Performance Characteristics of Commercially Available Gels for Protein Analysis by Capillary Gel Electrophoresis with UV Detection

Christian Wenz, Rainer Nitsche, Hans Brunnert and Martin Greiner

HPLC 2011
P1-S-404-TU

Agilent Technologies

Introduction

Capillary gel electrophoresis (CGE) is a widely used tool for the size-based analysis of proteins. Due to several advantages with regard to automation, reproducibility and resolution it has replaced the classical technique sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in many labs, especially in the biotechnology industry. CGE it is now routinely used in the quality control environment to assess purity and integrity of therapeutic proteins including monoclonal antibody. Commonly commercially available dextran-based separation matrices are used for these separations.

Here, two separation matrices for protein characterization by CGE with UV detection, the SDS Gel Buffer from Beckman Coulter and the recently introduced Protigels from Advanced Analytical, were compared in detail on the Agilent 7100 Capillary Electrophoresis system. Two different sample sets were analyzed: first, a protein size standard and BSA as a test protein for molecular weight determination; second, a reduced antibody standard for quantification of light chain, non-glycosylated heavy chain and heavy chain. Across these experiments, the gels from both suppliers showed a similar performance. Furthermore, impurity detection experiments were done with a low molecular weight protein spiked into a non-reduced antibody standard sample. With the gels of both suppliers it was possible to detect the low molecular weight protein down to a level of 0.1 %.

Experimental

Materials

SDS-MW Size Standard, IgG Control Standard, 10 kDa Internal Standard, SDS Sample Buffer and SDS Gel Buffer were from Beckman Coulter (Fullerton, CA, USA). Protigel A and P protein gels and Protein capillary conditioning solution were from Advanced Analytical (Ames, IA, USA).

Sample preparation

Reduced and non-reduced samples were essentially prepared as described in the IgG Purity/Heterogeneity Assay SOP from Beckman Coulter.

CGE

(1) *With the SDS Gel Buffer from Beckman Coulter:* capillary, 50 µm id bare fused silica, 33 cm total and 24.5 cm effective length; pre-run conditioning, flushes at 4 bar with 0.1 N NaOH for 3 min, 0.1 N HCl for 1 min, water for 1 min and SDS Gel Buffer for 10 min; injection, -5 kV for 20 s; separation, -16.5 kV (-500 V/cm) with 2 bar pressure applied to both inlet and outlet home vials; capillary temperature, 25°C; detection wavelength, 220 nm.

(2) *With Protigels A and G from Advanced Analytical:* capillary, 75 µm id bare fused silica, 33 cm total and 24.5 cm effective length; pre-run conditioning, flushes at 3.5 bar with Protigel for 5 min and then voltage equilibration at -10 kV for 5 min; injection, -5 kV for 20 s; separation, -10 kV (-303 V/cm) with 2 bar pressure applied to both inlet and outlet home vials; capillary temperature, 25°C; detection wavelength, 200 nm.

Results and Discussion

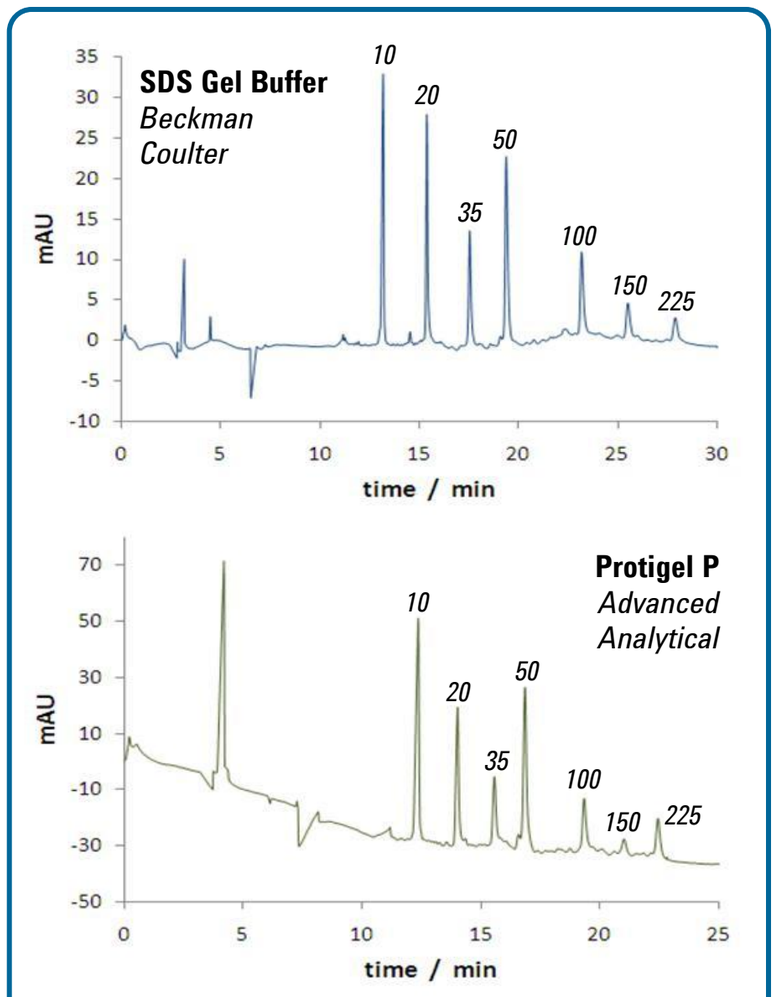

Fig. 1: CGE analysis of protein molecular weight standards with gels from Beckman Coulter (top) and Advanced Analytical (bottom). Molecular weights are indicated (in kDa).

Relative migration time repeatability's (n=6) across two capillary batches and 2 gel lots each were better than 0.2% RSD for both gel types. In both cases the target molecular weight of BSA was matched within an error of ± 10 %.

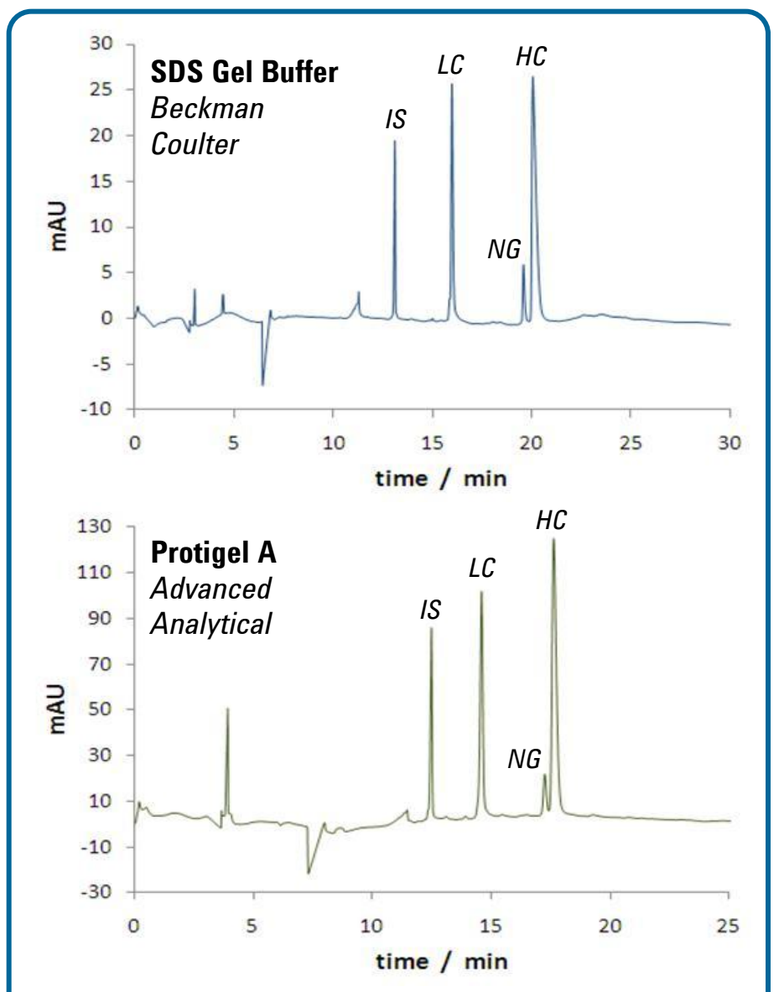

Fig. 2: CGE analysis of an IgG sample under reducing conditions with gels from Beckman Coulter (top) and Advanced Analytical (bottom). IS, internal standard; LC, light chain; NG, non-glycosylated heavy chain; HC, heavy chain.

The resolution of NG and HC peaks were better with the Beckman Coulter gel. However, peak area repeatability's (n=6) across two capillary batches and 2 gel lots each were 2% RSD or better for both gel types.

Results and Discussion

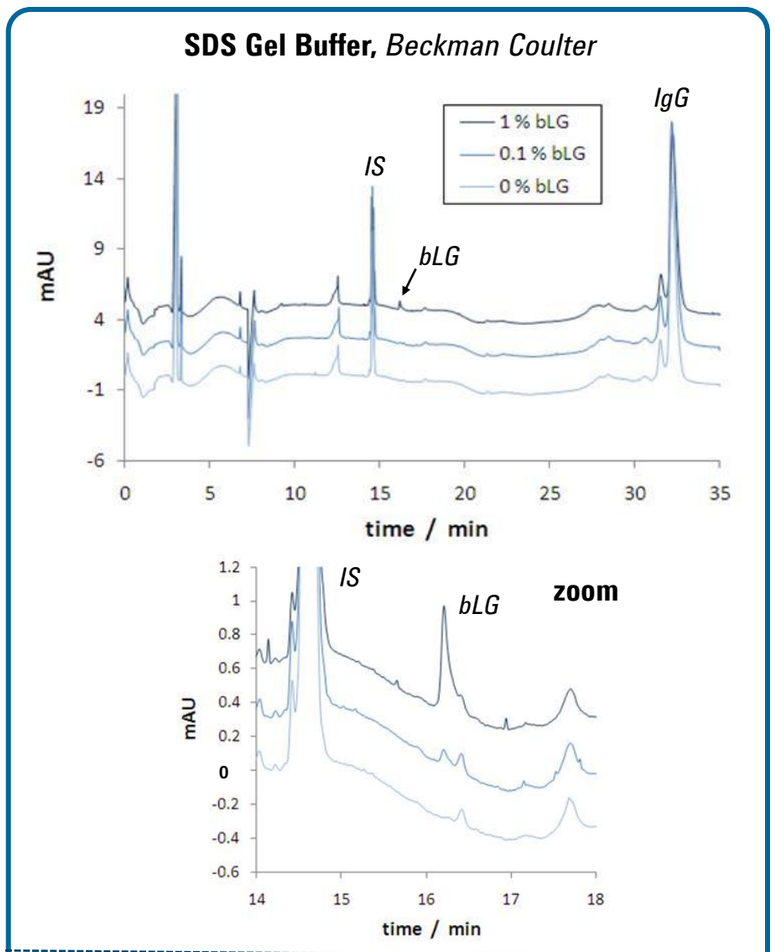

Fig. 3: Low level impurity detection with a non-reduced IgG sample with spiked-in bLG with gels from Beckman Coulter (top) and Advanced Analytical (bottom). Entire electropherograms and a zoom on bLG are shown for IgG samples containing 0 %, 0.1 % and 1 % bLG, respectively. IS, internal standard; bLG, beta-lactoglobulin.

The detection of the 0.1% spike was possible with both gel types.

Conclusions

Commercially available separation matrices are well suited for protein characterization by CGE with UV detection using the Agilent 7100 CE system. Gels from two different suppliers tested on this system showed a similar performance in typical CGE applications like molecular weight determination, IgG component quantification and impurity detection. Consistent results were obtained across all capillary batches and gel buffer lots tested. With the gels from both suppliers it is possible to detect low molecular weight protein impurities in a non-reduced IgG sample down to a level of 0.1 %.