

Influence of buffer on quantification of nucleic acids and proteins

Introduction

A common technique used to quantify nucleic acids and proteins is to measure their direct absorbance of light at 260 nm and 280 nm, respectively, through the application of UV spectrophotometry. A frequently overlooked factor that should be evaluated with each experiment is the buffer in which the sample is suspended. The sample buffer is typically used as the blanking solution, and should match in both composition and pH. However, some buffers that are commonly used with nucleic acids and proteins, such as some elution buffers and Triton™ X-100 lysis buffer, are not appropriate for absorbance-based quantification, as these buffers absorb substantially in the UV region and may interfere with direct measurements.

Buffer suitability can be assessed by blanking the spectrophotometer with pure, deionized water and then measuring the buffer as a sample. This procedure is referred to as a “blanking cycle” and reveals the absorbance contribution of the buffer at the analytical wavelength, which should vary no more than 0.02 absorbance units from the baseline. If the resulting absorbance at the analytical wavelength is too high for reliable quantification and the sample cannot be exchanged or dialyzed into a more suitable buffer, consider a fluorescence assay or a colorimetric assay.

Experimental procedures

Absorbance spectra of common buffers were measured against a deionized water (diH₂O) blank. Protein buffers included the following: PBS (1X); T-PER (1X); RIPA (1X); HEPES (25 mM); MOPS (100 mM); and Triton X-100 (1% v/v). Nucleic acid buffers included Tris-HCl, TE pH 7.5, TE pH 8.0, and an elution buffer from the Qiagen™ DNeasy™ Blood and Tissue Kit (Qiagen cat. no. 69504); these were measured neat. Samples were made by diluting bovine serum albumin (BSA) (Sigma-Aldrich™ brand, prod. no. A7284) or calf thymus dsDNA (Invitrogen™ UltraPure™ Calf Thymus DNA Solution, Thermo Fisher Scientific cat. no. 15633019) in a 1:1 ratio by volume with the associated buffers to generate final working concentrations of 1.0 mg/mL BSA and 50 ng/μL dsDNA.

Diluted samples were quantified using 2.0 μL aliquots, in replicates of five, on the microvolume pedestal of a Thermo Scientific™ NanoDrop™ Ultra[®] FL Spectrophotometer and Fluorometer. Concentrations were calculated by direct absorbance using the Protein A280 or dsDNA applications. BSA concentration in each buffer mixture was also quantified using the Thermo Scientific™ Pierce™ BCA Protein Assay Kit and the results were compared to concentrations determined by direct absorbance. Additionally, dsDNA concentration was quantified via fluorescence with the Thermo Scientific™ NanoDrop™ Ultra dsDNA Broad Range (BR) Assay Kit. The NanoDrop Ultra[®] FL instrument includes preprogrammed applications for fluorescence-based quantification of dyes bound to nucleic acids, and for proteins prepared using the BCA assay.

Results

The absorbance spectra of all tested buffers exhibited absorbance in the deep UV range, likely attributed to the presence of salts or other additives (Figure 1A, B). For most buffers, the absorbance profiles indicated minimal impact on the analytical wavelengths used for protein and nucleic acid quantification. These low-absorbance buffers included PBS, T-PER, HEPES, and MOPS for protein and TE and Tris HCl for dsDNA. In contrast, protein buffers that exhibited notable UV absorbance near 280 nm were RIPA and Triton X-100. For dsDNA, the elution buffer contributed approximately 0.4 absorbance units at 260 nm, which would correspond to an increase of 20 ng/ μ L to the reported dsDNA concentration.

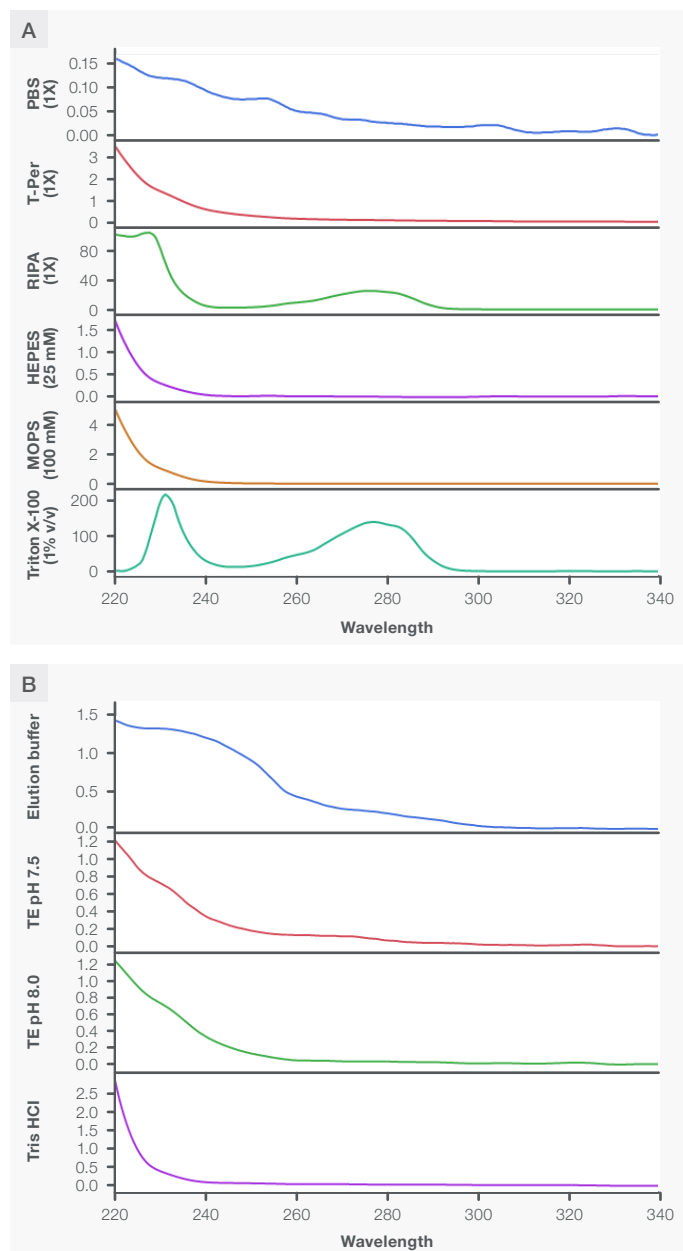


Figure 1. Absorbance spectra of (A) protein buffers; and (B) dsDNA buffers, measured against a diH₂O blank.

Most protein buffers did not affect absorbance quantification accuracy when compared with the BCA assay (Figure 2A). However, Triton X-100 and RIPA buffers interfered with direct 280 nm quantification because they contribute to a significant amount of absorbance at the analytical wavelength of proteins (Figure 2B). When blanking the NanoDrop Ultra[®] FL spectrophotometer and fluorometer with Triton X-100 and RIPA buffers, an error was displayed by the software indicating the absorbance was too high. This error occurred because blanking a spectrophotometer with a high-absorbing buffer limits the amount of light available for sample measurements, which can compromise measurement accuracy. For example, when BSA diluted in Triton X-100 was quantified by direct absorbance, the calculated concentration was nearly 10-fold higher than the concentration determined by the BCA assay because of the buffer's absorbance contribution at 280 nm. In cases where highly absorbing buffers are required, quantification via a colorimetric assay, such as the BCA assay, provides an acceptable alternative.

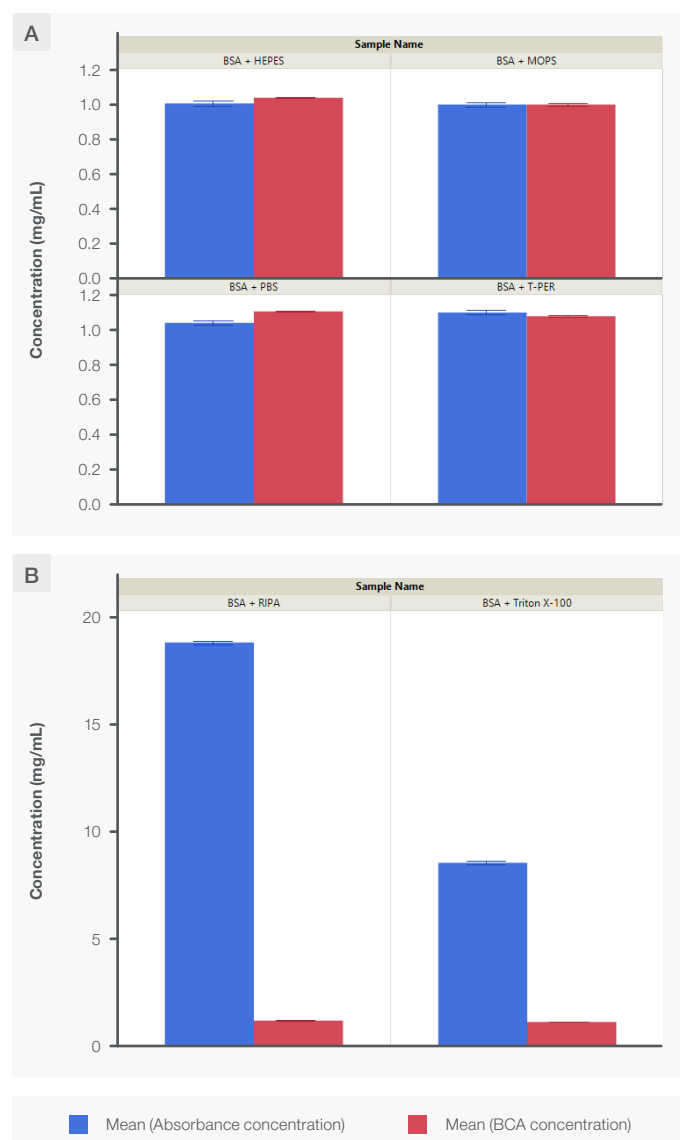


Figure 2. Comparison of 1 mg/mL BSA concentration determined via direct 280 nm absorbance (blue bars) and a colorimetric BCA assay (red bars). (A) BSA mixed 1:1 with HEPES, MOPS, PBS, and T-PER measured against a buffer blank; (B) BSA mixed 1:1 with RIPA and Triton X-100 measured against a water blank. Error bars represent $\pm$ standard deviation, $n=5$.

The differences between absorbance and fluorescence quantification results for dsDNA mixed with TE pH 8.0, Tris HCl, and elution buffer may be attributed to the use of TE pH 7.5 as the diluent for the fluorescence standards (Figure 3). Variations in buffer composition or pH for standards and samples may affect fluorescence quantification accuracy, but further studies would be required to confirm this effect. Additionally, the Thermo Scientific™ NanoDrop™ Ultra software triggered a “Possible Bad Blank” warning when the elution buffer was used as the blanking solution. This finding is consistent with the elution buffer spectral profile, which showed absorbance near the dsDNA analytical wavelength (Figure 1B).

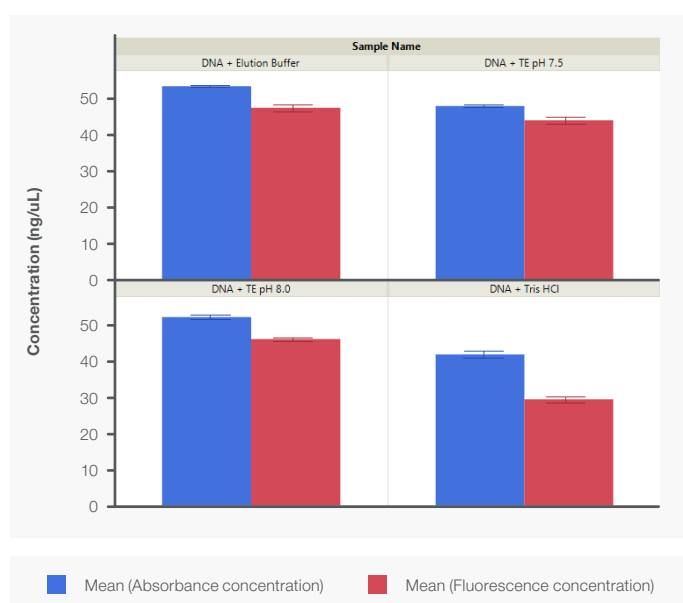


Figure 3. Comparison of 50 ng/uL dsDNA concentration measurements determined via direct 260 nm absorbance (blue bars) and fluorescence (red bars) when mixed 1:1 with elution buffer (top left); TE pH 7.5 (top right); TE pH 8.0 (bottom left); and Tris HCl (bottom right), with each measured against the corresponding buffer as a blank. Error bars represent $\pm$ standard deviation, $n=5$.

Conclusions

Direct absorbance measurements with the NanoDrop Ultra[®] FL spectrophotometer and fluorometer provide a convenient method for quantifying nucleic acids and proteins, but buffers should be carefully selected to ensure measurement accuracy. Common buffers such as TE and Tris HCl for dsDNA, and HEPES, MOPS, PBS, and T-PER for proteins, are acceptable for UV absorbance measurements. However, RIPA and Triton X-100 contributed to significant absorbance levels near 280 nm, causing an overestimation of protein concentration by direct absorbance. The tested dsDNA elution buffer also affected quantification accuracy because the buffer absorbed near 260 nm, as exhibited by the spectrum in Figure 1B. These results demonstrate the importance of evaluating buffer absorbance prior to direct UV absorbance quantification. Blanking with a high-absorbing buffer may trigger errors and might reduce measurement accuracy for the analyte of interest. When a highly absorbing buffer cannot be exchanged or dialyzed, an alternative analytical method such as a colorimetric BCA protein assay or a fluorescence assay for dsDNA quantification should be considered for improved concentration accuracy. By incorporating a blanking cycle into a UV absorbance workflow, buffer suitability can be evaluated, and an appropriate quantification method can be selected to ensure accurate biomolecule quantification. The NanoDrop Ultra[®] FL spectrophotometer and fluorometer, which includes preprogrammed applications for direct UV absorbance, colorimetric assays, and fluorescence assays, has demonstrated it can serve as a comprehensive tool for accurate protein and nucleic acid quantification.