Examination of the pH Profile in Simultaneous Chromatography and Electrophoresis

Brae Petersen, Kishor Prasain, Tim Rose, and David C. Collins
Chemistry Department, Brigham Young University - Idaho

Introduction
Simultaneous chromatography and electrophoresis (SCE) combines an electric field orthogonal to capillary action (Figure 1). Compounds are separated according to their charge-to-mass ratio and polarity. Electrolysis occurring at both electrodes creates acidic and basic regions. This work initiates an investigation into how these pH regions, in combination with the pH of the stationary and mobile phases, affect the chromatographic and electrophoretic separations.

Methods
Several buffer solutions of desired pH were prepared.
- **Acidic**: glycine (pH = 2.35)
- **Basic**: ammonia and NaOH (pH = 9.3 and 13)
- **Neutral**: phosphoric acid (6.5)

SCE was conducted using these buffers and silica gel stationary phase. Dyes (1 mg/mL) and buffer solutions were mixed (2:1) with propanol. Separation occurred in 14 min at 500 volts. Mobile phase traveled vertically solely through capillary action.

Results and Discussion

![Figure 2](image2.png)

Figure 2. Separations achieved with (a) glycine, (b) ammonia, and (c) phosphoric acid buffer solutions under similar conditions. Within experimental variation, these separations are virtually identical. (a) and (b) are spotted with bromocresol green indicator to show pH.

![Figure 3](image3.png)

Figure 3. Stationary phase sprayed with bromocresol green indicator. Acidic and basic regions created from electrolysis are identified.

![Figure 4](image4.png)

Figure 4. (a) Separation achieved with NaOH in propanol and sprayed with universal indicator. Even after separation, the silica gel stationary phase is acidic. (b) First dipping the stationary phase in NaOH to the "saturation line" resulted in a basic region, yet still acidic in the region of capillary action.

Conclusion
The acidic and basic regions produced at the electrodes do not travel the width of the stationary phase and do not significantly affect the separation of the dyes. The pH of the chosen mobile phase buffer also has little effect on the separation of the dyes. It appears that the acidic pH of the stationary phase predominately controls the pH of the separation when solely capillary action is involved. Thus, the charge-to-mass ratio of the dyes and the lateral separation is similar regardless of the identity of the mobile phase.

Acknowledgements
We gratefully acknowledge Brigham Young University - Idaho Chemistry Department and the College of Physical Sciences and Engineering for all financial support.

Experimental

**Apparatus**
- **Power supply**: Bio Rad Powerpac HV
- **Electrodes**: Platinum wire, copper wire
- **Solvent reservoir**: Four glass chambers

**Reagents and Materials**
- **TLC plates**: SIL G/UV plates, 0.20 mm
- **Separation chemicals**: propanol, ammonia, glycine, phosphoric acid, and sodium hydroxide.
- **pH indicators**: bromocresol green, universal indicator.
- **Dyes**: methylene blue, erioglaucine, tartrazine, crystal violet, allura red, methyl red, bromocresol green, and erythrosine

**Figure 1.** Diagram showing separation vectors for SCE with electrolysis occurring at the electrodes.