of oral streptococci to low surface free energy substrata is minimal. Hence, *in vivo* surface free energy may increase within 30 min of reduction by a surfactant, in this case aminefluorides. There is now convincing evidence, however, from Schakenraad et al. (1986) for cellular spreading *in vitro* and from Jendresen (1980) and van Dijk et al. (1986) for bacterial adhesion *in vivo*, that despite the presence of adsorbed protein films, which shield the physicochemical properties of a substratum, it is the surface free energy of the underlying substratum which determines the adhesion of cells and bacteria. In all probability adsorbed protein films are conditioned in an extremely complicated and subtle way to transfer the properties of the substratum towards the outer surface of the adsorbed protein film. A similar conclusion was reached by Dexter and Lucas (1985) in a study on protein adsorption to solid substrata using Photo Acoustic Spectroscopy.

Theoretically, it is expected that the positive zeta potential of the enamel after aminefluoride adsorption will

enhance adhesion instead of reducing it. It is difficult to estimate, however, how the combined effect of a reduced surface free energy, a positive zeta potential and salivary protein adsorption will influence bacterial adhesion *in vivo*.

Based on the results of this study, it would appear to be inappropriate to apply aminefluorides with solutions of concentrations above 1 mM, since there would be limited advantage in terms of reduction of the surface free energy (Fig. 2), and an increase zeta potential (Fig. 4).

The results of this study demonstrate that aminefluorides have a strong tendency to adsorb on enamel and to profoundly change its physicochemical properties.

References

- Adamson A. W. (1976) *Physical Chemistry of Surfaces*, 3rd edn. New York, Wiley.
- Arends J. (1979) Zeta potentials of enamel and apatites. *J. Dent.* **7**, 246–253.
- Arends J. and Jongbloed W. L. (1977) The enamel substrate characteristics of the enamel surface. *Swed. Dent. J.* **1**, 215–224.
- Azzam R. M. A. and Bashara N. M. (1977) In: *Ellipsometry and Polarized Light*. Amsterdam, North Holland Publishing.
- Balmelli O. P., Regolati B. and Mühlemann H. R. (1973) Inhibition of Streptococcal deposits on rat molars by aminefluoride. *Acta Odontol. Helv.* **8**, (Suppl.), 45–53.
- Busscher H. J., van Pelt A. W. J., de Jong H. P. et al. (1983) Effect of spreading pressure on surface free energy determination by means of contact angles. *J. Colloid Interf. Sci.* **95**, 23–27.
- Busscher H. J., Weerkamp A. H., van der Mei H. C. et al. (1984) Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. *Appl. Env. Microbiol.* **48**, 980–983.
- Busscher H. J., Uyen H. M., Weerkamp A. H. et al. (1986) Reversibility of adhesion of oral Streptococci to solids. *FEMS Microbiol. Lett.* **35**, 303–306.
- Busscher H. J., Uyen H. M., Kip G. A. M. et al. (1987) Adsorption of aminefluorides to glass surface free energy, zeta potential and adsorbed layer thickness. *Colloid Surfaces* **22**, 161–169.
- Darvell B. W., Murray M. D. and Ladizesky N. H. (1987) Contact angles: a note. *J. Dent.* **15**, 82–84.
- de Jong H. P., van Pelt A. W. J. and Arends J. (1982) Contact angle measurements on human enamel. *J. Dent. Res.* **61**, 11–13.
- de Jong H. P., de Boer P., Busscher H. J. et al. (1984 a) Surface free energy changes of human enamel during pellicle formation—an *in vivo* study. *Caries Res.* **18**, 408–415.
- de Jong H. P., van Pelt A. W. J., Busscher H. J. et al. (1984 b) The effect of topical fluoride application on the surface free energy of human enamel—an *in vitro* study. *J. Dent. Res.* **63**, 635–641.
- Dexter S. G. and Lucas K. E. (1985) The study of biofilm formation under water by photo acoustic spectroscopy. *J. Colloid Interf. Sci.* **104**, 15–27.
- Ericson T., Pruitt K. M., Arwin H. et al. (1982) Ellipsometric studies of film formation on tooth enamel and hydrophilic silicon surfaces. *Acta Odontol. Scand.* **40**, 197–201.
- Gehring F. (1983) Effect of amine and sodium fluorides on germs and plaque flora. *Dtsch. Zahnärztl. Z. (Sonderheft 1)* **38**, 36–41.
- Glantz P. O. (1971) The adhesiveness of teeth. *J. Colloid Interf. Sci.* **37**, 281–290.
- Jendresen M. D. (1980) Thesis, Department of Prosthetic Dentistry, Faculty of Odontology, University of Lund.
- Mühlemann H. R. (1983) Development and application of aminefluoride in caries prophylaxis. *Dtsch. Zahnärztl. Z. (Sonderheft 1)* **38**, 3–5.
- Olsson J., Glantz P. O. and Krasse B. (1976) Surface potential and adherence of oral streptococci to solid surfaces. *Scand. J. Dent. Res.* **84**, 240–242.
- Reynolds E. C. and Wong A. (1983) Effect of adsorbed protein on hydroxyapatite zeta potential and *Streptococcus mutans* adherence. *Infect. Immunol.* **39**, 1285–1290.
- Rutter P. R. and Vincent B. (1980) The adhesion of microorganisms to surfaces: physico-chemical aspects. In: Berkeley R. C. W., Lynch J. M. and Melling J. (eds), *Microbial Adhesion to Surfaces*. Chichester, Ellis Horwood, pp. 79–92.
- Schakenraad J. M., Busscher H. J., Wildevuur C. R. H. et al. (1986) The influence of substratum surface free energy on growth and spreading of human fibroblasts in the presence and absence of serum proteins. *J. Biomed. Mater. Res.* **20**, 773–784.
- Spitzer D. and ten Bosch J. J. (1975) The adsorption and scattering of light in bovine and human dental enamel. *Calcif. Tiss. Res.* **17**, 129–137.
- Uyen H. M., Busscher H. J., Weerkamp A. H. et al. (1985) Surface free energies of oral Streptococci and their adhesion to solids. *FEMS Microbiol. Lett.* **30**, 103–106.
- van Dijk L. J., Herkströter F., Busscher H. J. et al. (1987) Surface free energy and bacterial adhesion—an *in vivo* study in Beagle dogs. *J. Clin. Periodontol.* **14**, 300–304.
- van Pelt A. W. J., de Jong H. P., Busscher H. J. et al. (1983) Dispersion and polar surface free energy of human enamel (an *in vitro* study). *J. Biomed. Mater. Res.* **17**, 637–641.
- van Pelt A. W. J., Weerkamp A. H., Uyen H. M. et al. (1985) Adhesion of *Streptococcus sanguis* CH3 to polymers with different surface free energies. *Appl. Env. Microbiol.* **49**, 1270–1275.