Technical Overview

Agilent
Trusted Answers

Quality Analysis Using the Agilent ProteoAnalyzer System and SDS-PAGE

A comparison of sizing and quantification performance

Introduction

Quality control (QC) provides valuable information about the integrity of samples such as proteins before use in assays, experiments, or product release. Ensuring that samples are of high quality enhances the reproducibility of workflows, reduces variability, and minimizes the risk of data inconsistencies. Among the different attributes of proteins, the size and quantification of samples can be assessed with electrophoretic separations using sodium dodecyl sulfate (SDS). SDS denatures the sample and provides the proteins with a consistent mass-to-charge ratio, allowing for size-based separation.

Traditionally, protein QC is performed with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Alternatively, capillary electrophoresis sodium dodecyl sulfate (CE-SDS) uses the same principles of SDS denaturing and size-based separation, but uses a gel-filled capillary instead of a slab gel. In doing so, CE-SDS provides faster separation times, higher resolution, accurate sizing, and consistent quantification.

The Agilent ProteoAnalyzer system is an automated CE-SDS instrument that uses a 12-channel capillary array to analyze multiple samples in parallel and allows for multiple runs to be programmed at once. The system is designed to facilitate precise and accurate measurements of proteins and allow for the detection of impurities while only requiring 1 µL of sample. In this technical overview, quality assessment of different proteins was compared between automated CE-SDS using the ProteoAnalyzer system and conventional SDS-PAGE.

Experimental

Commercially available bovine serum albumin (BSA) (Sigma-Aldrich; part number A7906) and CAII (Sigma-Aldrich; part number C2273-1VL) were diluted in 1 x PBS (30 mM Tris-HCl, 26 mM NaH_2PO_4, 41 mM Na_2HPO_4, 79 mM NaCl, pH 8.5) to 2,000 ng/µL, and the concentration was verified using UV absorption. Both proteins were then serially diluted two-fold across the concentration range of the Agilent Protein Broad Range P240 kit (part number 5191-6640), as shown in Table 1. Each dilution was analyzed in triplicate under reduced conditions on the ProteoAnalyzer with the Protein Broad Range P240 kit, using the method for the optional addition of the upper marker. The samples were then assessed for sizing and quantification using Agilent ProSize data analysis software.

The serially diluted samples were also analyzed with SDS-PAGE using precast gels (Bio-Rad; part number 4569036) under reduced conditions. Each sample was diluted 3:1 with 4x Laemmli buffer (Bio-Rad; part number 161-0747), with a final concentration of 50 mM DTT. The samples were heat denatured at 90 °C for 5 minutes, then 10 µL of each concentration was loaded onto the SDS-PAGE gels. Then, 10 µL of Bio-Rad Precision Plus Protein Dual Color Standards (part number 161-0374) was added to the wells flanking the sample lanes. Separation was conducted at 200 V for approximately 40 minutes. The gels were fixed (10% acetic acid, 40% ethanol, 50% water) for 15 minutes with rocking, then rinsed with water. The gels were stained overnight in Bio-Rad QC Colloidal Coomassie stain (part number 1610803) and destained with de-ionized (DI) water for 3 hours. The experiment was repeated three times. Analysis was performed using GelAnalyzer software[2] for sizing and quantification.

Table 1. Agilent Protein Broad Range P240 kit specifications (LM: lower marker; UM: upper marker).

Analytical Specifications		**ProteoAnalyzer Protein Broad Range P240 kit**
Sizing Range	LM only LM and UM	10 to 240 kDa 10 to 200 kDa
Typical Sizing Accuracy (% Sizing Error)	LM only LM and UM	< 15% for BSA, CAII < 10% for BSA, CAII
Typical Resolution		< 10% molecular weight resolution between 15 to 150 kDa (based on ladder) R≥1 NIST mAb NGHC/HC (using reduced conditions)
Sizing Precision	LM only	< 8% CV for BSA, CAII, GREMLIN-1, and NIST mAb (using reduced conditions) < 10% CV for intact NIST mAb (using non-reduced conditions)
	LM and UM	< 5% CV for BSA, CAII, GREMLIN-1 and NIST mAb (using reduced conditions)
Quantitative Range		2 ng/µL to 2,000 ng/µL for BSA in PBS
Sensitivity (Signal/Noise > 3)		1 ng/µl for BSA, CAII in PBS
Quantification Reproducibility		<15 %CV for 20 – 2,000 ng/µL BSA <25 %CV for 2 – 20 ng/µL BSA

Results and discussion

Image analysis comparison

The ProteoAnalyzer and SDS-PAGE determine size by referencing to a ladder containing known protein species sizes. The ProteoAnalyzer thus has a sizing range from 10 to 240 kDa when using only the lower marker (LM), and 10 to 200 kDa when using the LM and upper marker (UM). In this technical overview, SDS-PAGE used a ladder that contained bands from 10 to 250 kDa.

To provide a comparison of protein separations and analysis between the ProteoAnalyzer and SDS-PAGE, serial dilutions of BSA and CAII were assessed on both systems. The ProteoAnalyzer collects data throughout electrophoresis as the sample migrates past a detector. Representative results from the ProteoAnalyzer are displayed in the Agilent ProSize data analysis software as electropherograms with time or size plotted on the X-axis and relative fluorescence on the Y-axis (BSA: Figure 1A; CAII: Figure 2A). A LM is used for alignment of the samples and ladder, allowing for accurate and precise sizing, along with an optional UM that can be added for improved alignment. Signals above the electropherogram baseline are equivalent to samples detected on a gel. The software also translates the electropherograms into a digital gel image for easy visualization. Digital gel images from the serial dilutions of BSA and CAII are shown in Figures 1B and 2B, respectively. By analyzing multiple samples in parallel, the ProteoAnalyzer provides easy, quick, and automated data analysis.

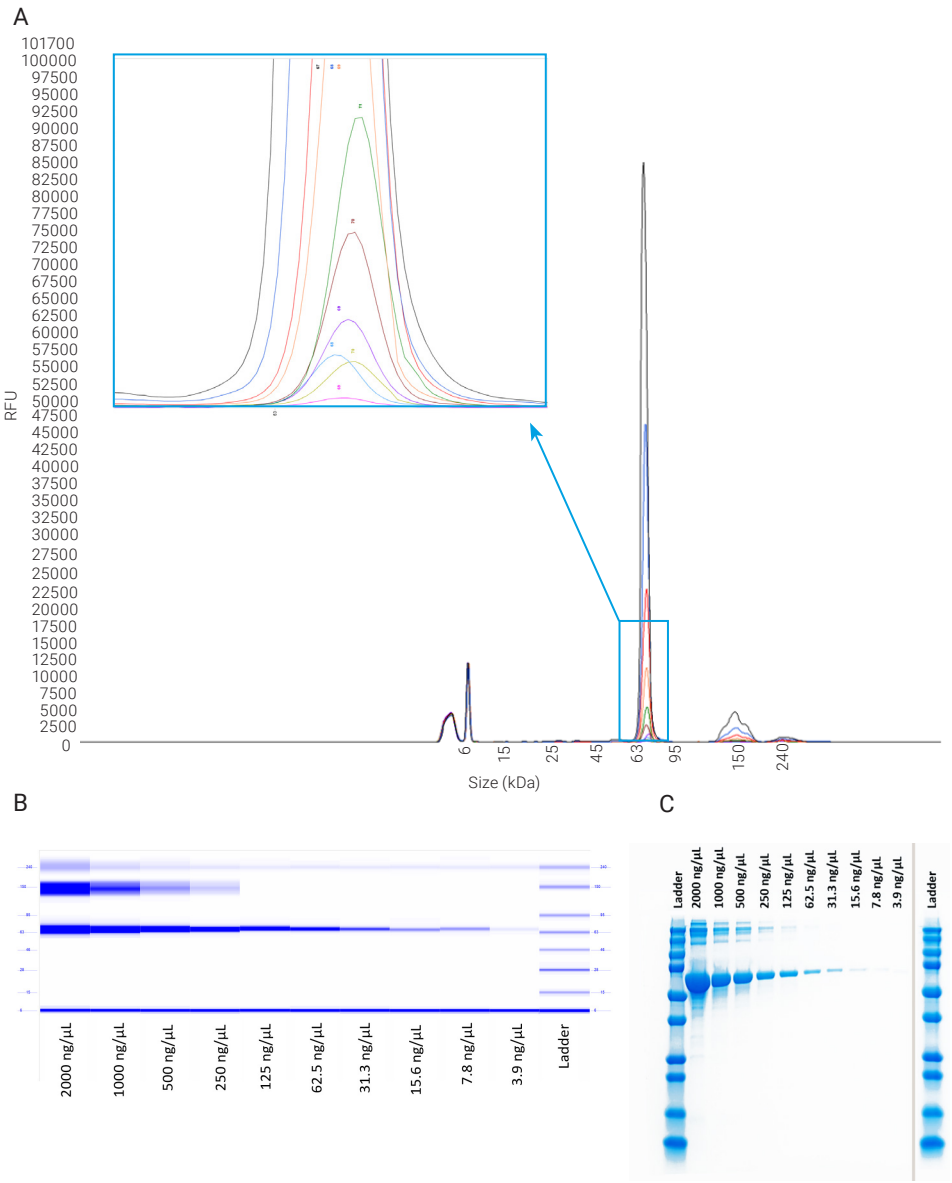


Figure 1. A serial dilution of BSA was analyzed on the Agilent ProteoAnalyzer system and SDS-PAGE gels for comparison. Results from the ProteoAnalyzer system are displayed as (A) an electropherogram overlay, with the smaller concentrations highlighted in the inset box, and (B) a digital gel image. (C) The same samples are shown on SDS-PAGE gel.

Alternatively, for SDS-PAGE, a final image of the gel is taken after electrophoretic separation and lengthy staining and destaining protocols. Interpretation of SDS-PAGE gels can be tedious and error prone but can be aided using software.[2] However, the analysis is still subject to errors that are inherent to SDS-PAGE separation. For example, the gels can be easily overloaded and thus misrepresent the size and quantification of the sample, as demonstrated in Figures 1C and 2C. The serially diluted samples were loaded onto the gel with the larger concentrations on the left and descending concentrations to the right. The left lanes at the highest concentrations (2,000 to 500 ng/µL) are overloaded, as indicated by the smearing pattern surrounding the sample bands. This smearing effect can make it difficult to achieve accurate quality or sizing analysis, as it is difficult to determine which part of the band to use to compare to the reference ladder. In these examples, sample lanes loaded at or below 250 ng/µL are at optimal concentrations and are visualized as single, sharp bands that can be easily compared to the ladder. In addition to overloading, issues with gel electrophoresis such as curving of the gel, often referred to as smiling[3], can lead to inaccurate sizing as the sample and ladder fail to migrate at the same time. Unlike the ProteoAnalyzer, SDS-PAGE does not use alignment markers with the samples to help with correcting migration issues. Although it is the traditional way of assessing protein samples, SDS-PAGE is still inaccurate prone and laborious to analyze. The ProteoAnalyzer streamlines the analysis process. and provides an improved form of protein QC that is not subject to the same errors as SDS-PAGE.

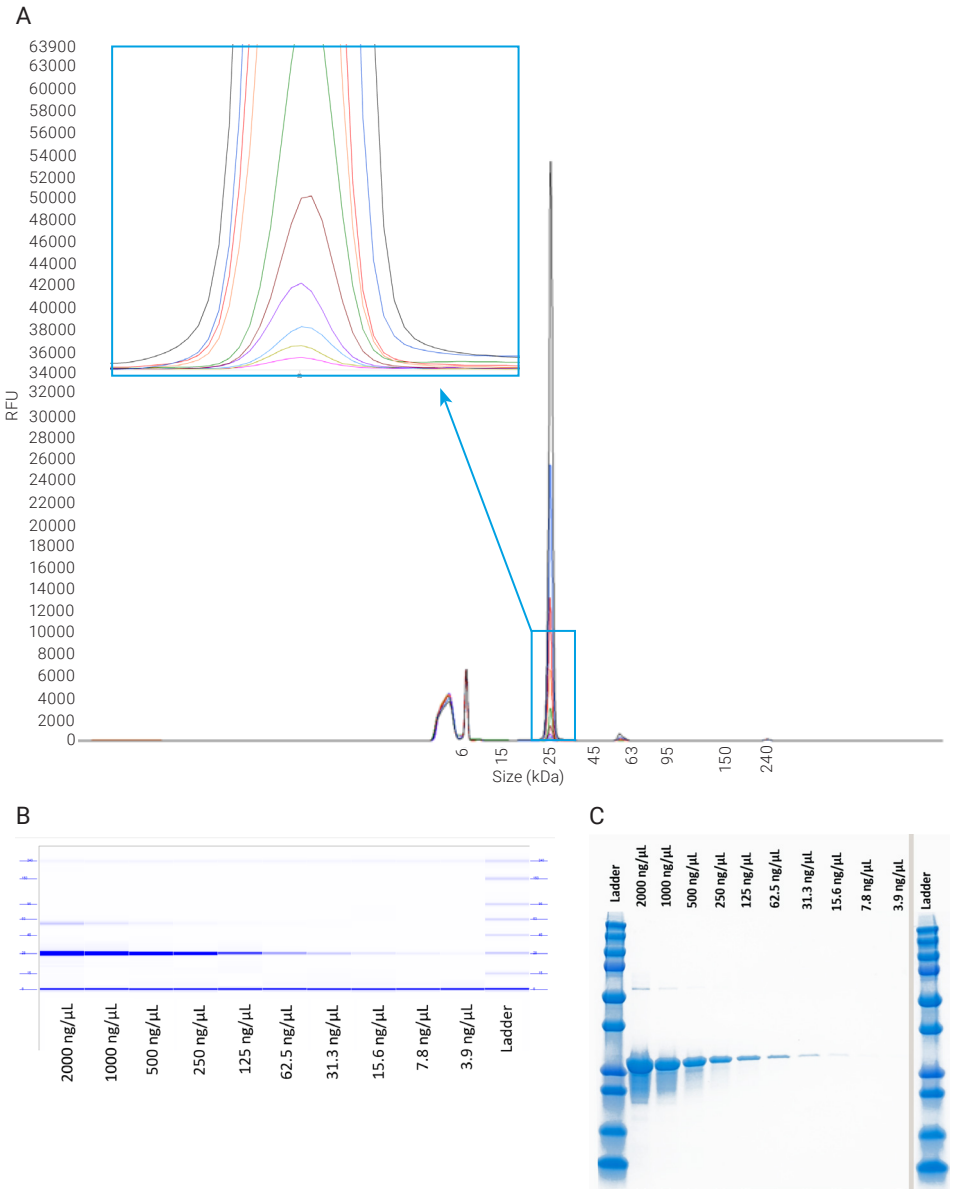


Figure 2. A serial dilution of CAII was analyzed on the Agilent ProteoAnalyzer system and SDS-PAGE gels for comparison. Results from the ProteoAnalyzer system are displayed as (A) an electropherogram overlay, with the smaller concentrations highlighted in the inset box, and (B) a digital gel image. (C) The same samples are shown on SDS-PAGE gel.

Sizing accuracy and precision of BSA

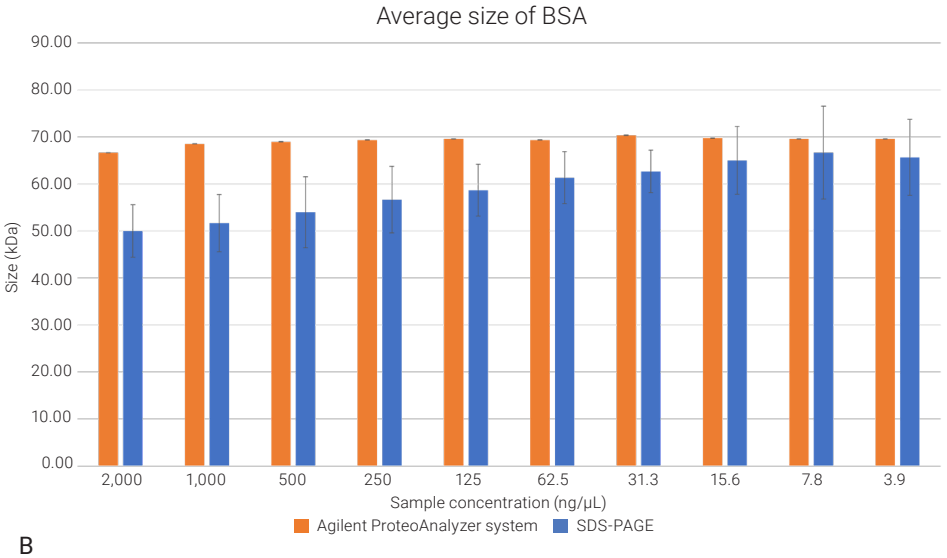
To compare fragment sizing between the ProteoAnalyzer and SDS-PAGE, BSA was assessed on both systems. BSA has a known size of 66 kDa. The sample was analyzed across the serial dilution and the average size observed was 69.2 kDa using the ProteoAnalyzer and 59.2 kDa using SDS-PAGE (Figure 3A).

The sizing accuracy between systems was assessed by comparison of their calculated percent errors for each dilution against the known sizes of the proteins (Figure 3B). Analysis of BSA with the ProteoAnalyzer displayed an average error of 4.81%, while SDS-PAGE had an average error of 10.25%. Further assessment of each concentration within the serial dilution showed that when proteins were assessed with SDS-PAGE, the percent error improved as the concentration decreased, revealing a concentration-dependent effect on sizing accuracy. Alternatively, the percent error achieved by the ProteoAnalyzer remained consistent despite the concentration of the sample, indicating that there is no concentration-dependent effect on sizing analysis (Figure 3B).

The dilution series was run three separate times on the ProteoAnalyzer and on three separate gels for SDS-PAGE to measure the precision of each system across different runs. The precision of each system was compared using the %CV calculated for each concentration analyzed across the serial dilution (Figure 3B). Overall, the ProteoAnalyzer had an average of 1.53% CV for BSA, while SDS-PAGE had an average of 13.71% CV. Precision for SDS-PAGE displayed a high %CV for samples between 3.9 and 15.6 ng/µL and samples ranging from 250 to 2,000 ng/µL. Alternatively, in the midrange of the tested concentration, samples from 31.3 to 125 ng/µL showed lower %CVs, indicating that this midrange was more precise than the flanking ranges. These results indicate that SDS-PAGE analysis can be inconsistent between gels and across concentrations. In contrast, the ProteoAnalyzer showed low %CVs, indicating high precision results across the dilution range, providing confidence that there is little run-to-run variation when analyzing samples.

Overall, for BSA, the ProteoAnalyzer showed high accuracy and excellent precision. However, SDS-PAGE displayed a large range of percent error values and varying precision throughout the dilution range. The data presented here provides assurance about the sizing capabilities of the ProteoAnalyzer.

A



B

BSA sizing assessment						
	Average Size (kDa)		Accuracy (% error)		Precision (%CV)	
Sample Concentration	ProteoAnalyzer	SDS-PAGE	ProteoAnalyzer	SDS-PAGE	ProteoAnalyzer	SDS-PAGE
2,000 ng/µL	66.7	50.0	1.01%	−24.24%	0.57%	11.14%
1,000 ng/µL	68.5	51.7	3.84%	−21.72%	0.59%	11.83%
500 ng/µL	69.0	54.0	4.49%	−18.18%	0.08%	13.98%
250 ng/µL	69.4	56.7	5.10%	−14.14%	0.46%	12.52%
125 ng/µL	69.6	58.7	5.40%	−11.11%	0.87%	9.39%
62.5 ng/µL	69.4	61.3	5.10%	−7.07%	0.46%	8.98%
31.3 ng/µL	70.4	62.7	6.62%	−5.05%	1.37%	7.20%
15.6 ng/µL	69.8	65.0	5.71%	−1.52%	0.54%	11.09%
7.8 ng/µL	69.6	66.7	5.40%	1.01%	0.79%	14.80%
3.9 ng/µL	69.6	65.7	5.40%	−0.51%	0.87%	12.31%
Overall	69.2	59.2	4.81%	−10.25%	1.53%	13.71%

Figure 3. (A) Average observed sizes for two-fold serially diluted BSA from both the Agilent ProteoAnalyzer system and SDS-PAGE. Error bars represent standard deviation. (B) Average size, % error, and %CV for each dilution of BSA using the ProteoAnalyzer and SDS-PAGE. (n = 3)

Sizing accuracy and precision CAII

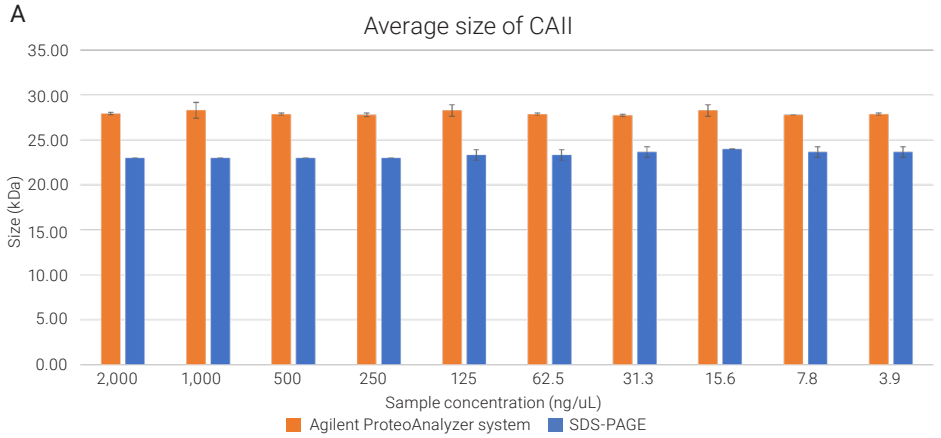
To further compare sizing of proteins, both the ProteoAnalyzer and SDS-PAGE were employed to assess CAII, which has a known size of 29 kDa. Across the dilution range, the average size observed from the ProteoAnalyzer was 28.0 kDa, while SDS-PAGE yielded an average size of 23.4 kDa (Figure 4A).

To compare the sizing accuracy that can be achieved by each of the two systems, the calculated percent errors for each concentration of CAII across the serial dilution was assessed (Figure 4B). The ProteoAnalyzer displayed an overall average error of 3.55%, indicating excellent accuracy, while SDS-PAGE exhibited a notably higher average error of 19.43%, indicative of lower accuracy. Evaluating each individual dilution indicated that the ProteoAnalyzer displayed high accuracy overall, while SDS-PAGE showed lower accuracy for each dilution.

The dilution series was run three separate times on the ProteoAnalyzer and on three individual gels for SDS-PAGE. Precision was assessed for both systems by analyzing the %CV for each serial dilution across runs (Figure 4B). The ProteoAnalyzer exhibited an overall average of 1.43% CV, indicative of a high level of precision. In contrast, the utilization of SDS-PAGE for the same sample dilution yielded 2.10% CV. Both methods had good precision, indicating consistent results.

Overall, for CAII, the ProteoAnalyzer showed low percent errors across the dilution range, indicative of excellent accuracy. Alternately, SDS-PAGE had high percent errors across all concentrations, revealing low accuracy. The ProteoAnalyzer and SDS-PAGE had low %CV values for each dilution, signifying highly consistent results each time the sample was analyzed. The ProteoAnalyzer displayed both high accuracy and high precision when

A



B

CAII sizing assessment						
	Average Size (kDa)		Accuracy (% error)		Precision (%CV)	
Sample Concentration	ProteoAnalyzer	SDS-PAGE	ProteoAnalyzer	SDS-PAGE	ProteoAnalyzer	SDS-PAGE
2,000 ng/µL	27.9	23	−3.68%	−20.69%	0.41%	0.00%
1,000 ng/µL	28.3	23	−2.41%	−20.69%	3.06%	0.00%
500 ng/µL	27.9	23	−3.91%	−20.69%	0.41%	0.00%
250 ng/µL	27.8	23	−4.14%	−20.69%	0.72%	0.00%
125 ng/µL	28.3	23.3	−2.53%	−19.54%	2.27%	2.47%
62.5 ng/µL	27.9	23.3	−3.91%	−19.54%	0.41%	2.47%
31.3 ng/µL	27.7	23.7	−4.37%	−18.39%	0.42%	2.44%
15.6 ng/µL	28.3	24	−2.53%	−17.24%	2.27%	0.00%
7.8 ng/µL	27.8	23.7	−4.14%	−18.39%	0.00%	2.44%
3.9 ng/µL	27.9	23.7	−3.91%	−18.39%	0.41%	2.44%
Overall	28.0	23.4	−3.55%	−19.43%	1.43%	2.10%

Figure 4. (A) Average observed sizes for two-fold serially diluted CAII from both the Agilent ProteoAnalyzer system and SDS-PAGE. Error bars represent standard deviation. (B) Average size, % error, and %CV for each dilution of CAII using the ProteoAnalyzer and SDS-PAGE. (n = 3)

Concentration

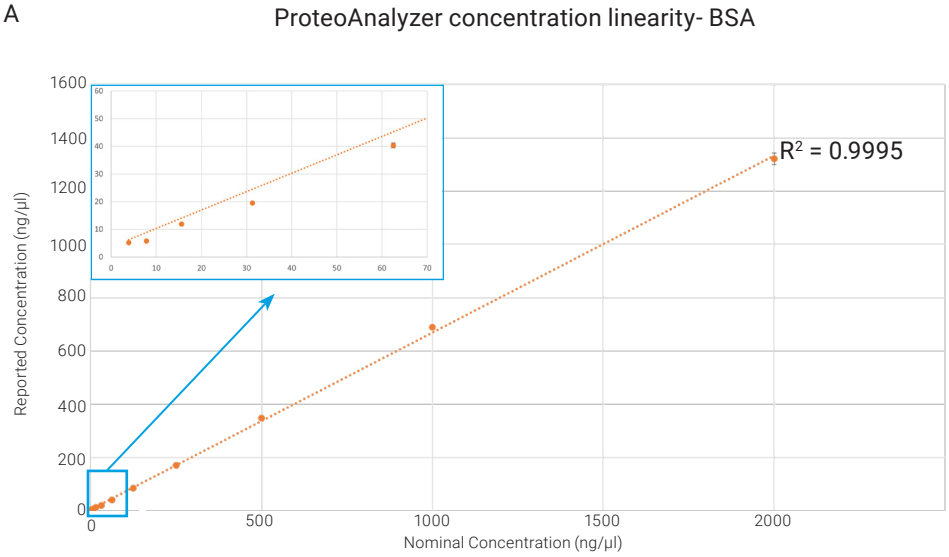
The ProteoAnalyzer measures the concentration of a sample by comparing the area of the sample peak to the area of the LM, which is used as an internal quantification standard. The ProteoAnalyzer measures the concentration of a sample by comparing the area of the sample peak to the area of the LM, which is used as an internal quantification standard. The system provides a three-log quantitative dynamic range, with a sample input concentration range of 2 to 2,000 ng/µL.

In contrast for SDS-PAGE separation, the gel is stained with Coomassie stain. This stain has a dynamic range of two logs or less. The larger dynamic range provided by the ProteoAnalyzer allows for lower levels of detection for better sample integrity analysis and impurity detection.

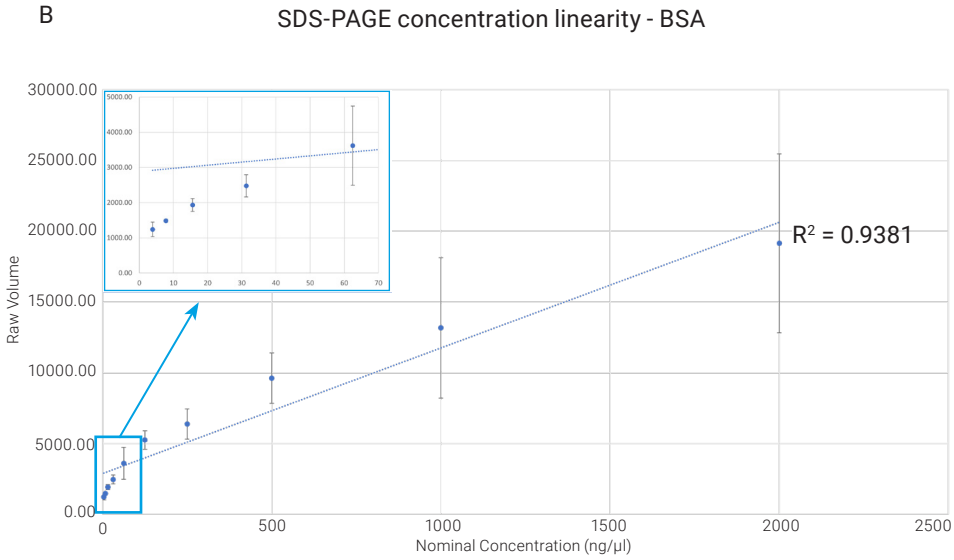
The quantification results that can be achieved with the ProteoAnalyzer and SDS-PAGE were compared by examining the linearity and precision calculated from each system's measurements. The ProteoAnalyzer outputs sample concentrations in ng/µL. GelAnalyzer, used for SDS-PAGE analysis, outputs densitometric data referred to as "raw volume." To compare the linearity from each system, nominal concentrations were plotted on the X-axis, with the Y-axis being the observed concentrations (BSA: Figure 5A and 5B; CAII: Figure 6A and 6B). A linear regression line was fit to each plot and the coefficient of determination, R^2, was calculated.

BSA and CAII results from the ProteoAnalyzer displayed strong linear correlations, while SDS-PAGE resulted in lower R^2 values for both proteins. The ProteoAnalyzer demonstrated a calculated R^2 of 0.9995 for BSA (Figure 5A), while SDS-PAGE resulted in a lower R^2 of 0.9381 (Figure 5B).

A



B



C

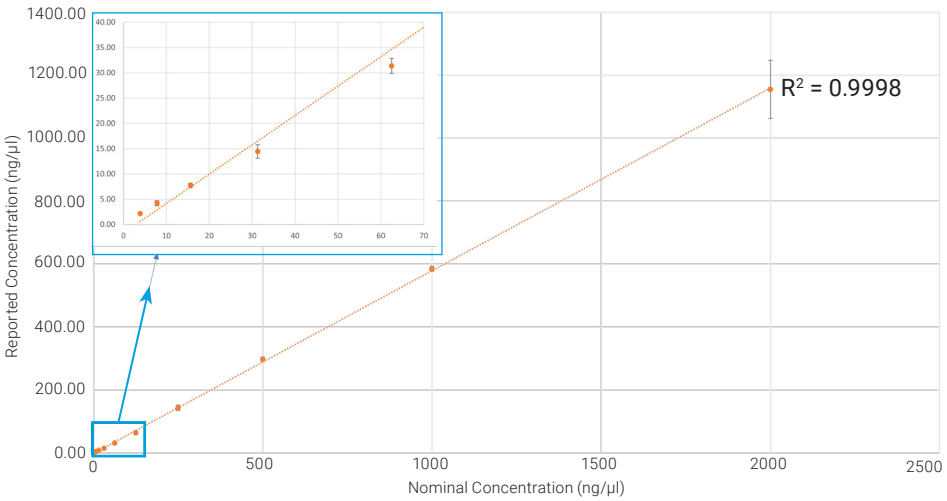
Concentration Precision (%CV) of BSA		
Nominal Concentration	ProteoAnalyzer	SDS-PAGE
2,000 ng/µL	1.28%	33.00%
1,000 ng/µL	1.45%	37.65%
500 ng/µL	0.74%	18.52%
250 ng/µL	1.33%	16.71%
125 ng/µL	1.99%	12.36%
62.5 ng/µL	2.23%	31.08%
31.3 ng/µL	1.35%	12.71%
15.6 ng/µL	3.09%	9.48%
7.8 ng/µL	4.46%	3.08%
3.9 ng/µL	7.84%	16.40%

Figure 5. Comparison between measured and nominal concentrations displayed as a linear correlation of serially diluted BSA samples with zoomed-in image of lower-concentration samples depicting the differences in linearity using (A) the Agilent ProteoAnalyzer system with the Agilent Protein Broad Range P240 kit and (B) SDS-PAGE. Error bars represent standard deviation. Also shown are the (C) resulting precision (%CV) values of measured concentrations for each serially diluted sample between each system. (n = 3)

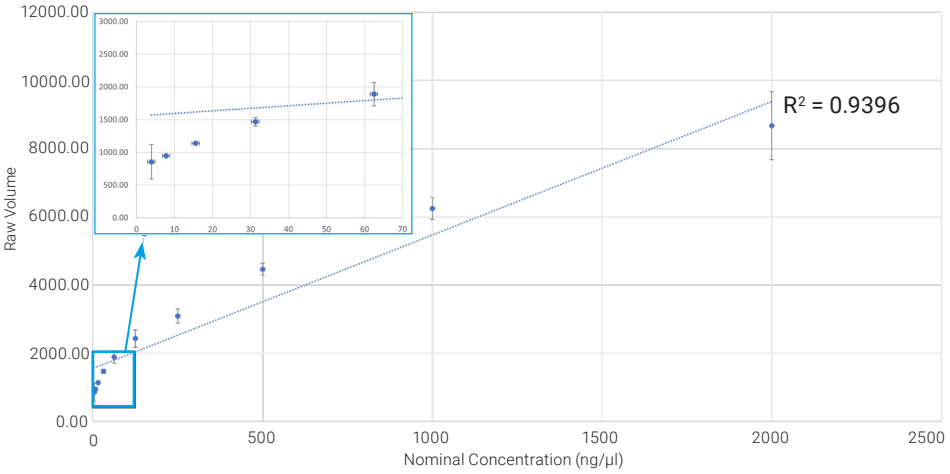
For CAII, the ProteoAnalyzer had an R^2 of 0.9998 (Figure 6A) and SDS-PAGE resulted in a lower R^2 of 0.9396 (Figure 6B). Close examination of the plots shown in Figures 5 and 6 account for the calculated R^2 values seen. For both proteins, the regression line for the ProteoAnalyzer ran through most of the points on the plots. Closer examination of the lower concentrations (inset images) shows that the regression line for the ProteoAnalyzer is plotted very close to the data points. In contrast, with SDS-PAGE, both proteins show a curved trend for all the data points but is especially apparent at the low concentrations, and caused a regression line that is not close to most of the data points. The R^2 and plots presented indicate that SDS-PAGE displays lower linearity compared to the ProteoAnalyzer, which showed excellent linearity overall.

To further assess the quantification capabilities of the systems, each protein sample was tested using three separate SDS-PAGE gels and analyzed three times on the ProteoAnalyzer to evaluate precision. The %CV values for BSA calculated from the ProteoAnalyzer data were 7.84% CV

A



B



C

CAII Concentration Precision (%CV)		
Nominal Concentration Range	ProteoAnalyzer	SDS-PAGE
2,000 ng/µL	8.02%	11.52%
1,000 ng/µL	1.34%	5.07%
500 ng/µL	2.37%	3.90%
250 ng/µL	6.03%	6.69%
125 ng/µL	2.35%	10.56%
62.5 ng/µL	4.67%	9.49%
31.3 ng/µL	9.27%	4.39%
15.6 ng/µL	6.19%	1.13%
7.8 ng/µL	11.98%	1.58%
3.9 ng/µL	8.95%	30.67%

Figure 6. Comparison between measured and nominal concentrations display a linear correlation of serially diluted CAII samples with zoomed-in image of lower concentration samples depicting the differences in linearity using (A) the Agilent ProteoAnalyzer system with the Agilent Protein Broad Range P240 kit system and (B) SDS-PAGE. Error bars represent standard deviation. Also, (C) resulting %CV values of measured concentrations for each serially diluted sample between each system. (n = 3)

or less, but up to 37.65% CV from SDS-PAGE (Figure 5C). For CAII, the ProteoAnalyzer had a calculated %CV of 11.98% or less, while SDS-PAGE had a %CV of 30.67% or less (Figure 6C). The %CV values determined from the ProteoAnalyzer were within the kit specifications for both proteins. Also, the %CVs from analysis with the ProteoAnalyzer for both proteins were significantly less than the SDS-PAGE analysis, indicating that the ProteoAnalyzer is more precise than SDS-PAGE.

Compared to SDS-PAGE, the ProteoAnalyzer displayed a stronger correlation between observed protein concentration and nominal concentrations as shown by higher R^2 values. The ProteoAnalyzer also showed greater precision when measuring BSA and CAII concentrations. SDS-PAGE results display more imprecise measurements of protein amounts, with lower R^2 values and more variability between replicates. The lower correlation seen from SDS-PAGE was expected due to the two-log dynamic range from the dye, which also limits detection of low-level impurities in a sample. This data indicates that users can confidently use the ProteoAnalyzer to detect their samples across a large concentration range with great accuracy and high precision.

Conclusion

This technical overview provides a comparison of the ProteoAnalyzer to SDS-PAGE and highlights the ability of the ProteoAnalyzer to provide consistent accuracy and precision for sizing proteins, with strong quantitative linear correlations. SDS-PAGE, while widely used, presents challenges due to its labor-intensive and error-prone nature, often impeding workflow efficiency. Although software tools are available to aid analysis, they are constrained by the inherent inaccuracies of the separation process. In contrast, the Agilent ProteoAnalyzer offers a streamlined, automated, and reliable approach to protein QC analysis. Moreover, the ProteoAnalyzer's quantification within a three-log quantitative dynamic range further shows its capabilities at assessing proteins. In conclusion, this comparative assessment displayed the differences between the ProteoAnalyzer and traditional SDS-PAGE for protein QC applications.

References

1. Protein Sizing and Quantification with the Agilent ProteoAnalyzer System. *Agilent Technologies technical overview*, publication number 5994-6718EN, **2023**.
2. Istvan Lazar Jr., PhD and Istvan Lazar Sr., PhD, CSc. *GelAnalyzer 23.1*. Gelanalyzer.com. http://www.gelanalyzer.com/index.html (accessed 2023-10-06).
3. Ventzki, R.; Stegemann, J.;Martinez, L.; de Marco, A. Automated Protein Analysis by Online Detection of Laser-Induced Fluorescence in Slab Gels and 3-D Geometry Gels. *Electrophoresis* **2006**, 27 (17), 3338–3348. https://doi.org/10.1002/elps.200600006

www.agilent.com/genomics/proteoanalyzer

PR7001-1861

Published in the USA, December 07, 2023
5994-6934EN

Agilent
Trusted Answers