

Figure 2

FID gas chromatogram from LC-GC clean-up and analysis of the same PAH standard mixture as in Figure 1, obtained after 6 months daily use of the epoxy-glued interface.

The gluing technique was further used in a GC-MS system to connect the fused silica capillary column from the gas chromatograph to the empty tubing acting as a transfer line to the mass spectrometer ion source. In such arrangements the low pressure in this part of the column normally leads to severe problems (increase in the background noise of the mass spectrometer and a reduction in the lifetime of the ion source filament) as a result of entry of air into the ion source through the leaking press-fit connection. Such problems were eliminated by use of the gluing technique. Air leakage was reduced to the levels normally observed when the capillary column is introduced directly into the ion source.

No background bleed from the epoxy glue could be detected by either flame ionization detection or mass spectrometry.

The high pressure stability of the glued press-fit connections was tested by use of an HPLC pump delivering pentane as mobile phase. A short piece (ca 0.5 m) of 0.25 mm i.d. fused silica tubing was glued into a press-fit connector which had been sealed by melting. The other end of the fused silica tubing was connected directly to the LC pump outlet by means of a Valco 1/16" union and Valco fused silica adapter. A flow of 0.1 ml/min was applied and the pressure allowed to rise to 300 bar in increments of 50 bar. At each level the pressure was monitored for 10 minutes to detect possible leaks; at 300 bar the pressure was monitored for 30 minutes. Several times during the testing the fused silica tubing cracked owing to the high pressure, but no leaks were detected in any of the press-fit connections.

Because of this stability at high pressure it would be possible to use glass press-fit connectors and the gluing technique for connection of capillaries in supercritical fluid chromatography.

References

- [1] E. R. Rohwer, V. Pretorius, and P. J. Apps, *HRC & CC* **9** (1986) 295.
- [2] B. Schilling, K. Grob Jr., P. Pichler, R. Dubs, and B. Brechbühler, *J. Chromatogr.* **435** (1988) 204.
- [3] A. D'Amato, C. Bicchi, and M. Galli, *HRC* **12** (1989) 349.
- [4] K. Grob Jr., *On-Line Coupled HPLC-GC*, Hüthig, Heidelberg (1991) 88.
- [5] P. Sandra, M. Schelfaut, and M. Verzele, *HRC & CC* **5** (1982) 50.
- [6] J. Roeraade, personal communication.

Ms received: July 3, 1991

Chemiluminescence Detection in Capillary Electrophoresis

Rajeev Dadoo, Luis A. Colón, and Richard N. Zare*

Department of Chemistry, Stanford University, Stanford, CA 94305, USA

Key Words:

Capillary electrophoresis
Chemiluminescence
Luminol
Photon counting

1 Introduction

Capillary electrophoresis (CE) has become a powerful analytical separation technique for the analysis of complex mixtures [1, 2]. By its capillary nature, the technique typically requires only nanoliter volumes of analyte, a characteristic that motivates the search for detection methods of high sensitivity. Most CE is performed with detection by UV absorption; typically, analyte concentrations of approximately 10^{-4} to 10^{-5} M may be detected by this method.

Highly sensitive detection in high performance liquid chromatography (HPLC) has been achieved by use of chemiluminescence:

several chemiluminescent reactions have been utilized, including the peroxyoxalate [3, 4], firefly luciferase [5, 6], and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) [7-10] reactions. Although the peroxyoxalate reaction is the most commonly applied, it requires the use of organic solvents because the solubility of the oxalates in aqueous solutions is poor. Luminol reacts with hydrogen peroxide in the presence of a catalyst in alkaline solution to emit light with a wavelength centered around 425-435 nm (**Figure 1**); the reaction has been used for chemiluminescence detection in HPLC, usually with the catalyst and hydrogen peroxide added post-column. Derivatives of luminol

such as isoluminol [10] and *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI) [7, 8] can also be used to label analytes such as carboxylic acids and amines: the labelled analytes (*e.g.*, amino acids) are separated, and then detected after post-column addition of the reagents.

Adaptation of chemiluminescence to CE appears promising because it is easily implemented. In this paper we present preliminary results obtained by application of the luminol chemiluminescent reaction, a reaction that provides sensitivity several orders of magnitude greater than that available from UV absorption. A photon counting system has been used for detection in order to measure the low levels of light generated in the capillary column.

2 Experimental

2.1 Electrophoresis Apparatus

A capillary electrophoresis system with a post-capillary reactor similar to that used by *Jorgenson* and coworkers [11] was modified for our purposes; a schematic diagram of the apparatus is shown in **Figure 2**. A +30 kV power supply (Spellman High Voltage Electronics, Bronx, NY, USA) was used to provide the separation voltage.

The electrophoretic capillary (48 cm × 75 μm i.d. × 375 μm o.d.), reagent capillary (75 cm × 200 μm i.d. × 375 μm o.d.), and reaction (outlet) capillary (65 cm × 150 μm i.d. × 375 μm o.d.), all purchased from Polymicro Technologies (Phoenix, AZ, USA), were held in place by a PEEK tee connector (Alltech, Deerfield, IL, USA).

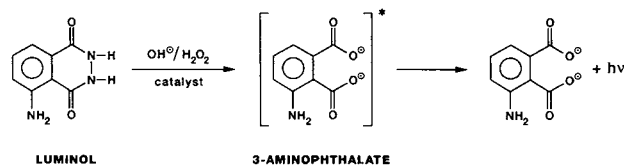


Figure 1
The luminol chemiluminescence reaction.

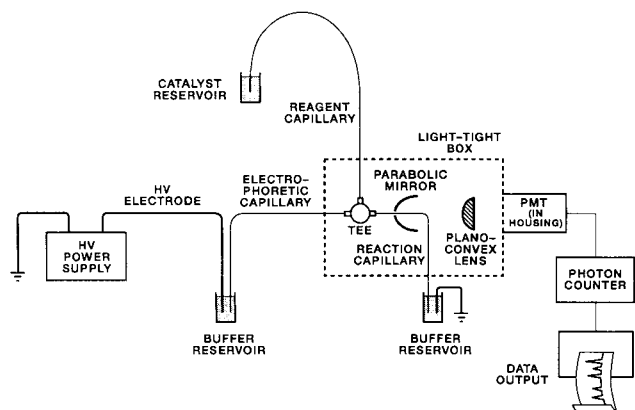


Figure 2
Schematic diagram of the CE – chemiluminescence detection apparatus.

A 3–4 cm section at the end of the electrophoretic capillary was etched to an outer diameter of approximately 100–120 μm by placing it in concentrated (48 %) hydrofluoric acid for 90–95 min; during this process the capillary was purged with helium. Approximately 2.5–3 cm of the etched portion of the electrophoretic capillary was inserted into the reaction capillary. A 2–3 mm detection window was made on the reaction capillary (starting at the point where the inner electrophoretic capillary terminated) by burning off the polyimide coating.

2.2 Detection Apparatus

The detection window of the reaction capillary was placed at the focal point of a parabolic reflector (Melles Griot, Irvine, CA, USA) with the help of micrometer translation stages. The light collimated by the reflector was focused by a planoconvex lens (Melles Griot) on to a photomultiplier tube (PMT) (Hamamatsu, San Jose, CA, USA) that was cooled to -20°C by means of a thermoelectric cooler (Products for Research, Danvers, MA, USA) connected to the PMT housing. The PMT was connected to a photon counting system (EG & G Princeton Applied Research, Princeton, NJ, USA) and an analog signal (proportional to the count rate) was monitored by means of a chart recorder (Linear Instruments, Reno, NV, USA).

The tee connector and detection optics were enclosed within an aluminum light-tight box, which had a hole for the PMT window. Except for the detection window, the portion of the reaction capillary inside the light-tight box was painted black to prevent extraneous light emitted after the detection region from reaching the detector.

2.3 Reagents

Potassium monohydrogen phosphate, potassium hydroxide, and potassium ferricyanide were purchased from J. T. Baker (Phillipsburg, NJ, USA), a 30 % solution of hydrogen peroxide from Fisher Scientific (Fair Lawn, NJ, USA), and luminol and ABEI from Aldrich (Milwaukee, WI, USA). All solutions were prepared in distilled and deionized water, and were filtered (0.2 μm) before use.

2.4 Procedure

Electrophoretic separations were performed in 10 mM phosphate buffer (pH 9.9, adjusted with 1 M potassium hydroxide) containing 0.2 M hydrogen peroxide as one of the reagents for the chemiluminescence reaction. Luminol and ABEI stock solutions (2.7 and 2.8 mM, respectively) were prepared by dissolving the appropriate amount of analyte in buffer solution containing 10 mM potassium hydroxide: addition of potassium hydroxide to the buffer was necessary for complete dissolution of the luminol and ABEI.

The reagent capillary was used to deliver the catalyst solution (25 mM potassium ferricyanide in pH 10.6 buffer) by gravity: the catalyst reservoir was placed 11 cm above the buffer reservoirs during the analyses. After making serial dilutions to obtain the required concentrations, the sample solutions were electrokinetically injected while maintaining the catalyst reservoir at the same height as the buffer reservoirs. The reagent capillary and the tee connector were initially filled with the catalyst solution by purging with a syringe; the separation voltage was then applied for 20–30 min, until the current stabilized.

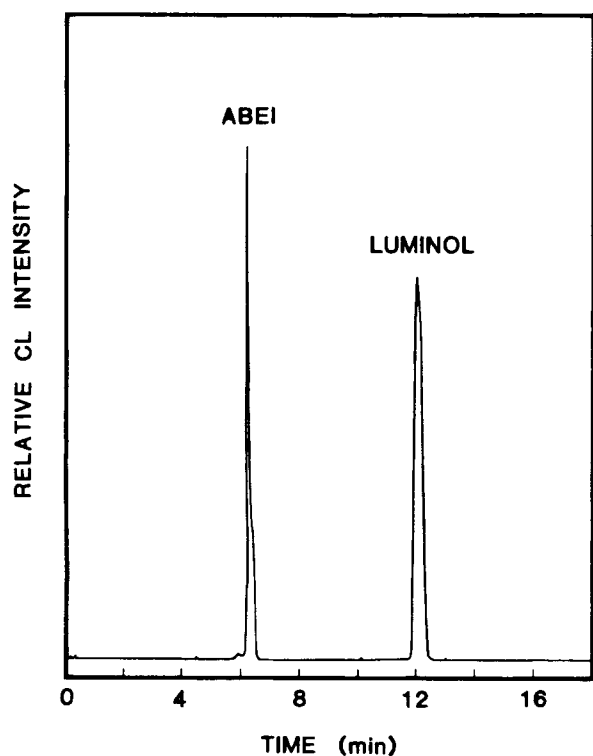


Figure 3

Electropherogram showing the separation of ABEI (7×10^{-7} M) and luminol (2.7×10^{-7} M) with chemiluminescence (CL) detection: separation voltage, 12.5 kV; sample injection, 10 s at 10 kV; other conditions are given in the text.

3 Results and Discussion

Fluorescence is the most sensitive means of detection currently employed with CE [12–14]. By using lasers as the excitation source, zeptomole (10^{-21} mol) quantities of analytes have been detected [13, 14]. To increase the detection sensitivity further, several sources of background noise need to be overcome; these include Rayleigh and Raman scattering, which may interfere with the fluorescence of the analyte of interest. Fluctuations in the intensity of the light source can also increase background noise levels, thereby reducing the signal-to-noise (S : N) ratio.

Since chemiluminescence is an inherently low background process, we decided to investigate its use as a potentially highly sensitive detection method for CE. The luminol – hydrogen peroxide reaction was adopted in this initial study because of its compatibility with aqueous solutions. The concentrations of the reagents employed (potassium ferricyanide and hydrogen peroxide) were similar to those used in work previously published on the use of luminol chemiluminescence detection in HPLC [7], and further optimization was not attempted for this work.

The detection technique was applied to the separation of luminol and ABEI; an electropherogram obtained from a standard mixture

is shown in **Figure 3**. For comparison purposes, the standard mixture was analyzed by CE with UV absorption (200 nm) as the detection method (experiments performed on a Beckman P/ACE 2000 system). With absorbance, we obtained a detection limit (S : N = 3) of 90 fmol and 10 fmol for luminol and ABEI, respectively, whereas employing chemiluminescence we obtained 100 amol (3×10^{-9} M) for luminol and 400 amol (7×10^{-9} M) for ABEI – an improvement in sensitivity of 2–3 orders of magnitude.

4 Conclusion

In this preliminary investigation, we have demonstrated the feasibility of using chemiluminescence as a detection technique for capillary electrophoresis. Owing to its sensitivity compared with other means of detection, chemiluminescence can be an attractive alternative detection method for CE. No excitation light source is required and the detection instrumentation used is relatively simple. Further studies will investigate the optimization of the overall system in order to enhance sensitivity and the determination of luminol-labeled compounds (e.g., amino acids).

Acknowledgment

We thank W. Howard Whitted, Robert J. Obremski, Xiaohua Huang, Jason B. Shear, and Harvey A. Fishman for many helpful discussions on this project. Support for this work by Beckman Instruments is gratefully acknowledged.

References

- [1] M. J. Gordon, X. Huang, S. L. Pentoney, Jr., and R. N. Zare, *Science* **242** (1988) 224.
- [2] A. G. Ewing, R. A. Wallingford, and T. M. Olefirowicz, *Anal. Chem.* **61** (1989) 292A.
- [3] S.-I. Kobayashi and K. Imai, *Anal. Chem.* **52** (1980) 424.
- [4] K. W. Sigvardson and J. W. Birks, *Anal. Chem.* **55** (1983) 432.
- [5] G. Mellbin, *J. Liq. Chromatogr.* **6** (1983) 1603.
- [6] K. Miyaguchi, K. Honda, and K. Imai, *J. Chromatogr.* **303** (1984) 173.
- [7] T. Kawasaki, M. Maeda, and A. Tsuji, *J. Chromatogr.* **328** (1985) 121.
- [8] K. Nakashima, K. Suetsugu, S. Akiyama, and M. Yoshida, *J. Chromatogr.* **530** (1990) 154.
- [9] A. MacDonald and T. A. Nieman, *Anal. Chem.* **57** (1985) 936.
- [10] S. R. Spurlin and M. M. Cooper, *Anal. Lett.* **19** (1986) 2277.
- [11] D. J. Rose, Jr. and J. W. Jorgenson, *J. Chromatogr.* **447** (1988) 117.
- [12] J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.* **53** (1981) 1298.
- [13] S. Wu and N. J. Dovichi, *J. Chromatogr.* **480** (1989) 141.
- [14] J. V. Sweedler, J. B. Shear, H. A. Fishman, R. N. Zare, and R. H. Scheller, *Anal. Chem.* **63** (1991) 496.

Ms received: December 23, 1991