

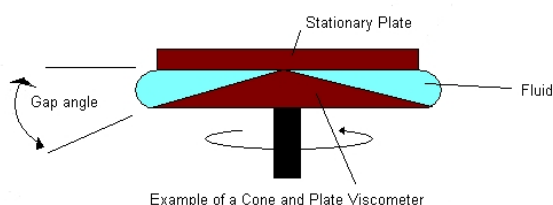
## Accurate Viscosity Measurement of Protein Solutions

### Application

Conventional rheometers are utilized for measuring the viscosities and the dynamic rheological properties of various liquids. However, due to the evaporation of solvents and/or film formation, an inevitable air/sample liquid interface poses a challenge to obtain accurate viscosity measurement. This application note addresses these challenges and offers an alternative method for viscosity measurement with higher accuracy.

### Air/liquid interface

As shown below, the cone and plate (or plate and plate) geometry combination is the most widely used tool. It is well known that the shear rate is constant throughout the sample in the gap between the cone and the plate (lubrication approximation).



A constant shear rate of the geometry makes viscosity measurement accurate, even for the non-Newtonian liquids: other viscosity measurement methods do not offer any shear rate unless the test liquid is Newtonian. However, cone and plate or plate/plate geometries must include air/liquid sample interface by design, which often makes viscosity measurement difficult or inaccurate. In particular, highly volatile solvents are difficult to measure in cone and plate, as they evaporate through the interface during measurement. This loss of solvent reduces the effective sample radius, which decreases the measured viscosity value.

Figure 1 clearly shows the decreasing trend in the measured viscosity for DI-water as a function of time (at 40°C). Due to evaporation, the viscosity of DI-water could be measured as shear thinning at varying shear rates. This leads to the erroneous conclusion that water is non-Newtonian.

The proper modification of the rheometer to slow the evaporation has also been evaluated in Figure 1. While the evaporation slows down significantly, it does not stop the behavior completely. For more information, see reference 1.

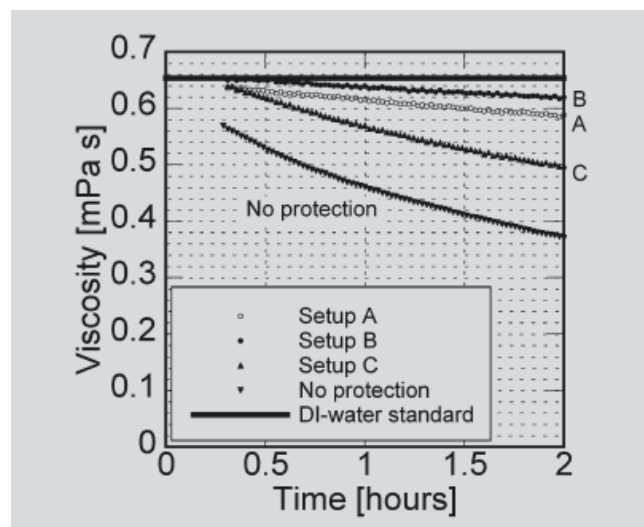


Figure 1: Measured viscosity of water with a rheometer with increasing duration at 40°C. Setups A, B, and C represent different levels of solvent prevention with the modified rheometer<sup>1</sup>.

### Air/liquid interface - protein solution

The viscosity measurement of aqueous protein solutions with cone and plate is more challenging. One issue: extreme caution and care must be exercised to ensure that viscosity data are reproducible and reliable. Challenges consist of preventing water evaporation during viscosity measurement, and the irreversible adsorption of protein molecules at the interface<sup>2</sup>. Irreversible adsorption of protein molecules could follow the mechanism described in Figure 2, according to EM Freer et.al. (ref 2):

- First protein molecules migrate to the interface to minimize the interface energy.
- Molecules partially unfold and aggregate
- Further forms gel-like network depending on proteins

<sup>1</sup> Adopted from J. Sato and V. Breedveld, "Evaporation Blocker for Cone & Plate Rheometry of Volatile Samples," *Applied Rheology*, 15, 6, 390 (2005).

<sup>2</sup> E. M. Freer, K.S. Yim, G.G. Fuller, and C. J. Radke, "Interfacial Rheology of Globular and Flexible Proteins at the Hexadecane/Water Interface: Comparison of Shear and Silatation Deformation," *J. Phys. Chem. B*, 2004, 108, 3835-3844.

# VROC<sup>TM</sup> - A MEMS Device for Newtonian and Non-Newtonian Viscosities

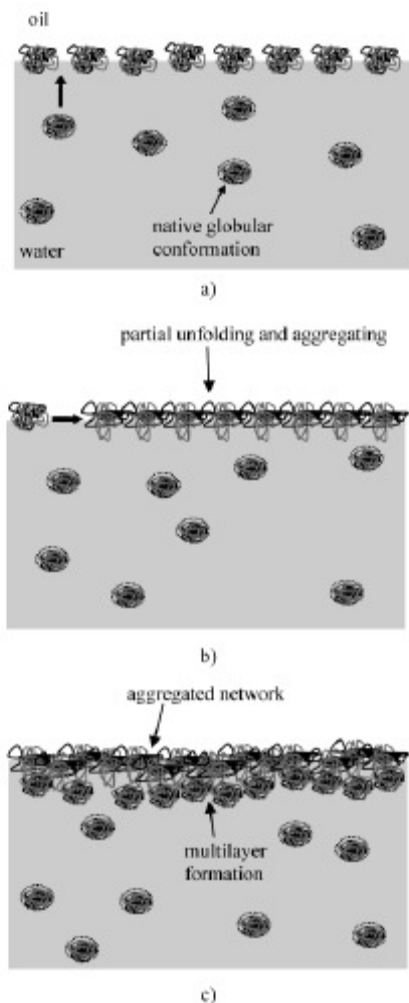


Figure 2: Sequence of irreversible adsorption of protein molecules at the oil/water interface. (Adopted from Reference 2.)

As protein molecules at the interface are more concentrated and may form a solid-like structure, its contribution to the viscosity measurement is significant. In cone and plate, viscosity is measured from the torque measurement following the simple relationship<sup>3</sup>:

$$torque = \frac{2\pi}{3} \sigma \times R^3$$

$\sigma$  is the shear stress and  $R$  is the radius of the cone.

As can be seen, the shear stress near the interface makes the greatest contribution to the measurement. For the same reason, water evaporation reduces the effective sample radius, which decreases the viscosity as shown in Figure 1. The high concentration of

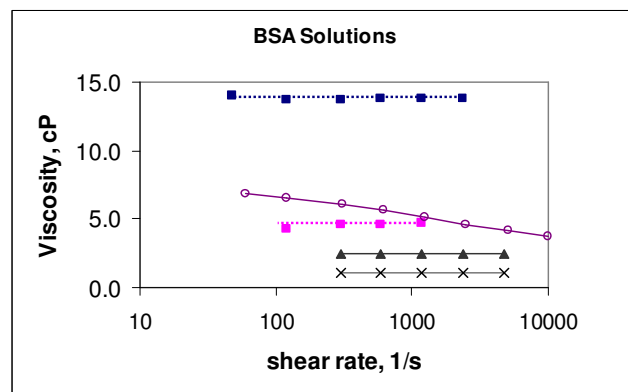
proteins or solid-like film at the interface would strongly bias the measurement. That bias results in a shear thinning behavior for Newtonians samples.

Tu and Breedveld observed a shear thinning (non-Newtonians) for a dilute BSA solutions (<10 mg/ml) with their rheometer<sup>1</sup>. Particle tracing with micro-rheology gave different viscosity trend. The authors suggested that elastic protein film at the air-water interface of the periphery of cone and plate geometry was responsible for the unusual behavior.

## VROC<sup>®</sup> - viscometer/rheometer-on-a-chip: No air/liquid interface

VROC<sup>®</sup> is an alternative for the rheometer as it measures viscosity for Newtonian and non-Newtonian liquids. However, volume requirement is much smaller (~ 50 ul) with VROC<sup>®</sup>.

Since there is no liquid/air interface, the viscosity measurement is not affected by the evaporation or protein film formation. With VROC<sup>®</sup>, the measured viscosities of BSA solutions at different concentrations show Newtonian viscosity: constant irrespective of shear rates as shown below.



Symbol	Sample description
■	404 mg/mL
■	300 mg/mL
▲	202 mg/mL
x	20.2 mg/mL
○	PEO 0.32%

<sup>3</sup> C. Macosko, "Rheology: Principles, Measurements, and Applications," Wiley, 1994.