

# Wettability Evaluation of Layer-by-layer Proteases Enzymatic Films for Biosensor Application

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Multilayer films were prepared through layer-by-layer self-assembly of proteases enzymes. Solutions of pepsin, lysozyme and trypsin at  $10^{-5}$  M (pH 6.4, pH 6.4 and pH 7.6 respectively) were used as precursors for film building. Wettability and contact angle analysis were carried out by sessile drop (volume around 5 $\mu$ L) of four different polar solutions: water; ethyleneglycol; formamide and diiodomethane (from Sigma). Images of the droplets on the film's surfaces were recorded using a video based optical device (capturing 30 frames per second) and the angles determined by FTA32 Image Software. All measurements were performed in air at room temperature (25-27 °C) and relative humidity of 74%. The surface free energy was calculated according to simplified Owens-Wendt equation [1].

The calculated surface energies show the different components of the surface energy for each sample condition: for bare glass the total contribution was measured as 66.9 mN/m corresponding closely to the surface tension of water (72.1 mN/m). For enzymatic films the surface free energy values were all similar and close to 34.5 + 1.07 mN/m. The dispersion components were also similar with reduced partial polar contributions, particularly to the trypsin surface. The differences in surface energy between substrates can be interpreted by means of the differences in their polar components. If the dipole moments of the molecules end groups are compared with the polar components of their respective surface energy, a correlation can be established [2]. Molecules with no or a very small dipole moment have a small polar contribution to surface energy whilst a high density of polar groups or bonds give rise to large polar surface energy components. Such behavior was revealed with trypsin: the increasing of water contact angle is accompanied by a reduction in the  $\gamma$  – surface polar component.

Keywords: enzymatic films; self-assembled films; surface wettability; multilayer film; AFM.

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