Introduction

Pulmonary surfactants cover the alveoli of the lungs and have a vital function in making the process of breathing easy. During inhalation, the surfactant reduces the surface tension of tissue by a factor of about 15 making it much easier to inflate the alveoli. During exhalation, the surface area of the alveoli decreases making the surfactant even more concentrated on the surface. This yields a near-zero surface tension at the end of exhalation, which prevents the alveoli from collapsing.

Dipalmitoylphosphatidylcholine (DPPC) is one of the phospholipids present on the alveoli surface. It is known that the highly ordered solid phase of DPPC sustains the near-zero surface tension on the alveoli during exhalation. [1] Other phospholipids cannot normally be compressed to such low surface tensions at close-to body temperatures. Traditionally selective enrichment, the so-called squeeze-out model, is used to explain how a dense film of DPPC is formed on the alveoli surface. In the model, phospholipids other than DPPC are gradually removed from the interfacial monolayer during compression (exhalation) and only a film consisting purely of DPPC is left.

In order to model the actual surfactant behavior in the alveoli, measurements at near-zero surface tensions are needed. Several groups have investigated the behavior of DPPC under low surface tensions by using Langmuir troughs [2,3], but with a conventional Langmuir trough it is challenging to measure near-zero surface tensions. Previously, captive bubble surfactometers and Langmuir systems with rhombic (diamond shape) frames have been used for these measurements [4-6] but they generally suffer from poor accuracy and/or control over the amount of the deposited surfactant.

In fact, there is a lack of highly controlled, direct experimental observations of natural surfactants at the physiologically relevant low surface tension range.

In this application note we show that a KSV NIMA Langmuir Ribbon Barrier Trough can be used to measure near-zero surface tensions of DPPC. In a Langmuir Trough, the measured surface pressure \( \pi \) is equal to the surface tension of the subphase in absence of a monolayer \( \gamma_0 \) minus the observed surface tension with the monolayer present \( \gamma \):

\[
\pi = \gamma_0 - \gamma
\]

As the surface tension of the monolayer approaches the surface tension of the water subphase (73 mN/m), surfactant begins to leak out between the barriers of the Langmuir trough. The KSV NIMA Langmuir Ribbon Barrier Trough prevents the leakage by using a Ribbon Barrier and controlled compression of the monolayer area.
Materials and methods
A Langmuir monolayer was created by spreading 15 µl of a 1 mg/ml DPPC solution in chloroform onto an ultrapure water surface using a KSV NIMA Langmuir Ribbon Barrier Trough. For reference, a similar monolayer of DPPC was also created using a regular KSV NIMA Langmuir Trough. After spreading, the films were left undisturbed for 15 minutes to allow the solvent to evaporate. The layers were compressed to a target surface pressure of 80 mN/m with a rate of 10 mm/min. During compression, the surface pressure - area (π-A) isotherms were recorded.

Results and discussions
The surface pressure isotherms obtained using the conventional KSV NIMA Langmuir Trough and the KSV NIMA Langmuir Ribbon Barrier Trough are presented in Figure 1. The compression of both films was started from pressures below 1 mN/m. With the regular Trough, a surface pressure of 62 mN/m was achieved, after which the barriers started leaking. With the Ribbon Trough, a stable surface pressure as high as 72 mN/m could be reached.

It has been shown that on the lung surface the squeeze-out of other phospholipids than DPPC happens at a surface pressure of 40-50 mN/m, where a clear plateau region in the surface pressure - area isotherm can be observed. [3] In the plateau region, the film reorganizes and transforms from a fluid monolayer to a condensed layer that can sustain high surface pressures without collapse. It can be seen from Figure 1 that the plateau region is very narrow, if not almost non-existent in the Ribbon Barrier Trough measurements. This indicates that the measurement itself does not significantly perturb the compression. Thus, the experiment clearly shows that very high DPPC packing densities can be achieved.

Conclusions
The study shows that the KSV NIMA Langmuir Ribbon Barrier Trough can be used to compress DPPC films on an ultrapure water surface to near-zero surface tensions. The method enables easy, highly controlled and reliable compression measurements on natural phospholipid surfactants and allows experimental examination of the phase transitions and the selective enrichment process of DPPC on the alveoli surface.

This note demonstrates that the KSV NIMA Langmuir Ribbon Barrier Trough is suitable for studying monolayers at high surface pressures. Such high pressures can be of interest in a number of applications (not only DPPC). Deposition onto solid samples of such high density monolayers can be achieved with the KSV NIMA Langmuir-Blodgett Ribbon Barrier Trough.

References: