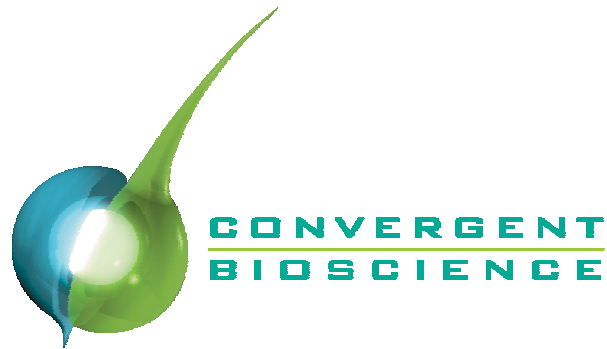


# Feasibility of Developing a Generic cIEF Method for the Determination of Charge Heterogeneity of All Mabs

*Jiaqi Wu*



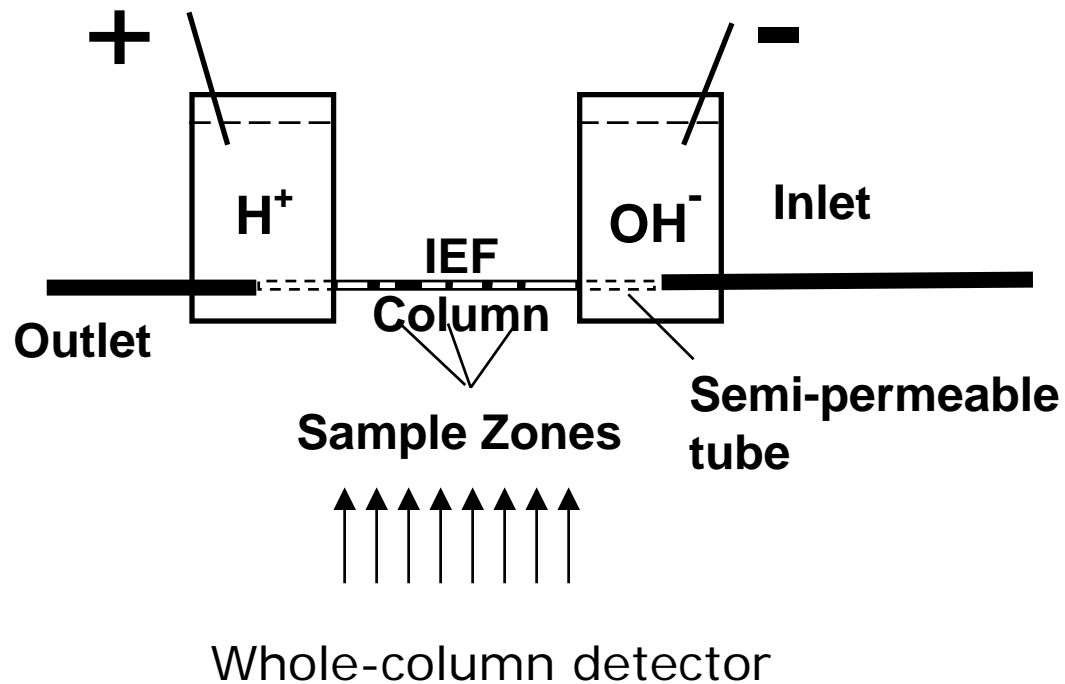
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# Introduction

- ◆ Methods to determine charge heterogeneity of Mabs
  - Slab gel IEF
    - » Time consuming, labor intensive and semi-quantitative
  - Conventional capillary IEF (cIEF performed on general CE)
    - » Long method development time (months), poor resolution and reproducibility
  - Ion exchange chromatography
    - » Long method development time (months)
    - » variation of resolution from column to column
- ◆ Whole-column detection cIEF
  - Short method development time (hours for Mabs),
  - Report of generic method validated for 20 Mabs\*

\*Xiaoping He, et. Al., CEPharm2006

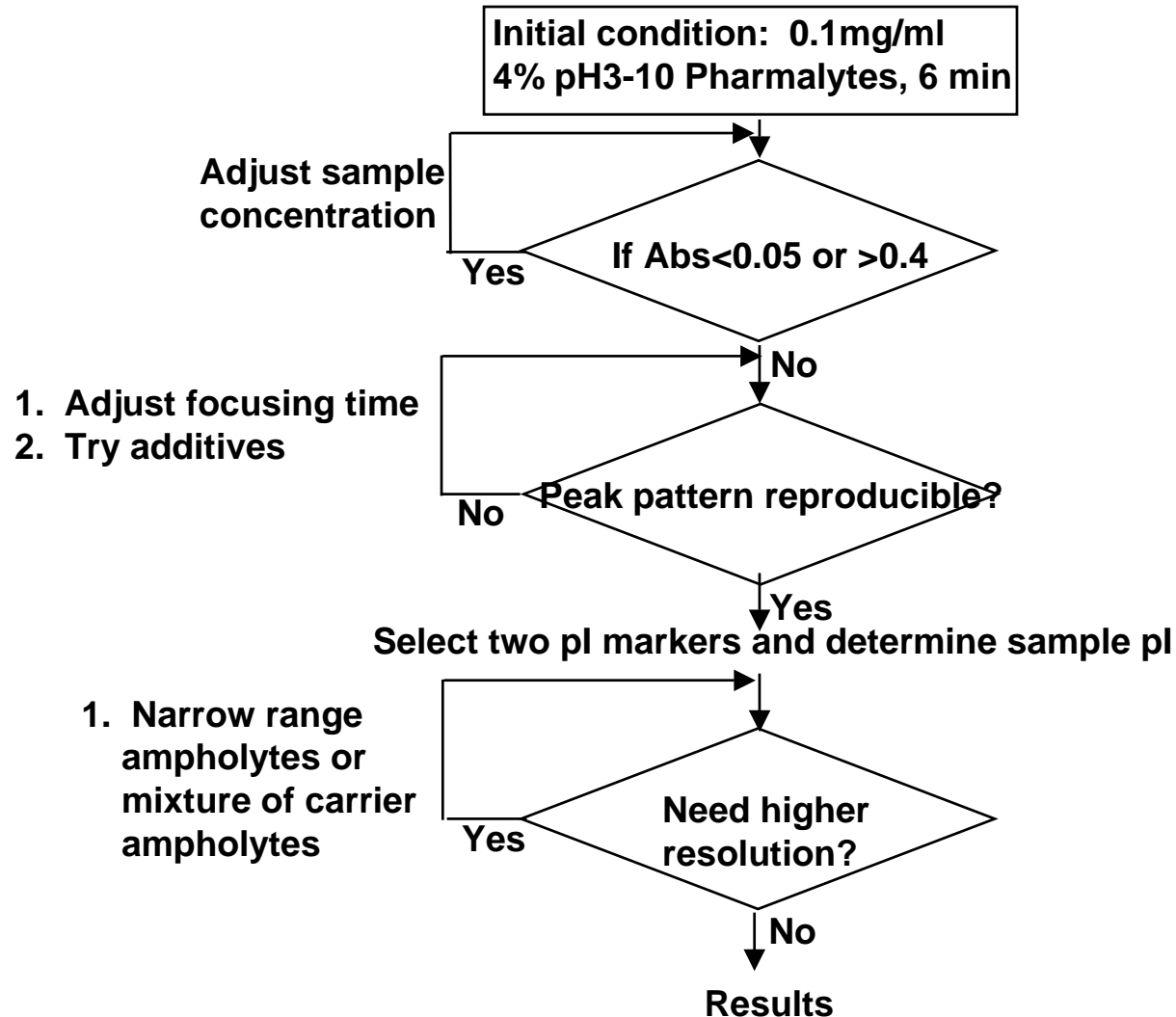
# Whole-Column Detection cIEF -cIEF without Mobilization – iCE280 Analyzer



# iCE280 Analyzer Features

- ◆ Mobilization process is eliminated
  - Only need to optimize focusing process
- ◆ Whole-column detector constantly monitors IEF process in entire column
- ◆ Short analysis time (up to 6 runs/hour)
- ◆ Accelerated method development (hours for Mabs)
- ◆ Reproducible peak pattern and high resolution
- ◆ Wide pI range (pH 2-11)

# IEF Method Development Using iCE280 Analyzer –Flow Chart



# IEF Method Development for Mabs Using iCE280 Analyzer

- ◆ Easy to achieve reproducible peak patterns for most Mabs
  - No additives are needed for >90% of Mabs
  - Focusing time is often optimized in a single run
- ◆ For most Mabs, only three conditions need to be optimized
  - Sample concentration
  - Selection of narrow pH range carrier ampholytes to achieve high resolution
  - Selection of pI markers

## Optimization of Three Conditions in IEF Method Development for Mabs

- ◆ Sample concentration
  - Sample concentration should be high enough to detect minor species, but not to saturate the detector
  - Major peak height reaches 0.5 Ab
- ◆ Selection of narrow pH range carrier ampholytes (CA)
  - Narrow pH range CAs are necessary to achieve satisfactory resolution
  - The CAs should cover the pIs of all samples
- ◆ Select pI markers
  - pI markers are needed for pI calibration
  - Marker peaks should bracket sample peaks

## Conditions in the Proposed Generic Method for All Mabs

- ◆ **4% pH 8 – 10.5 Pharmalytes**
  - pH range covers most Mabs
  - Provide the highest resolution possible for Mabs with pI ranging from 8 – 10
  - Good linearity in pH gradient means only two pI markers are needed
  - Lower background noise at 280 nm compared that of other brand name carrier ampholytes

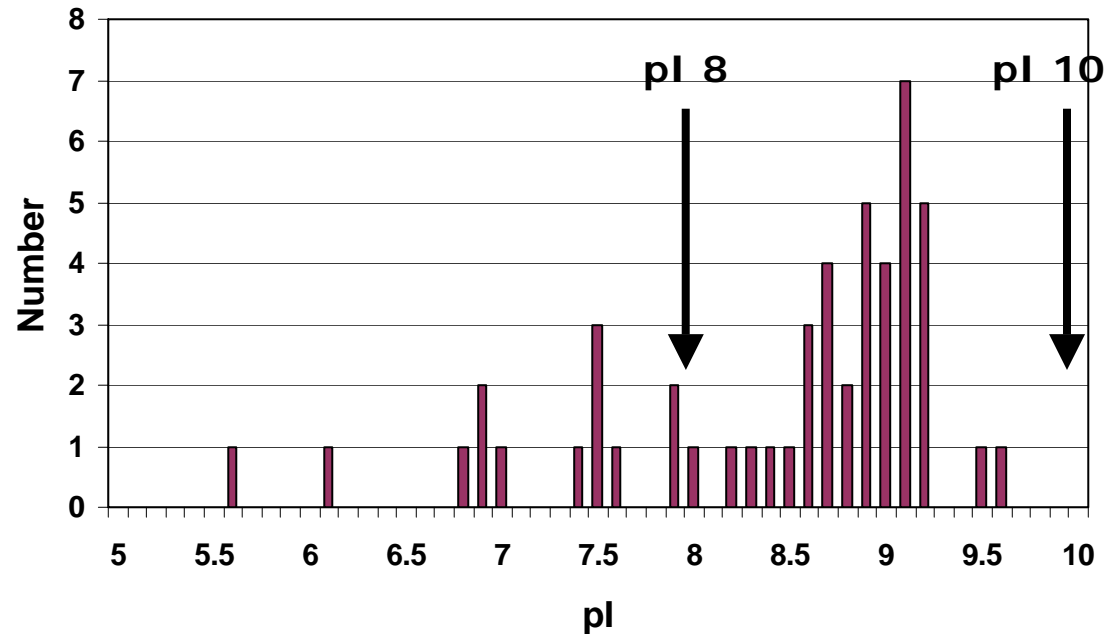
# Conditions in the Proposed Generic Method for All Mabs

- ◆ **pI 7.96 and 10.10 markers**
  - Cover the pH 8 – 10 range
- ◆ **10 min focusing time at 600 V/cm**
  - 1 minute pre-focusing at 300 V/cm
- ◆ **Sample concentration needs to be adjusted for each individual Mab**

***Will it work for all Mabs?***

# pI Values of Mabs

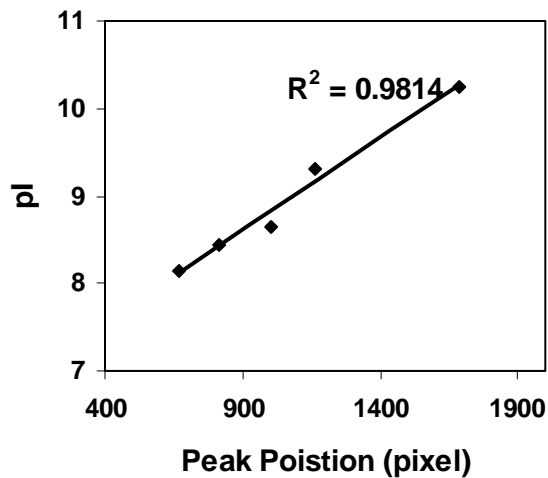
Mabs recently analyzed at Convergent (total 50)



**>75% of Mabs are in the range of pI 8 - 10**

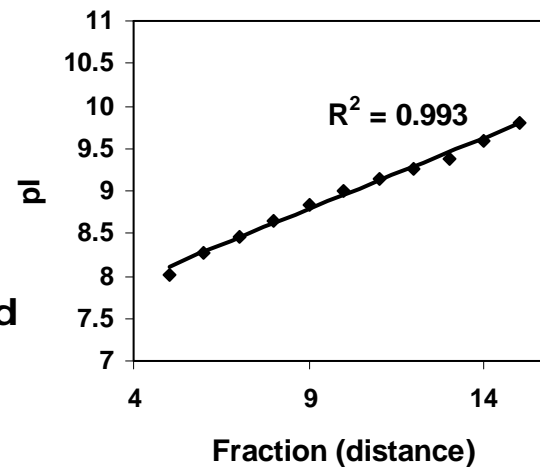
# Linearity of pH Gradient

## Pharmalyte 8 – 10.5



Determined by GE's protein pI markers

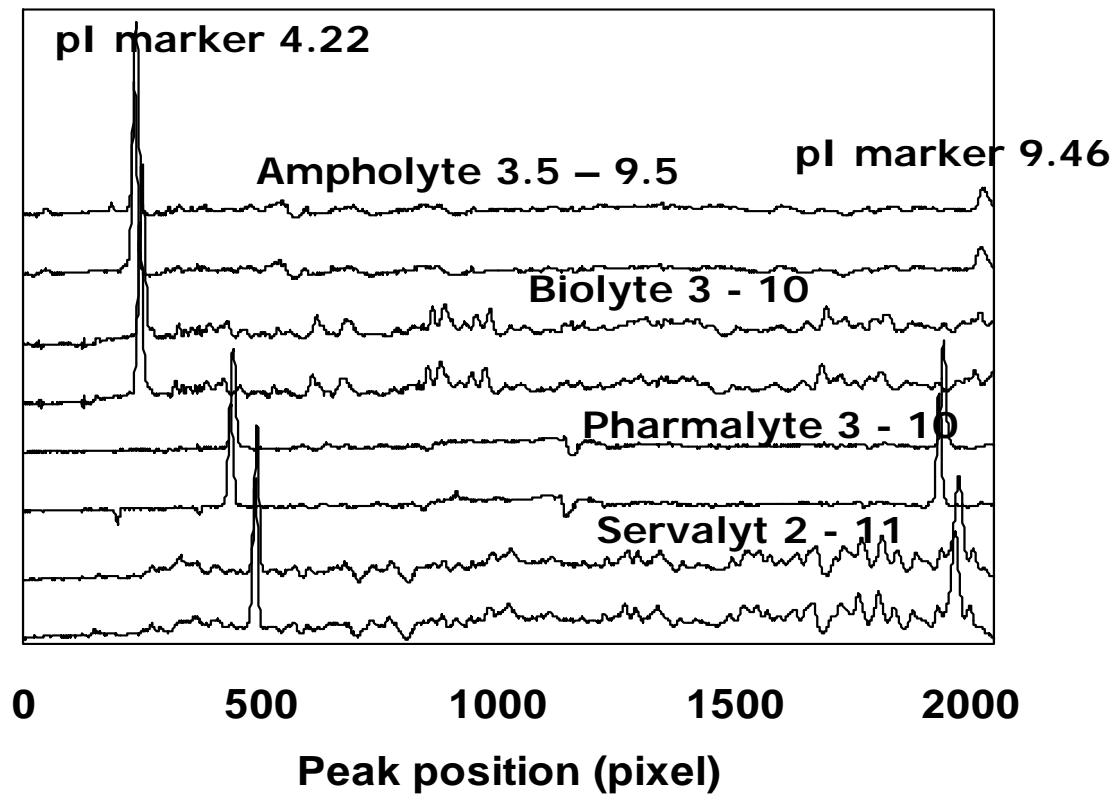
Determined by fractionation based on IEF \*



\*International Application PCT/US04/035466

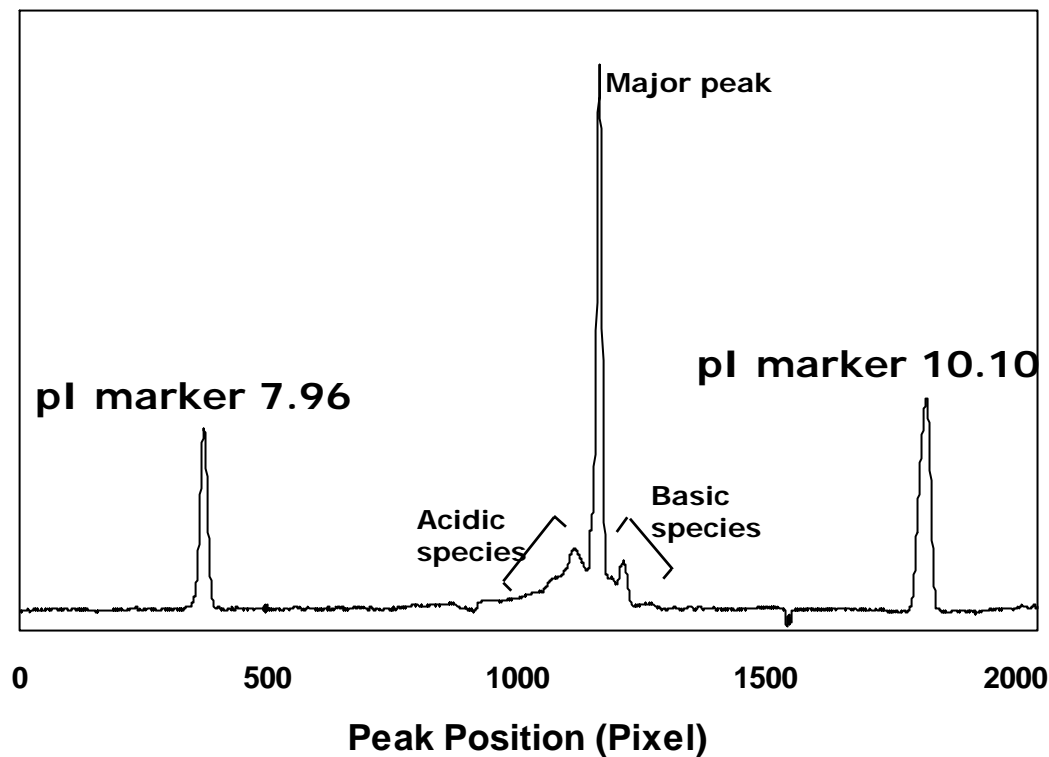
# Background Noise

CA concentrations = 8%



# Typical E-Gram of a Mab Using the Generic Method

4% Pharmalytes 8 – 10.5



## Results and Conclusion

- ◆ The pH range of the method covers 75% of Mabs (38 of 50)
- ◆ 2 Mabs of the 34 Mabs have aggregation problem during focusing
  - 0.5 M – 4 M urea solves the problem
  - Other conditions are all the same
- ◆ ***The proposed method is suitable for 32 of the 44 Mabs***