Wetting of biological lipids on aqueous substrates

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We study the dynamics and final wetting state of skin lipids on water and brine by fluorescence microscopy and ellipsometry. When a lipid droplet is brought into contact with the water surface, a lipid wetting film spreads out rapidly by a Marangoni effect. Subsequently, this film undergoes a dewetting instability. However, the final equilibrium is not partial wetting. The film breaks up into droplets with a mesoscopic ($\approx$50 Å) film in between. These observations result from a subtle interplay between short- and long-range forces: surfactants naturally present in the lipids favor wetting, while the van der Waals forces oppose it. In addition, this reveals the likely organization of the hydrolipid film that covers and protects the skin.

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In biological systems, surface layers of lipids, whether monomolecular or multilayer, greatly reduce the rate of surface evaporation [1]. A well-known example is the meibomian lipid layer preventing too fast evaporation of water from the tear film in the eye [2,3]. The presence of such lipid films on aqueous ''substrates'' may seem surprising, as these are mainly composed of long-chain hydrocarbons, and it is well known that long hydrocarbons do not wet water [4]. We therefore study the wetting behavior of a biological lipid on water.

The observation that long-chain hydrocarbons do not form films at the free surface of water follows immediately from considering the wetting properties of, say, an oil droplet (o) deposited on water (w) in the presence of their common vapor (v). Wetting is determined by the equation for the surface tensions [5]: $\sigma_{wv} \leqslant \sigma_{wo} + \sigma_{ov}$. At inequality, the oil droplet will have a finite contact angle with the surface; the oil partially wets the water. At equality, it becomes favorable to ''replace'' the water-vapor interface by an oil-water plus an oil-vapor interface. The droplet spreads out to form a wetting film and the oil is said to completely wet the water. Detailed experimental studies have shown that for long-chain oils especially the high value of σ_{wo} prevents complete wetting of the oils; no film is present at the water surface [4].

In addition to the question of the stability of lipid films, so far little is known about the structure and dynamics of the formation of such films. Arguably the most debated issue is the structure of the ''hydrolipid film'' [6,7], a layer that consists of both water and lipids and that covers the human skin surface. The lipid phase (sebum) is excreted by the sebaceous glands. The aqueous phase is present at the skin surface due to secretion by the sweat glands and transepidermal water loss. Sebum is a mixture of waxes and fatty acids; its excretion and biological function remain largely unexplained [8,9]. As far as the organization of the hydrolipid film is concerned, it is believed to be an emulsion [10,11]. In spite of the presence of fatty acids and phospholipids in the sebum, which may both play the role of surface-active components, there is no clear evidence for such a statement.

In this paper, we study the wetting properties of sebum on water and a variety of other aqueous phases. We show that when the two phases are brought into contact, first a wetting film of the lipid phase spreads out rapidly over the aqueous phase. Subsequently, this film breaks up, by a dewetting process, into droplets. We show that the resulting hydrolipid film is in fact a lipid film several molecular diameters thick, coexisting with lipid droplets, that completely covers the aqueous phase. The coexistence of droplets with a relatively thick film was observed earlier for the wetting of pure oils on water [4]: it is neither partial nor complete wetting, but an intermediate state called pseudopartial or frustrated complete wetting [4,12]. The results can consequently be understood as a subtle competition between the short-range forces that favor wetting and the long-range van der Waals forces that oppose it.

We use natural sebum and both natural and artificial sweat, brine, and pure water as substrates [13]. It turns out that the results are the same for the different aqueous subphases; we will consequently only show those obtained for pure water. The ''standard'' composition of sebum is the following [13]: triglycerides 54% (partially hydrolized into free fatty acids by bacteria present on the skin surface); wax esters 26% squalene 12%; cholesterol ester 3%; cholesterol 1%; traces of phospholipids, epidermal lipids (ceramids), and hydrocarbons. However, the actual composition may vary slightly between different subjects depending on, among other factors, age and diet.

The wetting dynamics are followed using fluorescence microscopy. For the fluorescence experiments, the sebum phase was stained with Nile Red (Aldrich) so that it is only this phase that is visible under the microscope: solubility of the fluorescent molecule in the aqueous phase is negligible. A small droplet (0.05 cm^3) of natural sebum at 37 °C is deposited gently at the surface of water, which is also kept at 37 °C by a thermostating stage.

We observe, as soon as the lipid droplet is brought into contact with the aqueous phase, a hydrodynamic flow that

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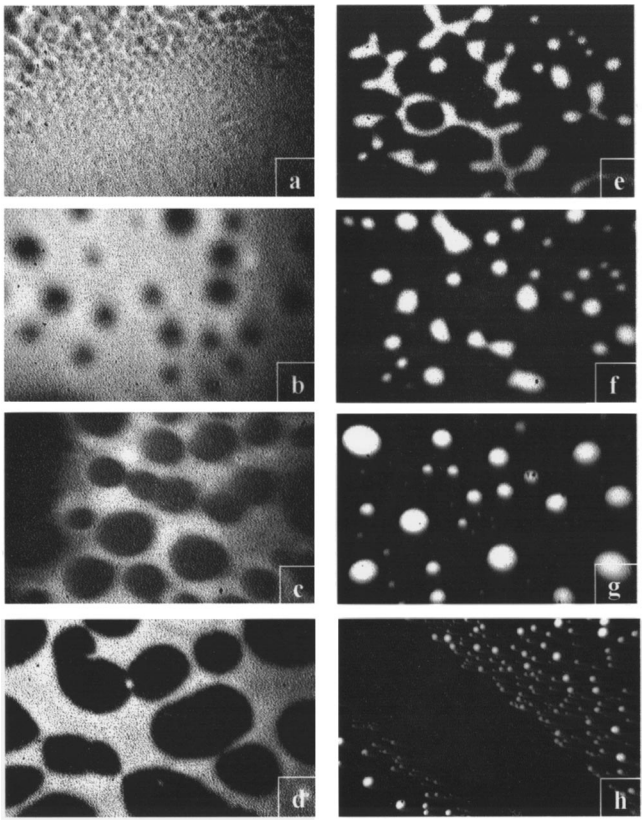

FIG. 1. Sequence of images of the aqueous-air surface after contact with the lipid phase obtained by fluorescence microscopy. Image sizes are ($240\times360\ \mu$m) A very thick film forms very rapidly after contact (a). Subsequently, holes form in the film (b)–(d). At later times, the lipid retracts (e), (f) and one observes the formation of lipid droplets (g), (h). The droplets appear to be interconnected, since they move in blocks when a slight air stream is passed over the free surface, in agreement with the finding that a film exists between the droplets. As the droplets are rather large, they produce so much fluorescence that the film of several molecules thickness connecting the droplets cannot be observed on these images. A fluorescent molecule has been added to the lipid phase, so that this is the only one observed in the experiments.

results in a very rapid coverage of the water surface with a thick lipid film. Subsequently, this thick film destabilizes. It is observed to undergo an instability, leading to the formation of holes in the film; the sebum appears to undergo a dewetting transition [15]. The remaining sebum retracts to form droplets, so that in the final stage, droplets of the lipid phase are observed to float on top of the aqueous phase (Fig. 1).

The observation that the lipids do spread over the water surface implies that the initial spreading coefficient S_i is positive. The spreading coefficient [5] $S=\sigma_{vw}-(\sigma_{wo}-\sigma_{ov})$ represents the difference in surface free energies between partial ($S<0$) and complete wetting ($S=0$). Although in equilibrium S can never exceed 0, when a droplet is put onto a substrate initially the spreading coefficient may be positive, which is the driving force for the spreading of the droplet. The experimental observations can easily be understood by realizing that the lipid phase contains surface-active agents, notably phospholipids. If this lowers σ_{wo} by a sufficient amount, covering the aqueous phase with a layer of sebum containing surface-active material will be favored by the system. For a positive initial spreading coefficient, the complete droplet will spread out to form a uniform thick film that covers the whole substrate surface. Depending on the size of the initial droplet, this film can easily be several micrometers thick.

The rapid spreading of the lipids over the surface also indicates that surface-active molecules are present. Due to the presence of surfactants, the Marangoni effect [14] causes a hydrodynamic flow of the lipid phase induced by a surface tension gradient. This surface tension gradient is, in turn, due to a surface gradient in surfactant concentration at the moment the two phases are brought into contact. This is a well-known effect for the spreading of surfactant-laden droplets on a variety of substrates and accounts for the rapid spreading into a thick lipid film [16].

However, it has been shown that in order for the thick film to be stable, the long-range van der Waals forces also need to favor the formation of a thick film [4,5,12,15]. The difference between the cohesive (sebum-sebum) and the adhesive (sebum-aqueous phase) interaction energy due to the intermolecular van der Waals forces can either favor or disfavor the formation of a thick wetting film. If the effective interaction disfavors wetting, but the surface tensions are such that fluid still spreads, these two effects enter in competition. The result of this competition is that a film does form, which, however, cannot grow very thick. This implies that an intermediate wetting state exists, in which droplets coexist with a relatively thick film. This film is neither molecularly thin (partial wetting, a few angstroms) nor very thick (complete wetting, several micrometers) [4,12].

The Hamaker constant A gives both the magnitude and the sign of the difference in cohesive and adhesive interaction energies due to the van der Waals forces. The total Hamaker constant A is the sum of a zero-frequency contribution $A_v=0$ given by the static dielectric constants $\varepsilon(0)$, and a dispersion contribution $A_v>0$ given by the refractive indices for visible light in the media 1 (water), 2 (lipids), and 0 (vapor) [5]:

$$A_{v=0}=\tfrac{3}{4}k_BT\frac{[\varepsilon_1(0)-\varepsilon_2(0)]}{[\varepsilon_1(0)+\varepsilon_2(0)]}\frac{[\varepsilon_0(0)-\varepsilon_2(0)]}{[\varepsilon_0(0)+\varepsilon_2(0)]},$$

$$A_{v>0}=\frac{3hv_e}{8\sqrt{2}}\frac{(n_0^2-n_2^2)(n_1^2-n_0^2)}{\sqrt{n_0^2+n_2^2}\sqrt{n_1^2+n_2^2}(\sqrt{n_0^2+n_2^2}+\sqrt{n_1^2+n_2^2})}$$

with k_B Boltzmann's constant, T the absolute temperature, h Planck's constant, and $v_e\approx3\times10^{15}\,\mathrm{s}^{-1}$ a typical absorption frequency in the ultraviolet [5]. For the lipid phase, one can take $\varepsilon_2(0)=n_2^2$; then, knowing the dielectric properties of the aqueous phase, A can be calculated if the refractive index n of the lipid phase is known [4,12]. Determining the latter experimentally on a standard refractometer ($n=1.426$), we find $A=-1.0\times10^{-20}$ J. The negative sign implies that the interaction energy decreases with decreasing film thickness. This shows that the total energy is minimal for thin, rather than thick films. Consequently, a thick wetting film of the

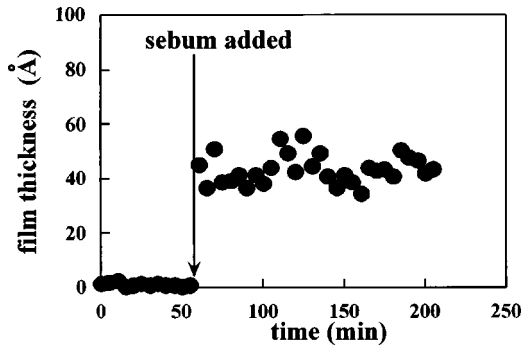

FIG. 2. Ellipsometry measurement of the layer thickness of the sebum film on an aqueous substrate. A Teflon dish contains the aqueous phase, onto which a droplet (0.05 cm^3) of natural sebum is deposited, and the ellipticity is monitored as a function of time. Due to the presence of the Teflon dish, only the film of several molecules thickness and not the oil droplets are in fact present at the surface: as the lipid phase wets the Teflon walls, a sebum reservoir is formed there, from which the observed film forms.

lipid at the aqueous-vapor interface is a thermodynamically unstable situation, which accounts for the observed destabilization of the sebum film after the initial spreading into a thick film, just after contact between the phases.

In order to see whether the lipids might be in a pseudopartial or frustrated complete wetting state, we performed a measurement of the thickness of the lipid film using ellipsometry. The ellipsometry experiments were performed on a standard phase-modulated ellipsometer operating at the Brewster angle. Details of the experimental setup are given in Ref. [4]. The well-known Drude equation is used to relate the measured ellipticity to the layer thickness [17]. Performing a measurement of the thickness of the sebum film that forms between the droplets observed in the fluorescence experiment, it follows that the system is indeed in the frustrated complete wetting state. After deposition of a small amount of sebum on the aqueous surface, a stable film forms of thickness 44 Å (Fig. 2), which is clearly a film of several molecular diameters thick: it is neither molecularly thin nor very thick. The conclusion is therefore that the ''hydrolipid film'' is in fact the coexistence of lipid droplets with a film of thickness of roughly five to ten molecules between the droplets, on top of the aqueous phase. The film is not observed in the fluorescence experiment, simply because of its relatively small thickness, implying that there are too few fluorescent molecules in the film to observe it if droplets are also present.

More quantitatively, the experiments on the wetting of alkanes on water [4] are in agreement with the theoretical prediction for the thickness of the pseudopartial wetting film $l=-3B/2A$, where B follows from the cohesive (lipid-lipid) interactions and the molecular size [4]. For our system, the former is given by the Hamaker constant of the lipid interacting with itself, which can again be calculated from the known refractive index. For the latter, we take a typical size of an oil molecule having the same refractive index as the sebum. Using these values, we find $B=3.3\times10^{-29}$ J, from which a film thickness of 48 Å follows, in excellent agreement with experiment.

These considerations therefore account for the experimental observations. As the lipid phase contains surface-active agents, notably phospholipids, covering the aqueous phase with a layer of sebum containing surface-active material will be favored by the system. Due to the presence of the surfactants, the Marangoni effect causes a hydrodynamic flow of the lipid phase induced by a surface tension gradient. This surface tension gradient is, in turn, due to a gradient in surfactant concentration at the moment the two phases are brought into contact. We thus observe the rapid spreading of the sebum on the aqueous phase, forming a lipid film that covers the surface. On the other hand, due to the long-range van der Waals forces (i.e., the Hamaker constant), the system is not in equilibrium with a thick lipid film on the aqueous phase. Once the Marangoni flow has stopped, the film will undergo an instability that will lead to the formation of the lipid droplets on the aqueous surface and that will coexist with a mesoscopic film.

There may also be some biological relevance to our findings. The spontaneous spreading of sebum observed here suggests mainly a physical control of the excretion process of the sebum; so far, its excretion has remained largely unexplained [8,9]. This is an important issue since sebum causes skin inflammations and is comedogenic [18]. Comparing with the monolayer lipid film on the eye [2,3], it follows that the detailed structure from the lipid film on the skin, as it follows from our experiments, is slightly different in that droplets coexist with the lipid film.

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