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Short communication

Off-column chemiluminescence detection in capillary electrophoresis

Yi-Ting Lee, Chen-Wen Whang*

Department of Chemistry, Tunghai University, Taichung 40704, Taiwan

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Abstract

An off-column chemiluminescence (CL) detection method for capillary electrophoresis (CE) is described. A cellulose acetate-coated porous polymer joint was created on-column near the end of the separation capillary to electrically isolate the CL detector from the CE system. This off-column detection scheme was found necessary in cases where the CL reagent or catalyst added at the column end will electrophoretically migrate into the separation capillary and decompose the analytes prior to their detection. Loss of efficiency due to the presence of a porous polymer joint was minimal. Application of off-column CL detection to CE of serotonin, catecholamines and catechol was demonstrated.

Keywords: Chemiluminescence detection; Detection, electrophoresis; Polymer joint, porous; Serotonin; Catechol; Catecholamines

1. Introduction

Chemiluminescence (CL) is a highly sensitive detection method in both flow injection analysis (FIA) and liquid chromatography (LC) [1–3]. Due to its simple optical system and low background nature, the feasibility of CL as a detection scheme in capillary electrophoresis (CE) has also been demonstrated recently [4–15]. Both direct and indirect detection modes can be employed for CE of various analytes. Basically, the CL methods for CE reported to date were all performed end-column, that is, the analytes emerge from the end of the separation capillary and mix with the CL reagent or catalyst in a reaction/detection zone at the column outlet. The reaction/detection zone is usually situated at the ground end of a high potential field. One of the requirements for a successful end-column detection is that the CL reagent or catalyst added at the column end must not stream back into the separation capillary. Otherwise, degradation or decomposition of analytes may occur inside the capillary before they reach the column end.

During our preliminary investigation of a permanganate-oxidized CL method for CE of catecholamines, we found that conventional end-column CL detection does not work. The oxidizing agent (permanganate) added at the column end will electrophoretically migrate towards the anode and preoxidize the analytes inside the capillary, therefore, no CL reaction occurs at the column end. In order to eliminate the backstreaming problem, the reaction/detection zone at the column outlet must be isolated from the effect of CE electric field. In this paper, we describe the design of an off-column CL detector for CE that can overcome the above-mentioned problem.

*Corresponding author.

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Its applicability to the CE of serotonin, catecholamines and catechol is demonstrated. To our knowledge, this is the first report on off-column CL detection in CE.

2. Experimental

2.1. Apparatus

The apparatus used for CE–CL detection experiments is shown in Fig. 1. A 0–30 kV power supply (Glassman High Voltage, Whitehouse Station, NJ, USA) provided the separation voltage. The capillary used for separation was 60 cm×100 μm I.D.×375 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA).

The CL detector was isolated from the CE electric field by a cellulose acetate-coated porous polymer joint created on-column near the cathodic end of the capillary, according to the procedure described previously [16]. In brief, a short section (ca. 3 mm) of polyimide coating was burned off 2 cm from the end of a prewashed and dried capillary. The exposed section was cleaned with methanol and then glued to a 2 cm×1 cm microscope slide. Using a glass-fibre cleaver, a small scratch was made on top of the uncoated silica. The capillary was then pushed up gently from below, directly under the scratch, until a fracture was produced. A small drop (ca. 3 μl) of 12% (w/v) cellulose acetate solution (in acetone) was carefully dripped onto the fracture. Under a gentle stream of air, a thin film of porous cellulose acetate was uniformly coated over the fracture region. Subsequently, the fracture assembly was vertically glued into a 10-ml plastic vial, with the short section of capillary stretching out of a small hole drilled in the bottom of the reservoir. The length of the stretched capillary was about 1.5 cm. With the capillary in position, the hole was sealed using epoxy glue. Before CE analysis, the vial was filled with buffer solution and a grounded platinum-wire electrode was dipped in the solution. An ammeter was connected in series to the electrode.

The interface for the CE–CL detector was constructed by directly inserting the stretched end of the separation capillary into a PTFE tube (80 cm×460 μm I.D.×920 μm O.D.) through a hole that was made ca. 5 cm from the end of the tube. The tip of the separation capillary was held in position by sealing the hole with epoxy glue. The oxidizing agent was fed into the PTFE tube from the other end by a syringe pump (Bioanalytical Systems, West Lafayette, IN, USA; Baby Bee). There was no tee or cross-connector used for coupling the separation capillary and the reaction/detection PTFE tube.

A 1-mm detection window on the PTFE tube (starting from the tip of the inner electrophoretic

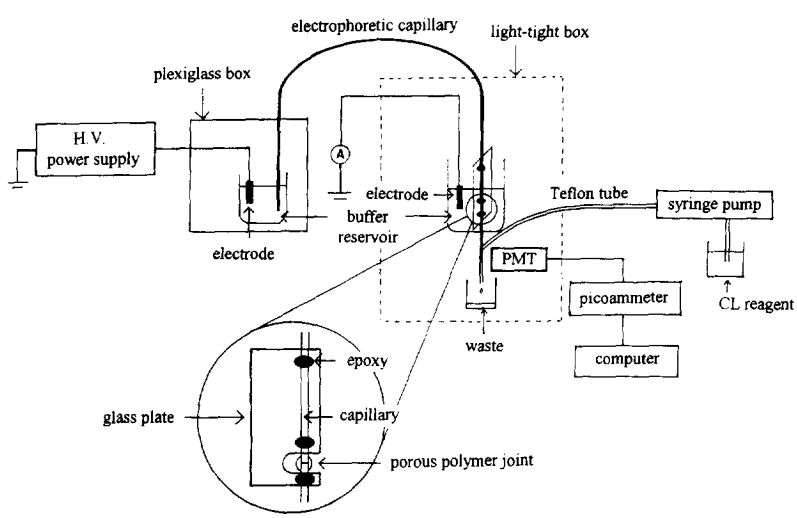

Fig. 1. Schematic diagram of the CE with off-column CL detection system.

capillary) was formed by wrapping the downstream tube with black tape. The emitted CL light was collected with a cooled (−10°C) photomultiplier tube (PMT) (Hamamatsu, Hamamatsu City, Japan; R-928) operated at −900 V. The photocurrent was fed to a picoammeter (Keithley Instruments, Cleveland, OH, USA; Model 485) and the signal was recorded using a Macintosh SE computer, equipped with a data acquisition interface. The whole CL detection system was held in a light-tight box that was constructed from black Plexiglass, to avoid ambient light.

2.2. Chemicals

Serotonin (5-hydroxytryptamine; 5-HT), dopamine (DA), epinephrine (EP), norepinephrine (NE) and catechol (CA) were obtained from Sigma (St. Louis, MO, USA). All other chemicals were of analytical-reagent grade. Water, purified using a Barnstead NANOpure II system (Dubuque, IA, USA), was used for all solutions.

2.3. Procedure

Electrophoretic separation of serotonin and catecholamines was performed in 10 m*M* sodium tetraborate buffer (pH 9.5). The oxidizing agent consisted of 1 m*M* potassium permanganate in 1 *M* aqueous sulfuric acid. All solutions were filtered through a 0.45-μm pore-size membrane filter before use. With the porous polymer joint immersed in the electrophoretic buffer, the separation capillary was carefully filled with the same buffer using a syringe. The oxidizing agent was fed at a rate of 2.5 μl/min by a syringe pump. A 15-kV voltage was first applied for ca. 30 min, until the measured background stabilized. The sample was then electrokinetically injected for 5 s. In order to avoid siphoning air into the separation capillary, the syringe pump was turned off during injection. Monitoring was started after the high voltage resumed and the pump turned on.

3. Results and discussion

Oxidation of catecholamines by potassium permanganate in an acidic medium is known to exhibit CL. This feature has already been employed in the CL detection of catecholamines in FIA [17,18], but, as yet, it has not been used in LC or CE. During our preliminary investigation of a CE–CL method for catecholamines, the conventional end-column detection mode was first attempted. The end of a separation capillary (50 μm I.D.×150 μm O.D.) was directly inserted into a short piece of a fused-silica reaction/detection capillary (180 μm I.D.×360 μm O.D.), and a tee connector was used to join the separation capillary, oxidant capillary and reaction/detection capillary together [13]. Acidic permanganate solution was fed through the oxidant capillary and mixed with analytes at the end of the separation capillary, which was inside the reaction/detection capillary. The downstream end of the reaction/detection capillary was dipped in a grounded buffer reservoir to complete the CE electric circuit. A 5-mm window was formed on the reaction/detection capillary (starting at the point where the inner separation capillary terminated) by burning off the polyimide coating. However, CL emission of catecholamines was not observed. Also, the colorless buffer solution in the anodic reservoir turned pink gradually, with successive CE experiments. This is presumably caused by a backstream migration of permanganate anion towards the anodic end of the separation capillary. The analytes will be preoxidized by permanganate inside the capillary before they reach the column outlet, which may explain the absence of an end-column CL emission.

In order to prevent the permanganate from backstream migrating into the capillary, the reaction/detection zone at the column end must be separated from the high-voltage electric field. A similar problem has been observed in CE with electrochemical detection (ED) and various methods have been developed to electrically isolate the ED cell from the CE system [19]. In our study, we attempt to solve this electric field problem by the same strategy. We had previously reported the design and application of a cellulose acetate-coated porous polymer joint for CE–ED [16,20,21]. The porous polymer joint can be easily constructed by fracturing the capillary followed by covering the fracture with a thin layer of cellulose acetate membrane. The porous joint, rather than the end of the capillary, was submerged in a buffer reservoir along with the cathode (see Fig. 1).

The applied voltage was dropped across the capillary prior to the porous joint and the resulting electroosmotic flow (EOF) acted as a pump to push the analytes through the short section (ca. 2 cm) of capillary after the joint. The analytes mixed with permanganate and emitted CL in a field-free region at the column outlet. This scheme is characterized as "off-column" detection. Fig. 2 shows the electropherogram of a test mixture containing 5-HT, DA, NE and CA that was obtained with the off-column CL detection. The four analytes can be efficiently detected. EP was not included in the test mixture because complete separation of EP and NE could not be achieved with the present buffer composition. No attempt was made to optimize their separation.

In a CE system with an on-column porous joint, some sample loss and band broadening are expected. In order to examine the effect of a porous polymer joint on the performance of CE, two capillaries of the same I.D. and length were prepared for UV detection, with one containing a porous polymer joint that is 3 cm before the detection window and the other without a joint. Under the same field strength, sample loss and band broadening were determined by comparing the peak area and half-peak width of a test compound, DA, obtained using a porous joint-based CE–UV system with those obtained from a bare column CE–UV system. The loss of peak area was about 5% and the loss of theoretical plate number (N) was about 8%. In all CE–CL detection methods reported to date, separation efficiencies were always lower than those typically found in CE with an on-column detection method, e.g. UV detection. Major causes of efficiency loss probably include the relatively large detection zone and the turbulence generated at the column outlet, which are believed to be the inherent limitations of the CE–CL detection technique. The average N value in Fig. 2 is ca. 8000 while the average N value obtained using a CE–UV system is ca. 20 000. This significant loss of efficiency may be attributable to the relatively large I.D. (460 μm) of the reaction/detection tube used, in addition to the turbulence created at the column outlet due to mismatch between the pump-driven oxidant flow and EOF. In comparison with these major band broadening factors, the effect of a porous polymer joint on the loss of separation efficiency is obviously minimal.

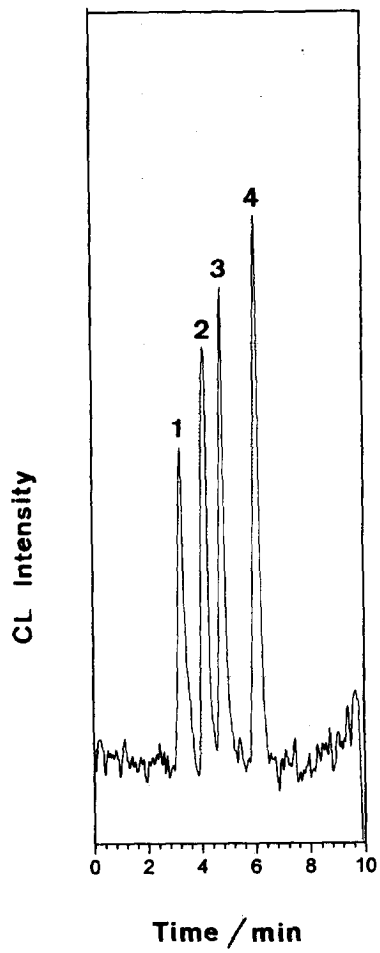

Fig. 2. Electropherogram of serotonin and catechol compounds with off-column CL detection. Separation capillary, 60 cm×100 μm I.D.×375 μm O.D. fused-silica tube; reagent/detection column, 80 cm×460 μm I.D.×920 μm O.D. PTFE tube; electrophoretic buffer, 10 mM sodium tetraborate (pH 9.5); separation voltage, 15 kV (56 μA); oxidant, 1 mM $KMnO_4$ in 1 M H_2SO_4; oxidant flow-rate, 2.5 μl/min; sample injection, 5 s at 15 kV; sample concentration, 1 mM of each analyte; PMT voltage, −900 V. Peak identities: (1) serotonin; (2) dopamine; (3) norepinephrine and (4) catechol.

The reproducibility of migration times and peak areas of the test analytes was determined based on ten replicate injections of a mixture containing 1 mM of each species. The results are listed in Table 1. The relative standard deviations (R.S.D.s) on migration times range from 0.30% for NE to 0.62% for DA. The R.S.D.s on peak areas range from 4.90% for DA

Table 1
Reproducibility of migration times and peak areas with CE–off-column CL detection

Analyte	Migration time (min)	R.S.D.[a] (%)	Peak area[b]	R.S.D.[a] (%)
Serotonin (5-HT)	3.21	0.46	2137	6.04
Dopamine (DA)	3.98	0.62	34 667	4.90
Norepinephrine (NE)	4.60	0.30	29 230	8.33
Catechol (CA)	5.71	0.44	38 307	5.30

[a] $n=10$.
[b] Peak area is expressed in arbitrary units.

to 8.33% for NE. The limit of detection ($S/N=3$) was estimated to be about 100 μM (10 pmol) for each test analyte. Due to low emission characteristics of the CL reaction between catecholamines and permanganate, both a low temperature (−10°C) and a high bias voltage (−900 V) were applied to the PMT for monitoring the weak CL signal. The relatively large noise level on the baseline, seen in Fig. 2, was due mainly to thermal and shot noises of the PMT. The poor sensitivity also may be attributable to the low optical transparency of the PTFE tube, which is about 25% less than that of a fused-silica tube to the emitted CL light.

4. Conclusion

The feasibility of using off-column CL detection in CE has been demonstrated. Off-column detection can be performed by decoupling the CL detector from the CE system with a porous polymer joint. In comparison with conventional end-column CL detection, an off-column detection scheme is necessary in cases where the CL reagent or catalyst added at the column end will electrophoretically migrate into the separation capillary and decompose the analytes before their detection. The effect of a porous polymer joint on efficiency loss is minimal. The only limitation to the off-column detection is that at least some EOF is needed to push the analytes past the grounded joint. From our preliminary investigation, this detection method is applicable to the CE of serotonin, catecholamines and catechol. Further studies will investigate the optimization of the overall system in order to enhance the sensitivity and efficiency.

Acknowledgments

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