Quantitation of High Concentration Protein Formulations Using LSPR Biochips

Exemplary for Bovine Serum Albumin

SUMMARY

- Refractometric protein quantitation on a conventional microplate reader
- High throughput measurement based on the SBS format
- Linear response in a very broad concentration range obviates the need for dilution
- No requirement for method calibration using expensive protein standards

INTRODUCTION

The accurate determination of total protein concentrations in therapeutic formulations is of high importance. Spectrophotometers are commonly used for protein quantitation. However, depending on the amino acid sequence molar extinction coefficients vary greatly for different proteins and a calibration with standards is necessary. In contrast, LSPR Biochips detect small changes in refractive index (RI) of a solution and convert it to an optical response, which can be read with a monochromator or CCD based microplate reader or spectrophotometer. The RI of a protein solution varies much less for different proteins and linearly depends on the concentration. Moreover, the linear dependence covers a very broad range of concentrations, which eliminates the need for dilution. The SBS format of the LSPR Biochips (see Figure 1) allows to measure a high number of samples in a short time on widespread laboratory instruments. Therefore, no additional instrument, i.e. refractometer, is required.

ABOUT THE TECHNIQUE

Localized surface plasmon resonance (LSPR) sensors possess several so called active areas with arrays of highly uniform metallic nano-structures. These structures exhibit specific absorption peaks due to the particles’ geometry, arrangement, size and material properties. Even very small changes of the dielectric environment of the metallic nano-structures lead to detectable shifts in the plasmon resonance frequency. Therefore, the RI of the analyte solution can be obtained from a simple absorbance measurement. LSPR AG has industrialized Localized Surface Plasmon Resonance (LSPR) spectroscopy into a sensitive, versatile and powerful technique for protein quantification and probing biological interactions on the molecular level. The dedicated software PLASMON for data collection and post-processing works with most commercial microplate readers, makes the method easy to use and yields presentable results in a short time.

MATERIALS AND EQUIPMENT

- 24 datapoint LSPR biochip (Part No. 0101-01)
- State of the art microplate reader
- PLASMON microplate reader control software
- LSPR biochip holder
- 0.85% NaCl solution
- Bovine serum albumin (BSA, Sigma Aldrich A2153)

Figure 1 Photograph of LSPR Biochips in a holder compatible with the SBS format
PREPARATION OF SOLUTIONS

A 250mg/ml stock of bovine serum albumin was prepared in 0.85% NaCl solution. A two-fold serial dilution in the same diluent followed until five solutions were obtained with BSA concentrations ranging from 15.6mg/ml to 250mg/ml.

ASSAY PROCEDURE

1. 25µL of 0.85% NaCl solution was pipetted into each well, then an absorbance scan of each data point was set with PLASMON and acquired with a state of the art microplate reader.
2. The NaCl solution in the wells was replaced with 25µL of each of the prepared BSA solutions (in triplicate).
3. Another absorbance scan was set in PLASMON and measured.
4. Data analysis: Shifts were calculated in reference to the blank measurement and converted to refractive index values.

RESULTS

Figure 2 represents the obtained curve for the prepared BSA solutions, obtained in less than 30 minutes in a label-free manner. The sensor response, shown as the refractive index of the sample solution is linearly proportional to the BSA concentration in the range from 15.6mg/ml to 250mg/ml.

CONCLUSIONS

This application note demonstrates the viability for refractometric protein quantitation on a standard microplate reader using the example of bovine serum albumin. A linear response for a wide concentration range from 15.6mg/ml to 250mg/ml is obtained without the need for sample dilution. The method provides a straightforward way for high throughput measurements of high concentration protein solutions especially for laboratories already equipped with a standard microplate reader.

![Figure 2](image-url)