Automation of PA 800 *plus* CE-SDS Assays

Technical Information Bulletin

Automation of CE-SDS Sample Preparation for PA 800 *plus* IgG Purity / Heterogeneity Assays Using a Biomek 4000 Automation Workstation

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Abstract

The Beckman Coulter PA 800 *plus* Pharmaceutical Analysis System was designed in collaboration with the pharmaceutical industry to maximize the advantages of capillary electrophoresis (CE) and has become the industry standard for CE-SDS analysis. However, CE-SDS sample preparation for the PA 800 *plus* is still a manual process, which can absorb significant operator time and remains a potential source of human error. Automation of the manual steps on the Biomek 4000 Workstation has resulted in reduced hands-on time and elimination of potential operator variability while delivering a robust assay.

In this application note, we demonstrate the automation of a CE-SDS workflow to prepare IgG protein samples on the Biomek 4000 Workstation. The fully automated denaturation and reduction of IgG controls resulted in highly reproducible peak migration times with CVs <2% and corrected peak areas with CVs <3% using UV absorbance at 220nm. These results were accomplished using both β-mercaptoethanol (n=48) and TCEP (n=24) as reducing agents and demonstrated no cross contamination between samples.

4000 Workstation, buffer trays and

Introduction

Although the PA 800 *plus* (Figure 1) replaces the manual process of pouring and running slab gels, sample preparation must still be performed manually. Manual sample preparation is time-consuming and remains a potential source of operator variation and human error. Automation of the sample preparation process ahead of CE-SDS analysis by the PA 800 *plus* has the potential to address these issues.

The sample preparation for CE-SDS analysis of MAbs on the PA 800 *plus* requires denaturation of the proteins by heating the samples in detergent, either in the presence or the absence of a reducing agent. Precise reagent and sample transfers are essential for accurate protein quantification and robustness of mixing and heating are essential for accurate migration times. To run the prepared samples, the PA 800 *plus* requires vials in the inlet and outlet buffer trays be filled with various kit buffers and this process is a potential source of human error. The sample and buffer trays are then loaded into the PA 800 *plus* for analysis (Figure 2).

The Biomek 4000 Workstation (Figure 1) was used to automate the sample and buffer preparation for the IgG Purity and Heterogeneity Assay kit from Beckman Coulter. The automation method drives the denaturation/reduction of 1 to 24 samples (with optional normalization) as well as transfer of the PA 800 *plus* working buffers to buffer vials. Adapter plates were designed to hold the PA 800 *plus* buffer trays on the deck of the Biomek 4000 Workstation (Figure 1). Test samples consisted of a prepared IgG Control Standard (Beckman Coulter) that contains a controlled quantity of the non-glycosylated heavy chain. Results with two different reducing agents showed excellent consistency in peak migration time and peak area across experiments with no cross contamination between samples.

Discovery *in motion.*

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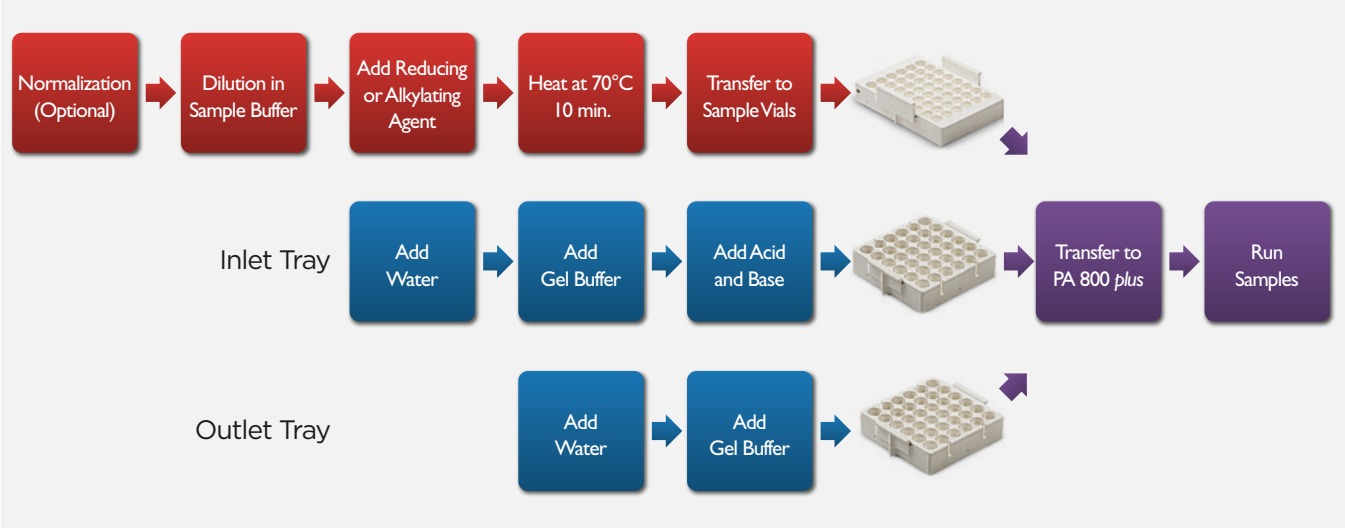

Figure 2. Process Workflow. The Biomek 4000 Workstation is used to automate the preparation of samples (red) and buffer trays (blue). The user transfers the sample tray and buffer trays to the PA 800 *plus* and runs the samples (purple).

Materials and Methods

IgG control samples (Beckman Coulter) were prepared using the IgG Purity and Heterogeneity Assay kit (Beckman Coulter). The SDS-MW kit (Beckman Coulter) is also compatible with this method. Reducing agents used were 2-mercaptoethanol (βME, Sigma Aldrich) and Tris (2-carboxyethyl) phosphine (TCEP, Sigma Aldrich). The Biomek 4000 Workstation was placed in a fume hood for the sample preparation to mitigate the inhalation hazard of βME.

The automation method features a user interface that allows users to set reagent volumes and then select sample locations using a run-time pattern (Figure 3). If normalization is required, a .csv file (i.e. Microsoft Excel) that contains sample volume and location information is used. A reagent calculator indicates the necessary total volumes of each reagent and the locations in the inlet/outlet trays that require buffer vials (Figure 3). The Biomek 4000 Workstation combines up to 24 samples with sample buffer, internal standard (optional) and either reducing agent or alkylating agent in a PCR plate. A typical deck layout is shown in Figure 4. This plate is then heated to 70° C for 10 minutes on an integrated shaking Peltier device. Inlet and outlet buffer trays are held on the deck of the instrument by an adapter plate (Figure 1) and buffer vials are filled with kit buffers (water, gel buffer, acid, and base). Denatured samples are transferred to sample vials for analysis on the PA 800 *plus*.

All samples were run on a PA 800 *plus*. All separations were performed using 50 µm ID bare fused silica capillaries with a 30.2 cm total length and 20.2 cm effective length from the sample introduction inlet to the detector window. All runs were performed using the Beckman Coulter PA 800 *plus* IgG Purity/Heterogeneity Assay standard protocol (Application Guide A51967AC).

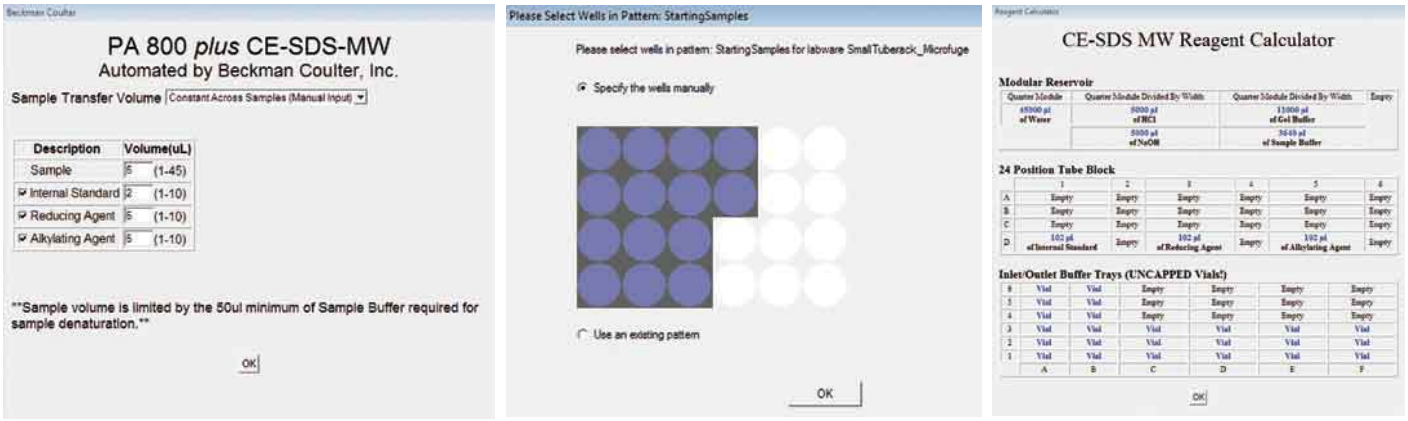

Figure 3. User Interface Screenshots. A) The user interface allows the selection of reagent and sample volumes and (B) sample locations using either a runtime pattern or a worklist file. C) The reagent calculator shows the location and total reagent volumes required, as well as the buffer tray locations that require buffer vials.

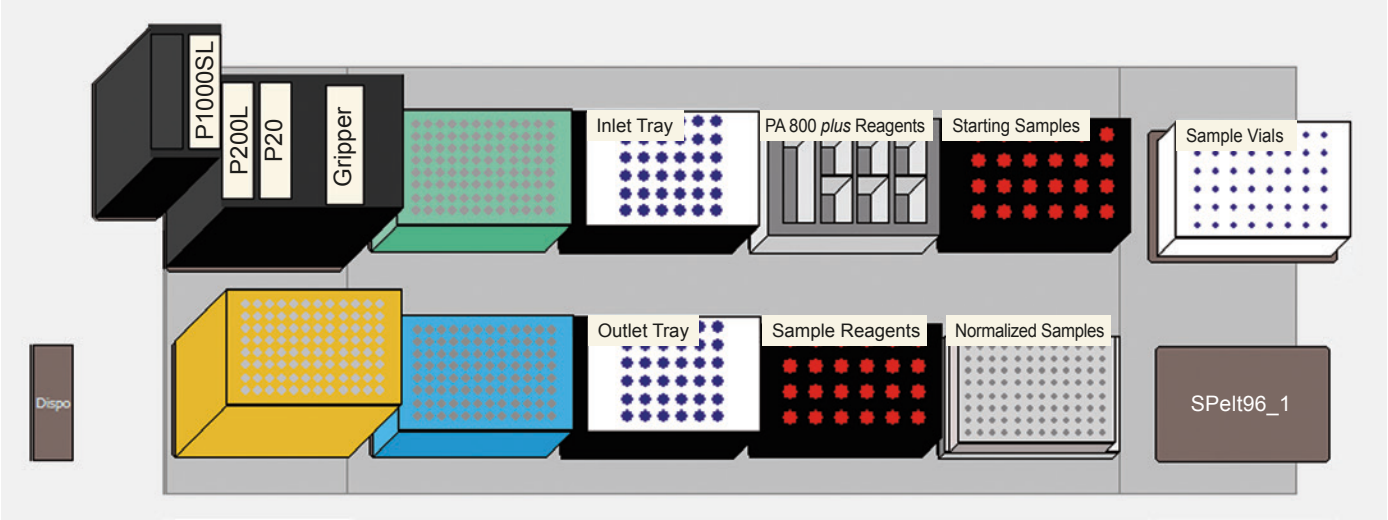

Figure 4. Biomek 4000 Workstation's Deck Layout. This software screenshot represents a typical deck layout for the IgG Purity and Heterogeneity Assay workflow. This setup includes the pipetting tools and gripper (upper left), tips (1000µL – yellow, 200µL – green, 20µL – blue), PA 800 *plus* inlet and outlet buffer trays, a modular reservoir for large volume reagents, tube racks for samples and small volume reagents, and the PA 800 *plus* sample vial tray. Samples are heated in a PCR plate ("Normalized Samples") on the integrated shaking Peltier device ("SPelt96_1").

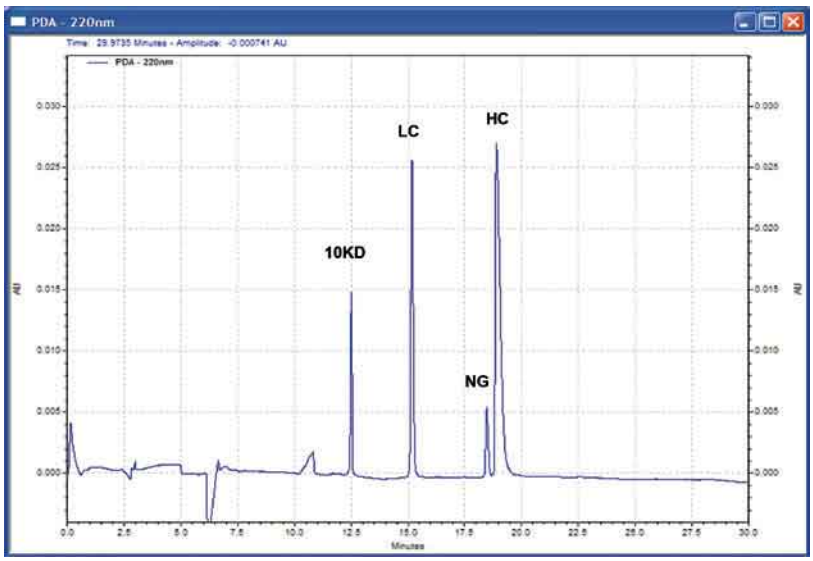

Figure 5. Electropherogram of IgG analysis using the PA 800 *plus*. Peaks: internal standard (10KD), light chain (LC), nonglycosylated heavy chain (NG), glycosylated heavy chain (HC).

Results and Discussion

Precise and Reproducible IgG Analysis

Figure 5 shows a representative PA 800 *plus* electropherogram that illustrates the expected peaks for the internal standard, light chain (LC), nonglycosylated heavy chain (NG), and heavy chain (HC) peaks found in a typical reduced IgG sample. We analyzed the peak migration time, peak area, and corrected peak area (area/time) for multiple experiments of 24 replicate samples. The robustness of the combined automated sample preparation and PA 800 *plus* analysis is demonstrated by the low coefficient of variation (% CV) for these parameters. The variability in migration time was <2%, while peak area and corrected peak area variability were <3% (Table 1 and data not shown). In addition, the values for mobility, resolution, and relative peak areas all surpassed IgG control specification limits (i.e. mobility variation <1%, data not shown).

To further characterize the automated method, we processed alternating samples and blanks and determined that there was no cross-contamination between wells (data not shown). We also processed samples with 100mM TCEP as an alternative reducing agent due to its substantially lower toxicity than β-mercaptoethanol. Table 1 shows that denaturation and reduction of IgG in the presence of TCEP gives equivalent peak migration and area as when reduced with βME. The 24 samples were processed in this fashion and PA 800 *plus* buffers were dispensed to vials in a total of 90 minutes, reducing hands-on preparation time by roughly this amount.

A) βME – Biomek Run #1 (n=24)

Migration Time	10 kd	LC	NG	HC
Mean (Min.)	12.86	15.61	19.00	19.44
Stdev (Min.)	0.10	0.12	0.16	0.16
% CV	0.80	0.79	0.83	0.82

Corrected Peak Area	LC	NG	HC
Mean	11284.15	2043.03	20532.93
Stdev	183.35	42.13	441.77
% CV	1.62	2.06	2.15

B) βME – Biomek Run #2 (n=24)

Migration Time	10 kd	LC	NG	HC
Mean (Min.)	12.54	15.22	18.52	18.95
Stdev (Min.)	0.12	0.16	0.21	0.22
% CV	0.97	1.06	1.13	1.14

Corrected Peak Area	LC	NG	HC
Mean	11688.91	2189.35	21467.08
Stdev	95.76	50.39	498.02
% CV	0.82	2.30	2.32

C) TCEP – Biomek Run #3 (n=24)

Migration Time	10 kd	LC	NG	HC
Mean (Min.)	12.58	15.26	18.57	18.98
Stdev (Min.)	0.14	0.18	0.23	0.25
% CV	1.15	1.21	1.24	1.32

Corrected Peak Area	LC	NG	HC
Mean	11923.49	2297.47	21582.15
Stdev	331.39	53.02	515.93
% CV	2.78	2.31	2.39

Table 1. IgG migration times and corrected peak areas following automated processing with either βME (A, B) or TCEP (C) as the reducing agent. The mean, standard deviation, and coefficient of variation values from 24 replicate samples are shown. High reproducibility was seen in both migration times (CV <2%) and corrected peak areas (CV <3%).

Ordering Information	
B37830	Biomek 4000 Workstation CE SDS-MW Configuration
A66528	PA 800 *plus* Pharmaceutical Analysis System
A66527	PA 800S *plus* Pharmaceutical Analysis System
390953	SDS-MW Analysis Kit
A10663	IgG Purity and Heterogeneity Assay
391734	IgG Control Standard

Conclusion

Automation of buffer and sample preparation of recombinant proteins for molecular weight analysis by CE-SDS on the PA 800 *plus* provides significant advantages. High liquid transfer precision results in excellent assay reproducibility and low variability in peak area, thereby conferring high confidence in the data. By automating the complete process, significant walk-away time is gained and sample preparation throughput can be increased as scientists are freed from the bench. Finally, by automating up to 144 transfer steps, the likelihood of user error is reduced and sample tracking provides the assurance necessary for critical protein analysis.

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