

Application of Imaged CIEF in Clinical QC Testing

Kimia Rahimi, John De Los Santos, Wenni Gao, Koman Joe and Sarah Du
Protein Analytical Chemistry, Genentech, Inc., South San Francisco, CA, USA

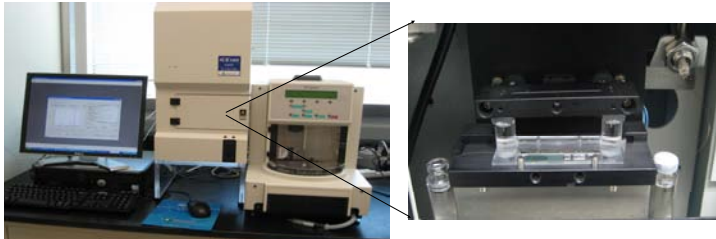
ABSTRACT

Imaged Capillary Isoelectric Focusing (ICIEF) is a protein characterization tool for determination of pI and quantitation of charge heterogeneity. Under an electric field, charged proteins and peptides migrate through a pH gradient created by zwitterionic ampholytes and focus at their pI value. This ICIEF method was optimized by Oscar Salas-Solano, et al., and implemented in Clinical QC at Genentech, Inc. as an alternative to Ion Exchange Chromatography. This poster presents an overview of the practical application of a multiproduct, quantitative ICIEF method which is used for Drug Substance, Drug Product and stability testing, and some case studies of issues, observations and troubleshooting.

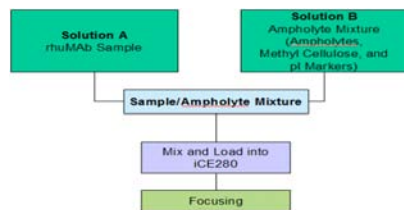
Advantages of ICIEF in Clinical Testing

- Advantages of ICIEF method:
- Simple method, multiple-product suitability
 - Time savings compared to IEC
 - Instrument set-up
 - Run times
 - Low volume of reagents and waste
 - Reproducible and stability-indicating

Convergent Bioscience ICIEF Instrument

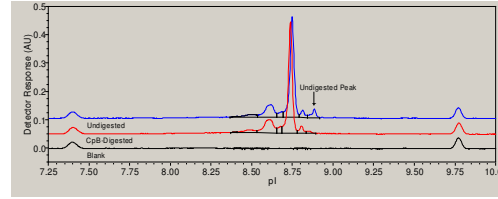


PROCEDURE

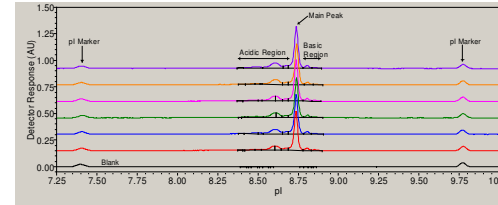


Example Electropherograms

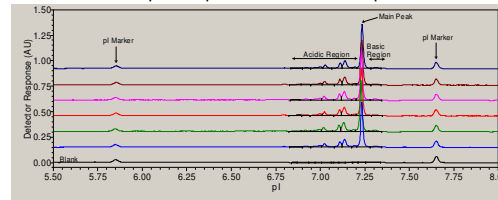
rhuMAB 1: Effect of Carboxypeptidase B Digestion



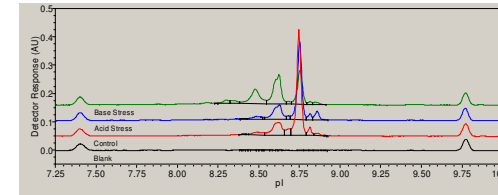
rhuMAB 1: Multiple Preparations of a "High-pI" Protein



rhuMAB 2: Multiple Preparations of a "Low-pI" Protein



rhuMAB 1: Stressed Profiles



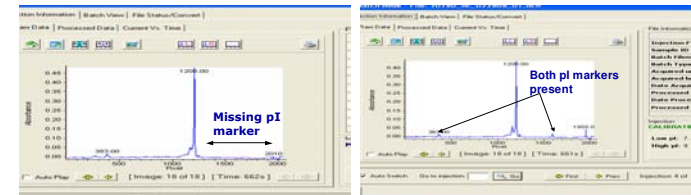
Summary of ICIEF Method Validation

	rhuMAB 1 % Peak Area			rhuMAB 2 % Peak Area		
	Acidic Region	Main Peak	Basic Region	Acidic Region	Main Peak	Basic Region
N	56			48		
Mean	26.94	71.28	1.78	38.55	59.28	2.17
SD	1.2	1.2	0.2	0.5	0.6	0.2
RSD (%)	4.3	1.7	11.3	1.2	1.0	11.2
Minimum	24.22	68.53	1.34	37.78	57.89	1.57
Maximum	29.38	73.99	2.24	39.91	60.56	2.60
Mean + 3SD	23.46	67.54	1.18	37.20	57.56	1.44
Mean - 3SD	30.43	75.01	2.38	39.90	60.99	2.90
Mean + SSD	21.14	65.05	0.78	36.30	56.42	0.95
Mean - SSD	32.75	77.50	2.78	40.81	62.13	3.39

Case I: Missing pI Marker

Possible Causes:

- Was the pI marker added?
- Imbalance? Profile drift to the right side due to siphoning from waste vial to balance vial
- Marker degradation at room temperature
- Use of non-glass tube to prepare Ampholyte mixture (adsorption of marker)
- Marker digestion by sample prep components



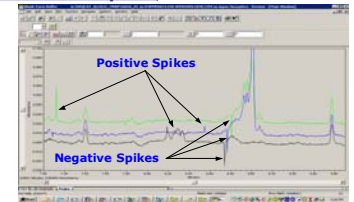
Case II: Observation of Spikes

Possible Causes:

- Dust on cartridge window
- Dust on detector lenses
- Particulates in sample
- Flaws in cartridge window

Remedies:

- Blow away dust with canned air
- Centrifuge samples carefully
- Rinse cartridge with water
- Change cartridge



ACKNOWLEDGEMENTS

Will McElroy
David Michels
Toby Reichenberg
Zara Safarian
Oscar Salas-Solano
Reed Harris

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