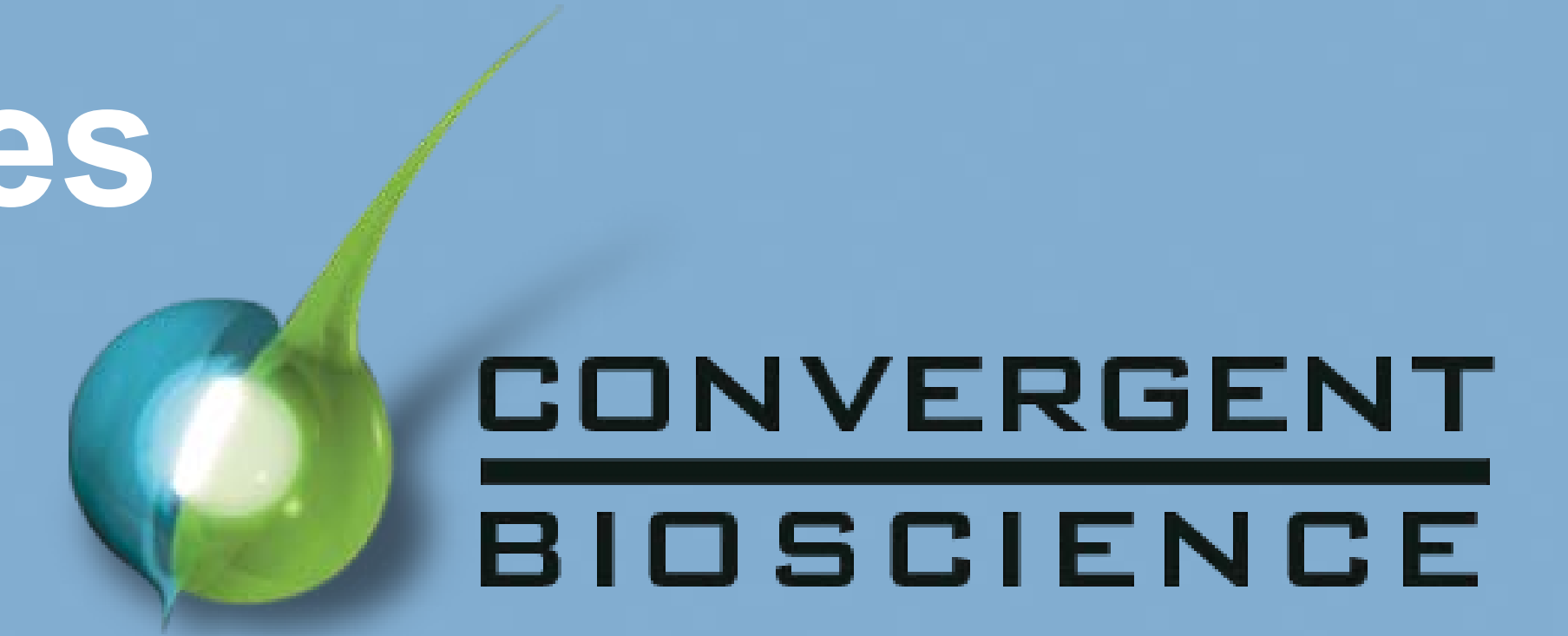


The Linearity and Resolving Power of High pH Range Carrier Ampholytes

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INTRODUCTION

Capillary isoelectric focusing (cIEF), which provides the highest resolution for protein separation, is widely used for charge-based separations, from early discovery to product QC, in the biopharmaceutical industry.

cIEF, like slab gel IEF, is a special electrophoresis technique performed in a pH gradient that is generated by carrier ampholytes (CAs). The linearity of CAs determines the pI calibration and the resolving power of CAs decides protein separation in cIEF. Limited by the current synthesis methodologies, commercial CAs at the high pH range from different vendors may have different separation performance.

In this poster, a set of well calibrated pI markers and the whole column detection cIEF, iCE280 IEF Analyzer were used to study the linearity and resolving power of two commercial brands of high pH range CAs.

cIEF of pI MARKERS with SERVALYTES pH 9-11

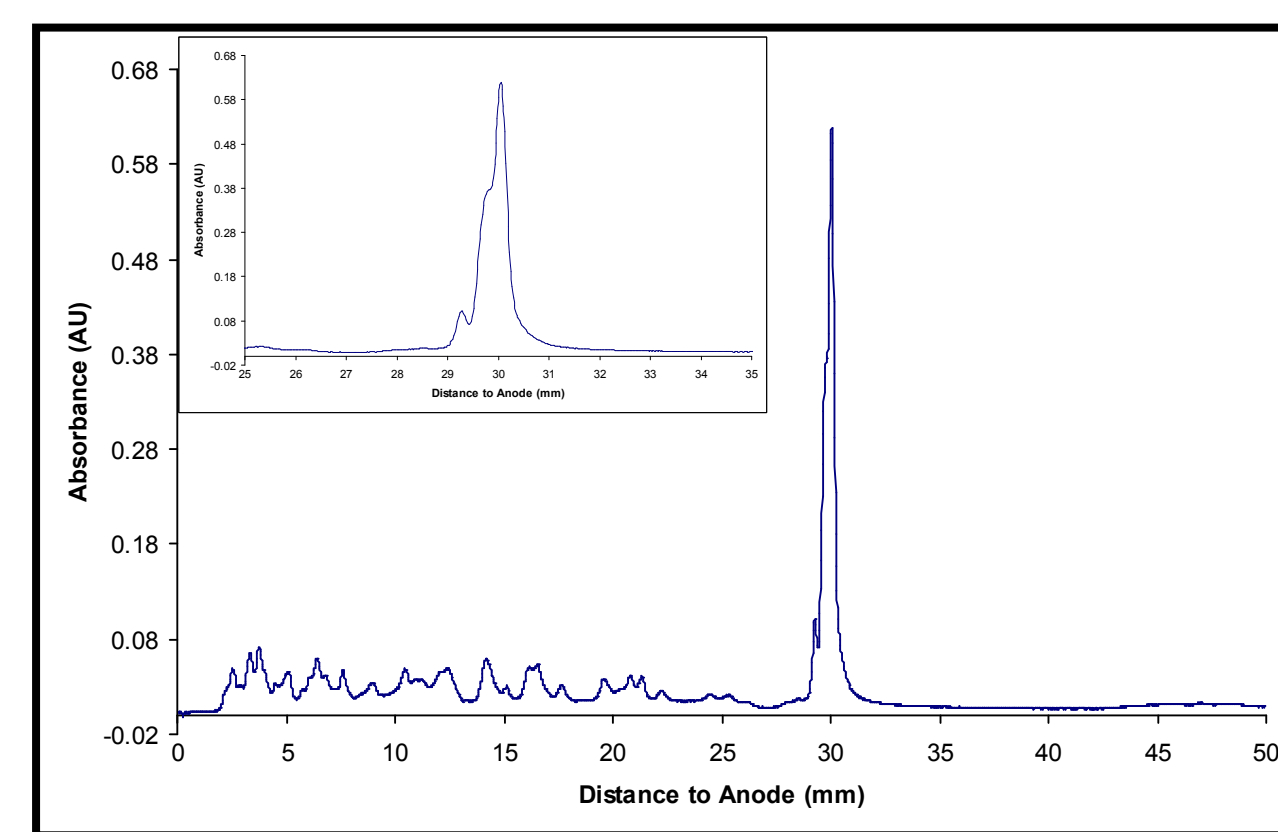
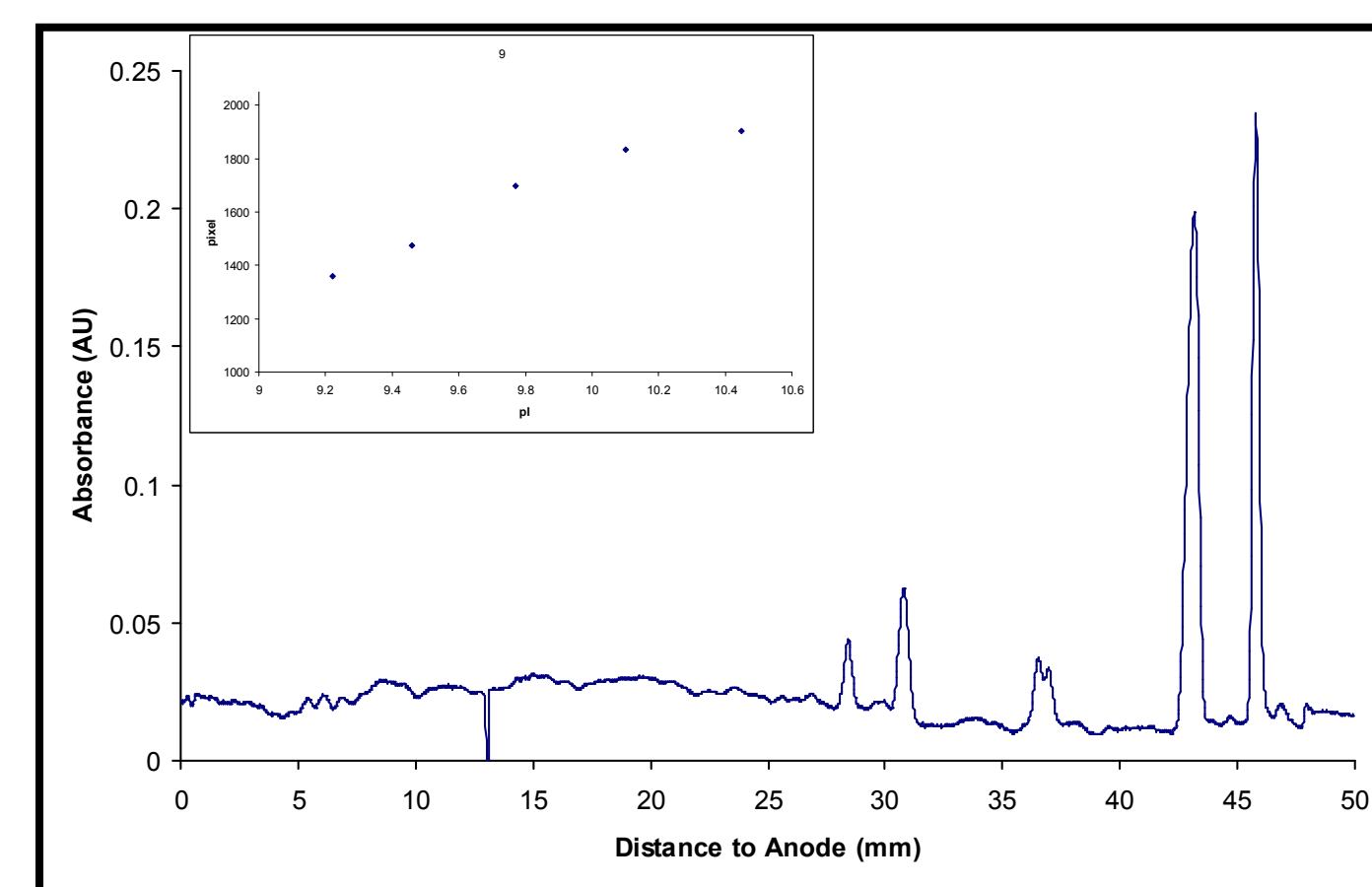


Figure 1. Focusing of pI markers (9.22, 9.46, 9.77, 10.10 and 10.45) with Servalyts pH 9-11. Experimental Conditions: pI markers were mixed with 4 % (V/V) CAs and 0.35% (W/V) methyl cellulose. Focusing voltage was 60V/mm for 5.5 minutes.

From Figure 1 and its insert, the poor linearity and resolving power of Servalyts pH 9-11 failed to separate the five pI markers into individual peaks.



cIEF of pI MARKERS with PHARMALYTES pH 8-10.5

Figure 2. Focusing of pI markers (9.22, 9.46, 9.77, 10.10 and 10.45) with Pharmalytes pH 8-10.5.

Experimental Conditions: pI markers were mixed with 4% (V/V) CAs and 0.35% (W/V) methyl cellulose. Focusing voltage was 60V/mm for 5.5 minutes.

From Figure 2, the five pI markers were separated very well (the insert shows the linearity) and demonstrated the relatively good and resolving power of pH 8-10.5 Pharmalytes. However, the much smaller and split pI 9.77 peak indicates possible interaction between Pharmalytes 8-10.5 and pI 9.77.

cIEF of pI MARKERS with PHARMALYTES pH 3-10

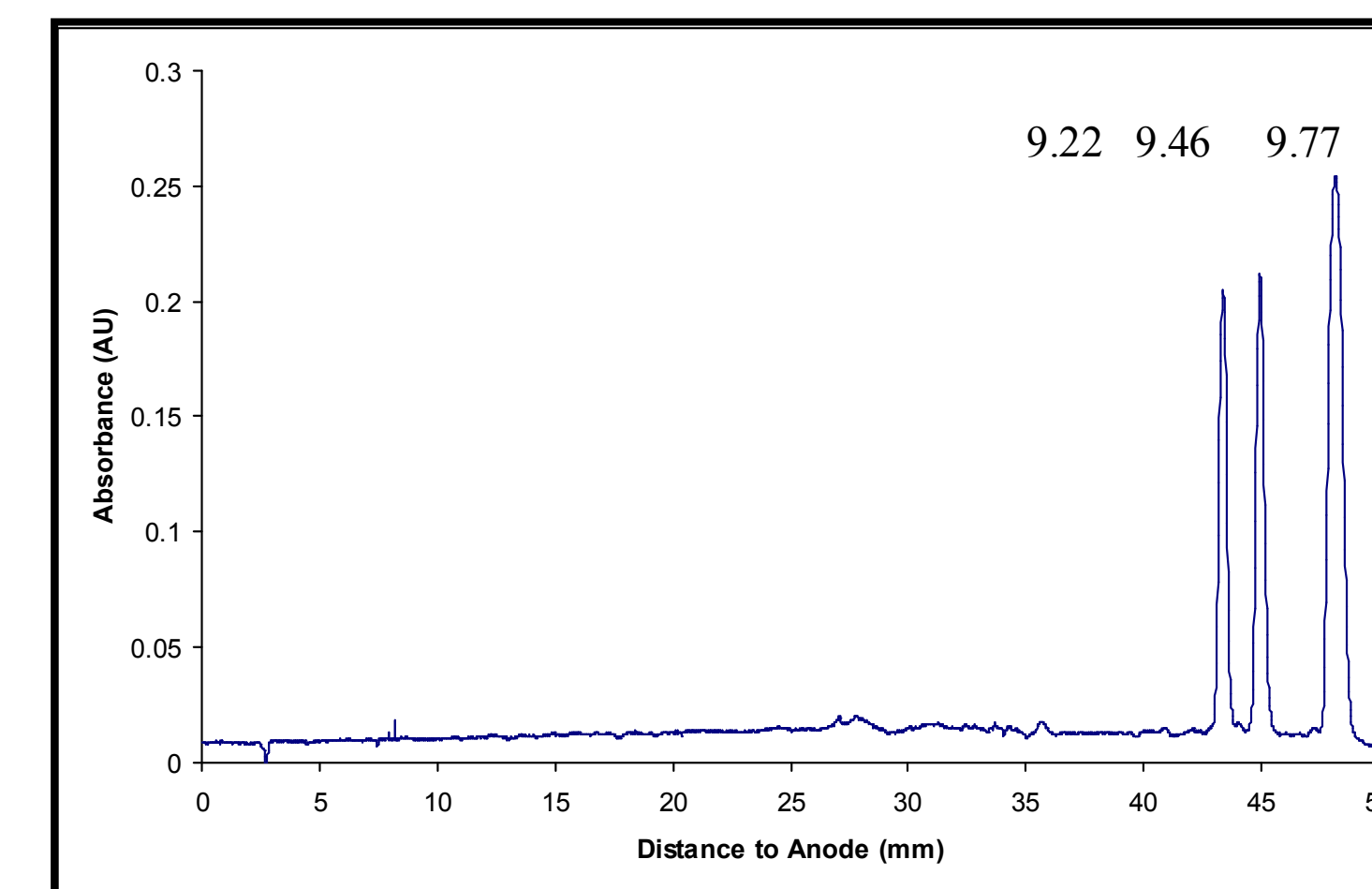


Figure 3. Focusing of pI markers (9.22, 9.46, 9.77, 10.10 and 10.45) with Pharmalytes pH 3-10.

Experimental Conditions: pI markers were mixed with 4% (V/V) CAs and 0.35% (W/V) methyl cellulose. Focusing voltage was 60V/mm for 5.5 minutes. The two most basic pI markers (pI 10.10 and pI 10.45) were out of the detection window of the pH 3-10 range.

cIEF of pI MARKERS with PHARMALYTES pH 3-10 and BASE

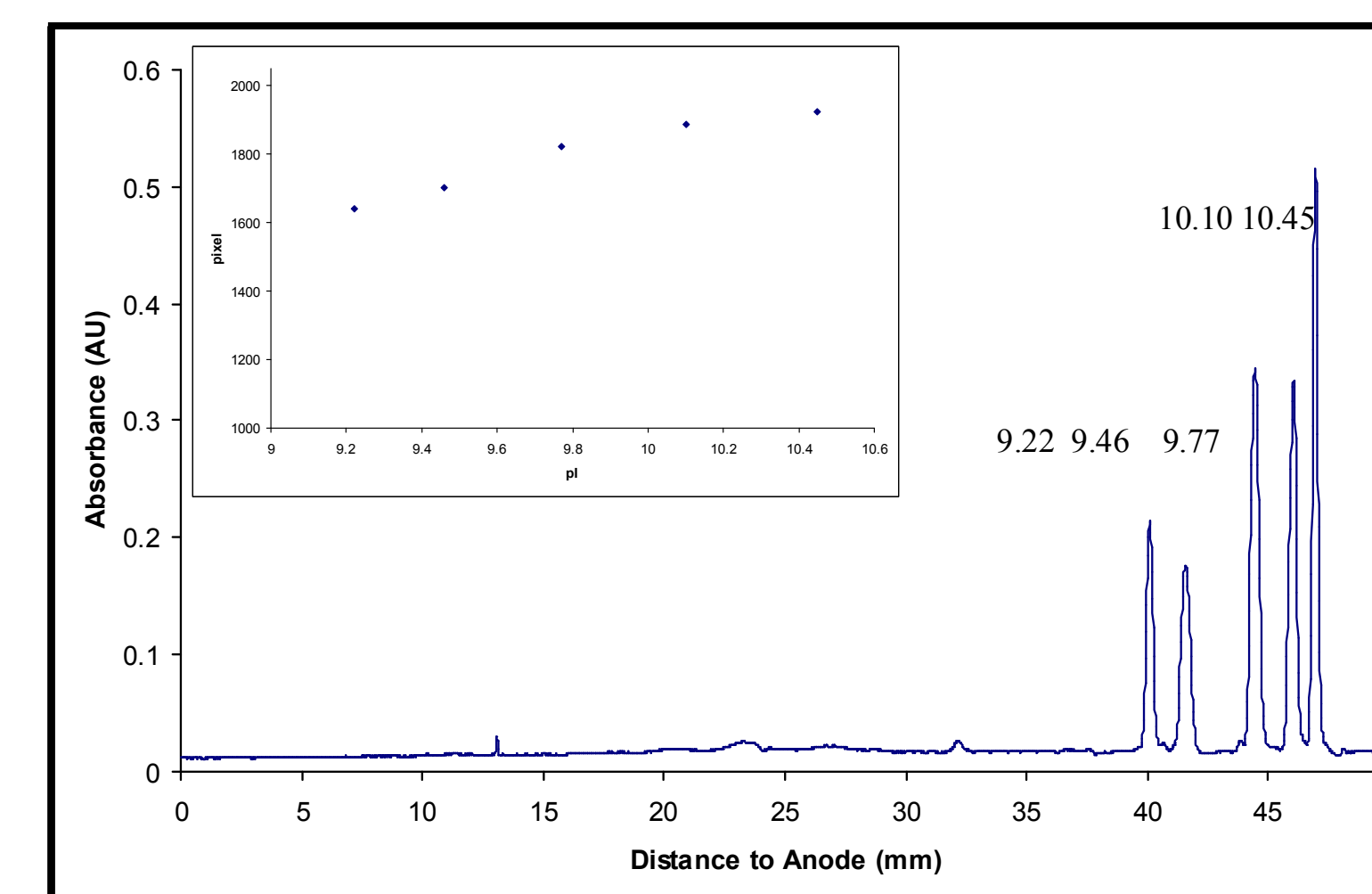


Figure 4. Focusing of pI markers (9.22, 9.46, 9.77, 10.10 and 10.45) with Pharmalytes 3-10 and base. Experimental Conditions were the same as in Figure 3 except the sample mixture also contained 20 mM NaOH.

When a base such as NaOH is present in the sample mixture, it will squeeze the pH gradient toward the anodic end. As a result, the extremely basic pI markers (10.10 and 10.45) that were previously out of the detection window are now detected.

Surprise observation! Pharmalytes 3-10 showed very good linearity in the high pH range comparable to Pharmalytes 8-10.5. In addition, they don't interfere with pI 9.77!

REFERENCES

1. Huang T, Liu Z, Pawliszyn, J, Anal. Bioanal. Chem 2005, 783-788.
2. Righetti P.G, Simó C, Sebastiano R., Citterio A., Electrophoresis 2007, 28, 3799-3810.

MATERIALS

1. pI Markers - Convergent Bioscience, Toronto, ON, Canada
2. Servalytes® - Crescent Chemical, Islandia, NY
3. Pharmalytes® - Sigma- Aldridge, Oakville, ON, Canada

cIEF of pI MARKERS with PHARMALYTES pH 3-10 and pH 8-10.5

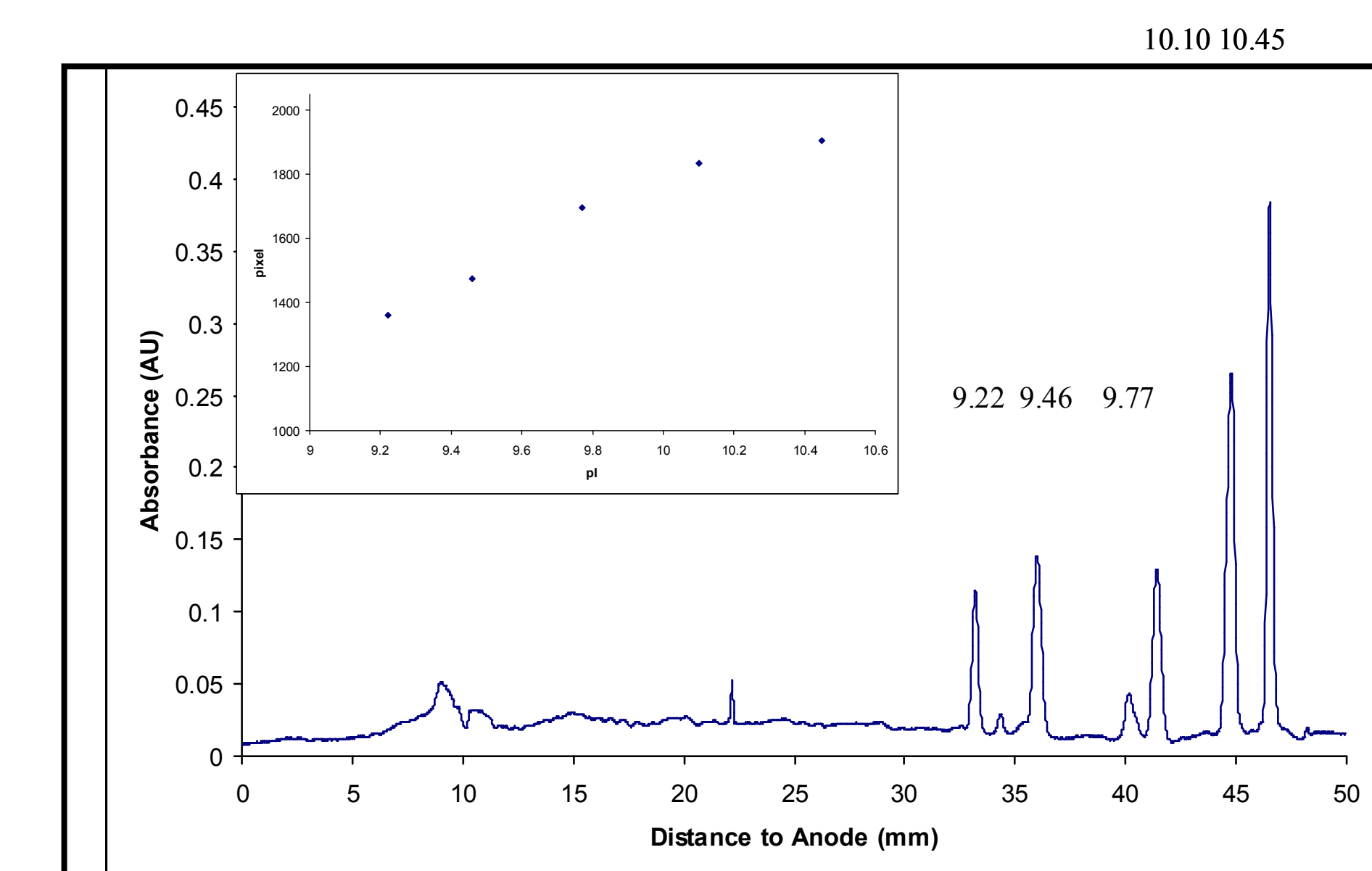


Figure 5. Focusing of pI markers (9.22, 9.46, 9.77, 10.10 and 10.45) with Pharmalytes pH 3-10 and pH 8-10.5.

Experimental Conditions: pI markers were mixed with 2 % (V/V) Pharmalytes pH 3-10 and 8-10.5 each, and 0.35% (W/V) methyl cellulose. Focusing voltage was 60V/mm for 5.5 minutes.

cIEF of pI MARKERS with PHARMALYTES pH 8-10.5 and SERVALYTS 9-11

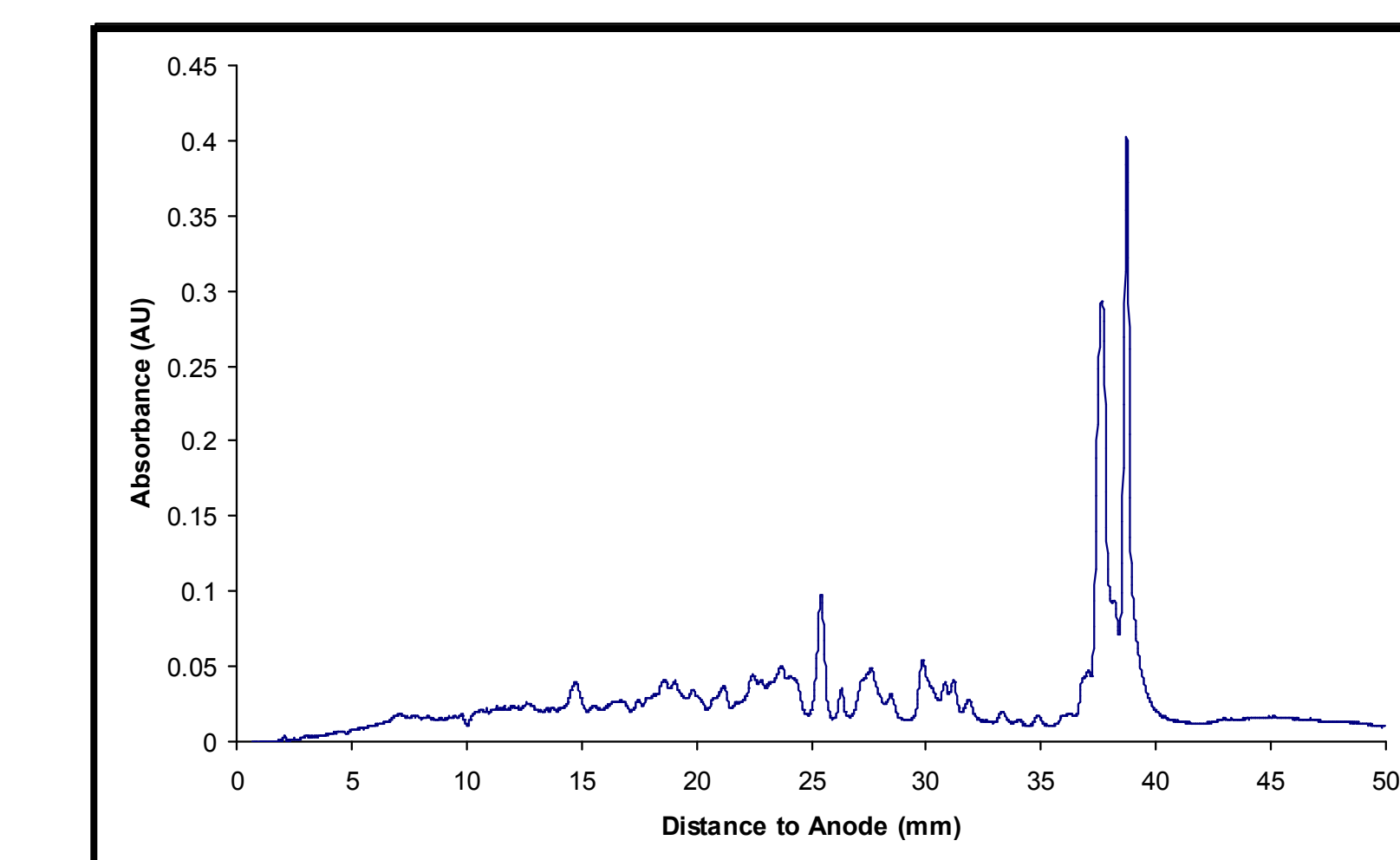


Figure 6. Focusing of pI markers (9.22, 9.46, 9.77, 10.10 and 10.45) with Pharmalytes pH 8-10.5 and Servalyts pH 9-11.

Experimental Conditions: pI markers were mixed with 2% (V/V) Pharmalytes 8-10.5 and Servalyts 9-11 each, and 0.35% (W/V) methyl cellulose. Focusing voltage was 60V/mm for 5.5 minutes.

Compared with Figure 1, somewhat resolving power improvement was achieved for Servalyts 9-11 when it was mixed with Pharmalytes pH 8-10.5. However, compared with Figure 2, a much worse resolving power was observed for Pharmalytes pH 8-10.5 when it was mixed with Servalyts pH 9-11!

CONCLUSIONS

1. The iCE280 protein Analyzer provides direct observation of the cIEF process, allowing quick identification of complicate issues related to the complex synthetic carrier ampholytes.
2. Synthetic carrier ampholytes might not provide good linearity or resolving power even within the range of the nominal value provided by the vendors!
3. There exists potential interaction between synthetic carrier ampholytes and proteins and small molecules, such as pI markers!
4. It might not be always true that better resolving power can be achieved by mixing synthetic carrier ampholytes from different vendors!