Spread films of human serum albumin at the air–water interface: optimization, morphology and durability

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The non equilibrium nature of spread films of proteins at the air/water interface has attracted a lot of attention during the last century. It is known that a lower surface tension can be achieved when spreading concentrated droplets of protein solution [1] than by adsorption for a given total bulk concentration. This approach exploits non-equilibrium effects to form kinetically-trapped, loaded films. Over a narrow range of concentrations, protein solutions show a decrease in surface tension up to ~ 20 mN/m. For these systems the application of the Gibbs adsorption isotherm to derive the surface excess leads to values that exceed the one of a closed-pack layer [2], thus a new picture is needed. In the present work we apply several surface sensitive techniques (neutron and X-ray reflectometry, ellipsometry, Brewster angle microscopy and surface pressure vs. area isotherm), directly at the air/water interface, to characterize fully the surface behaviour of spread films of defatted human serum albumin (HSA), comparing them with those formed by bulk adsorption [3]. Our results show clearly that the main mechanism controlling the interfacial stoichiometry of the spread films is the Gibbs-Marangoni spreading, which is activated only if the concentration of the spreading solution is high enough to ensure a sufficient surface tension gradient between the droplets and the subphase. We also show that the mechanical properties of the interface are strictly dependant on the morphology of the formed layer. The annealing of the surface during consecutive compression/expansion cycle leads to a more stable, durable and homogeneous layer due to the coalescence of HSA islands. Protein films have already a wide range of applications: from the food industry to coating based technologies. In most cases, proteins are used as surface stabilizer/destabilizer agents. We believe that our findings could be applied to the optimization of protein use for technological applications.

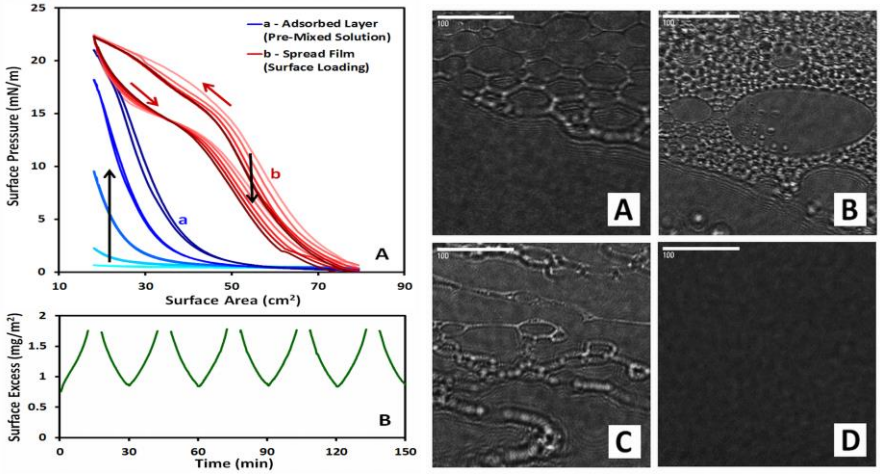

Figure 1- left: (A) surface pressure vs. area isotherm during five consecutive compression/expansion cycles of spread (red shaded) and adsorbed (blue shaded) HSA films. (B) surface excess of the spread film measured by ellipsometry during the pressure vs area experiment; **right:** Brewster angle microscopy images of spread films from (A) 10 droplet of 0.01 mg/cm^3 and (B) 1 droplet of 0.1 mg/cm^3 HSA solution on standard phosphate buffer (SPB) and adsorbed layers of (C) 0.00080 and (D) 0.020 mg/cm^3 HSA in SPB.

[1] Gorter E. et al., *Faraday Soc.* 1926, **22**, 477
[2] Fainerman V. et al., *Proteins at Liquid Interfaces,* 1998, p.51
[3] Campbell R. A. et al., *Langmuir,* 2015, **31**, 13535