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ARTICLE

Dimo Kashchiev · Dotchi Exerowa

Structure and surface energy of the surfactant layer on the alveolar surface

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Abstract The surface energy of the alveolar surfactant layer is determined in the scope of a modification of the structural model of Larsson et al. [(1999) J Disp Sci Technol 20:1–12], according to which this layer is built up of a lipid monolayer adsorbed at the hypophase/air interface and supported by a network of lipid bilayers immersed into the hypophase, i.e., the alveolar liquid. Formulae are derived for the dependence of the specific surface energy of the surfactant layer on the distance between the bilayers constituting the layer. It is shown that at equilibrium this energy can have values comparable with or less than 1 mJ/m^2 needed for normal functioning of the alveolus during the respiration cycle. The specific surface energy of the surfactant layer with monolayer-bilayer structure can have such low values only if the layer is of optimal thickness and if the specific line energy of the monolayer-bilayer contact lines is negative and that of the bilayer-bilayer contact lines is positive. It is found that in dynamic regime the change in the specific surface energy of the alveolar surfactant layer with bilayer-monolayer structure is in qualitative agreement with that determined experimentally during lung inflation and deflation.

Key words Alveolar surfactant layer · Lipid monolayer · Lipid bilayer · Alveolar surface energy · Specific line energy

Introduction

The surfactant layer covering the surface of the alveoli is built up mostly of the dipalmitoylphosphatidylcholine and phosphatidylglycerol phospholipids. Along with the specific alveolar surfactant proteins, the amphiphilic molecules of these two lipids are organized into a particular structure which plays an important role in lowering the specific surface energy of the alveolar surface. This structure is usually modeled by that of a monolayer of lipid molecules adsorbed at the interface between the alveolar liquid, termed the hypophase, and air (e.g., Clements et al. 1958; King and Clements 1985; Scarpelli 1988; Schürch et al. 1998). However, a major shortcoming of the monolayer model of the alveolar surfactant layer is that this model leads to a rather high equilibrium value, 25–30 mJ/m^2, of the specific surface energy of the alveolar surface. The actual value of this energy is less than about 1 mJ/m^2 and has to be so small in order for the alveoli to avoid collapse in the course of the respiration cycle.

Since lipid bilayers and multilayers can also exist in the hypophase, Exerowa and Lalchev (1986) proposed these layers as a model of the alveolar surfactant layer (see also Exerowa and Kruglyakov 1998). A number of parameters characterizing the bilayer stability can be introduced on the basis of the theory of Kashchiev and Exerowa (1980, 1983, 1998) of bilayer rupture mediated by hole nucleation. Values of these parameters were determined experimentally for bilayers of model phospholipids (Exerowa et al. 1992; Exerowa and Kruglyakov 1998), for bilayers of alveolar surfactants extracted from amniotic fluid (Exerowa et al. 1984; Exerowa and Lalchev 1986; Nikolova and Exerowa 1996; Exerowa and Kruglyakov 1998) or rabbit lung (Exerowa and Lalchev 1986; Exerowa and Kruglyakov 1998), and for bilayers of therapeutic surfactants (Cordova et al. 1996; Scarpelli et al. 1997; Exerowa and Kruglyakov 1998). The bilayers studied were microscopic foam bilayers formed under conditions (temperature, electrolyte concentration in the aqueous solution, capillary pressure, bilayer area) modeling those of the lung alveoli in vivo. These studies substantiated a new clinical diagnostic method for assessment of fetal lung maturity (e.g., Exerowa et al. 1986; Exerowa and Kruglyakov 1998). Owing to the good correlation

D. Kashchiev · D. Exerowa (✉)
Institute of Physical Chemistry,
Bulgarian Academy of Sciences,
ul. Acad. G. Bonchev 11, Sofia 1113, Bulgaria
E-mail: exerowa@ipchp.ipc.bas.bg
Fax: +3592-9712662

between the results for the stability of bilayers of surfactants of amniotic fluid at different gestation weeks and the results for development of respiratory distress syndrome in newborns, it was concluded that rather than being merely an adsorbed monolayer, the alveolar surfactant layer is an ordered structure of bilayers and multilayers (Exerowa and Lalchev 1986; Exerowa and Kruglyakov 1998). Recently, the existence of such layers was confirmed by atomic force microscopy and special microscopic techniques for solution/air interface studies (Cosgrove and Zasadzinski 1998). Multilayer structures at high concentration of liquid extract surfactant were registered also by Schürch et al. (1998) with the help of captive bubble surfactometry.

Considering the lining of the lung, Scarpelli (1988) introduced the hypothesis of intraalveolar bubble formation. The existence of bubbles in the alveoli at birth was established by Scarpelli and Mautone (1994) by a stereomicroscopic technique of lung cuts in situ (see also Scarpelli 1998). Regarding their results as evidence for the presence of a foam bilayer at the "mouth" of the alveolus, they proposed the closed lipid monolayer as a model of the alveolar surfactant layer (Scarpelli and Mautone 1994).

Recently, Larsson et al. (1999) proposed a new model of the structure of the alveolar surfactant layer. Their model, like that of Clements et al. (1958), also allows for the presence of an adsorbed lipid monolayer at the hypophase/air interface. However, in the model of Larsson et al. (1999) the monolayer is connected with a network of lipid bilayers immersed into the hypophase. The bilayers themselves are organized into a tetragonally symmetric phase of close-packed tubules extending parallel to the monolayer. Morphological observations (e.g., Nichols 1976; Sanderson and Vatter 1977; Stratton 1977; Williams 1977; Weibel 1986; Scarpelli 1988; Larsson et al. 1999) are in support of this organization of the lipid bilayers, which is known also as tubular myelin.

Assuming that the monolayer-bilayer model of Larsson et al. (1999) describes realistically the structure of the surfactant layer on the alveolar surface, we can ask the question: what is the equilibrium value of the specific surface energy of the hypophase/air interface, which results from this model? The answer to this question is important, for if this value is not small enough (see above), the monolayer-bilayer model can hardly be regarded as a substantial improvement of the known monolayer one.

The aim of this paper is to answer the above question. In doing that we shall also modify to a certain extent the original monolayer-bilayer model of Larsson et al. (1999) by making it contain the monolayer model as a limiting case.

Discussion

Monolayer-bilayer model

Following Larsson et al. (1999), we consider the alveolar surfactant layer as constituted of an adsorbed lipid monolayer which is supported by regularly ordered lipid bilayers immersed into the hypophase. The structure of the whole surfactant layer is schematized in Fig. 1. The monolayer is represented by the shaded plane, and the bilayers are depicted hatched. As shown by Stratton (1977), they are organized in a network with tetragonal symmetry. This network is formed of close-packed prismatically shaped tubules with square cross section, d (m) being the length of the cross-sectional edge or, in other words, the interbilayer distance ($d \gg d_m$ where $d_m \approx 0.7$ nm is the diameter of the head of a lipid molecule). The tubules are oriented parallel to each

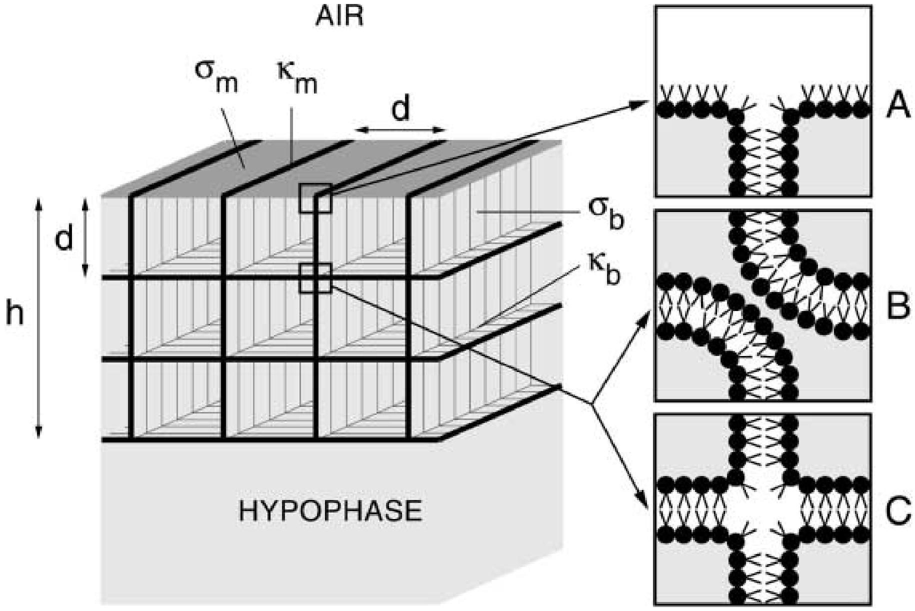

Fig. 1 Monolayer-bilayer model of alveolar surfactant layer of thickness *h*. The monolayer (*the shaded plane*) at the hypophase/air interface is supported by a network of bilayers (*the hatched planes*) at a distance *d* from each other. Cross sections of MB and BB contact lines (*the small squares*) are shown enlarged on the right. Square *A* represents the molecular structure of the MB contact line (the *circles* and the *lines* schematize, respectively, the heads and the tails of the lipid molecules). Squares *B* and *C* illustrate two possible molecular structures of the BB contact line

other and to the hypophase/air interface. The total thickness h (m) of the surfactant layer depends on how many layers of tubules are involved in supporting the adsorbed monolayer. By definition, $h \geq d$.

The monolayer-bilayer (MB) contact lines on the alveolar surface form a grid of parallel straight lines at a distance d from each other. The d values reported (Gil and Weibel 1969/1970; Nichols 1976; Sanderson and Vatter 1977; Stratton 1977; Williams 1977; Weibel 1986; Larsson et al. 1999) are about 50 nm so that h may be expected to range from 50 to 500 nm (this corresponds to $h/d = 1$–10 tubular layers). Clearly, in the limiting case of $d = \infty$ the MB contact lines are infinitely apart from each other, the lipid monolayer is not supported by the bilayers in the hypophase, and the whole MB construction seen in Fig. 1 degenerates into a monolayer one. Thus, the considered MB model of the alveolar surfactant layer appears as a generalization of the known monolayer model (Clements et al. 1958; King and Clements 1985).

The molecular structure of the monolayer, the bilayers, and the lines of MB and bilayer-bilayer (BB) contact is illustrated in squares A, B, and C in Fig. 1. Square A is a cross section of the MB contact line and shows that the lipid molecules in the monolayer have their hydrophilic heads (the circles) in the hypophase and their hydrophobic tails (the lines) in air. At the MB contact line the molecules arrange into tail-tail contact to form a bilayer which extends into the hypophase along the normal to the monolayer. As to the BB contact line, as discussed by Sanderson and Vatter (1977), it can have one of the two structures whose cross sections are depicted by squares B and C in Fig. 1. The first possibility is the bilayers to be really in BB contact through an ultrathin liquid layer (square B). Alternatively, the two bilayers can cross each other along the BB contact line (square C), so that this line is in fact a crossing rather than a contact line. We see that this structure is congenial with that of the MB contact line (square A). Experimentally, both structures of the BB contact line were observed (Nichols 1976; Sanderson and Vatter 1977), but it seems that the structure schematized in square B is a transient one (Sanderson and Vatter 1977). As we shall see in the next section, the actual structure of the BB contact line is of no importance for the thermodynamic description of the surface energy of the surfactant layer on the alveolar surface. Concerning the $\perp$-shaped BB contact lines appearing on that side of the surfactant layer, which faces the hypophase, we note that their molecular structure differs from the structure of the $+$-shaped ones in that the lower part of the vertical bilayer in square C in Fig. 1 is missing.

Surface energy of the alveolar surfactant layer at equilibrium

We consider a surfactant layer occupying area A (m^2) of the alveolar surface. The surfactant layer is presumed to be in thermodynamic equilibrium, which means that the chemical potentials of the surfactant molecules in the hypophase and in the layer are equal.

According to the monolayer model, the total surface energy Φ (J) of the surfactant layer is given by:

$$\phi = \sigma_{\mathrm{m}} A \tag{1}$$

where σ_{m} (J/m^2) is the specific surface energy of the monolayer. This is so, because this model treats the surfactant layer as a monolayer of molecules adsorbed at the hypophase/air interface.

Now, let us determine Φ in the case of the MB model under the assumption that the surfactant layer is with a fixed thickness h. Clearly, along with σ_{m}, the specific surface energy σ_{b} (J/m^2) of the bilayers will also contribute to Φ. Moreover, the MB and BB contact lines are characterized by specific line energies κ_{m} (J/m) and κ_{b} (J/m), respectively, and these two energies have also to be taken into account in the determination of Φ. An important distinction between the σ's and the κ's is that while the former are necessarily positive quantities, the latter can have either positive or negative values. Indeed, σ_{m} and σ_{b} refer to the contact between only two phases (hypophase and air in the case of σ_{m}, hypophase and hypophase in the case of σ_{b}), and thermodynamics tells us that these phases can coexist solely provided $\sigma_{\mathrm{m}} > 0$ and $\sigma_{\mathrm{b}} > 0$. Typically, $\sigma_{\mathrm{m}} \approx 25$–$30$ mJ/m^2 and $\sigma_{\mathrm{b}} \approx 1$ mJ/m^2. The physical reason for which κ_{m} and κ_{b} can be of either sign is that they are the energies per unit length of the contact line between more than two phases. As known from thermodynamic considerations (Toshev 1991), this line can exist regardless of whether κ_{m} and κ_{b} are positive, zero, or negative. The possibility for the specific line energy to be negative was noted already by Gibbs (1928), and Platikanov et al. (1980) demonstrated experimentally that this energy can change sign in dependence on the concentration of ions in one of the contacting phases. Experimentally determined absolute values of the specific line energy in various cases range between 0.1 and 10 nJ/m (for reviews see, e.g., Toshev et al. 1988; Exerowa et al. 1994).

Let the surfactant layer be built up of n layers of tubules and let the monolayer have N MB contact lines each of length L (m). The total surface energy Φ of the surfactant layer can then be written down as:

$$\phi = \sigma_{\mathrm{m}} A + n\sigma_{\mathrm{b}} A + N\sigma_{\mathrm{b}} hL + N\kappa_{\mathrm{m}} L + nN\kappa_{\mathrm{b}} L \tag{2}$$

Here the first summand is the contribution to Φ of the monolayer surface, and the σ_{b} terms allow for the surface energy of the bilayers: the surfactant layer contains n bilayers parallel and N bilayers normal to the monolayer, which have individual areas A and hL, respectively. The κ_{m} term represents the contribution to Φ of the N lines of MB contact on the alveolar surface. Likewise, the last summand in Eq. (2) takes into account the energy of the nN lines of BB contact in the hypophase (we ignore the possible difference in the values of

κ_b for the +-shaped and the ⊥-shaped BB contact lines). For the determination of the specific surface energy σ (J/m^2) of the surfactant layer, it is convenient to use the geometrical relationships:

$$A = NdL \tag{3}$$

$$h = nd \tag{4}$$

in order to cast Eq. (2) in the equivalent form:

$$\phi = \sigma_m A + (2\sigma_b h/d)A + (\kappa_m/d)A + (\kappa_b h/d^2)A \tag{5}$$

Thus, recalling the thermodynamic definition $\sigma \equiv \partial\Phi/\partial A$, from Eq. (5) we find that σ is given by:

$$\sigma = \sigma_m + (\kappa_m + 2\sigma_b h)/d + \kappa_b h/d^2 \tag{6}$$

This formula represents the specific surface energy of the alveolar surface as a function of the distance d between the MB contact lines, i.e., between the bilayers constituting the surfactant layer. In it, σ_m, σ_b, and h have more or less known values and only κ_m and κ_b are unknown parameters. As seen from Eq. (6), $\sigma = \sigma_m$ in the limit of $d = \infty$. This means that, as already noted, in this limit the MB model passes into the monolayer one [cf. also Eqs. (1) and (5) at $d = \infty$].

Thermodynamics requires that Φ be minimum under conditions of equilibrium. When the area A of the surface of the surfactant layer is fixed, this means that at equilibrium σ must be minimum, too. The equilibrium specific surface energy σ_e of the surfactant layer is thus the lowest value of σ and corresponds to the equilibrium distance d_e between the MB or BB contact lines. Physically, d_e and σ_e are important, as they represent the most probable spacing between the bilayers building up the surfactant layer and the thermodynamically favored value of the specific surface energy of this layer. Employing σ from Eq. (6) in the extremum condition $\mathrm{d}\sigma/\mathrm{d}d = 0$ at $d = d_e$, accounting that h is constant and treating the σ's and the κ's as d-independent, we obtain:

$$d_e = -2\kappa_b h/(\kappa_m + 2\sigma_b h) \tag{7}$$

$$\sigma_e = \sigma_m - (\kappa_m + 2\sigma_b h)^2/4\kappa_b h \tag{8}$$

Equation (8) is the sought formula for the equilibrium specific surface energy of the alveolar surfactant layer in the scope of the MB model. It says that at a fixed layer thickness h this model is physically relevant only if $\kappa_b > 0$, for only then is $\sigma_e < \sigma_m$. When this is the case, i.e., when $\kappa_b > 0$, taking into account that $d_e > 0$, from Eq. (7) we conclude that $\kappa_m + 2\sigma_b h < 0$, i.e., that $\kappa_m < 0$ (as already noted, σ_b and h are positive quantities). Thus, the negative line energy of the MB contact lines is the physical reason for which the specific surface energy of the alveolar surfactant layer at equilibrium is lower than σ_m when this layer is structured according to the MB model. This negative energy [the κ_m/d term in Eq. (6)] is counterbalanced by the positive surface and line energies [the $\sigma_b h/d$ and the $\kappa_b h/d^2$ terms in Eq. (6)] of the bilayers which form the network supporting the monolayer at the hypophase/air interface. Clearly, any physicochemical factor that makes $\kappa_m > 0$ (more accurately, $\kappa_m > -2\sigma_b h$) and/or $\kappa_b < 0$ will have an adverse effect on the MB structure of the alveolar surfactant layer.

Using Eqs. (7) and (8), we can eliminate the material parameters in the d terms in Eq. (6) and represent the $\sigma(d)$ dependence in the form:

$$\sigma/\sigma_m = 1 - (1 - \sigma_e/\sigma_m)[2d_e/d - (d_e/d)^2] \tag{9}$$

This expression reveals that the dimensionless specific surface energy σ/σ_m of the surfactant layer is a universal function of the dimensionless interbilayer distance d/d_e. This is illustrated in Fig. 2 at $\sigma_e/\sigma_m = 0.05$, 0.5, and 0.95 (as indicated). Curve 0.05 is that of practical interest, since at $\sigma_m = 25$ mJ/m^2 it corresponds to $\sigma_e = 1.25$ mJ/m^2. We see that the minimum of this curve is rather narrow, the implication being that the actual interbilayer distance cannot differ too much from the equilibrium distance d_e.

In some cases it may be convenient to express σ in terms of the surface pressure π (N/m) of the surfactant layer. If $\sigma_0 \approx 70$ mJ/m^2 is the specific surface energy of the hypophase/air interface in the absence of adsorbed surfactant, as known (Adamson 1976), $\pi \equiv \sigma_0 - \sigma$. Hence, Eq. (9) can be presented equivalently as:

$$\pi/\pi_m = 1 + (\pi_e/\pi_m - 1)[2d_e/d - (d_e/d)^2] \tag{10}$$

Here $\pi_m \equiv \sigma_0 - \sigma_m$ is the surface pressure of the monolayer, and $\pi_e \equiv \sigma_0 - \sigma_e$ is the equilibrium value of π. In conformity with Eq. (8), π_e is given by:

$$\pi_e = \pi_m + (\kappa_m + 2\sigma_b h)^2/4\kappa_b h \tag{11}$$

Equations (7) and (8) can be used for estimation of the line energies κ_m and κ_b with the aid of known values of d_e, h, σ_m, σ_b, and σ_e. Solving Eqs. (7) and (8) for κ_m and κ_b yields:

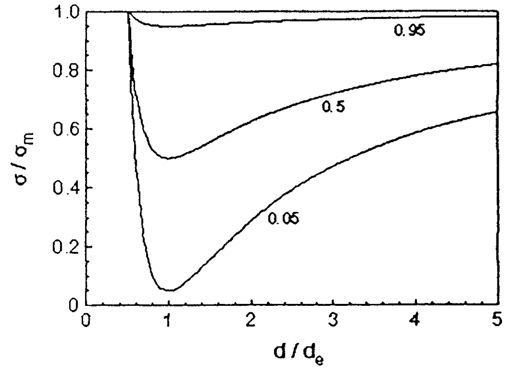

Fig. 2 Dimensionless specific surface energy of the alveolar surfactant layer as a function of the dimensionless distance between the bilayers supporting the monolayer. The *curves* are drawn according to Eq. (9) at $\sigma_e/\sigma_m = 0.05$, 0.5, and 0.95 (as indicated)

$$\kappa_{\mathrm{m}} = -2[(\sigma_{\mathrm{m}} - \sigma_{\mathrm{e}})d_{\mathrm{e}} + \sigma_{\mathrm{b}}h] \quad (12)$$

$$\kappa_{\mathrm{b}} = (\sigma_{\mathrm{m}} - \sigma_{\mathrm{e}})d_{\mathrm{e}}^2/h \quad (13)$$

With $d_{\mathrm{e}}=50$ nm, $h=250$ nm, $\sigma_{\mathrm{m}}=25$ mJ/m^2, $\sigma_{\mathrm{b}}=1$ mJ/m^2, and $\sigma_{\mathrm{e}}=1$ mJ/m^2, these equations lead to $\kappa_{\mathrm{m}} = -2.9$ nJ/m and $\kappa_{\mathrm{b}}=0.24$ nJ/m. These values of the specific line energies of the MB and BB contact lines are comparable with line-energy values known in the literature for other cases of contact along a line between more than two phases (Platikanov et al. 1980; Toshev et al. 1988; Exerowa et al. 1994).

Shown in Fig. 3 are the $d_{\mathrm{e}}(h)$ and $\sigma_{\mathrm{e}}(h)$ dependences of Eqs. (7) and (8). The calculation is done with the σ_{m}, σ_{b}, κ_{m}, and κ_{b} values given above. We observe that while the equilibrium interbilayer distance d_{e} increases almost proportionally to the thickness h of the surfactant layer, the equilibrium specific surface energy σ_{e} of the layer also increases with h, but in an essentially non-linear way. The important point is that only a thin enough surfactant layer can have the required sufficiently low $\sigma_{\mathrm{e}} \approx 1$ mJ/m^2. However, at a certain critical thickness h_{c} of the surfactant layer, σ_{e} vanishes, and below this thickness it becomes negative, because the surface and line energies of the bilayers in a too thin surfactant layer cannot counterbalance the negative line energy of the MB contact lines. Since $\sigma_{\mathrm{e}} \leq 0$ implies vanishing of the hypophase/air interface, this result means that the surfactant layer is subject to destruction if it is not sufficiently thick. It is worth noting that such a destruction might have some connection with the known respiratory distress syndrome caused by surfactant deficiency in the hypophase. Defining h_{c} as the solution of the equation $\sigma_{\mathrm{e}}(h_{\mathrm{c}})=0$, from Eq. (8) we find that:

$$h_{\mathrm{c}} = (\kappa_{\mathrm{b}}\sigma_{\mathrm{m}}/2\sigma_{\mathrm{b}}^2 - \kappa_{\mathrm{m}}/2\sigma_{\mathrm{b}}) \times [1 - (1 - 2\kappa_{\mathrm{m}}\sigma_{\mathrm{b}}/\kappa_{\mathrm{b}}\sigma_{\mathrm{m}})^{1/2}/ (1 - \kappa_{\mathrm{m}}\sigma_{\mathrm{b}}/\kappa_{\mathrm{b}}\sigma_{\mathrm{m}})] \quad (14)$$

As an example, using the above σ_{m}, σ_{b}, κ_{m}, and κ_{b} values in this formula yields $h_{\mathrm{c}}=243$ nm (the arrow in Fig. 3 indicates this value).

The effect of the thickness of the surfactant layer on the values of d_{e} and σ_{e} is illustrated also in Fig. 4, which displays the $\sigma(d)$ dependence at $h=250$, 350, and 450 nm (as indicated). The curves are drawn according to Eq. (6) with the σ_{m}, σ_{b}, κ_{m}, and κ_{b} values used for Fig. 3. As seen, at these σ and κ values only the 250 nm thick surfactant layer has the low enough equilibrium specific surface energy $\sigma_{\mathrm{e}}=1$ mJ/m^2. In this case, $d_{\mathrm{e}}=50$ nm and the distance d between the MB contact lines can depart from d_{e} without making σ increase excessively over σ_{e}. This result is of importance, as it indicates that the MB structure of the alveolar surfactant layer has the ability to ensure normal functioning of the alveolus in dynamic regime, i.e., during the respiration cycle which involves a certain variation of d.

The sensitivity of d_{e} and σ_{e} to the values of the specific line energies κ_{m} and κ_{b} is illustrated in Figs. 5 and 6, respectively. The calculation is done according to Eqs. (7) and (8) at fixed $h=250$ nm and again with the help of the σ_{m}, σ_{b}, κ_{m}, and κ_{b} values used for Figs. 3 and 4. We see that κ_{m} and κ_{b} must be neither too high nor too low in order for the σ_{e} value to be close enough to the physiologically relevant value of about 1 mJ/m^2. In other words, any factor (e.g., the concentration of ions in the hypophase) affecting sufficiently strongly the values of κ_{m} and/or κ_{b} can cause a malfunctioning of the alveolus by making $\sigma_{\mathrm{e}} \gg 1$ mJ/m^2 (then the alveolus can collapse) or $\sigma_{\mathrm{e}} \leq 0$ (then the alveolar surfactant layer is subject to destruction).

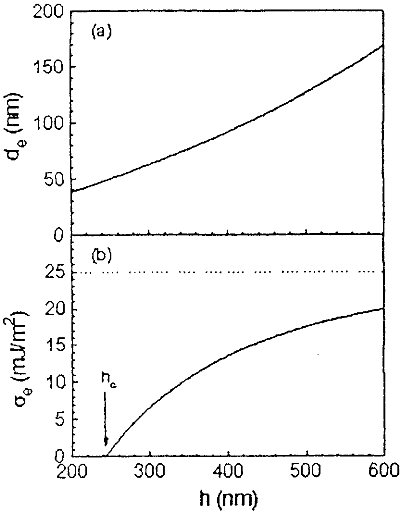

Fig. 3 Dependence of **a** the equilibrium interbilayer distance and **b** the equilibrium specific surface energy of the alveolar surfactant layer on the thickness of the layer. The *curves* are drawn according to Eqs. (7) and (8), the *dotted line* visualizes the σ_{m} value, and the *arrow* indicates the value of the critical thickness h_{c}

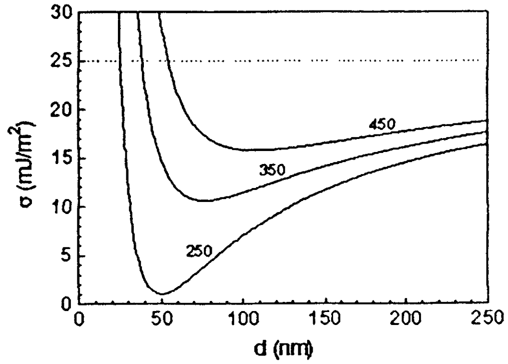

Fig. 4 Dependence of the specific surface energy of the alveolar surfactant layer on the distance between the bilayers supporting the monolayer. The *curves* are drawn according to Eq. (6) at $h=250$, 350, and 450 nm (as indicated), and the *dotted line* visualizes the σ_{m} value

Surface energy of the alveolar surfactant layer in dynamic regime

The specific surface energy σ of the alveolar surface layer varies during the respiration cycle. There exists experimental evidence (e.g., Bachofen et al. 1987; Scarpelli 1988) that σ increases with increasing lung volume and that while it is below 1 mJ/m^2 when the lung is less than 40% of maximum inflation, it is already 15–20 mJ/m^2 at around 80% of the maximum inflation of the lung. We shall now see what is the variation of σ predicted by the MB model of the alveolar surfactant layer in dynamic regime characterized by the condition $d \geq d_e$. Physically, this condition corresponds to the surfactant layer in a state of equilibrium at the onset of inspiration (then $d = d_e$, Fig. 7a) and in a state of lateral expansion (then $d > d_e$, Fig. 7b) at a later moment of inspiration.

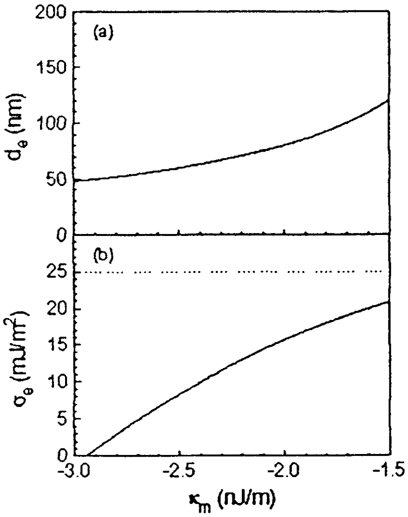

Fig. 5 Dependence of **a** the equilibrium interbilayer distance and **b** the equilibrium specific surface energy of the alveolar surfactant layer on the specific line energy of the MB contact line. The *curves* are drawn according to Eqs. (7) and (8), and the *dotted line* visualizes the σ_m value

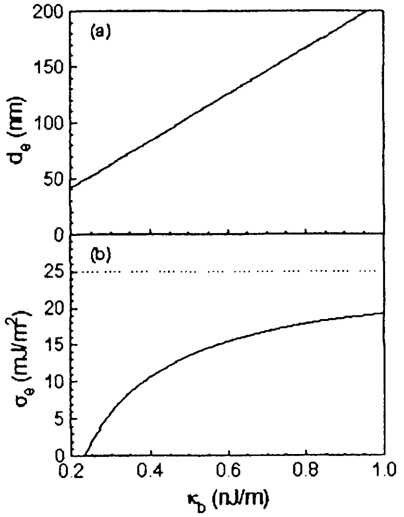

Fig. 6 Dependence of **a** the equilibrium interbilayer distance and **b** the equilibrium specific surface energy of the alveolar surfactant layer on the specific line energy of the BB contact line. The *curves* are drawn according to Eqs. (7) and (8), and the *dotted line* visualizes the σ_m value

To determine the $\sigma(d)$ dependence in dynamic regime we can employ Eq. (2) upon accounting that now the number n of the bilayers parallel to the monolayer is invariable. To simplify the treatment we shall assume (1) that the distance between these bilayers remains fixed and equal to the equilibrium distance d_e (see Fig. 7) and (2) that the change in the area A of the surface of each of these bilayers is negligible. The former assumption means that in dynamic regime in lieu of Eq. (4) we have:

$$h = nd_e \tag{15}$$

and the latter assumption is consistent with the suggestion of Larsson et al. (1999) that the bilayers building up the surfactant layer can have a wavy surface (such a surface can keep its area the same while d varies with time). Thus, using Eqs. (3) and (15) in Eq. (2) and recalling that $\sigma \equiv \partial\Phi/\partial A$, we obtain:

$$\sigma = \sigma_m + \sigma_b h/d_e + (\kappa_m + \sigma_b h + \kappa_b h/d_e)/d \tag{16}$$

This formula describes the change of the specific surface energy of the alveolar surfactant layer in dynamic regime, i.e., under non-equilibrium conditions involving variation of d with time. At equilibrium $d = d_e$ and in view of Eq. (7), as it should be, σ from Eq. (16) assumes its equilibrium value σ_e given by Eq. (8). We emphasize that in Eq. (16) σ_m may or may not depend on the momentary value of the distance d between the MB contact lines. Indeed, in the limit of no adsorption/desorption of molecules in the surfactant monolayer during respiration, σ_m increases with increasing d, because a greater d corresponds to a larger area A_m per molecule in the monolayer. In the opposite extreme, i.e., when adsorption/desorption is a sufficiently fast process, the area

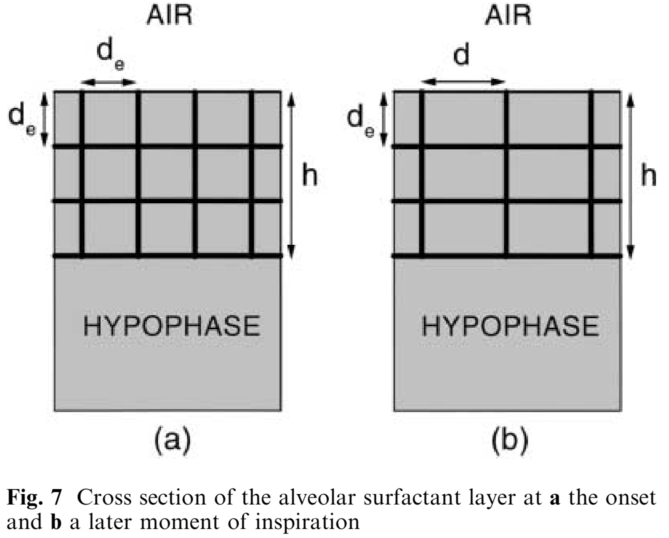

Fig. 7 Cross section of the alveolar surfactant layer at **a** the onset and **b** a later moment of inspiration

A_m occupied by a molecule in the monolayer is the same at any value of d so that σ_m is d-independent. Clearly, determination of the $\sigma_m(d)$ function and, hence, of the complete d dependence of σ from Eq. (16) requires knowledge of the rate of the adsorption/desorption process and, if this is too slow, of the $\sigma_m(A_m)$ isotherm of the virtually insoluble monolayer.

To get a feeling about the $\sigma(d)$ dependence of Eq. (16) we shall consider it in the case of d-independent σ_m. In this case, with the aid of Eqs. (7) and (8) we can represent Eq. (16) in the equivalent form:

$$\sigma = \sigma_e + \sigma * (1 - d_e/d) \tag{17}$$

where $\sigma^* > 0$ is a constant factor given by:

$$\sigma^* = -(\kappa_m + \sigma_b h + \kappa_b h/d_e)/d_e \tag{18}$$

With $d_e = 50$ nm, $h = 250$ nm, $\sigma_b = 1$ mJ/m^2, $\kappa_m = -2.9$ nJ/m, and $\kappa_b = 0.24$ nJ/m, from Eq. (18) it follows that $\sigma^* = 29$ mJ/m^2.

The $\sigma(d)$ dependence of Eq. (17) is displayed in Fig. 8, the calculation being done with $\sigma_e = 1$ mJ/m^2 and the above value of σ^*. We observe that, for instance, a 50% increase of the distance d over d_e leads to a considerable increase of σ. This behavior of σ is in qualitative agreement with that determined experimentally in the course of lung inflation (e.g., Bachofen et al. 1987; Scarpelli 1988). Physically, σ increases with d, since the MB contact lines at the hypophase/air interface carry less negative energy per unit interface area when they are more distanced from each other than at equilibrium.

Finally, we note that the general d-dependence of the surface pressure π of the alveolar surfactant layer in dynamic regime can be represented by the formula:

$$\pi = \pi_m - \sigma_b h/d_e - (\kappa_m + \sigma_b h + \kappa_b h/d_e)/d \tag{19}$$

This formula follows from Eq. (16) and in it, like the monolayer specific surface energy σ_m in Eq. (16), the monolayer surface pressure π_m may or may not depend on d. The $\pi_m(d)$ dependence has to be accounted for in the case of virtual absence of adsorption/desorption in the monolayer during respiration and is determined by the $\pi_m(A_m)$ isotherm of the monolayer. When π_m is d-independent, i.e., when adsorption/desorption is fast enough, Eq. (19) turns into the expression:

$$\pi = \pi_e - \sigma * (1 - d_e/d) \tag{20}$$

which can be obtained also from Eq. (17) and in which the constants d_e, π_e, and σ^* are given by Eqs. (7), (11), and (18).

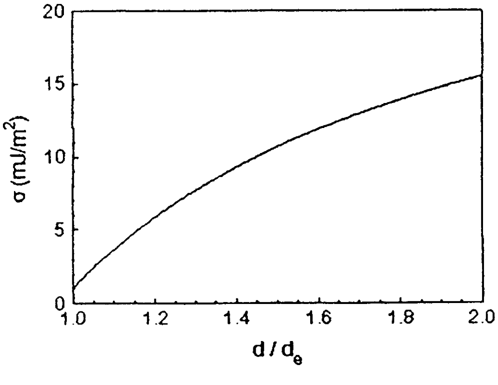

Fig. 8 Dependence of the specific surface energy of the alveolar surfactant layer on the distance between the MB contact lines in dynamic regime. The curve is drawn according to Eq. (17)

Conclusion

The MB model considered here appears as a generalization of the known monolayer model of the structure of the alveolar surfactant layer. The MB model provides a more realistic description of this layer, as it leads to values of the specific surface energy of the layer which are low enough to allow normal functioning of the alveolus during the respiration cycle. Also, the MB model unites in a simple way the known monolayer and bilayer models of the alveolar surfactant layer.

The dependence of the equilibrium interbilayer distance d_e and specific surface energy σ_e on the thickness h of the surfactant layer and the specific line energies κ_m and κ_b of the MB and BB contact lines is given by Eqs. (7) and (8). These equations reveal that σ_e can have values comparable with or lower than 1 mJ/m^2 only if the layer is thin enough and if $\kappa_m < 0$ and $\kappa_b > 0$. However, if the layer is too thin, κ_m is too negative, and/or κ_b is too close to zero, σ_e becomes negative and the layer is subjected to destruction. Thus, the MB model of the alveolar surfactant layer leads to the conclusion that normal respiratory activity of the alveolus is possible under rather stringent physicochemical conditions.

In dynamic regime, the dependence of the specific surface energy σ of the alveolar surfactant layer on the distance d between the MB contact lines is described by Eq. (16) in general and by Eq. (17) in the particular case of fast enough adsorption/desorption in the surfactant monolayer. The change in σ predicted by Eq. (17) is in qualitative agreement with that determined experimentally during lung inflation and deflation.

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References

Adamson AW (1976) Physical chemistry of surfaces, Wiley, New York

Bachofen H, Schürch S, Urbinelli M, Weibel ER (1987) Relations among alveolar surface tension, surface area, volume and recoil pressure. J Appl Physiol 62:1878–1887

Clements JA, Brown ES, Johnson RP (1958) Pulmonary surface tension and the mucus lining of the lungs: some theoretical considerations. J Appl Physiol 12:262–268

Cordova M, Mautone AJ, Scarpelli EM (1996) Rapid in vitro tests of surfactant film formation: advantages of the Exerowa black film method. Pediat Pulmonol 21:373–382

Cosgrove T, Zasadzinski JA (1998) Imaging and other techniques. Seeing is believing: the role of microscopy in determining morphology and kinetics of colloids. Curr Opin Colloid Interface Sci 3:123–124

Exerowa D, Kruglyakov PM (1998) Foam and foam films. Elsevier, Amsterdam

Exerowa D, Lalchev Z (1986) Bilayer and multilayer foam films – model for study of the alveolar surface and stability. Langmuir 2:668–671

Exerowa D, Lalchev Z, Kashchiev D (1984) Stability of foam lipid bilayers of amniotic fluid. Colloids Surf 10:113–121

Exerowa D, Lalchev Z, Marinov B, Ognyanov K (1986) Method for assessment of fetal lung maturity. Langmuir 2:664–667

Exerowa D, Kashchiev D, Platikanov D (1992) Stability and permeability of bilayers. Adv Colloid Interface Sci 40:201–256

Exerowa D, Kashchiev D, Platikanov D, Toshev BV (1994) Linear energy with positive and negative sign. Adv Colloid Interface Sci 49:303–339

Gibbs JW (1928) Collected works, vol. 1. Longmans-Green, New York

Gil J, Weibel ER (1969/1970) Improvements in demonstration of lining layer of lung alveoli by electron microscopy. Respir Physiol 8:13–36

Kashchiev D, Exerowa D (1980) Nucleation mechanism of rupture of Newtonian black films. I. Theory. J Colloid Interface Sci 77:501–511

Kashchiev D, Exerowa D (1983) Bilayer lipid membrane permeation and rupture due to hole formation. Biochim Biophys Acta 732:133–145

Kashchiev D, Exerowa D (1998) Lifetime of a foam bilayer in contact with an insoluble monolayer. J Colloid Interface Sci 203:146–152

King RJ, Clements JA (1985) Lipid synthesis and surfactant turnover in the lungs. In: Fishman AP, Fisher AB, Geiger SR (eds) Handbook of physiology. The respiratory system, vol 1. American Physiology Society, Bethesda, pp 309–327

Larsson M, Larsson K, Andersson S, Kakhar J, Nylander T, Ninham B, Wollmer P (1999) The alveolar surface structure: transformation from a liposome-like dispersion into a tetragonal CLP bilayer phase. J Disp Sci Technol 20:1–12

Nichols BA (1976) Normal rabbit alveolar macrophages. I. The phagocytosis of tubular myelin. J Exp Med 144:906–919

Nikolova A, Exerowa D (1996) Foam bilayer of amniotic fluid: formation and phase state. Langmuir 12:1846–1850

Platikanov D, Nedyalkov M, Nasteva V (1980) Line tension of Newton black films. II. Determination by the diminishing bubble method. J Colloid Interface Sci 75:620–628

Sanderson RJ, Vatter AE (1977) A mode of formation of tubular myelin from lamellar bodies in the lung. J Cell Biol 74:1027–1031

Scarpelli EM (1988) Surfactants and the lining of the lung. The Johns Hopkins University Press, Baltimore

Scarpelli EM (1998) The alveolar surface network: a new anatomy and its physiological significance. Anat Rec 25:491–527

Scarpelli EM, Mautone AJ (1994) Surface biophysics of the surface monolayer theory is incompatible with regional lung function. Biophys J 67:1080–1089

Scarpelli EM, Mautone AJ, Lalchev Z, Exerowa D (1997) Surfactant liquid and black foam films: formation, stability and correlative function in vivo. Colloids Surf B 8:133–145

Schürch S, Green FHY, Bachofen H (1998) Formation and structure of surface films: captive bubble surfactometry. Biochim Biophys Acta 1408:180–202

Stratton CJ (1977) The three-dimensional aspect of the mammalian lung surfactant myelin figure. Tissue Cell 9:285–300

Toshev BV (1991) On Gibbs' phase rule. Langmuir 7:569–571

Toshev BV, Platikanov D, Scheludko A (1988) Line tension in three-phase equilibrium systems. Langmuir 4:489–499

Weibel ER (1986) Functional morphology of lung parenchyma. In: Macklem PT, Mead J (eds) Handbook of physiology. The respiratory system, vol 3. American Physiology Society, Bethesda, pp 89–111

Williams MC (1977) Conversion of lamellar body membranes into tubular myelin in alveoli of fetal rat lungs. J Cell Biol 72:260–277