



WCBP

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*San Francisco, January, 2006*

# **Applications of iCE280 Analyzer in Formulation Development (Excerpt)**

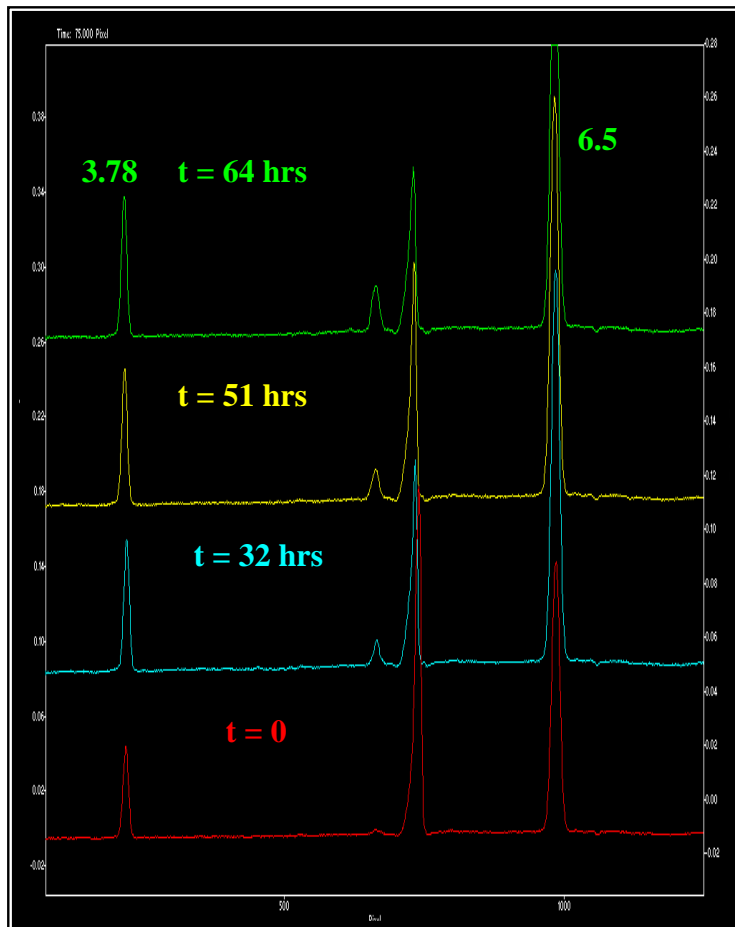
Ning Li,

Kendall Kessler and David Zeng

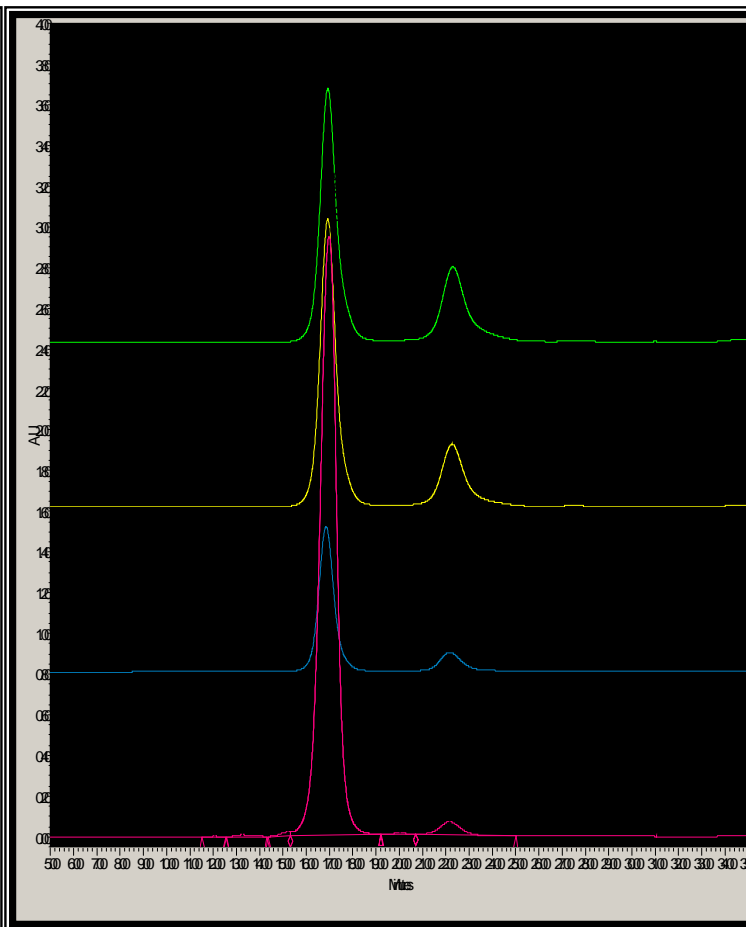
Pharmaceutical R&D, Global Biologics

St. Louis, MO

## Electropherogram

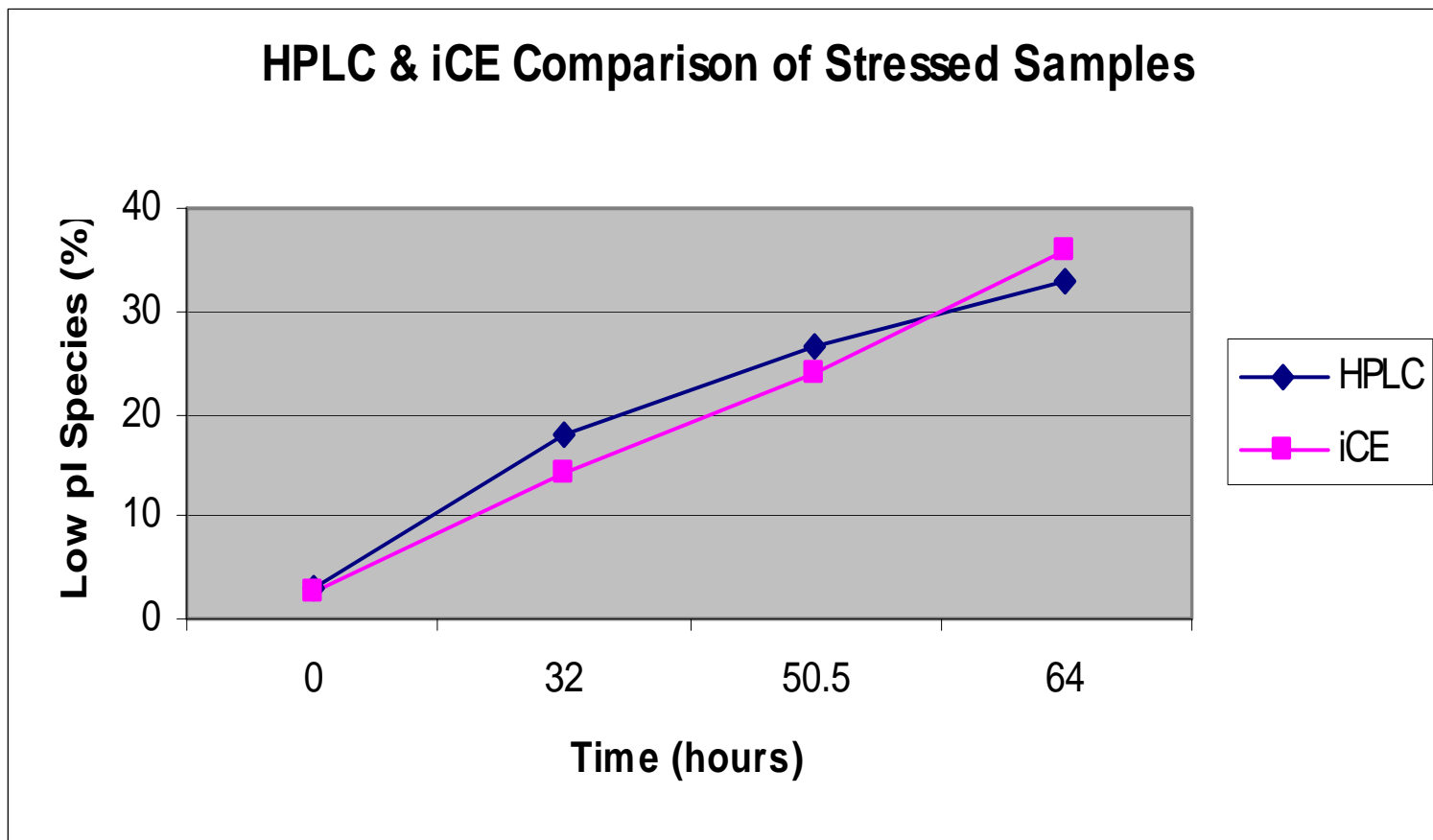


## AEX HPLC Chromatogram

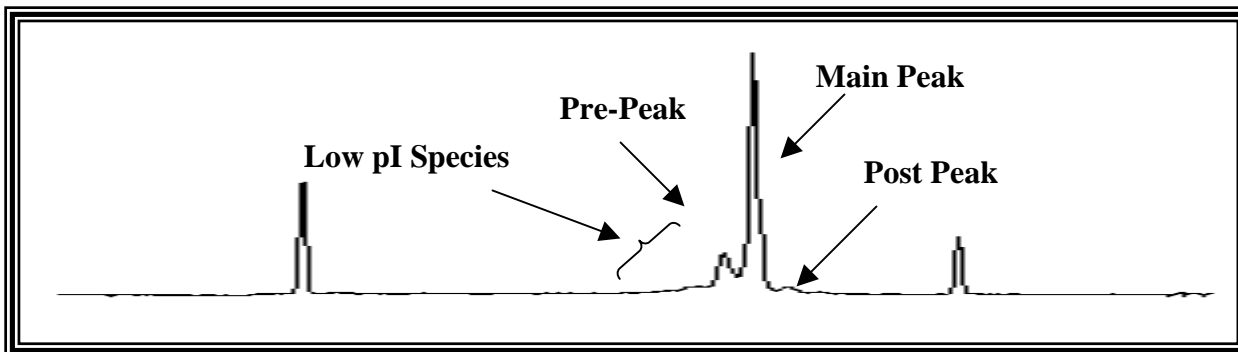


Protein stressed in pH ~9 buffer

# Correlation between iCE and HPLC analysis



# Precision (Repeatability)



## pI Measurement, 6 injections/day

	Pre-Peak	Main-Peak	Post-Peak
Average pI	8.11	8.28	8.51
%RSD	0.1	0.1	0.1

## Area distribution measurement, 6 injections/day

	Low pI (%)	Main-Peak (%)	Post-Peak (%)
Averaged Area Distribution	26.2	69.3	4.6
%RSD	0.3	0.5	6.1

**Method demonstrated good intra-day precision**



## Precision (Intermediate)

### pI Measurement, 3 injections/day for 3 days

	PrePeak	MainPeak	PostPeak
Average pI	8.11	8.27	8.50
%RSD	0.1	0.1	0.2

### Area distribution measurement, 3 injections/day for 3 days

	Low pI (%)	MainPeak (%)	PostPeak (%)
Averaged Area Distribution	26.1	69.1	4.7
%RSD	1.4	0.6	5.7

**Method demonstrated good inter-day precision**



## Precision (Reproducibility)

### pI Measurement, 6 injections in lab 1 and 6 injections in lab 2

	Pre-Peak	Main-Peak	Post-Peak
Average pI	8.10	8.27	8.50
%RSD	0.1	0.1	0.2

### Area distribution measurement, 6 injections in lab 1 and 6 injections in lab 2

	Low pI (%)	Main-Peak (%)	Post-Peak (%)
Averaged Area Distribution	26.3	69.2	4.6
%RSD	1.3	0.5	5.7

**Method was reproducible**

### **pI Measurement under intentionally changed MC concentrations**

	<b>Pre-Peak</b>	<b>Main-Peak</b>	<b>Post-Peak</b>
<b>5+ % Methyl Cellulose</b>	<b>8.06</b>	<b>8.23</b>	<b>8.46</b>
<b>Desired</b>	<b>8.11</b>	<b>8.28</b>	<b>8.51</b>
<b>5- % Methyl Cellulose</b>	<b>8.11</b>	<b>8.27</b>	<b>8.50</b>
<b>% RSD</b>	<b>0.3</b>	<b>0.4</b>	<b>0.3</b>

### **Area distribution measurement under intentionally changed MC concentrations**

	<b>Low pI (%)</b>	<b>Main-Peak (%)</b>	<b>Post-Peak (%)</b>
<b>5+ % Methyl Cellulose</b>	<b>26.9</b>	<b>68.8</b>	<b>4.3</b>
<b>Desired</b>	<b>26.2</b>	<b>69.2</b>	<b>4.6</b>
<b>5- % Methyl Cellulose</b>	<b>26.1</b>	<b>68.7</b>	<b>5.2</b>
<b>% RSD</b>	<b>1.6</b>	<b>0.4</b>	<b>9.2</b>

**pI Measurement under intentionally changed pharmalyte concentrations**

	<b>Pre-Peak</b>	<b>Main-Peak</b>	<b>Post-Peak</b>
<b>5+ % Pharmalyte</b>	<b>8.24</b>	<b>8.38</b>	<b>8.61</b>
<b>Desired</b>	<b>8.11</b>	<b>8.28</b>	<b>8.51</b>
<b>5- % Pharmalyte</b>	<b>8.12</b>	<b>8.28</b>	<b>8.51</b>
<b>% RSD</b>	<b>0.9</b>	<b>0.7</b>	<b>0.6</b>

**Area distribution measurement under intentionally changed pharmalyte concentrations**

	<b>Low pI (%)</b>	<b>Main-Peak (%)</b>	<b>Post-Peak (%)</b>
<b>5+ % Pharmalyte</b>	<b>24.9</b>	<b>69.1</b>	<b>6.0</b>
<b>Desired</b>	<b>26.2</b>	<b>69.2</b>	<b>4.6</b>
<b>5- % Pharmalyte</b>	<b>26.0</b>	<b>69.3</b>	<b>4.7</b>
<b>% RSD</b>	<b>2.8</b>	<b>0.1</b>	<b>15.4</b>

### **pI Measurement using 3 lots of cartridges**

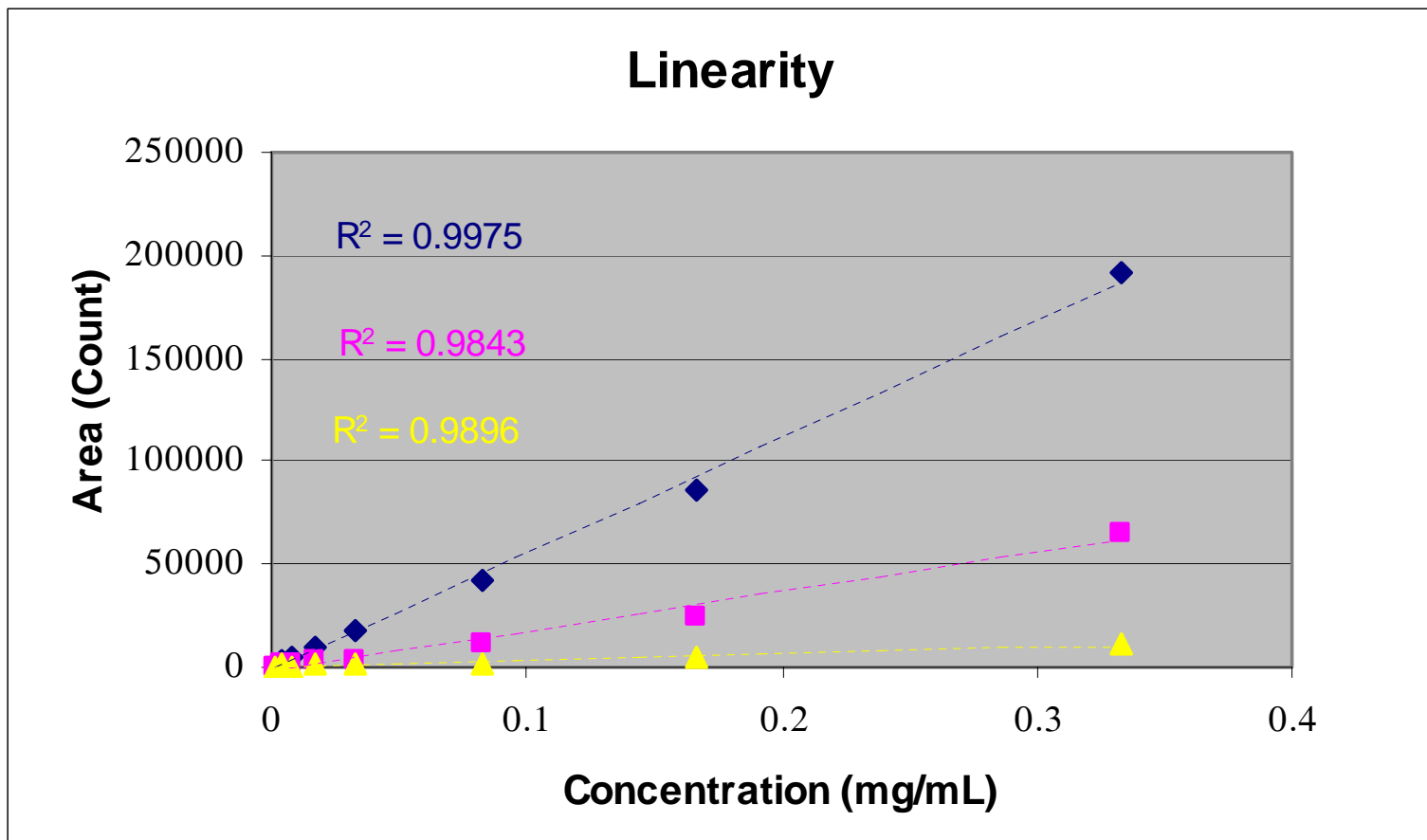
<b>Cartridge Lot #</b>	<b>Pre-Peak</b>	<b>Main-Peak</b>	<b>Post-Peak</b>
<b>99574</b>	<b>8.11</b>	<b>8.28</b>	<b>8.51</b>
<b>99417</b>	<b>8.09</b>	<b>8.26</b>	<b>8.49</b>
<b>99653</b>	<b>8.02</b>	<b>8.20</b>	<b>8.43</b>
<b>% RSD</b>	<b>0.6</b>	<b>0.5</b>	<b>0.4</b>

### **Area distribution measurement using 3 lots of cartridges**

<b>Cartridge Lot #</b>	<b>Low pI (%)</b>	<b>Main-Peak (%)</b>	<b>Post-Peak (%)</b>
<b>99574</b>	<b>26.4</b>	<b>69.1</b>	<b>4.5</b>
<b>99417</b>	<b>26.2</b>	<b>69.2</b>	<b>4.6</b>
<b>99653</b>	<b>26.1</b>	<b>69.1</b>	<b>4.8</b>
<b>% RSD</b>	<b>1.2</b>	<b>0.4</b>	<b>5.6</b>

**Total 12 injections, 6 in lab 1 and 6 in lab 2**

	<b>Low pI (Count)</b>	<b>Main Peak (Count)</b>	<b>Post-Peak (Count)</b>
<b>Averaged</b>	<b>82975</b>	<b>218458</b>	<b>14393</b>
<b>%RSD</b>	<b>2.8</b>	<b>2.5</b>	<b>7.0</b>



**Linear response was achieved in concentration range of 0.2 to 300 ug/mL**

## Glycosylated molecule

Autosampler	Instrument plumbing time + focusing time	Total run time	Autosampler capacity
Alcott	3 min 5 sec + 1 min + 12 min	16.1 min	8 hrs/syringe pump
Prince	9 min 50 sec + 1 min + 12 min	22.8 min	40 samples
IEF Gel	10 samples per gel	2 days	N/A
2 Step cIEF <sup>1</sup>	6 min + 8 min + 28 min (mobilization)	42 min	

## Non-glycosylated molecule

Autosampler	Instrument plumbing time + focusing time	Total run time	Autosampler capacity
Alcott	3 min 5 sec + 1 min + 5 min	9.1 min	8 hrs/syringe pump
Prince	9 min 50 sec + 1 min + 5 min	15.8 min	40 samples
IEX HPLC	N/A	65 min	100 samples
2 Step cIEF <sup>2</sup>	6 min+20 min+30 min (mobilization)	56 min	

1) L.C. Santora et al., *Analytical Biochemistry*, 275, 98-108 (1999)

2) A.M. Lasdun et al., *Journal of Chromatography A*, 917, 147-158 (2001)



## Summary

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- ❖ Resolutions of charge related heterogeneity of glycosylated and non-glycosylated molecules were achieved
- ❖ Sources of charge heterogeneity were identified
- ❖ Precisions and robustness were demonstrated
- ❖ Method was linear in concentration range of 2 to 300 ug/ml
- ❖ Throughput was higher than other techniques
- ❖ Instrument can be used as both characterization and quantitation tool