Experimental report

08/04/2026

Proposal: **INTER-613** **Council:** 4/2025

Title: The complexity of commercial and model pulmonary surfactants under neutron reflectometry

Research area:

This proposal is a new proposal

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Samples: Curosurf, lipids and proteins solutions in chloroform

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	30/08/2025	01/09/2025

Abstract:

FINAL REPORT

Proposal: INTER-613

Introduction

Pulmonary surfactant is a lipid/protein complex that coats the respiratory surface in mammals minimizing the surface tension at the end of expiration, what is crucial for preventing alveolar collapse [1]. This system is organized as an interfacial monolayer connected to 3-D subphase reservoirs being very dynamic and versatile in order to sustain the demanding conditions of breathing mechanics.

3D structures associated to the interfacial layer are formed at high enough surface pressures to allow for the rapid adsorption of lipids to the interface when needed, as demonstrated in previous NR experiments with synthetic samples [2] only in the presence of SP-B, a hydrophobic surfactant protein in charge of prompting membrane-membrane and membrane-interface contacts by forming lipid channels able to mobilize phospholipids [3]. However, in the presence of only SP-C, the other hydrophobic and biophysically active surfactant protein, no reservoirs were detected.

Aim

In this project we aimed at studying the structure of interfacial films formed by synthetic samples in presence of SP-B and SP-C without its previous separation, as native interactions between them could be present and the synergy that is reported to occur between them may still persist, obtaining different NR profiles from the ones corresponding to the proteins separately [3].

Furthermore, we also analyzed the behavior of the clinical surfactant Curosurf® in optimal and suboptimal (expired) conditions. Curosurf® is a clinical surfactant developed by Chiesi Farmaceutici, from Parma, Italy, widely administered at the neonatology services of European hospitals to treat respiratory disorders that curse with surfactant malfunctioning and require supplementation with exogenous therapeutic surfactant formulations. These experiments were performed to determine to what extent currently used therapeutic formulations can efficiently mimic operative surfactants. This is crucial in order to design and produce better clinical surfactants to treat respiratory pathologies still unresolved.

Experimental design and samples analysed

Our experimental design was the following: hydrogenated or deuterated version of phospholipid mixtures mimicking lung surfactant composition

(DPPC/POPC/POPG 50:25:15 w:w:w) in the presence or absence of physiological or supra-physiological concentrations of the hydrophobic protein fraction of surfactant (containing both SP-B and SP-C) were injected directly onto the air-liquid interface of a Langmuir trough to reach a surface pressure of 20-25 mN/m. The aqueous subphase employed was a buffer containing Tris 5mM NaCl 150mM pH7.4 in D_2O or Air Contrast Match Water. Prior to beginning with the experiment, 10 minutes were left for chloroform evaporation. Afterwards, the barrier of the Langmuir trough was compressed until reaching a surface pressure of first 45 mN/m and then 50 mN/m and neutron reflection was recorded at both surface pressures.

With Curosurf® samples, measurements at 45 mN/m were performed, although very different amounts of sample upon spreading were needed depending on whether the sample was expired or not. When trying to reach higher surface pressures of 55 or 60 mN/m, we reached the collapse.

Results

In all cases, reservoirs were detected with the x10 amount of protein, while not for the native protein amount. These results agree with previous measurements in FIGARO (#8-02-1004) in which the same synthetic samples but with proteins that had been previously isolated from one another were measured. Only when the amount of protein was increased up to 10 % of each protein, the formation of reservoirs was detected. In these experiments, we studied films compressed to surface pressures of 35 and 45 mN/m, before and after surface cycling between 35 and 45 mN/m, as higher surface pressures were not reachable due to overflow. Only at 45 mN/m the appearance of 3D reservoirs was observed. That is why, experiments were only performed at 45 mN/m or higher surface pressures in this proposal.

Native surfactant was also characterized in one previous proposal (#8-02-865) not being able to detect any 3D structure due to low compression velocity, low surface pressures reached and low amount of protein within this sample. In Curosurf® samples, formation of 3D structures was detected for the first time in a commercial surfactant only when experiments were performed starting from a very concentrated surfactant solution (40 mg/ml). However, data analysis is still undergoing.

In an already granted beam time that will take place next June (#8-02-1078) the organic extract from which Curosurf ® is elaborated before and after its filtration and same samples compressed to the highest surface pressures of 50, 60 and 70 mN/m taking advantage of the leak-proof design of the quadrotrough [4].

References: [1] Pérez-Gil, J. 2022, Biomed J, **45**(4), 615-628. [2] **(2)** B. Olmeda, B. Garcia-Alvarez, M.J. Martinez, M. Martinez-Calle, A. Cruz, J. Perez-Gil. 2015, FASEB J. **29**, 42364247. [3] Collada, A. et al. 2025, J Colloid Interface Sci., **701**, 138769. [4] Tein et al. 2022, Rev Sci. Instrum, **93**, 093903.