

# Method Development for cIEF Collaboration Project: Carrier Ampholyte and Additive Selection for the IgG Sample

Jiaqi Wu, Convergent Bioscience Ltd., Toronto, Ontario, Canada M8Z 2L8

## INTRODUCTION

In the method development for the IgG sample used in the cIEF collaboration project, different carrier ampholytes (CAs) were evaluated for the sample based on their baseline noise, peak pattern reproducibility and resolution. Urea was used in the cIEF method to stabilize sample peak patterns. This poster discusses the criteria in selecting the CAs and urea concentration in the method development for two lots of the IgG sample (6/4/07 and 7/27/07).

## SELECTION OF CARRIER AMPHOLYTES (CAs)

### Brand Names

- Ampholine
- Pharmalytes
- Servalyts

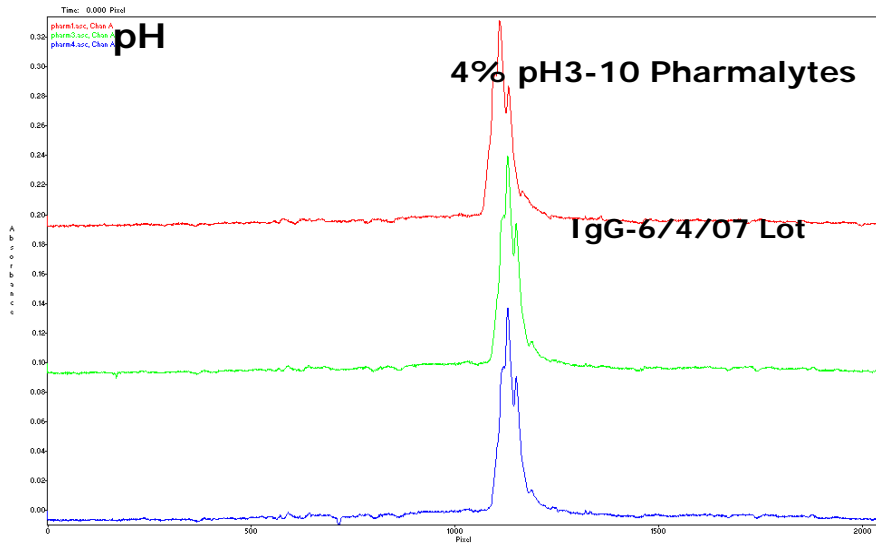
### Criteria in Evaluation of CAs

- Sample peak pattern reproducibility
  - Sample may aggregate
  - Sample may interact with CAs
- Baseline noise
  - All CAs absorb UV light at 280 nm
- Resolution
- pH gradient linearity

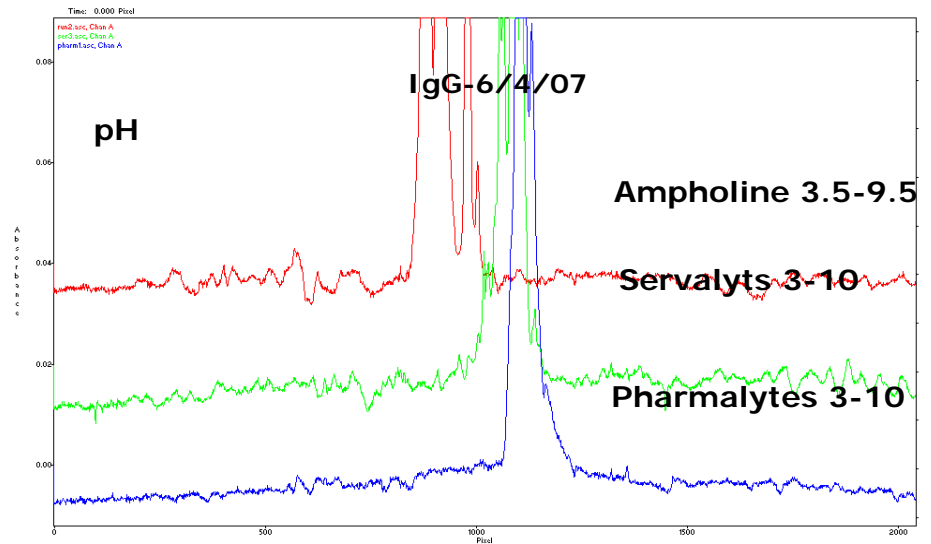


# BASELINE NOISE AND IgG (LOT 6/4/07) PEAK PATTERN REPRODUCIBILITY IN DIFFERENT CAs

## Pharmalytes pH 3 - 10

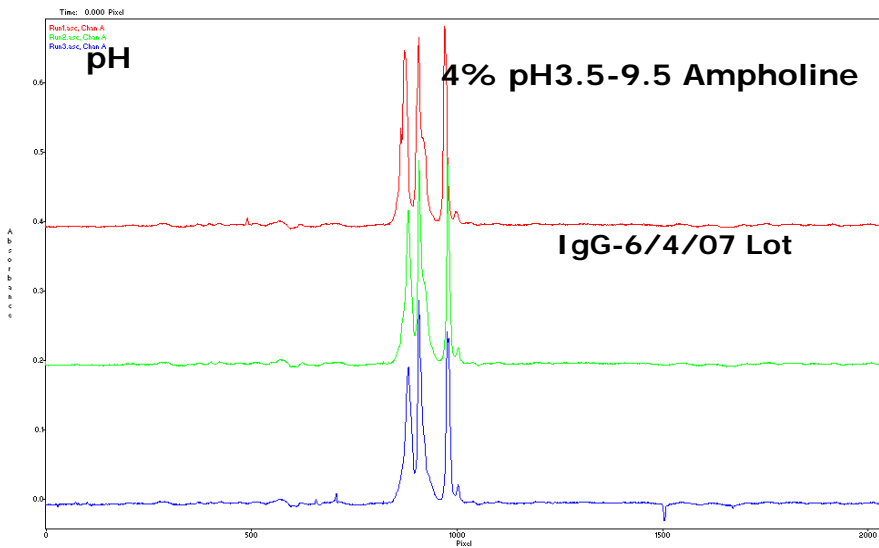


## Baseline noise level of the three CAs

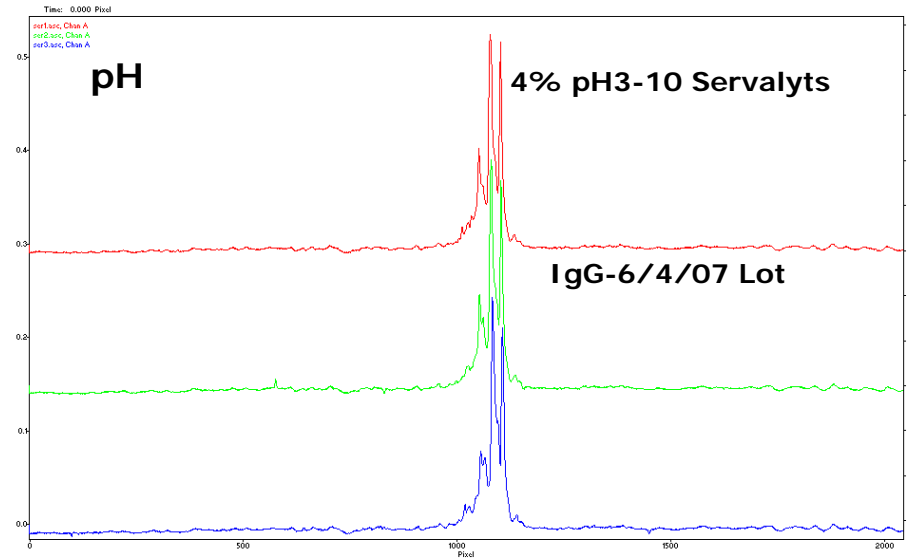


# BASELINE NOISE AND IgG (LOT 6/4/07) PEAK PATTERN REPRODUCIBILITY IN DIFFERENT CAs

## Ampholine pH 3.5 – 9.5



## Servalvys pH 3 - 10



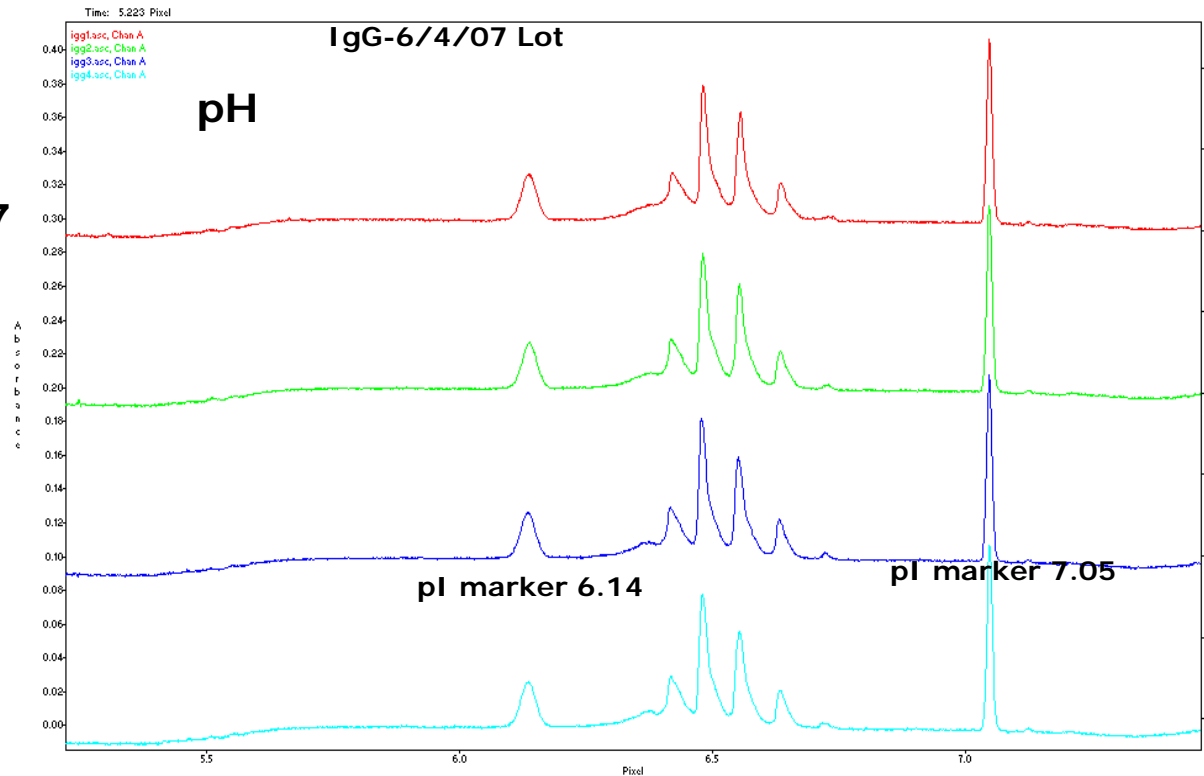
## Narrow pH range Pharmalytes pH 5 – 8 is used for higher resolution

### Selection of CA's

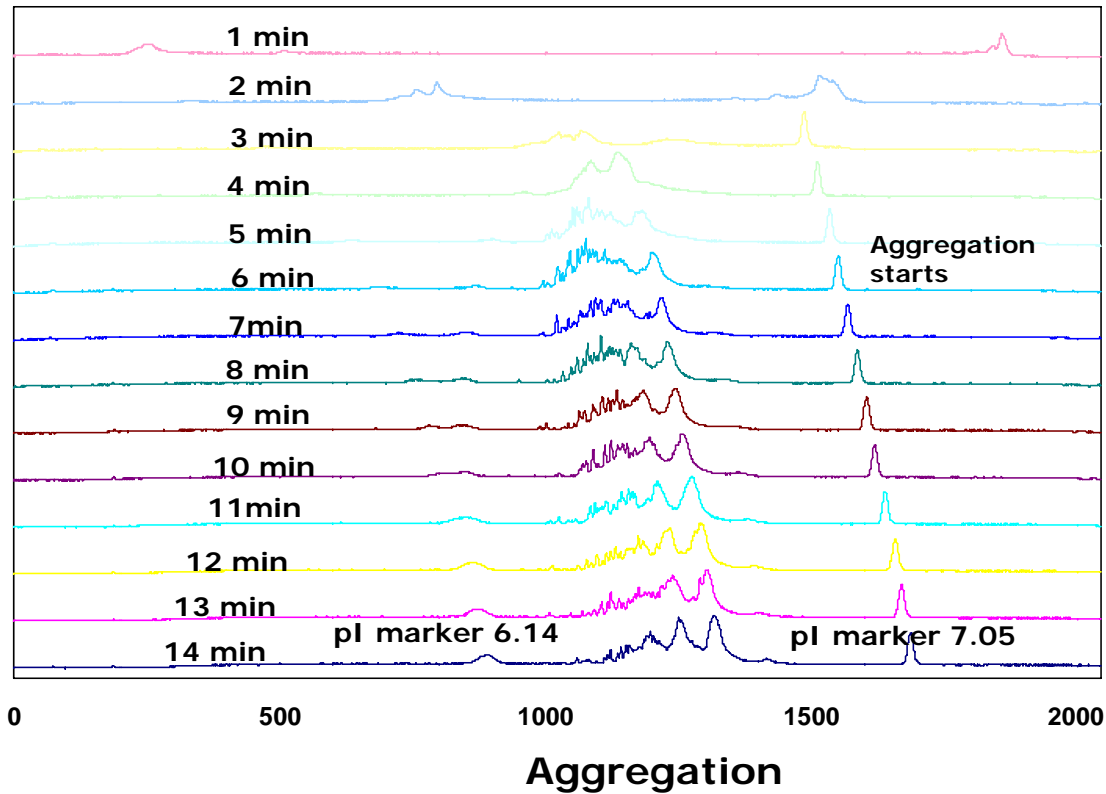
- Peak pattern of the sample is only reproducible in Pharmalytes
- Pharmalytes have the lowest baseline noise

### Final Conditions for Lot 6/4/07

- 4% pH5-8 Pharmalytes
- 0.35% M. C.
- Focusing at 1.5 kV for 1 min, then at 3 kV for 19 min



## IEF process of IgG (7/2707) in Pharmalytes pH 5 - 8

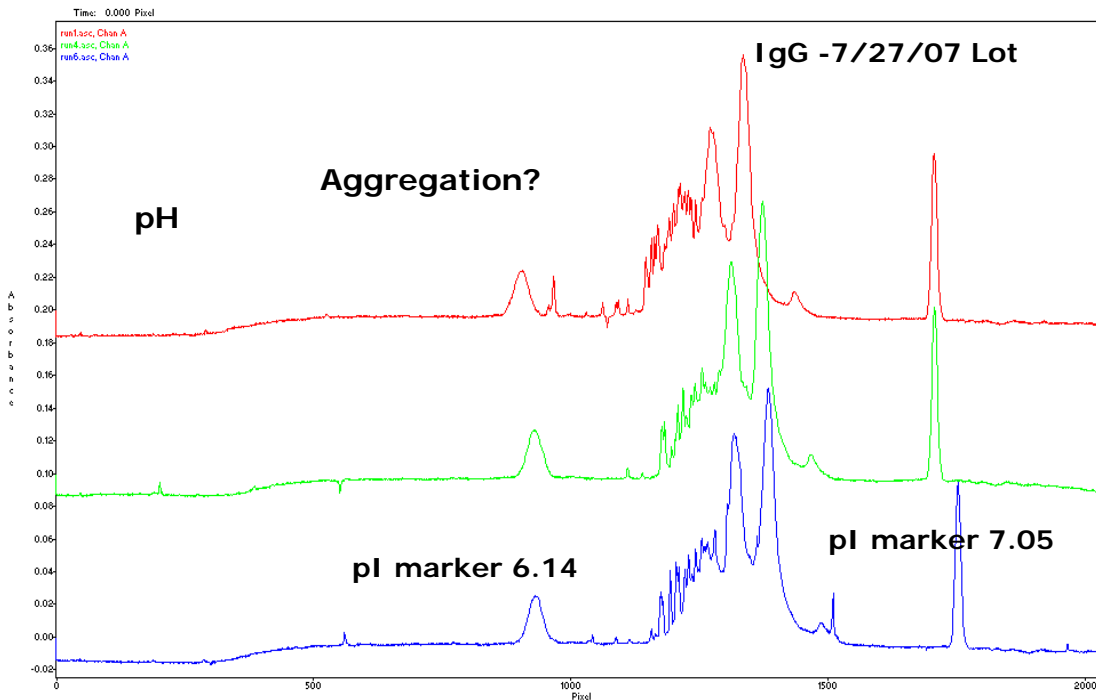


The spikes start to appear when the protein focuses

- Sign of sample aggregation during IEF

# APPLY THE CONDITIONS TO THE SECOND LOT (7/27/07)

## Peak pattern becomes non-reproducible



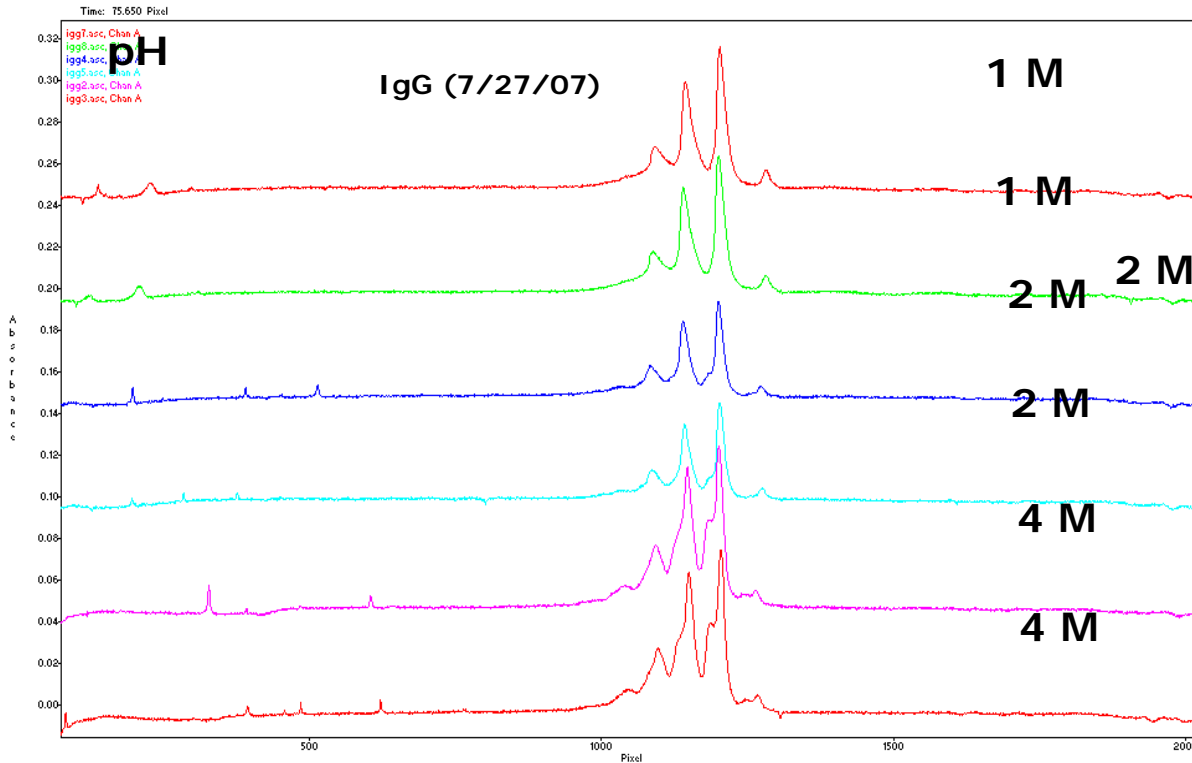
### Cause of non-reproducible peak pattern

Aggregation during IEF?

Whole-column detection is a powerful diagnostic tool.

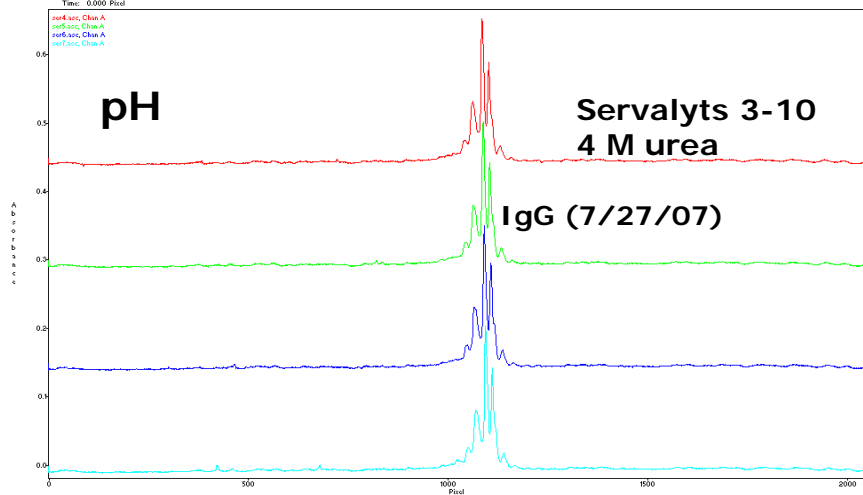
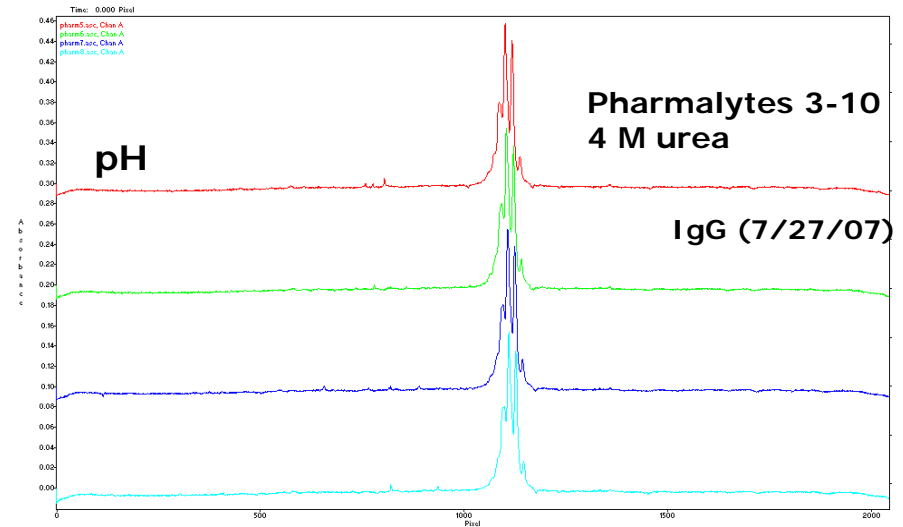
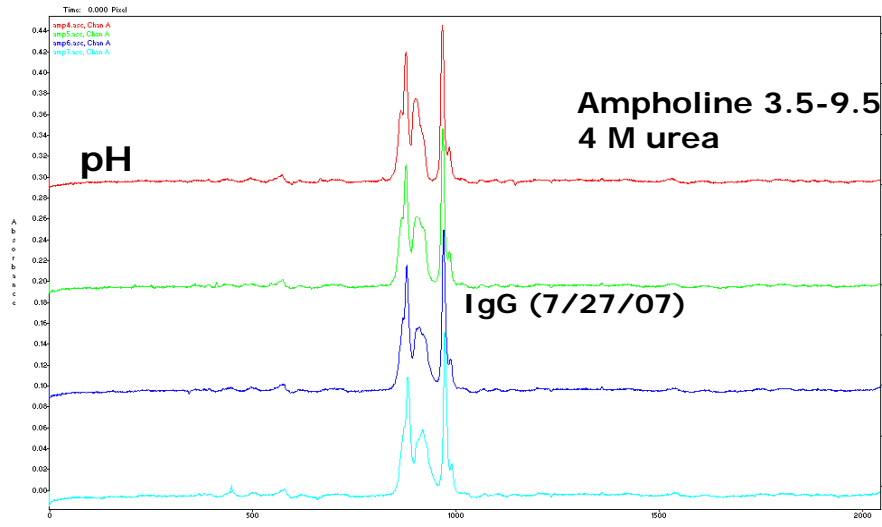


# 1 M – 4 M urea eliminates the aggregation



- 0.5 M – 4 M urea usually eliminates the problem
- Up to 4 M urea also eliminate the interaction between proteins and CAs

# WILL UREA IMPROVE PEAK PATTERN REPRODUCIBILITY FOR OTHER CAs?

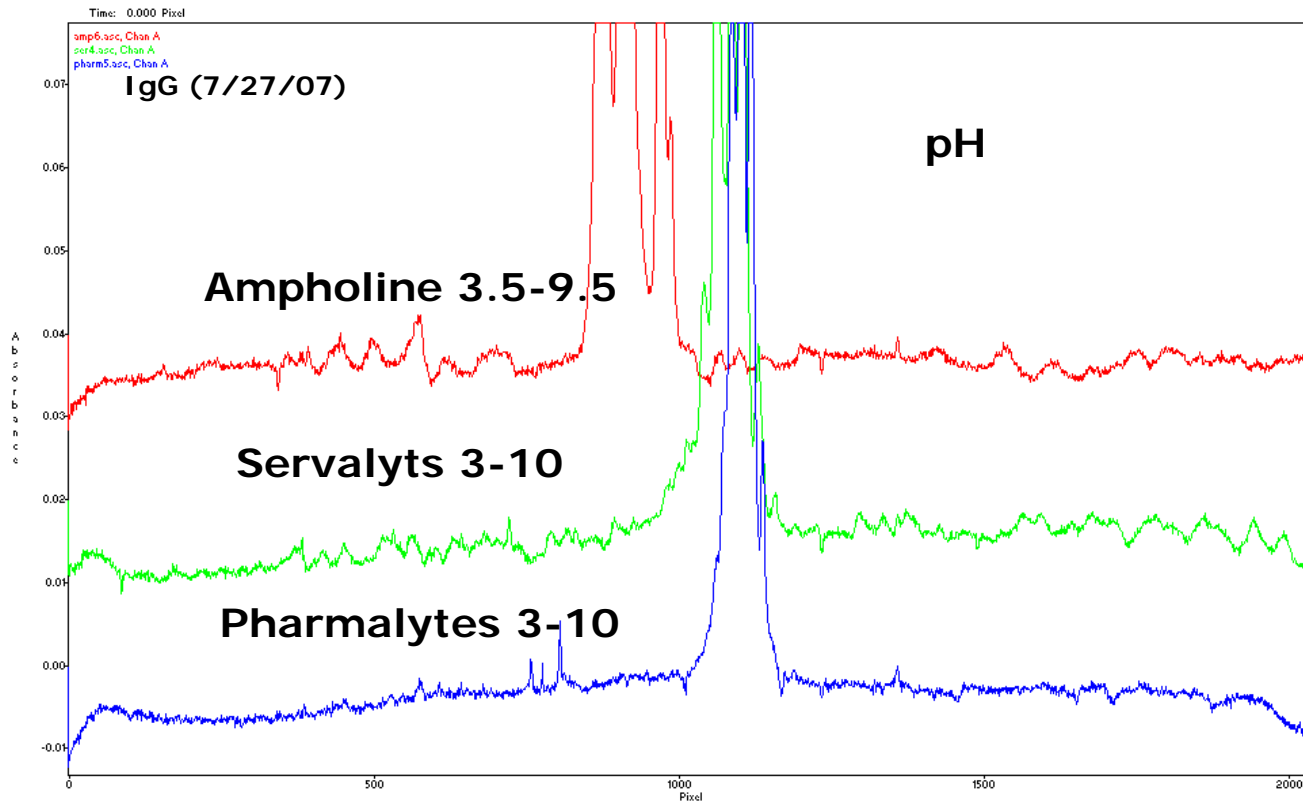


**Peak patterns of the sample are reproducible for the three CAs when 4 M urea are added**

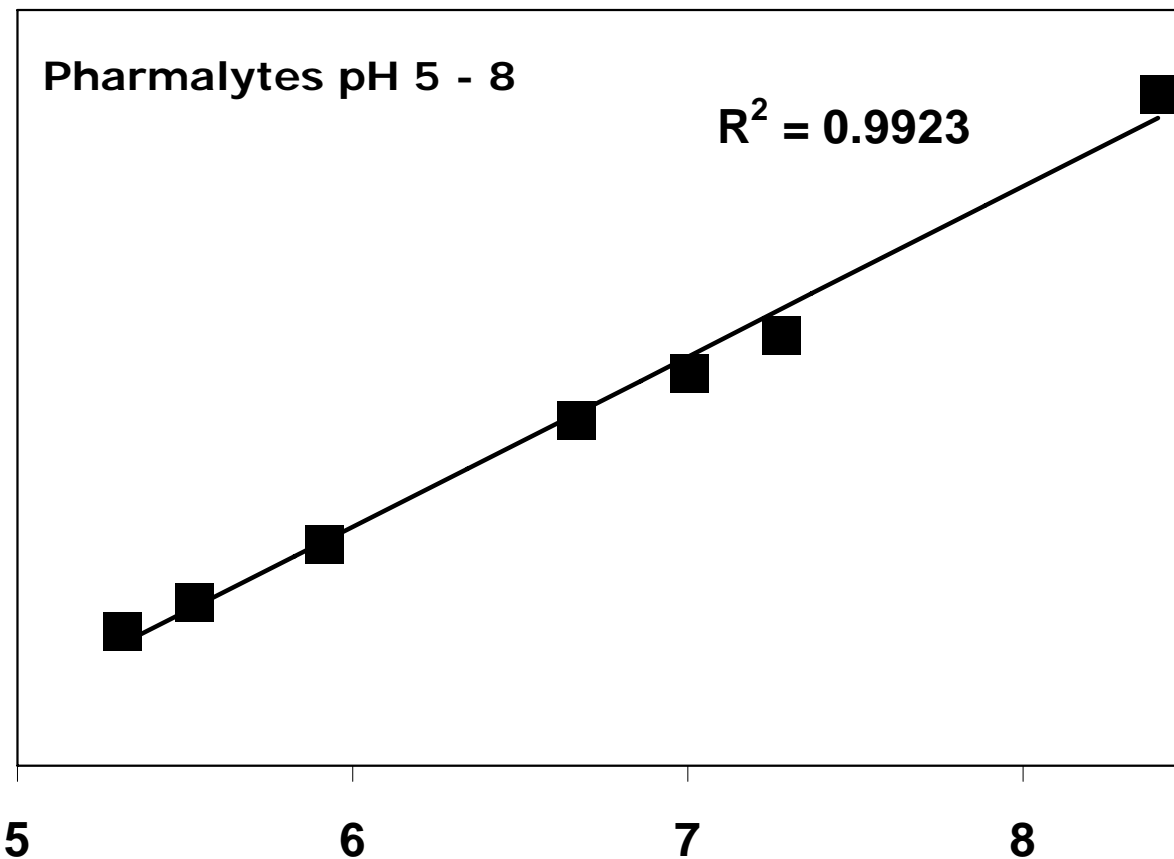
- Urea breaks the interaction between the protein and CAs?

# BASELINE NOISE OF THE THREE CAs IN 4 M UREA

Pharmalytes have the lowest noise level



## pH GRADIENT LINEARITY OF PHARMALYTES



## MATERIALS

pI Markers: Convergent Bioscience  
Servalyt®: Crescent Chemical  
Pharmalytes®, Ampholine®: Sigma- Aldridge

## CONCLUSIONS

1. The time required to optimize conditions for lot (6/4/07) was 2 hours and for lot (7/27/07) was 4 hours.
2. Pharmalytes had the lowest baseline noise with/without urea.
3. Pharmalytes pH 5-8 provided satisfactory resolution for the IgG samples.
4. Less than 4 M urea eliminated sample aggregation and interaction between sample and CAs.

## ACKNOWLEDGEMENT

Dr. Chitra K. Ratnayake of Beckman Coulter for providing samples.



# FINAL CONDITIONS FOR IgG LOT (7/27/07) AND THE RESULTS

4% pH5-8 Pharmalytes  
0.35% M.C.  
2 M urea  
Focusing at 1.5 kV for  
1 min then at 3 kV for 15 min

