Performance Comparison of the Experion™ Automated Electrophoresis System and a Competing Automated System for Protein Analysis

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Introduction

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is an established technique employed for protein separation. However, this traditional gel-based method involves multiple manual steps and several hours to complete. New microfluidics-based technologies, such as the LabChip platform developed by Caliper Life Sciences, Inc., have advanced protein separations by miniaturizing and automating the time-consuming separation, staining, imaging, and analysis steps required to complete the SDS-PAGE process. Microfluidic chip-based automated electrophoresis systems, such as the Experion system and the Agilent 2100 bioanalyzer, utilize the LabChip platform to perform SDS-PAGE-like separation and analysis of protein samples.

The Experion system uses the Experion Pro260 analysis kit for the separation of 10–260 kD proteins under denaturing conditions. Typically, a Pro260 separation and analysis of up to ten samples is accomplished in approximately 30 min. This automated system can be used to perform a variety of tasks, such as monitoring purification procedures (by measuring the concentration and checking the purity of protein fractions) and performing one-dimensional differential expression studies, degradation assessments, cleavage investigations, and analysis of immunoprecipitation experiments.

This tech note presents a comparison of the performance of the Experion Pro260 analysis kit with the Agilent protein 200 plus LabChip kit, which is employed by the 2100 bioanalyzer, for protein analysis. Specifically, the accuracy and reproducibility of molecular weight (MW) estimation and quantitation of both assays were assessed, as well as their resolution, linear dynamic range, sensitivity, and salt compatibility. Overall, the data show that the performance of the Experion Pro260 analysis kit is equivalent to or exceeds that of the protein 200 plus LabChip kit for each of the parameters tested.

Methods

Protein samples included an *E. coli* protein lysate (catalog #163-2110), purified bovine serum albumin (BSA) purchased from the National Institute of Standards and Technology (NIST), and bovine carbonic anhydrase (CA) purchased from Sigma-Aldrich. Unless otherwise indicated, samples were prepared in 1x phosphate-buffered saline (PBS). Concentrations of BSA and CA were independently determined using UV spectroscopy and extinction coefficients of 0.667 (at 279 nm) and 1.73 (at 280 nm), respectively, for 1 mg/ml solutions.

The 200 plus LabChip kit, 2100 bioanalyzer, priming station, and 2100 expert software (Rev B.01.02 Si136) were purchased from Agilent Technologies. The Experion Pro260 analysis kit was used for protein analyses with the Experion system, and the protein 200 plus LabChip kit was used with the Agilent 2100 bioanalyzer; samples and microfluidic chips were prepared and loaded according to the protocols described in the instruction manuals for each kit.

Results and Discussion

Molecular Weight Estimation (Sizing)

Knowledge of a protein's MW is fundamental to many applications. For example, in studies of proteolytic or deglycosylation processes, the MW shift of a cleaved protein relative to that of the intact protein is monitored over the course of an experiment. In these and other cases where sizing is critical, it is important to obtain reliable sizing data over a broad MW range.

The Pro260 and protein 200 plus assays were examined for their ability to size the proteins in both the Pro260 and protein 200 plus ladders. In these experiments, each chip-based assay used its respective ladder as the standard by which sizing estimates were made. Results were analyzed with regard to sizing accuracy, which was measured as the percent difference from the expected MW, and sizing reproducibility, which was evaluated using the coefficient of variation, or CV ([standard deviation/mean] x 100), as a statistical measure. The results of these experiments are summarized in Table 1.

The published separation ranges of the Pro260 and protein 200 plus assays are 10–260 kD and 14.4–210 kD, respectively, and, as could be expected, each system produced the most accurate sizing for its own ladder proteins (Table 1). When the Pro260 ladder was used as sample, the Pro260 assay produced size estimates that deviated from expected values by less than 1%, as compared to deviations as high as 5% produced by the protein 200 plus assay. In addition, the protein 200 plus assay was unable to resolve the 10 kD and 260 kD proteins of the Pro260 ladder, as both were outside of its analysis range. In contrast, when the protein 200 plus ladder was used as sample, the Pro260 assay produced less accurate size estimates (deviations as high as 8%) than the protein 200 plus assay (deviations ≤3%). The data in Table 1 also show that the Pro260 analysis kit consistently produced highly reproducible results (CV ≤1.1%) regardless of the ladder used, while the protein 200 plus LabChip kit produced highly reproducible results only when its own ladder was analyzed (CV ≤1%, as compared to a CV of 0.9–7.2% with the Pro260 ladder).

Overall, both systems appear to acceptably size each other's ladder proteins, though the Experion system offers the advantages of better reproducibility and the capability to resolve and size fragments smaller than 14.4 kD and larger than 210 kD. This broader separation and sizing range is advantageous because it offers the potential for analysis of proteins encompassing a larger range of MW at one time.

Quantitation

For many downstream applications and investigations of the physical and chemical properties of proteins, concentration information is useful and often crucial. The Pro260 assay automatically determines the relative concentration of protein samples using a single-point calibration, wherein the peak area of a protein of interest is compared to the peak area of a 260 kD internal upper marker, which is present in each sample at a known concentration. Additionally, the user has the option to obtain an absolute protein concentration by using known concentrations of a purified protein to create a calibration curve on the chip.

To evaluate the accuracy and reproducibility of relative and absolute quantitation by the Pro260 and protein 200 plus assays, 100 ng/µl stock solutions of BSA and bovine CA were prepared, quantitated by UV spectroscopy, and analyzed on the two systems. The results, shown in Table 2, reveal that the Pro260 assay delivered highly reproducible relative quantitation (CV of 4.3% and 10.7% for BSA and CA, respectively). In contrast, the relative quantitation generated by the protein 200 plus assay and bioanalyzer was less reproducible (CV of 19.0% and 18.2%) for the same two sample preparations. To further illustrate the difference in quantitation reproducibility for the two systems, all data points acquired for BSA quantitation were plotted (Figure 1). The clustering of the Pro260 assay data indicates that the interchip and intrachip reproducibility of quantitation by this assay was superior to that observed with the protein 200 plus assay.

For both protein analysis systems, the accuracy of relative quantitation was much greater for the BSA sample than for CA. The Pro260 assay produced deviations in quantitation of approximately 7% and 71% for BSA and CA, respectively, and the protein 200 plus assay quantitated these same two protein samples to within approximately –20% and 48% of the spectroscopically measured concentration (Table 2).

Table 1. Comparison of accuracy* and reproducibility of sizing the proteins in the Pro260 and protein 200 plus ladders.** The Experion Pro260 and Agilent protein 200 plus ladders were separated with the Experion Pro260 analysis kit and the Agilent protein 200 plus LabChip kit using the Experion system and the Agilent 2100 bioanalyzer, respectively. The 260 kD and 210 kD proteins in the Pro260 and protein 200 plus ladders, respectively, were not included in the data because they served as internal standards in all wells.

	Experion				Bioanalyzer			
Sample	# of Wells	Mean MW (kD)	Accuracy	Reproducibility	# of Wells	Mean MW (kD)	Accuracy	Reproducibility
Pro260 Ladder								
10 kD band	29	10.0	–0.4%	0.5%	—	—	—	—
20 kD band	29	20.1	0.4%	0.9%	25	19.3	–3%	7.2%
25 kD band	29	25.1	0.3%	1.1%	25	23.9	–4%	2.1%
37 kD band	29	37.1	0.4%	1.0%	25	35.2	–5%	4.8%
50 kD band	29	50.2	0.4%	1.0%	25	47.7	–5%	3.2%
75 kD band	29	75.5	0.7%	1.0%	25	72.7	–3%	3.2%
100 kD band	29	100.4	0.3%	0.7%	25	100.0	0%	1.9%
150 kD band	29	150.4	0.3%	0.5%	25	142.9	–5%	0.9%
Protein 200 Plus Ladder								
14.4 kD band	25	15.4	7%	0.7%	24	13.9	–3%	1%
21.5 kD band	25	22.6	5%	0.7%	24	20.8	–3%	1%
29 kD band	25	30.5	5%	0.8%	24	28.2	–3%	0.7%
32.5 kD band	25	34.3	5%	0.7%	24	31.9	–2%	0.8%
53 kD band	25	57.1	8%	0.8%	24	52.8	–0.3%	0.6%
66.7 kD band	25	71.2	7%	0.7%	24	66.6	–0.1%	0.8%
97.4 kD band	25	98.2	0.8%	0.6%	24	97.8	0.5%	0.7%
117 kD band	25	122.4	5%	0.4%	24	116.9	0.0%	0.5%

* Calculated as % difference relative to expected.

** Calculated as % CV.

Table 2. Comparison of accuracy and reproducibility of relative and absolute protein quantitation. BSA and CA at the concentrations indicated were separated with the Experion Pro260 analysis kit and Agilent protein 200 plus LabChip kit using the Experion system and the Agilent 2100 bioanalyzer, respectively. Absolute quantitation data were analyzed using the calibrated curve function of each system's software.

		Experion				Bioanalyzer			
Sample	UV-Based Conc. (ng/µl)	# of Wells	Measured Conc. (ng/µl)	Accuracy	Reproducibility	# of Wells	Measured Conc. (ng/µl)	Accuracy	Reproducibility
Relative quantitation									
BSA	102	29	109	7.0%	4.3%	25	82	–19.6%	19.0%
CA	94.2	30	161	70.6%	10.7%	25	139	48.0%	18.2%
Absolute quantitation									
CA	93.6	15	93.4	–0.2%	17.3%	15	85.5	–8.7%	14.0%
CA	502	9	581.2	15.8%	16.9%	10	554.5	10.5%	6.0%

Such differences in quantitation accuracy for different proteins also occur with other commonly used protein quantitation methods, such as the Bradford and Lowry assays (bulletin 1069, Bradford 1976, Lowry et al. 1951), and are often due to differences in protein staining efficiency. Unique protein characteristics, such as primary sequence, amino acid composition, isoelectric point, and presence of modified amino acids and prosthetic groups, can affect the levels of staining and colorimetric signals produced. Both microfluidic systems perform quantitation using a single-point calibration to an internal upper marker protein; thus, relative quantitation accuracy is often highest for proteins that stain with similar efficiency to the upper marker protein.

Because proteins exhibit differences in staining behavior, an alternative protocol exists for their quantitation. By loading a chip with a series of known concentrations of a purified protein, the user can generate a calibration curve. An equation derived from the linear fit of the data points comprising this curve is then used to quantitate each protein in the other sample wells. This form of absolute quantitation performs best when the protein used for calibration is the same as that undergoing quantitation.

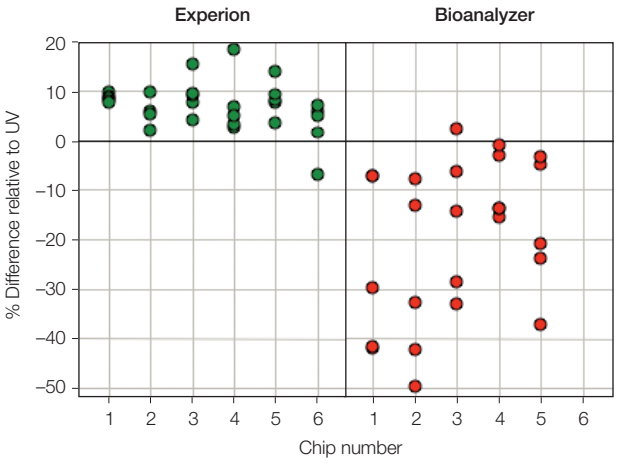

Fig. 1. Scatter plot comparison of quantitation accuracy. Graphical representation of intrachip and interchip data for BSA. Left panel, data generated by the Experion Pro260 analysis kit using the Experion system and 6 chips for a total of 29 data points; right panel, data generated by the protein 200 plus LabChip kit using the Agilent 2100 bioanalyzer and 5 chips for a total of 25 data points. Each dot represents data recorded from one well, and each column indicates a separate chip run.

We applied this absolute quantitation approach using both microfluidic systems to see if the quantitation accuracy for CA could be improved. The software of each system was directed to generate a calibration curve from a dilution series, and then use a linear fit of the data to quantitate two concentrations of CA. Using this approach, accuracy of quantitation by the Pro260 assay was significantly improved, to within 15.8% of the UV-determined concentration, and the protein 200 plus assay, to within 10.5% (Table 2).

Resolution

Another important parameter that defines the overall performance of a protein separation system is its capacity for resolving one protein from another. Identification and quantitation improve as a protein of interest is separated to a greater degree from neighboring proteins.

To evaluate the resolution capabilities of each automated electrophoresis system, a 2 mg/ml *E. coli* protein lysate was separated with the Pro260 and protein 200 plus LabChip kits, and the total number of peaks detected by the software was recorded. An average of 35 protein peaks were identified with the Pro260 assay upon separation of this complex protein sample; essentially the same number of protein bands were observed when the same sample was separated by SDS-PAGE on 4–20% gradient gels (data not shown). On the other hand, the protein 200 plus assay detected an average of only 23 protein peaks from the same sample. Representative electropherograms are shown in Figure 2 to demonstrate the superior resolving capabilities of the Experion system.

The resolution of two symmetrical peaks can also be defined using a resolution value, R_s, which is defined by the equation $R_s = 1.17(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 = migration times of the protein peaks, and w_1 and w_2 = widths of the peaks at half height. Using this equation, $R_s > 1.5$ indicates that two adjacent peaks are baseline resolved.

To calculate the R_s that might theoretically be achieved with both systems, we used the migration time and peak width data collected from the Pro260 ladder separations as reference points. For this analysis, the R_s achievable by each system was calculated for each ladder peak (within each system's respective separation range) and a hypothetical protein 10% larger in MW but producing a peak of the same shape. Experion software exported the migration times and peak width data necessary for solving the equation; however,

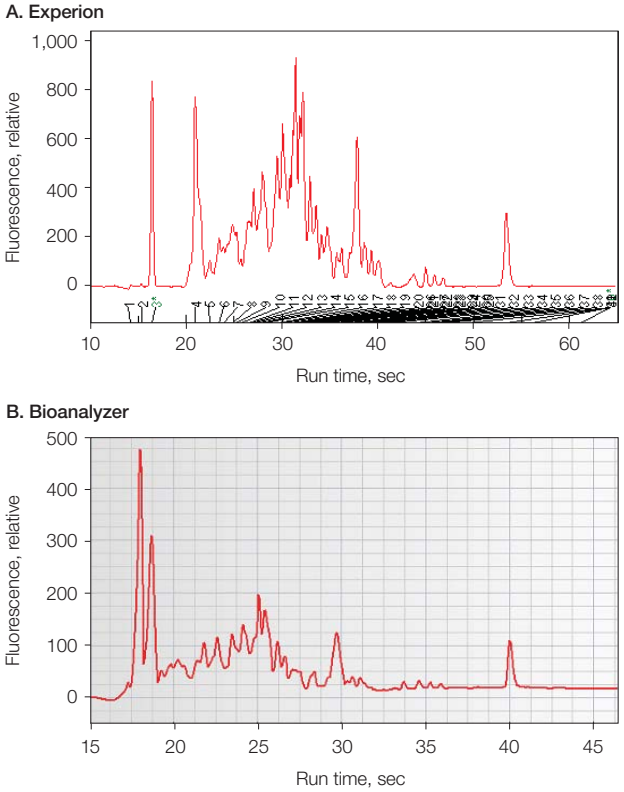

Fig. 2. Comparison of protein detection from an *E. coli* lysate. A, electropherogram of separation with the Experion Pro260 analysis kit using the Experion system; B, electropherogram of separation with the Agilent protein 200 plus LabChip kit using the Agilent 2100 bioanalyzer. The Pro260 analysis displays better resolution and a greater number of peaks.

these data had to be manually determined for the bioanalyzer. Note that resolution numbers obtained were theoretical and that, in reality, the apparent resolution for chip separation is dependent on and limited by several factors. Such factors include the heterogeneity of a protein population, and modification of proteins in the sample, which can cause peak broadening and can alter protein migration.

The calculated R_s values were plotted as a function of the expected MW of each protein in the ladder (Figure 3). With the Pro260 assay, R_s increased with increasing protein size, from 1.2 for a 10 and 11 kD protein pair to 2.0 for a pair of proteins of 260 and 286 kD. The Experion data reveal the point at which two proteins differing in MW by 10%, and with peak shapes similar to those of the Pro260 ladder proteins, can be completely resolved by the system ($R_s > 1.5$). These criteria are met with proteins larger than 25 kD. The R_s values associated with the protein 200 plus separations also increased with increasing MW, but to a lesser extent ($R_s < 1.7$) and with resolution at the baseline occurring for proteins larger than about 55 kD (Figure 3). The data again show that the Pro260 analysis kit achieves improved resolution of proteins compared to the protein 200 plus assay across a broad range of MW.

Linear Dynamic Range

Of interest to users of any protein quantitation assay is its linear dynamic range, or the concentration range over which

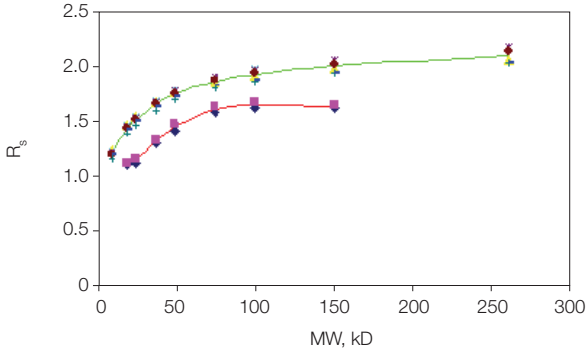

Fig. 3. Plot of the theoretical resolution (R_s) achievable by each system. Data were calculated for the resolution of Pro260 ladder proteins from theoretical proteins of the same peak shape and of 10% larger MW. Upper trace (green), Experion Pro260 analysis; lower trace (pink), protein 200 plus assay. R_s values for the 10 kD and 260 kD ladder proteins were not calculated for the protein 200 plus assay as both these proteins fell oustide of the separation range of this assay.

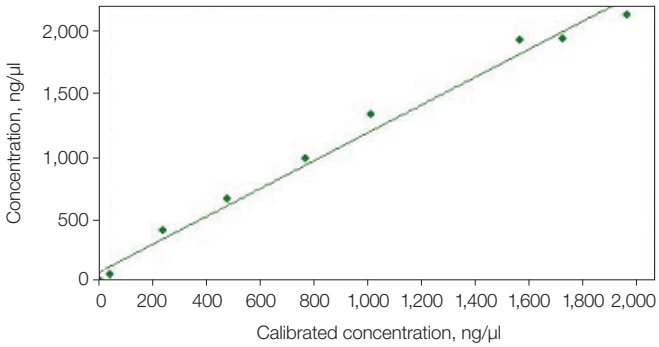

Fig. 4. Linear dynamic range of protein separation with the Experion Pro260 analysis kit. Samples of CA at the concentrations indicated were separated with the Experion Pro260 analysis kit, and the relative concentration obtained was plotted as a function of spectroscopically derived concentration. The r^2 value determination derived from the linear fit of this data set was 0.99.

the relative detection signal and concentration remain directly proportional to each other. A wide linear dynamic range permits the analysis of a wide range of sample concentrations.

This parameter was examined using the Experion Pro260 analysis kit and the protein 200 plus LabChip kit by loading a chip with increasing concentrations of CA covering nearly three orders of magnitude (2.5–2,000 ng/µl). Using the calibration curve function in each software package, a linear fit of the data was created and a coefficient of determination, r^2, was calculated. On average, the 10-point concentration series analyzed on each Experion chip displayed an r^2 value of 0.98. A representative plot of the data from one Pro260 analysis is shown in Figure 4. Though plots of the data from the same samples run on the bioanalyzer system also yielded an average r^2 of 0.98, the protein 200 plus assay did not detect the 2.5 ng/µl peak or clearly distinguish the 10 ng/µl peak; therefore, graphs drawn from these data covered the smaller 50–2,000 ng/µl range rather than the full range tested.

Sensitivity

The sensitivity, or limit of detection, of each system is also an important performance specification and is one factor that distinguishes the two automated electrophoresis systems.

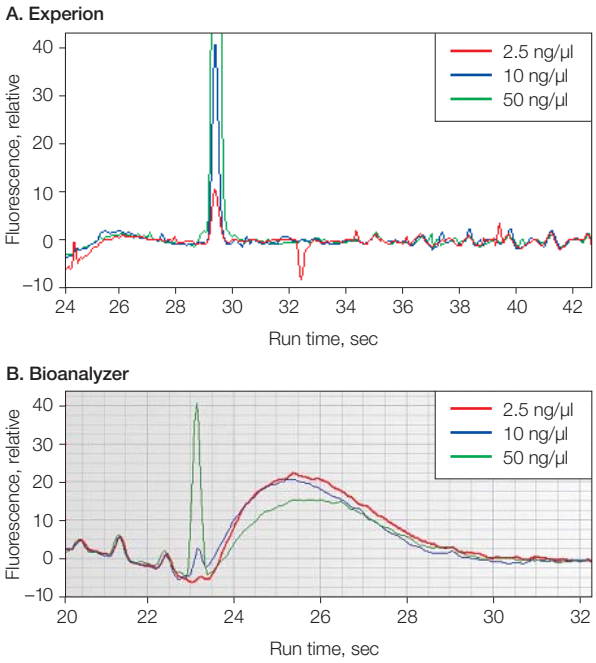

Fig. 5. Comparison of sensitivity. Electropherograms obtained from separations of CA with the Experion system (A) and the bioanalyzer (B). The region near the CA peak has been expanded. The vertical fluorescence scale is the same in both plots, but migration times vary for the two systems and could not be exactly compared. Note that the Experion Pro260 analysis of the 2.5 ng/µl sample generated relative fluorescence units similar to those obtained for the 10 ng/µl sample analyzed with the protein 200 plus assay. The same trend was observed for the 10 and 50 ng/µl samples, suggesting that the Pro260 analysis is approximately 4-fold more sensitive than the protein 200 plus assay. Additionally, the large broad peak (24–28 sec) in the bioanalyzer electropherogram, which may be generated by contaminants in the sample buffer, adds to the difficulty in detecting the smaller CA peak.

Sensitivity becomes particularly important when protein concentrations are low or when purity is being evaluated. The separation of the *E. coli* lysate on both systems (Figure 2) illustrates that the Experion system is more sensitive than the bioanalyzer; when an identical sample was loaded onto both systems, the peaks in the Experion electropherogram were of a much higher intensity than those in the bioanalyzer electropherogram.

The sensitivity of both systems was also evaluated by comparing the signal-to-noise ratio produced by a dilute sample of CA (2.5 ng/µl). The peak generated by this sample was not identified by the protein 200 plus assay (Figure 5); however, based on the peak height generated on the Experion system, a mean signal-to-noise ratio of 20.5 was calculated. The electropherogram shown in Figure 5A illustrates the extent to which the fluorescence signal associated with this CA sample rises above the background with the Experion system. The low detection limit of the Experion Pro260 analysis reveals that this assay has a sensitivity comparable to that of colloidal Coomassie Blue G-250 stain, and enables the detection of impurities that are present in a sample at levels as low as 0.1–0.2% of the target protein concentration.

Since the protein 200 plus assay could not detect the 2.5 ng/µl CA sample, an electropherogram showing an overlay of the 2.5, 10, and 50 ng/µl CA samples was used to estimate the relative sensitivity of both assays (Figure 5). The Experion Pro260 analysis of the 2.5 ng/µl sample generated relative fluorescence units similar to those obtained for the 10 ng/µl sample analyzed with the protein 200 plus assay. The same trend was observed for the 10 ng/µl and 50 ng/µl samples. These data imply an approximately 4-fold greater sensitivity of the Pro260 assay over the protein 200 plus assay, though working near the limit of detection of any instrument or assay is rarely advisable.

In the above analyses, bovine CA was used in determining the sensitivity of both systems. However, when other protein samples are used, various factors may influence the overall assay sensitivity for either system. First, sensitivity may vary depending on the staining efficiency of a particular protein, as discussed previously. Thus, a protein with poor affinity for the stain would have to be present at concentrations above 2.5 ng/µl to be detected above the baseline. Second, the shape of a peak may affect the lower detection limit, as broader peaks are more difficult to identify above the baseline. Finally, and probably most significantly, the buffer composition plays a large role in setting the limit of detection for a protein sample. Samples are loaded into the separation channel of the chip by an electrokinetic injection process; therefore, the amount of protein injected depends on the ionic strength of the buffer in which the sample is solubilized. A buffer conductivity similar to that of 1x PBS is recommended. If the protein sample has a higher conductivity, then less protein will be injected and the limit of detection will rise. The opposite should also be true — for samples of low conductivity, the limit of detection will likely decrease, thereby enhancing sensitivity.

Effects of Salt

The fact that the sensitivity of an assay decreases with increasing sample conductivity may be problematic because many chromatographic procedures involve eluting fractions with a salt gradient, or in a stepwise fashion with increasing salt concentration. It is, therefore, important that the protein assay used to analyze these chromatographic fractions be able to handle varying levels of salt without affecting the accuracy and reproducibility of sizing or quantitation.

To examine the effect of salt concentration on the ability of each system to correctly determine the MW and concentration of each CA sample, alternating wells of each chip were loaded with 100 ng/µl CA in 10 mM sodium phosphate buffers (pH 7.4) containing either 0.15 M NaCl (1x PBS), 0.25 M NaCl, or 0.5 M NaCl. With both systems, no obvious salt-related effects on the accuracy and reproducibility of sizing were observed (Table 3). Increasing salt concentrations did, however, have a pronounced effect on the accuracy of quantitation by the protein 200 plus assay (Table 4), shown by deviations from expected values at the 0.5 M NaCl level that were almost double those displayed by the controls. For the Pro260 analysis, the accuracy and reproducibility of quantitation remained unchanged regardless of the level of salt in the protein sample.

Table 3. Comparison of accuracy and reproducibility of sizing in buffers of increasing salt concentration. CA (100 ng/µl) prepared in 1x PBS buffer (0.15 M NaCl) or in 1x PBS buffer containing NaCl at final concentrations of 0.25 M or 0.5 M was separated with the Experion Pro260 analysis kit and Agilent protein 200 plus LabChip kit using the Experion system and the bioanalyzer, respectively.

	Experion				Bioanalyzer			
[NaCl]	# of Wells	Mean MW (kD)	Accuracy	Reproducibility	# of Wells	Mean MW (kD)	Accuracy	Reproducibility
0.15 M	20	30.46	5.04%	0.92%	20	28.12	–3.03%	0.77%
0.25 M	15	30.50	5.16%	0.52%	15	28.18	–2.83%	0.65%
0.50 M	15	30.47	5.08%	0.66%	15	28.34	–2.28%	0.61%

Table 4. Comparison of accuracy and reproducibility of quantitation in buffers of increasing salt concentration. Analysis as in Table 3.

		Experion				Bioanalyzer			
[NaCl]	UV-Based Conc. (ng/µl)	# of Wells	Measured Conc. (ng/µl)	Accuracy	Reproducibility	# of Wells	Measured Conc. (ng/µl)	Accuracy	Reproducibility
0.15 M	99.4	20	191	92%	10%	20	157	58%	28%
0.25 M	98.3	15	190	93%	12%	15	184	87%	31%
0.50 M	103	15	195	90%	9%	15	221	114%	20%

To further emphasize the minimal variance of the Pro260 analysis in the presence of higher salt concentrations, data from individual chips were plotted (Figure 6). Evident from the plot are the consistent CA concentration determinations and clustering of the data points for the Pro260 analysis at all three salt concentrations.

Conclusions

Overall, the Experion Pro260 analysis was equivalent to or even superior to the protein 200 plus assay in every perfor`mance category examined. The Pro260 analysis displayed a broader separation range, allowing for the analysis of a larger population of proteins, and delivered accurate and reproducible sizing and relative quantitation of proteins, even in the presence of high concentrations of salt.

Accurate absolute quantitation was also achieved using the internal calibration curve function in the Experion software. Moreover, the Experion system provided enhanced resolution capabilities and up to 4-fold higher sensitivity, permitting the separation and detection of more sample proteins. The Experion system also provided a linear response over a concentration range of nearly three orders of magnitude, giving greater flexibility with sample loads. Taken together, the integrated, high-quality components of the Experion system and Pro260 analysis kit offer an automated platform that provides superior data quality for a variety of protein applications.

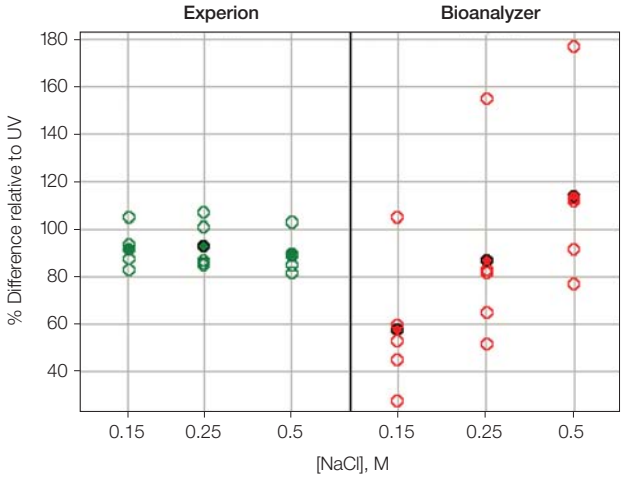

Fig. 6. Scatter plot comparison of quantitation accuracy under various salt concentrations. Graphical representation of intrachip data (open circles) and interchip data (closed circles) for 100 ng/µl CA in 1x PBS buffer (0.15 M), and in 1x PBS buffer containing NaCl at final concentrations of 0.25 M or 0.5 M. Left panel, data generated by the Experion Pro260 analysis kit using the Experion system; right panel, data generated by the protein 200 plus LabChip kit using the Agilent 2100 bioanalyzer.

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