

Experimental report

11/07/2024

Proposal: 9-10-1787

Council: 4/2023

Title: Effect of pH on the internal structure of protein stabilized foams studied by SANS

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: casein
Bovine Serum Albumin
Beta Lactoglobulin

Instrument	Requested days	Allocated days	From	To
D33	3	2	08/12/2023	10/12/2023

Abstract:

Foams stabilized by proteins will be investigated by small angle neutron scattering (SANS) in order to elucidate the internal foam structure. Therefore, foams stabilized by three different proteins Beta Lactoglobulin, Casein and Bovine Serum Albumin at varying pH values will be investigated. From studies at single foam films, it is known that the different types of proteins show different tendencies for aggregation and network formation which is affected by the pH value. We want to know how the aggregation/networks affect the inner foam structure and the foam stability. In addition, structures with different liquid volume fractions in the foam will be studied. In this context the question of thinning of the foam films during foam drainage will be addressed. This experiment will be realized by probing a steady state foam at different heights in our home-built sample environment, which was designed to study macroscopic foams by SANS.

Effect of pH on the internal structure of protein stabilized foams studied by SANS

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Date Performed: 08.12.2023 – 10.12.2023

Proposal number: **9-10-1787**

Beamline: **D33**

ABSTRACT Foams stabilized by proteins will be investigated by small angle neutron scattering (SANS) in order to elucidate the internal foam structure. Therefore, foams stabilized by three different proteins Beta Lactoglobulin, Casein and Bovine Serum Albumin at varying pH values will be investigated. From studies at single foam films, it is known that the different types of proteins show different tendencies for aggregation and network formation which is affected by the pH value. We want to know how the aggregation/networks affect the inner foam structure and the foam stability. In addition, structures with different liquid volume fractions in the foam will be studied. In this context the question of thinning of the foam films during foam drainage will be addressed. This experiment will be realized by probing a steady state foam at different heights in our home-built sample environment, which was designed to study macroscopic foams by SANS.

EXPERIMENT DESCRIPTION AND OBJECTIVES Small-angle neutron scattering experiments were conducted on the D33 beamline at the Institute Laue Langevin (ILL, Grenoble, France). Using a neutron wavelength of $\lambda = 6.0017 \text{ \AA}$ (resolution $\Delta\lambda/\lambda = 0.1$) and sample-detector distances of 1.7 m to 10.3 m we covered a q-range of 0.0036 to $0.3370 \text{ \AA}^{-1}$. Sample foams were produced in a Teclis FoamScan cell (different to what was mentioned in the proposal) with a path-length of 3.5 cm by sparging N_2 gas with 10 ml/min up to 50ml/min through a porous glass disk. After foam creation, the N_2 flow was adjusted for every sample to counteract the foam collapse and guarantee a constant foam height. The data reduction was done on site using the “grasp” code.

MATERIALS The globular protein Beta Lactoglobulin (BLG) was kindly provided by the group of Ulrich Kulozik (TU München, Germany), the protein Bovine Serum Albumin (BSA) was provided by Santa Cruz Biotechnology Inc. (San Diego, California, United States), the protein whole Casein (CN) was provided by EMD Chemicals Inc. (San Diego, California, United States) and Lupine Protein Isolate was provided from Piowald GmbH (Mühbrook, Germany). All samples were prepared with D_2O , purchased from Sigma Aldrich (Darmstadt, Germany). The pH was set via addition of HCl (Sigma Aldrich (Darmstadt, Germany)) or NaOH (Sigma Aldrich (Darmstadt, Germany)) around the isoelectric point (IEP) of the individual protein solution. The chemicals were used without further purification. Before use, all glassware was cleaned by soaking in aqueous Q9 solution and rinsed extensively with ultrapure milliQ water ($18.2 \text{ M}\Omega\text{cm}$ resistivity, Merck, Darmstadt, Germany). The samples were prepared from stock solutions that were not older than one day and stored in a cooled environment.

DATA ANALYSIS The data are fitted using a code provided by Leonardo Chiappisi, which is based on a code described in Kühnhammer et al. [1]. The fit-model consists of (incoherent) superposition of individual reflectivity curves with normal distributed film thicknesses, a Porod- and a background-contribution. In the present work the Porod-contribution was further refined by a superposition of small angle contribution generated by the proteins and plateau borders ($I \sim N \cdot \Phi$) as introduced by

Lamolinaire et al. [2], where N is the protein number density and Φ is the liquid volume fraction. From conductivity measurements it is known that the liquid volume fraction depends on the pH and is drastically reduced at the IEP.

RESULTS We measured 4 systems (BLG, BSA, CN and LPI) with varying pH values (minimum 4 values) at three different foam heights (separated 5 cm from each other, named bottom, middle and top) flawlessly. Cleaning of the sample cell is hard when proteins are used and therefore time consuming. It was beneficial to have two identical sample cells were available, thus one cell could be cleaned/prepared while the other was in the beam. This provided maximum efficient beam time use.

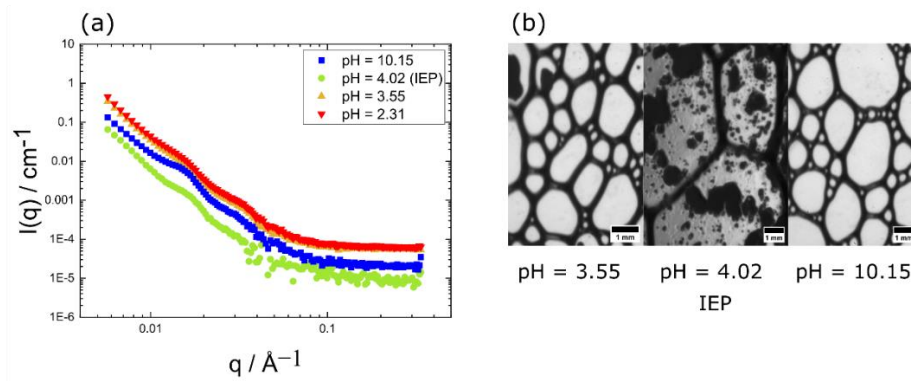


Figure 1 (a) SANS data of foams prepared from a CN solution of $c = 116.87 \mu\text{M}$ at varying pH (2.31 to 10.15). (b) Corresponding in situ images during SANS measurements (a) of foams around the IEP. The scale bars represent 1 mm.

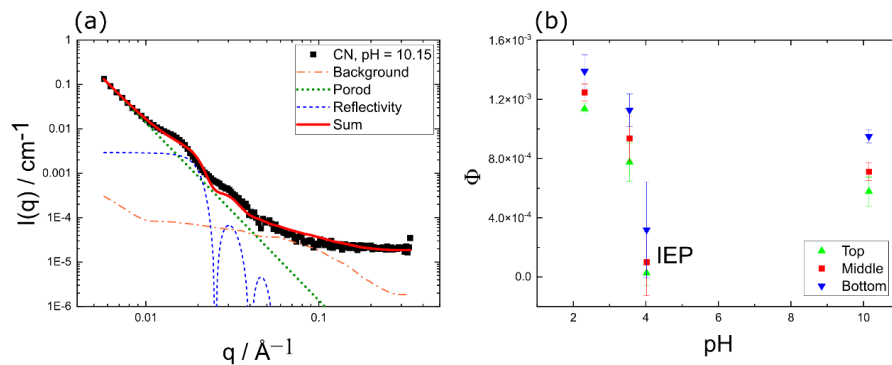


Figure 2 (a) SANS data of a foam prepared from a CN solution of $c = 116.87 \mu\text{M}$ at a pH of 10.15 measured at the bottom position of the column. Black dots represent the data, the orange dashed line represents the background, the green dotted line represents the Porod contribution and the blue dashed line is the contribution of the reflectivity. The sum is represented by the full red line. (b) Foam liquid fraction Φ of CN foams in dependence of pH for different height positions in the steady state foam, resulting from the fits.

Figure 1 (a) shows SANS data for four different foams from a CN solution with $c = 116.87 \mu\text{M}$ at various pH values. The feature expression as well as the overall intensity depends on the pH. Around the isoelectric point ($\text{pH}_{\text{IEP, CN}} = 3.9$) the intensity is the lowest due to the larger bubbles (and therefore reduced amount of foam lamellae in the beam) as seen in the middle image of Figure 1 (b). Additionally, at the IEP the protein is hydrophobic resulting in large agglomerates in the mm range [3], thus the protein concentration in the foam is reduced drastically but does not vanish completely, as indicated by the existence of large non-spherical bubbles. The absence of small bubbles hints to fast disproportion. Nevertheless, the existence of bubbles in general requires proteins in the foams, which are obviously non-agglomerated. Around the IEP all investigated protein stabilized foams show a shift of the features towards higher q values and in addition a minimum intensity consistently, indicating a drastically reduced protein concentration. Below and above the IEP the proteins are more charged and

therefore more hydrophilic which prevents agglomeration. Therefore, more proteins are available in the liquid part of the foam lamella, resulting in more and smaller foam bubbles with reduced disproportion. Also, the foam aging, due to drainage, can be observed using Φ at different foam height positions: young foam portions at the bottom of the column have a consistent higher liquid fraction, as older foam portions at higher positions. Table 1 summarizes the parameters extracted from the SANS model fits. Fitted parameters are: Surface area per unit volume of the Plateau borders S_V , the surface area per unit volume of the foam lamellae A_{foam} , the film roughness σ_{film} , the liquid fraction of the foam Φ and the background bkg, while the film thickness d_{film} and the solution SLD were fixed parameters.

Table 1 Fit parameters for the fit of CN ($c = 116.87 \mu\text{M}$ at $\text{pH} = 10.15$) represented in Figure 2 (a). Fixed values are marked with *.

$S_V / 10^{-9} \text{ \AA}^{-1}$	$d_{\text{film}} / \text{nm} *$	$A_{\text{foam}} / 10^{-9} \text{ \AA}^{-1}$	$\sigma_{\text{film}} / \text{\AA}$	Φ	$\text{SLD} / 10^{-6} \text{ \AA}^{-2} *$	$\text{bkg} / 10^{-6}$
7.18	35	1.16	25	0.00094	6.3	16.7

In order to investigate the shift of the features towards higher q values around the IEP, a new set of fits was produced with a variable value of the foam film thickness d_{film} . The resulting values for d are shown in Figure 3 for all investigated systems BLG, BSA, CN and LPI.

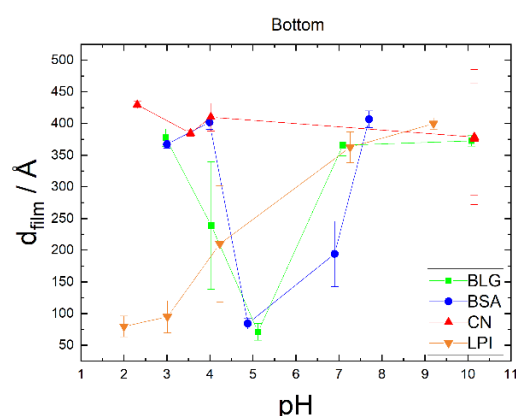


Figure 3 Thickness d_{film} of the foam films in dependence of pH for the investigated systems BLG (100 μM), BSA (34.6 μM), CN (116.9 μM) and LPI (52.9 μM) resulting from the fits.

BLG and BSA show a clear minimum of d around their IEP ($\text{pH} = 5.12$ and $\text{pH} = 4.88$, respectively). With values below 100 $\text{\AA}$ this indicates so called Newton Black Films, which could also be observed for these two systems within a freestanding foam film inside a Thin Film Pressure Balance in [3]. For CN, no minimum of d around the IEP ($\text{pH} = 4.02$) could be observed. In [3] it could be shown that CN foam films rarely show NBFs and when they do, the single CN foam films rupture within seconds after NBF formation. So, no NBFs within the macroscopic foams could be observed for CN. LPI shows an increase of the film thickness d with increasing pH but no minimum at the IEP. The reason for this could be the poor data quality, since LPI creates very unstable foams due to a high precipitation of agglomerates and flakes resulting in a low concentration of free proteins able to stabilize the foam films.

[1] M. Kühnhammer, L. Braun, M. Ludwig, O. Soltwedel, L. Chiappisi and R. v. Klitzing, *Journal of Applied Crystallography*, 2022, **55**:758-768

[2] J. Lamolinairie, B. Dollet, J. L. Bridot, P. Bauduin, O. Diat and Leonardo Chiappisi, *Soft Matter*, 2022, **18**:8733-8747

[3] K. Gräff, R. v. Klitzing et al. *Front. Soft. Matter*, 2021, **2**:1035377