

## Adsorption and interfacial gelation of proteins at air-water and oil-water interface

This application note demonstrates how the KSV NIMA Interfacial Shear Rheometer can be used to study monolayer viscoelastic properties at the air-water and oil-water interface.

### Introduction

The adsorption and network formation of proteins to air-liquid and liquid-liquid interfaces is a commercially important process in food, cosmetics and pharmaceutical industries. Especially in food industries where natural components of food fats, oils, proteins and water usually form colloidal solutions and the colloidal structure is partially stabilized by amphiphilic proteins. In some cases the proteins can form a two dimensional network at the interface while denaturing, increasing the stability of the emulsion or dispersion dramatically. This effect, also called interfacial gelation, can be easily detected from the viscoelastic properties of the interface, but the phenomena has not been extensively investigated yet due to the lack of sensitive enough methods to perform rheological experiments on interfaces.

The KSV NIMA Interfacial Shear Rheometer (ISR) uses a floating magnetic probe to detect the viscoelasticity of the interface. The method is highly sensitive because the probe inertia is small compared to the measured changes, and the method has been proven suitable for many different viscoelastic interfaces. [1-5]

Dynamic shear rheological experiments are capable of separating the storage (elastic) and loss (viscous) modulus of complex rheological behavior. When time dependent network formation is measured, it is possible to measure the changes of the viscoelastic components quantitatively. It is also possible to determine the gel point of the system after which the elastic properties start to dominate over viscosity and a two dimensional gel has been formed at the interface.

### Materials and methods

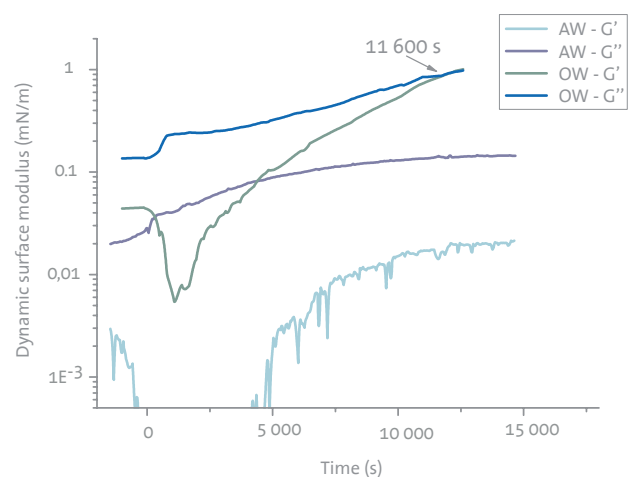
A 20 mg/ml solution of Lysozyme (hen egg, Sigma Aldrich) was prepared in PBS buffer. Two distinct measurements were conducted, one at the air-water interface, the other at the oil-water interface. For the air-water (AW) interface measurement, a low volume measurement cell was filled with PBS buffer. In the oil-water (OW) interface experiments Brassica rapa oil was carefully poured on the buffer phase, which was otherwise prepared the same way as for the AW experiments. For both experiments, after a short background measurement the Lysozyme solution was

injected to the buffer phase so that the final concentration of the lysozyme compared to buffer was 1 mg/ml. The ISR measurements were performed in a single frequency mode using a frequency of 0.1 Hz and shear of less than 3%.

### Results and discussions

The dynamic surface modulus ( $G'$  storage and  $G''$  loss) is shown in Figure 1 in a semi-logarithmic plot. The adsorption to the air-water interface had only a slight effect on the viscoelastic properties. There was no network formation, the adsorption ended to a plateau and the viscosity dominated during the whole experiment. In the oil-water experiment the interfacial elasticity clearly developed faster than the interfacial viscosity and a gel point was reached after approximately 11 600 seconds (3.2 hours).

In both AW and OW experiments the apparent elasticity of the interface dropped after the injection of the protein while viscosity increased. This can be explained by the globular proteins



**[Figure 1]:** The storage ( $G'$ ) and loss ( $G''$ ) modulus of both AW and OW experiments. The lysozyme injection was done at time 0 s. The gel point of the OW experiment is marked at 11 600 s.

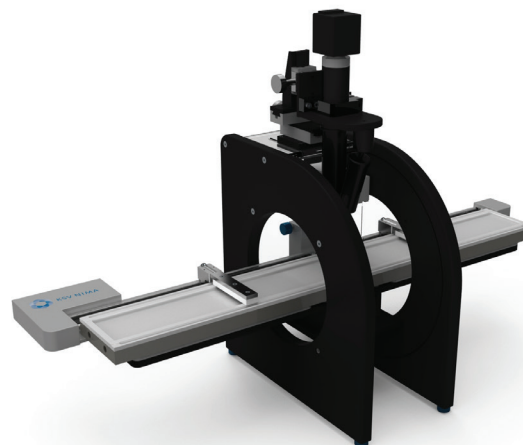
adsorbing to the interface and increasing the viscosity. The proteins had not started to interact with each other and did not therefore show significant elasticity yet. Only after the proteins started to denature and entangle the layers began to form elastic properties.

The viscoelasticity of the AW interface remained several decades smaller than the OW interface during the whole experiment. This would suggest that the AW interface absorbed only a small amount of material compared to the OW interface, and the proteins kept their native conformation. In the OW interface the proteins denature and a network formation takes place. These findings were similar to those shown in literature of OW protein adsorption earlier. [2]

## Conclusions

Lysozyme was shown to form viscoelastic gels at the oil-water interface, opposed to air-water interface where no such reaction took place. It was possible to determine the gel point for the reaction, and quantify the storage and loss modulus of the interfacial systems.

The KSV NIMA ISR was shown to be an excellent instrument for studying interfacial gelation and network formation at different interfaces. The dynamic experiments yielded information on the reaction times, rates and magnitudes of viscoelastic properties in simple experiments.



KSV NIMA ISR

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