

Refractive index based determination of detergent concentration and its application to the study of membrane proteins

The concentration of detergent in membrane protein purifications can have a critical role on protein stability, function, and crystallization. Unfortunately, dialysis or protein concentration can lead to an unknown amount of detergent in the final membrane protein solution.

We have developed a method for the determination of detergent concentration based on measuring the refractive index of the detergent solution with an Optilab RI instrument. We applied this method to quantitate the *amount* of detergent remaining in solution *after* concentration in various concentrators. We also quantitated the amount of unbound detergent in protein:detergent mixtures.

When coupled with size exclusion chromatography and light scattering, RI detection can be used to determine the oligomeric state of the membrane protein, the size of protein-associated micelle, as well as the amount *and* size of the unbound detergent micelle.

Refractive index measurements were performed using an Optilab instrument with a P10 cell. The standard curves for C₁₂E₉, DDM, FOS14, and LDAO were obtained by injecting 5 ml of detergent solution through the refractometer at 0.5 ml/min. For the determination of excess detergent in protein:detergent mixtures, bovine serum albumin (1 mg/ml) was mixed with 0.25 %, 0.5 %, 0.75 %, and 1.0 % LDAO and 100 µl was injected over a size exclusion column (Shodex-804) prior to the refractive index measurement.

In our experiments, standard curves were constructed and the dn/dc values were determined for C₁₂E₉, DDM, FOS14, and LDAO detergents. In addition to the protein concentrator analysis, protein:detergent mixtures were separated on an SEC column, and the amount of unbound detergent, as well as the micelle sizes were obtained for several detergents.

The high accuracy and sensitivity of the RI can be used in membrane protein purification to *improve* protein preparative protocols and yield membrane proteins with improved function, stability, and an increased chance of crystallization.

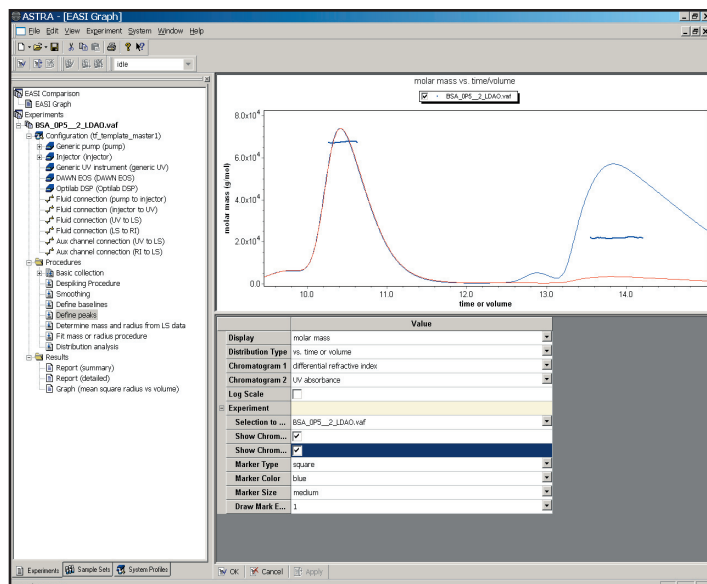


Figure 1. BSA was injected over a Shodex 804 SEC column either with 0.5% LDAO (blue line), or without LDAO (red line). The molecular size across the peak was then calculated with ASTRA for both the BSA ($dn/dc=0.185$) and LDAO (dn/dc value determined from previous experiments).

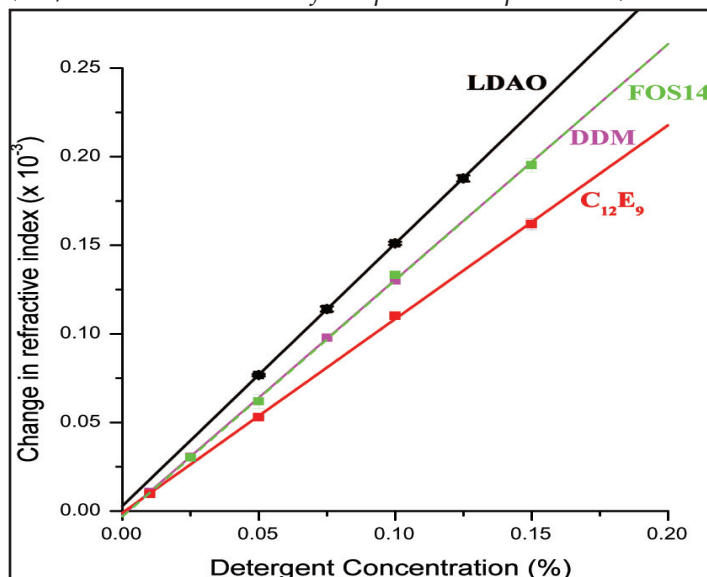
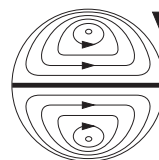


Figure 2. Change of refractive index as a function of detergent concentration. Standard curves are shown with error bars for commonly used detergents C₁₂E₉, DDM, FOS14, and LDAO. The slope of the standard curve is the dn/dc value for each detergent.

This note graciously submitted by Pavel Strop, Clark Center E300, Stanford University, Stanford, 94305



**Wyatt
Technology**
CORPORATION

6300 Hollister Avenue • Santa Barbara, CA 93117
TEL (805) 681-9009 • FAX (805) 681-0123
E-mail: info@wyatt.com • URL <http://www.wyatt.com>