Influence of Buffer on Choice of Protein Quantification Method

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Introduction

The most common protein quantification methods include direct absorbance at 280 nm, colorimetric assays, and fluorescent assays. The choice of quantification method depends on several factors, including the approximate protein concentration and whether it has been purified. A frequently overlooked factor that should be taken into consideration when choosing a protein quantification method is the buffer in which the protein is suspended.

This study examines the spectra of several commonly used protein buffers, particularly with respect to their absorbance contribution at 280 nm. By measuring the buffer in which a protein is suspended against a pure, deionized water blank, the absorbance spectral profile of the buffer can be observed. The amount of absorbance at 280 nm can then help to determine whether the buffer is suitable for protein quantification by direct A280 measurement.

This study also examines the accuracy of direct A280 quantification when measuring a protein suspended in a RIPA buffer. These buffers have substantial absorbance in the UV region and therefore are examples of buffers that may not be suitable for direct A280 quantification. The A280 method and BCA colorimetric assay were used to quantify bovine serum albumin samples prepared in either PBS or RIPA buffers as a means of assessing the impact of unsuitable buffers.

Materials and Methods

In order to determine absorbance spectra of common buffers, typical working concentrations of PBS, M-PER, T-PER, HEPES and RIPA were measured against a water blank. Typical working concentrations of Triton X-100, CHAPS and NDSB-201, reagents often used in protein buffers, were also measured against a water blank.

A 2 mg/mL BSA protein stock (Thermo Scientific Pierce Products Cat # 23209) was diluted 1:1 with either 0.05M PBS or RIPA buffer containing 25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS (Sigma Cat # R0278) to create 1 mg/mL standards in each buffer (final buffer concentration of 0.5x). Standard curves for use in the BCA assay were then created by serial dilution of these standards in each of the 0.5x buffer described.

Two "test" BSA samples with the same concentration were prepared, one in 0.5x PBS and the other in 0.5x RIPA buffer. Both test samples were then quantified using a Thermo Scientific NanoDrop 2000c spectrophotometer by direct A280 measurement and by using a BCA colorimetric assay with a standard curve generated with protein standards diluted in the respective buffer.

Results

The absorbance spectra of all tested protein buffers and components showed some absorbance in the lower UV region (fig. 1); this absorbance typically decreased to zero by ~230 nm. Notable exceptions to this included RIPA, NDSB, and Triton X-100 buffers, which did have significant absorbances at 280 nm (fig. 1 and 2, respectively).

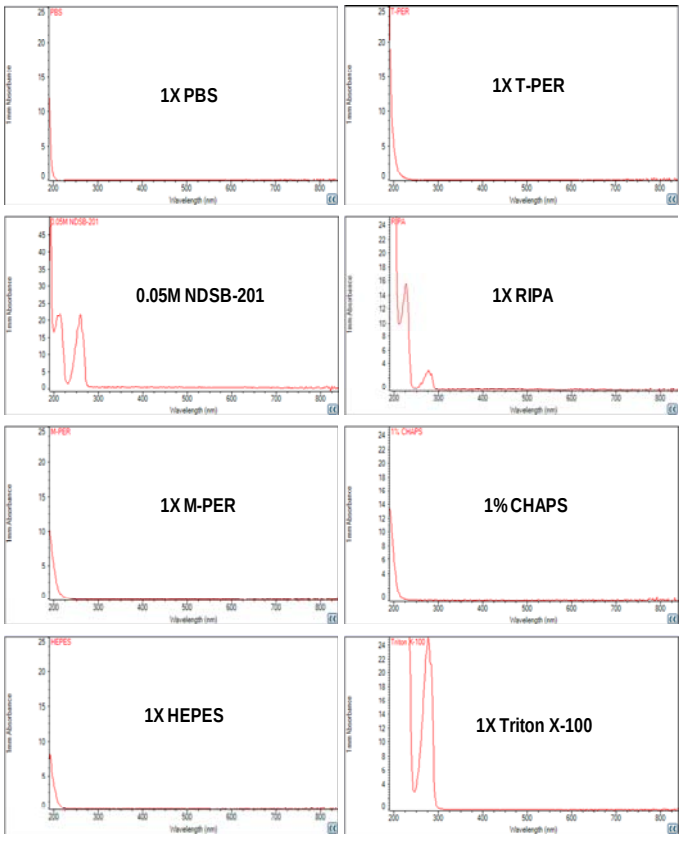

Figure 1: Absorbance of various buffers and buffer components (instrument blanked using deionized water).

When the absorbance of the BSA samples in 0.5x PBS or 0.5x RIPA buffers was measured, a deviation between the two spectra was observed across the monitored wavelength range (fig. 2), even though each protein sample was measured against a blank of the same buffer.

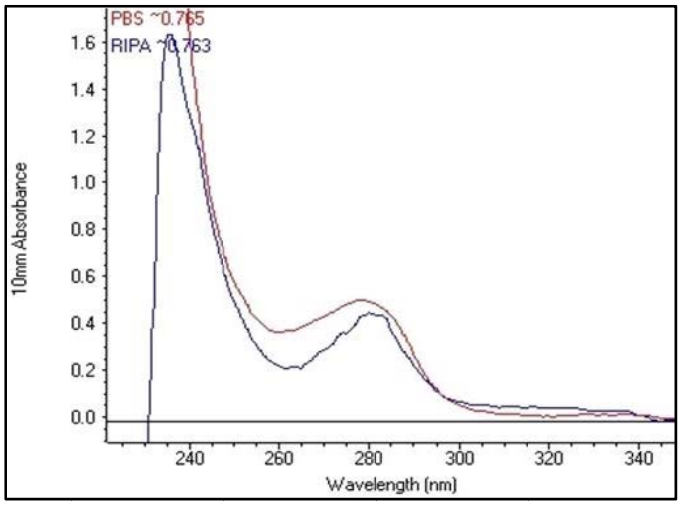

Figure 2: Absorbance spectra of 0.76 mg/ml protein solutions in PBS (red) and RIPA buffer (blue).

The use of a RIPA buffer resulted in a greater than 20% error in concentration measurement for a 0.76 mg/ml BSA sample and compromised measurement precision (fig. 3). Conversely, quantification of the two protein samples using the BCA colorimetric assay showed the test samples to have the same concentration, regardless of buffer (fig. 3).

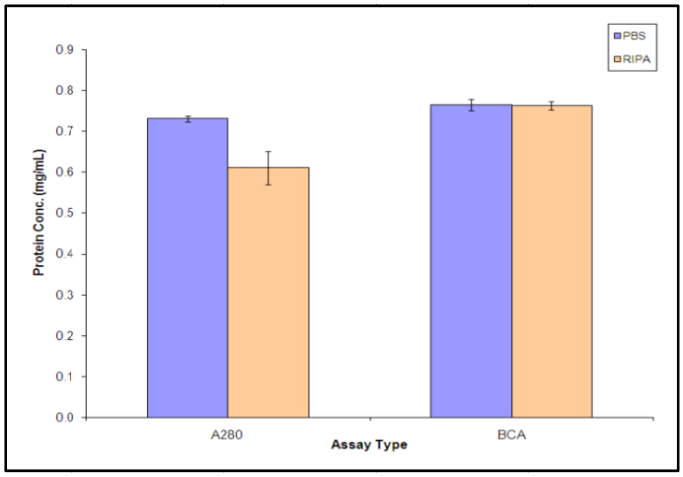

Figure 3: Quantification of the same protein sample in either 0.5x PBS or 0.5x RIPA buffer. n=3 for all; error bars represent standard deviations.

How to Assess the Suitability of a Buffer

Follow the steps below to determine if a buffer exhibits significant absorbance in the region of interest:

1. Ensure both the lower and upper pedestal measurement surfaces are clean.

2. Open the A280 application. Load an aliquot of dH_2O onto the lower measurement pedestal and lower the sampling arm.

3. Click **Blank**. After the measurement is complete, use a dry, lint-free lab wipe to remove the water from both the top and bottom measurement surfaces.

4. Pipette an aliquot of the sample buffer onto the pedestal, lower the arm and click **Measure**.

The result should be a spectrum that varies no more than 0.04 absorbance (10 mm absorbance equivalent) from the baseline at 280 nm. If not, consider using a colorimetric method to quantify the protein samples.

Conclusion

Most buffers show significant absorbance only in the lowest UV region, likely caused by various salts present in the buffer. These buffers do not affect the accuracy of A280 quantification.

The large absorbance at 280 nm of the RIPA buffer is most likely due to the NP-40 or Triton X-100 content of the RIPA buffer, as surfactants such as these strongly absorb UV light. Similarly, the ring structure in the NDSB molecule likely causes this buffer's strong absorbance in the UV region.

The use of buffers with large absorbances at 280 nm results in quantification errors, as blanking on any spectrophotometer when using the direct UV A280 method may not fully compensate for the absorbance of the buffer. *It is good practice, however, to always blank using the buffer in which a sample is suspended.*

This study has shown that while the majority of commonly used buffers are suitable for protein quantification using absorbance at 280 nm, exceptions to this rule exist. In cases where the buffer exhibits a substantial absorbance at 280 nm, alternative methods such as colorimetric assays should be used.