

Abstract:

Capillary isoelectric focusing (cIEF) is frequently a method of choice for the separation of charged variants and impurities of proteins, and for the determination of isoelectric point (pI) inherent to each protein or peptide. This presentation will discuss parallel studies conducted on two capillary electrophoresis instruments, the Beckman P/ACE MDQ and the Convergent Bioscience iCE280 system, with additional analyses performed on the Beckman's PA800. Historically, cIEF separations were performed on instruments using UV/Vis detection on one end of the capillary. Such an approach requires mobilization of proteins after the focusing step, as in the case of the MDQ & PA800. Capillary isoelectric focusing performed on the iCE280 module requires no mobilization and utilizes a whole column imaging capture of the entire separation, being equipped with UV deuterium lamp, a Camera & a CCD sensor. Common issues encountered with the cIEF technology are related to training, method development, method transfer, and the level of expertise required for running the instruments and achieving quality performance and reproducibility of the assay. Additional issues are related to peak diffusion and/or protein precipitation during the cIEF separation. Our studies were focused on the determination of optimal instrument parameters to minimize troubleshooting, and provide faster turnaround time. Additional objective was to identify instrumentation that is user-friendly and amenable to high-throughput performance.

Introduction

Capillary electrophoresis has made significant progress as a tool in protein separation and the characterization of charge variants. The traditional methods of running gel IEF & SDS-PAGE electrophoresis are progressively being replaced by capillary isoelectric focusing methods (cIEF) and LabChip assays. The cIEF methodology is also becoming more "main stream" and also an important/significant complement to other analytical techniques such as HPLC, LC-MS, Peptide Mapping, Biacore/Elisa binding assays, Biological assays, and release and stability testing of biological products. Common applications of cIEF are determination of the pI of the protein or peptide, identity of the product, separation of charged variants & impurities, and detection of disassembled, or other modified species. The data shown in this report were generated using either a Beckman MDQ/PA800 (Fig.1) instrument or an Imaged ICE280 Analyzer from Convergent Bioscience (Fig.2). Schematic diagrams for each instrument/technique are shown below (Fig.3). Each of these instruments has its own advantages and disadvantages which are discussed in this poster.



Fig.1



Fig.2

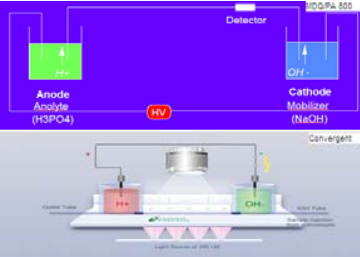


Fig.3

Materials & Methods

P/ACE/MDQ, Beckman Coulter

Capillary electrophoresis was performed on Beckman MDQ/PA800, controlled by a 32 Karat software, operating on Windows 2000. Fluorocarbon (FC), Divinylbenzene (DB1), and Neutral capillaries, with effective lengths ranging from 30cm to 50cm, were evaluated and used for analysis of monoclonal antibodies. Analysis and processing were performed using the Turbochrome and, more recently, Empower data collection system(s).

iCE280, Convergent

Capillary Isoelectric Focusing system (cIEF): Acquisition and analysis were performed on an iCE280 Convergent Bioscience system equipped with CFR Software Version 2.3, cIEF cartridge-FC coating (convergent # 101701), an Alcott 719L autosampler, and a Maximizer Lab Alliance pump for continuous delivery of methylcellulose solution. Calibration of pI markers was performed by the iCE software followed by the conversion and processing of data by Empower or EZChrom version 6.8. Reagents used for sample preparation were 10M Urea solution, dithiothreitol (DTT), HPLC grade water, Pharmalyse(s) from Sigma, and synthetic pI markers ordered from Sigma. Other reagents include 1% methylcellulose from Convergent, 90mM Phosphoric acid & 100mM Sodium hydroxide solutions in water.

Sample preparation

Preparation of monoclonal antibodies, non-reduced conditions:

Samples were prepared for cIEF analysis on the iCE280 Convergent system by the addition of 190µL of water, 250µL of 1% methylcellulose, 20µL of a 10mg/mL sample, 20µL of ampholyte mix, 3-10/8-10.5 (1:1, vol/vol) or 3-10 only & 2µL of pI markers, 7.05/9.5 (1:1, vol/vol).

Preparation of 10M Urea solution:

Measure 30.0g +/- 0.3g Urea using an analytical balance. Transfer urea to a 50mL polypropylene graduated tube. Add 25mL of Milli-Q water and mix until dissolved. Add Milli-Q water to the final volume of 50mL. Mix well until well dissolved.

Preparation of a sample dilution buffer (reduced & denaturing conditions):

A sample dilution buffer is prepared by mixing 2mLs of 10M Urea solution, 15mg solid Dithiothreitol (DTT), 120µL of 5-8 ampholyte, and 10µL of synthetic markers pI 5.30/6.60/7.00. The solution is mix well and filtered using a millipore filter.

Reference STD & Sample (70kDa recombinant protein) preparation:

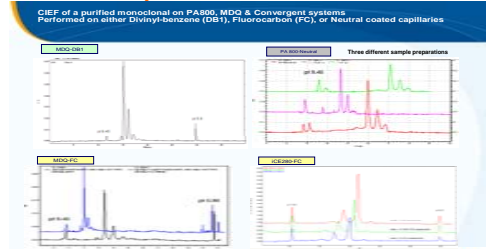
The 70kDa protein is first diluted to approximate concentration of 4mg/mL with HPLC grade water. Forty microliters of the above is further diluted with 48µL of the freshly prepared sample buffer. The sample is mixed and spun briefly for 1min at high speed.

Studies on Convergent iCE280 and Beckman P/ACE MDQ Compared:

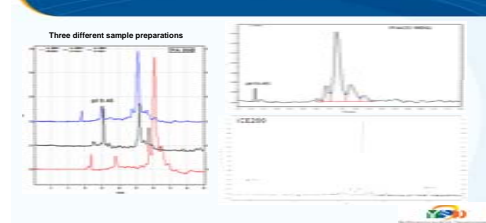
	Beckman P/ACE/MDQ	Convergent iCE280
Capillary	FC capillary, 30-50cm in len	FC capillary, 5 cm, precast
Focusing Time	8-16min	6-16min
Mobilization	YES	NO
Run Time	~ 30-60min	15-20min
Method Development	MDQ (not automated) PA 800 (Automated)	Automated
Detection	single channel uv/vis detector	whole column, image detection
pI Determination	Automated	Automated
Data converted to Empower	Yes	Yes
21 CFR Part 11 Compliant	Yes	Yes

cIEF of Monoclonal Antibody Products

Non-reduced assays:



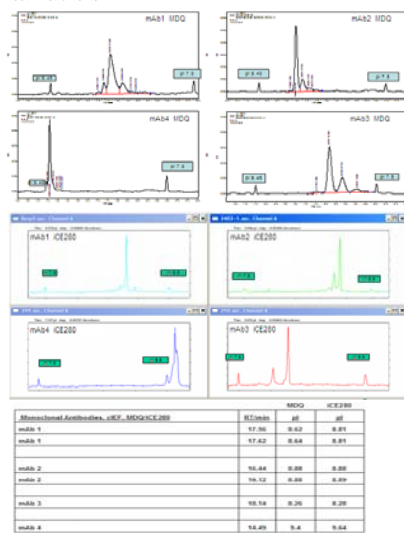
Capillary Isoelectric Focusing of a GSK mAb on PA800, MDQ & Convergent iCE280 System



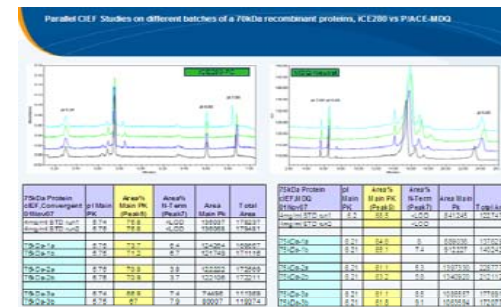
pI Assessment:

Four monoclonal antibodies were compared to determine their isoelectric point (pI) values. cIEF separations were run on Beckman P/ACE MDQ (shown below on the top 4 electropherograms) & on the iCE280 Convergent systems (bottom 4 electropherograms).

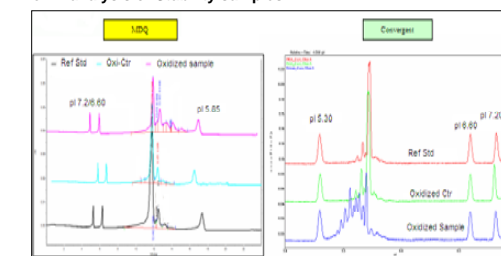
The table below shows good correlation of cIEF values obtained on both instruments.



cIEF of a 70K recombinant protein cont'd:



cIEF analysis of Stability samples



Conclusions - Advantages/Disadvantages:

MDQ vs Convergent:	MDQ	iCE280 from Convergent
Advantages	Amenable to: CE/cIEF, SDS Separations, Carbohydrate Analysis, CE/MS, Empower data Acquisition	Whole-column light absorption at @ 280nm, Requires less time for method development, User friendly
Disadvantages	Performance & some procedural difficulties encountered, Requires proficiency training to run the assays, UV-Vis detection located at one end of capillary, Requires slightly longer time for method development, Occasional mis-injection of samples & shift in retention times	Semi-automated pI assignments and quantitative analysis of products, Amenable to high throughput sample analysis, Applicable only for cIEF method of protein/peptide analysis, Requires proficiency training to run the assays, Occasional shift in retention times of peaks, Data not directly collected on Empower, Occasional air bubbles in the system

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