Graphic Analysis of Contraction-Distension Isotherms for Pulmonary Surfactant Monolayers

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A new method for analyzing the surface-active properties of pulmonary surfactants is proposed, based on calculating a "local stability index" and then plotting this index as a function of S/S_0, where S_0 and S are, respectively, the initial and contracted areas of the surfactant monolayer formed at the interface. The course of the curves thus obtained allows for reasonably precise and easy comparisons of alterations in the surface-active properties of pulmonary surfactants in response to different factors acting on the body.

Key Words: *pulmonary surfactants; surface-active properties; surface tension; analytical methods*

One of the most informative approaches to evaluating the functional state of the pulmonary surfactant system is to study the surface activity of surfactants and how their monolayers formed on a liquid support behave [3,9]. A standard study of surface-active properties of the pulmonary surfactant system involves analysis of isotherms of surface tension of pulmonary surfactant monolayers. Such isotherms are obtained during the cyclic contraction (up to 20% of the initial area) and distension of these monolayers (Fig. 1). Subsequent processing of the isotherms consists, in most cases, in determining the maximal and minimal surface tension values and then calculating a stability index (SI) [7] and a loop-shape index (ρ) [9], which are regarded as the most useful indices for evaluating surface-active properties of surfactants [2]. It should be noted that changes with time in the isotherms of contraction-distension of surfactant monolayers are taken into account only when the ρ index is calculated.

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However, the classic methods of examining the properties of surface-active substances using the monolayer technique presuppose the need for analyzing dynamic isotherms [1,8].

We have developed a method that takes into consideration temporal changes in surface tension during the cyclic contraction and distension of pulmonary surfactant monolayers and makes graphic representation of the results possible. This method is described below.

MATERIALS AND METHODS

Wistar rats weighing 200-250 g were used. They were subjected to total-body irradiation in a dose of 1 Gy. Surfactants were isolated as previously described [3].

Contraction-distension isotherms for pulmonary surfactant monolayers formed at the 0.9% NaCl solution-air interface were recorded using a device consisting of (1) a glass Langmuir cuvette (290×145×25 mm, 1.1 liters) with a movable Teflon barrier and (2) a modified Wilhelmi balance (based on an electromechanical converter connected to a half-submerged 45×10×0.1 mm rough platinum plate permitting automatic recording of dynamic isotherms (sensitivity 0.1 mN/m) [4-6]. Physiological saline

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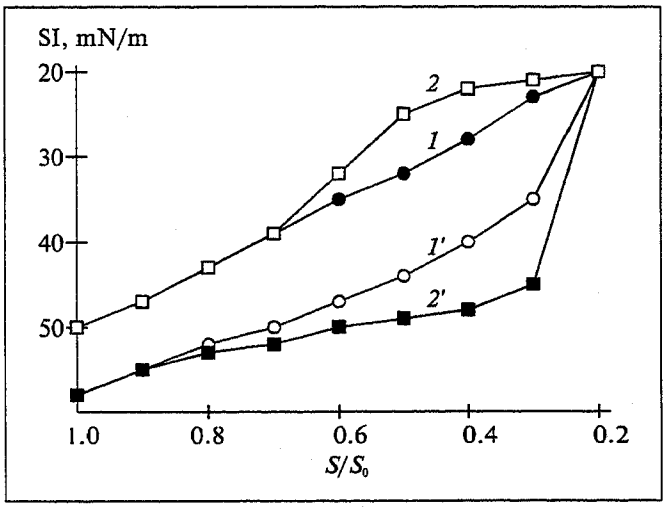

Fig. 1. Contraction–distension isotherms for pulmonary surfactant monolayers.

was prepared with NaCl of analytical grade and twice-distilled water. The subphase was continuously stirred. The barrier moved at a rate of 6.2 cm/min.

The hysteresis loop resulting from the first contraction-distension cycle was separated into segments by vertical lines intersecting the abscissa at points to which decimal values of the ratio between the altered (S) and initial (S_o) areas of the monolayer corresponded (Fig. 1). At points where the lines intersected the isotherms, surface tension values during monolayer contraction and distension (σ_{con} and σ_{dis}, respectively) were determined. The resultant σ_{con} and σ_{dis} pairs corresponding to particular S/S_o ratios were then used to calculate the "local" SI by the formula:

$$SI=2(\sigma_{dis}-\sigma_{con})/(\sigma_{dis}+\sigma_{con}).$$

From the data obtained, plots of SI as a function of (S/S_o) were constructed in Cartesian coordinates. The plots obtained for each of the contraction-distension isotherms shown in Fig. 1 are depicted in Fig. 2.

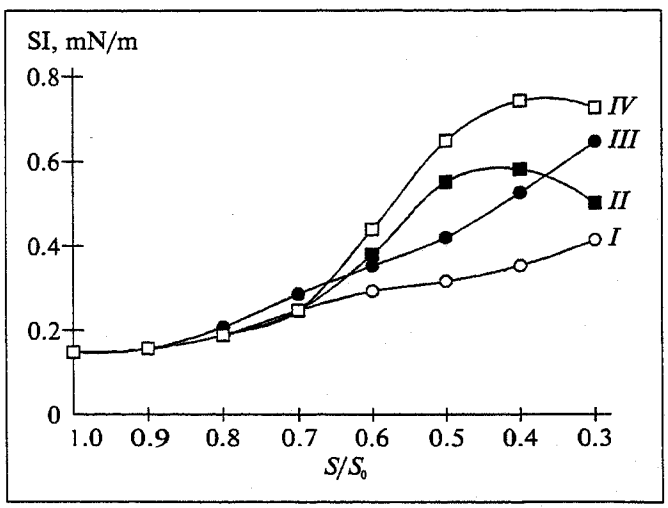

Fig. 2. Stability index (SI) as a function of S/S_o.

RESULTS

Figure 1 shows a schema of contraction-distension isotherms for equilibrium surfactant monolayers, both normal (*1*, *1'*) and after their surface-active properties were altered at different times after exposure to ionizing radiation (*2*, *2'*). In our experiments on rats we obtained four different shapes of hysteresis loops for pulmonary surfactant monolayers (unpublished data). They are described by the following combinations of isotherms: *1* and *1'* (normal) and *2* and *1'*, *1* and *2'*, and *2* and *2'* after exposure to ionizing radiation. Curve *2*, which reflects a reduction in surface tension at a lower value of monolayer contraction, may be indicative of an altered structure of the monolayer components and, consequently, of qualitative changes in monolayer composition, e.g., an increase in the amount of cholesterol. The course of distension isotherm *2'* - a rapid rise of surface tension in the initial stage of monolayer distension - is an indication that considerable amounts of water-soluble components are present in the surfactant monolayer. The combination of curves *2* and *2'* characterizes the behavior of a monolayer with densely packed water-soluble surfactant components.

In line with the traditional approach, Table 1 shows values of minimal surface tension (ST_{min}) and the results of calculating the indices SI and ρ for the following isotherm combinations: *1* and *1'* (*I*), *2* and *1'* (*II*), *1* and *2'* (*III*), and *2* and *2'* (*IV*). It is easy to see that the heterodirectional changes in the shape of hysteresis loops, which characterize not only alterations in surface-active properties, but also changes in the composition of surfactant monolayers, have little or no effect on the ρ value. Thus, even the "loop-shape index" ρ does not necessarily reflect the true direction of changes in the shape of hysteresis loops in surfactant monolayers. As for the SI, it does not change at all, despite marked changes in the shape of the loops.

With the graphic approach it is possible to avoid errors in interpreting the results from a study of surface-active properties of surfactants. Indeed, Fig. 2 presents plots of SI as a function of S/S_o calculated for each of the loops shown in Fig. 1. SI values were calculated as described in *Materials and Methods* for the same isotherm combinations, i.e., *1* and *1'* (*I*), *2* and *1'* (*II*), *1* and *2'* (*III*), and *2* and *2'* (*IV*). By comparing these figures, we may conclude that the course of the curves clearly reflects any changes in the shape of hysteresis

TABLE 1. Standard Characteristics of Pulmonary Surfactant Monolayers with Altered Surface–Active Properties

Isotherm combination	ST_{min}, mN/m	SI	ρ
I	20	0.857	0.227
II	20	0.857	0.281
III	20	0.857	0.280
IV	20	0.857	0.327

Note. ST = surface tension.

loops of surfactant monolayers and, consequently, changes in their surface-active properties.

The method proposed, by which the contraction-distension isotherms for surfactant monolayers are represented in graphic form, may be a useful adjunct to the traditional techniques, especially when a large quantity of experimental data have to be analyzed. It is hoped that this method will find practical application in studies of surface-active properties of the pulmonary surfactant system.

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