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Short communication

Apparatus and method for interfacing capillary electrophoresis and chemiluminescence nitrogen detection for the analysis of nitrogen-containing compounds ☆

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Abstract

We have developed an interface that allows the specific detection of nitrogen-containing compounds by using a chemiluminescence nitrogen detector. The feasibility of using this interface was demonstrated by separating and detecting two nitrogen-containing compounds, *p*-aminosalicylic acid and L-phenylalanine. Although baseline separation was achieved, the theoretical plates were lower when compared to UV detection (25 000 vs. ~85 000). A sensitivity of 75 ng (~500 pmol) per injection was achieved with this system which is adequate for pharmaceutical and biotech applications.

Keywords: Interfaces; CE–chemiluminescence nitrogen detection; Instrumentation; Detection, electrophoresis; Aminosalicylic acid; Phenylalanine

1. Introduction

Capillary electrophoresis (CE) is a rapidly growing separation technique that is capable of high resolution, low sample and reagent usage, resolving neutral and charged analytes, and rapid analysis. One of the main advantages of CE is its wide range of applications. Since its inception many forms of classical (molecular sizing, isoelectric focusing, isotachophoresis) and non-classical (micellar electrokinetic chromatography, capillary electrochromatography) electrophoresis applications have been developed. Although quite versatile in terms of separation ability, CE is limited in the number of ways to conduct highly sensitive analysis. Thus, considerable effort has been directed towards improvement of sensitivity. Much of this effort has been concentrated in two areas: (i) increasing injection volume (bigger internal diameter capillaries), sample stacking [1–4], or expanding the detector path length (bubble cells, Z-cells, etc.) and (ii) coupling it with sensitive detection techniques, such as fluorescence, electrochemical and chemiluminescence [5–10].

The detection techniques showing the most potential for enhancing sensitivity also have considerable disadvantages. For instance, laser-induced fluo-

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rescence (LIF) is extremely sensitive but the analyte must either be fluorescent, which most analytes are not, or be made fluorescent by tagging with a suitable fluorophore. In addition, there are only two lasers with emission wavelengths 325 and 488 nm that are economically feasible to use in commercially available CE instruments. Using these lasers limits the analytes to fluorescein or danzyl derivatized compounds. Even with these shortcomings, LIF is being used increasingly to detect amino acids, proteins, and nucleotides [11].

Electrochemical detection (ED) is potentially an ideal method of detection for microcolumn-based separations. Since detection is based on an electrochemical reaction at the surface of a working electrode, the reaction volume can be very small with no appreciable loss in sensitivity. This is contrary to optical detectors where response is dependent on path length. Two approaches have been reported to give femtomole detection limits, amperometric detection at constant potential (ADCP) [12,13] and pulsed amperometric detection (PAD) [14–16]. Although sensitive, ED suffers from limitations imposed by the highly alkaline conditions (pH>12), poor reproducibility due to the difficulty associated with the electrode/capillary alignment, and difficulty in electrically isolating the working electrode from the high potential field present during electrophoresis.

Another way to increase sensitivity is to increase the signal-to-noise (S/N) ratio. The S/N ratio has been shown to be increased in CE by using a time-resolved detector [17]. However, this requires the use of labels having a long fluorescent lifetime, such as the lanthanides. Alternately, the S/N ratio can be improved through the use of chemiluminescence. Dadoo et al. first demonstrated the feasibility of luminol-based chemiluminescence in CE in 1992 [18]. The same year Ruberto and Grayeski achieved a sensitivity of 10^{-7} M using acridinium chemiluminescence [19]. Both of these methods are solution based and require prior analyte derivatization, restricting their application to a relatively few classes of compounds. Chemiluminescence can also be based on the gas phase reaction of nitric oxide and ozone that has been used to detect nitrogen-containing compounds post-GC and HPLC separations with sensitivities in ng/injection [20–23]. The detection mechanism for chemiluminescence nitrogen detection (CLND) involves the high-temperature oxidation of nitrogen-containing species to form nitrogen oxides. The resulting nitrogen oxides then react with ozone to form excited nitrogen dioxides that undergo spontaneous deactivation to the ground state by emitting a photon of light. GC– and HPLC–CLND has been used for many years to analyze nitrogen containing pesticides, pharmaceuticals, light petroleum, nitrosoamines, and food flavors [22]. Although CLND has been interfaced to GC and HPLC, it has not to the best of our knowledge been interfaced to CE. We report for the first time the development of an interface for CE and CLND.

2. Experimental

2.1. Apparatus

CE analysis was performed on a PrinCE system equipped with Caesar's data analysis software (Helena Labs., Beaumont, TX, USA). Separation was done on a 80 cm×50 μm I.D.× 375 μm, O.D. fused capillary tube (Polymicro Technologies, Phoenix, AZ, USA). Samples were pressure injected (0.5 p.s.i.; 1 p.s.i.=6894.76 Pa) for 20 s. Antek Instruments (Houston, TX, USA) provided the CLND system. Typically, a CLND system comprises of an electrically heated combustion furnace with inlets for oxygen for complete combustion of organic matter and uses argon as a carrier gas. The furnace is maintained at 1040°C through out the analysis process. The gases from the furnace are passed through a drying chamber to remove formed water and then into a quartz detection cell. The dried nitrogen oxides from the furnace are then mixed with ozone in the cell and the resulting chemiluminescence is measured at ~600 nm.

2.2. CE–CLND interface

Fig. 1 shows the schematic diagram of the CE–CLND interface. As shown in the figure the interface is comprised of two polyether ether ketone (PEEK) Tees (Upchurch Scientific). The first T joint holds the fused-silica capillary and sheathing fluid tube (stainless steel tube, 400 μm I.D.×1587 μm O.D.).

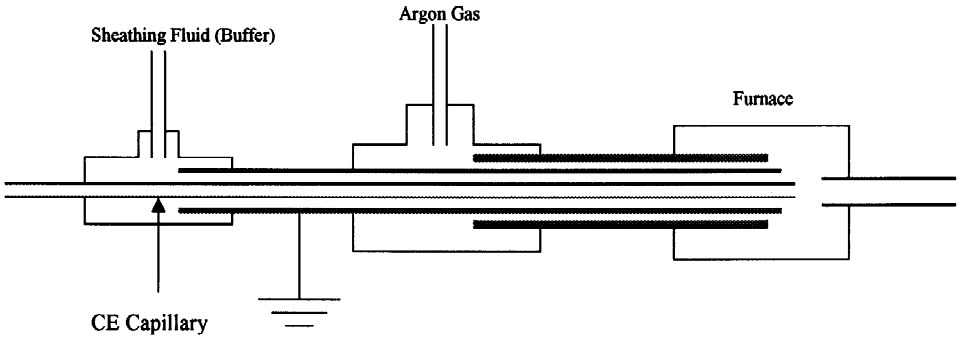

Fig. 1. Schematic representation of the CE–CLND interface.

The sheathing fluid (run buffer) is pumped at a flow-rate of 5 μl/min via a Beckman HPLC pump (Beckman, Fullerton, CA, USA). The sheathing fluid tube is connected to the negative electrode thereby completing the circuit. Both the sheathing fluid tube and CE capillary enters into a third carrier gas tubing (stainless steel tubing, 530 μm I.D.×1587 μm O.D.) and are held in place by second PEEK Tee, the inside of this tube is grooved so as to allow a circular concentric flow of argon gas that ensures minimum band broadening. Argon gas is introduced through the second T at a flow-rate of 0.4 l/min, the flow-rate is adjusted to provide maximum nebulization.

2.3. CE separation

The run buffer is composed of 0.4% (w/v) boric acid and 0.3% (w/v) sodium borate. A constant potential of 30 kV was applied across the two electrodes giving a steady current of 9–10 μA. A stock solution of 0.01 *M* *p*-aminosalicylic acid (PAS) and L-phenylalanine (PHE) in deionized water was used to make standards containing different concentrations of these two compounds.

3. Results and discussion

Fig. 2 shows the baseline separation of PAS and PHE by CE and their detection by CLND. The interface described here is similar to the direct injection nebulizer (DIN) described by Liu et al. [24]. The main challenge for this interface was to avoid the evaporation of run buffer in the combustion furnace and prevent the deposition of salt at the end of the fused-silica capillary. We were able to achieve this by using a concentric flow of argon gas to assist nebulization and keeping the capillary surface cool thereby preventing evaporation of the run buffer. The other advantages provided by this interface included (i) 100% sample introduction

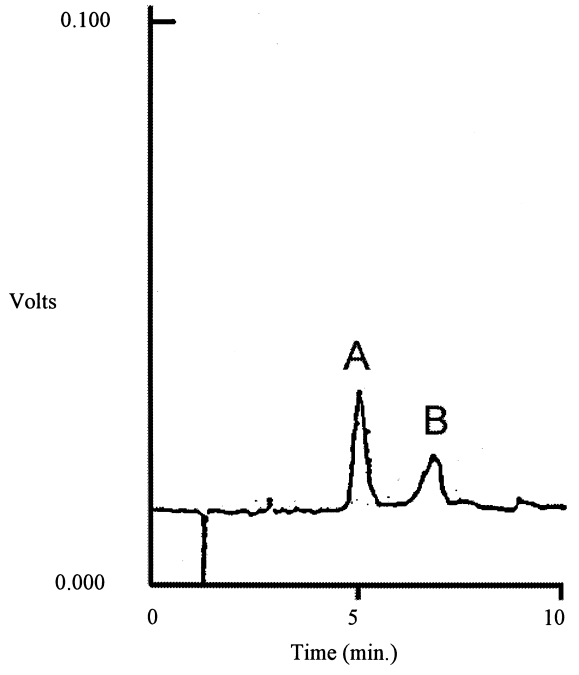

Fig. 2. Electropherogram of *p*-aminosalicylic acid and L-phenylalanine with CLND. Peaks: (A) *p*-aminosalicylic acid and (B) L-phenylalanine; capillary, 80 cm×50 μm I.D.×375 μm, O.D. fused-silica capillary tube; run buffer is composed of 0.4% (w/v) boric acid and 0.3% (w/v) sodium borate; applied voltage (A) constant 30 kV; pressure injection (0.5 p.s.i.) for 20 s.

efficiency into the combustion furnace the CLND system; (ii) accommodation of low sample introduction flow-rates (1–10 μl/min); and (iii) fast sample wash out with minimal memory effect.

The sheathing fluid introduced at 5 μl/min serves two purposes. First it provides a continuous and stable electrical contact at the grounding end of CE capillary completing the electrical circuit. It also helps maintain the capillary at temperatures below the boiling point of water thus preventing the salt deposition and capillary blockage. The effectiveness of this interface is validated by the fact that even the polyimide coating on the capillary surface is not burned off, although the interface is introduced into the furnace where the temperatures ranges between 400 and 500°C.

The low flow-rates in CE did not produce any significant back pressure in the quartz combustion tube in the furnace as seen in HPLC–CLND systems. The sensitivity of this system was evaluated by injecting 0.01 *M* solution of mixture for different time interval. The quantity of compound injected was calculated by using Eq. (1) [25].

$$Q = \Pi r^2[\Delta P\, r^2 t_{\text{int}}/(8\eta L)]C_i \quad (1)$$

where ΔP is the pressure difference, r is the capillary inner radius, t_{int} is the introduction time, η is the viscosity of sample solution, L is the length of the column and C_i is the concentration of the sample solution. Assuming a solution viscosity of 0.9548 cP at 22°C the minimum detectable amount was found to be 76 ng or approximately 500 pmol ($S/N > 3$) per injection. Although this system is not as sensitive as HPLC–CLND (sensitivity 14 ng per injection) this is mainly due to low quantities of sample that can be introduced into the capillary. Using on-column preconcentration techniques, such as transient isotachophoresis, should give sensitivities comparable to HPLC–CLND. Although a baseline separation was achieved for the two test compounds the theoretical plates obtained using this interface were considerably lower than one observed for an on-line UV detector (Table 1). This is due to the band broadening that occurs during combustion of eluted analyte. Increasing the carrier gas flow-rate did give better peak shape but with a loss in sensitivity due to incomplete combustion resulting from reduced residence time of the eluted compounds in the furnace.

Table 1
Comparison of theoretical plates using UV absorbance and chemiluminescence nitrogen detection

Compound	Theoretical plates	
	UV	CLND
PAS	85 788	25 393
PHE	80 687	20 299

4. Conclusion

The interface between CE and CLND described in this application is capable of detecting only nitrogen-containing compounds. One of the main drawbacks of this system is its inability to use biological buffers since they all contain nitrogen. Thus, the choice of buffers that can be used for this electrophoresis is limited to non-nitrogen-containing compounds such as borate, acetates and carbonates. Loss of resolution in the combustion phase can be overcome by using longer capillary columns and modifying the run buffer. Alternately, a packed quartz tube in the furnace can be used instead of open tubing, thereby permitting higher flow-rates for the carrier gas. A linear relationship was found for the peak area and injected quantity over two orders of magnitude, allowing the quantitation of percent nitrogen in the sample. This technique will potentially be very useful for quantitating nitrogen-containing compounds in pharmaceutical and biotechnology applications.

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