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Simplified Sheath Flow Cuvette Design for Ultrasensitive Laser Induced Fluorescence Detection in Capillary Electrophoresis

Oluwatosin O. Dada, Bonnie J. Huge, and Norman J. Dovichi

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana. 46556, USA

Abstract

We present a design for a sheath-flow cuvette that uses a relatively inexpensive quartz cuvette. The cuvette has a high optical quality square flow chamber that is fused to quartz tubes at each end. PEEK/TEFZEL fittings hold and seal the quartz flow chamber without putting strain on the cuvette. The performance of the cuvette is evaluated as a laser-induced fluorescence detector for capillary electrophoresis. The cuvette produces mass detection limits of 50 yoctomoles (30 copies) for 5 – carboxyl tetramethylrhodamine (5 TAMRA SE) with separation efficiency of 400,000 theoretical plates.

INTRODUCTION

The sheath-flow cuvette is an important component of a high-sensitivity post-column laser-induced fluorescence detector for capillary electrophoresis. It provides outstanding sensitivity and resolution for the detection of fluorescently labeled amino acids, proteins, lipids, glycolipids, carbohydrates, and DNA.^{1–10} Previous reports on the use of sheath flow cuvette as a detector commonly employ a square quartz cuvette with a 250- μm square flow chamber and 2 mm thick windows. As one exception, Nirode *et al.* reported a round cross-section sheath flow cuvette.¹¹ The square cuvette was originally developed for flow cytometry where a suspension of cells is injected into the center of a flowing sheath stream.¹² In application as a detector for capillary-based separations, the square geometry provides an ideal optical system for excitation and collection of fluorescence from the narrow sample stream. By locating the sample stream far from the cuvette windows, light scatter from the window-sample interface is easily blocked with an opaque aperture. Manipulation of the relative flow rates of the sample stream and sheath stream can produce very narrow sample streams that prevent sample loss on the cuvette wall.¹³

The original design of the sheath-flow cuvette was based on the cuvette used in a flow cytometer. That design employs a stainless-steel fixture to hold the flow chamber. Besides requiring complex machining that is far beyond the skill of amateurs, the metal fixture demands serious care in sealing the cuvette because the quartz cuvette can be easily broken or chipped. Sobhani *et al.* described an alternative Teflon holder that uses compression to seal without inducing torque on the cuvette,¹⁴ but that design is still relatively complex to construct. Most recently, Skinner reported a round and a square channel PDMS sheath flow cuvette for capillary electrophoresis with laser induced fluorescence detection.¹⁵ That system requires sophisticated fabrication technology, and PDMS can generate a fair amount of autofluorescence, which can result in degraded performance compared with a cuvette manufactured from optical quality quartz.

In this report, we describe a sheath flow cuvette design that uses a special-order cuvette and inexpensive PEEK/TEFZEL fixtures. The fixtures are simple to assemble or disassemble, and can be easily integrated with commercial capillary-based instruments. Detection limits

produced by a fluorescence detector with this cuvette are 50 yoctomoles (30 copies) for 5 – carboxyl teramethylrhodamine (5 TAMRA SE) in borate buffer.

EXPERIMENTAL METHOD

Instrument Overview

Several versions of sheath-flow cuvette based laser-induced fluorescence detectors in capillary electrophoresis have been described in the literature.^{16–20} The current system was constructed on a 2-ft × 2-ft optical breadboard. The sample was excited with a CW 532 nm diode-pumped laser (Model GCL-025-M, CrystaLaser LC, Reno, NV) operating at 25 mW output power. The beam from this laser was reflected from a mirror, centered on a 6.3X, 0.2 NA microscope objective (22.5 mm focal length, Melles Griot, Rochester, NY), and then focused into the cuvette. The cuvette and the holder are described below.

Fluorescence was collected at right angles with a microscope. The microscope housing was made from commercial parts available from ThorLabs, Inc., with the exception of an aluminum riser block that was fabricated locally. A 60X, 0.7 NA, 1.44 mm working distance, achromatic microscope objective (Universe Kogaku) collected fluorescence, which was passed through a 590 DF 10 nm bandpass interference filter (Thorlabs, Inc) and imaged through an adjustable iris diaphragm (ThorLabs) onto a GRIN-lens coupled 50 μ m diameter, 60 cm long optical fiber (ThorLabs). The fiber directed fluorescence to a single-photon counting avalanche photodiode module (PerkinElmer). Photocounts were acquired at 50 Hz with a National Instruments DAQ card (PCI-6035E) driven by a home built Labview program. Further data processing was performed with Matlab procedures running on a Macintosh computer. Capillary electrophoresis data were first treated with a three-point median filter to remove spikes generated by particles passing through the laser beam. The data were then convoluted with a Gaussian function. This function had a standard deviation of 5 points (100 ms) and a width of 44 points.

Cuvette and Cuvette Holder

The cuvette design is based on a quartz flow cytometry cuvette with a 250 μ m square flow chamber (NSG Precision Cells, Farmingdale, NY). The cuvette was custom-made with top and bottom quartz tubes bonded at both ends, Fig. 1a. The quartz tubes (2.5 mm OD, 1 mm ID, 15 mm L) allow connection with commercial PEEK/TEFZEL adapters. The cuvette dimension is 2.5 mm × 2.5 mm × 20 mm and matches the tube outer diameter.

The cuvette holder was constructed from two TEFZEL polymer adapters (IDEX Health and Science, WA). A TEFZEL cross adapter (part number P-634) was used at the top of the flow chamber and a TEFZEL tee adapter (part number P-714) at the bottom, Fig 1b. One of the nuts from each adapter was replaced with a PEEK nut (part P-335, used P-353 ferrule). The cuvette top and bottom tubes were fastened into the adapters via the TEFZEL ferrule. The tubes were polymer-sleeved or Teflon taped to enhance sealing. The remaining three nuts on the cross adapter connect the separation capillary, sheath liquid inlet, and sheath liquid outlet while the remaining two nuts on the tee adapter connect the electrical ground and the waste line (Fig. 1c). The separation capillary was fastened into the cross adapter via a TEFZEL ferrule and was centered inside the sheathing quartz with the aid of a microscope placed opposite the collection objective and consisted of a 10X, 0.25 NA microscope objective (Melles Griot) and a 10X eyepiece. The cuvette assembly was mounted on the CE system as shown in Figure 2. Note that only the top cross is fixed to a solid support. Enhanced stability would be expected if the bottom tee was also fixed.

Materials and Chemicals

Unless noted, reagents were purchased from Sigma-Aldrich (St. Louis, MO). Deionized water was obtained with a Barnstead NANOpure deionization/sterilization unit (Thermo Scientific Model 7146). 5 – carboxyl teramethylrhodamine (5 TAMRA SE) was purchased from AnaSpec (Fremont, CA). Uncoated fused-silica capillary were obtained from Polymicro Technologies (Phoenix, AZ).

Electrophoresis

A 10 mM borate buffer was used for the experiment. Electrophoresis was performed in a 30 cm long, 30 μ m ID, 150 μ m OD fused silica capillary. A high-voltage power supply provided a potential of 20 kV during electrophoresis. The sample was injected at 1000 V for 1 s. The bottom tee adapter of the sheath-flow cuvette was held at ground potential.

RESULTS AND DISCUSSION

Figure 3 presents an electropherogram generated by injection of 50 pL of a 100 pM solution of 5 – carboxyl teramethylrhodamine in 10 mM borate buffer. The data were treated with a 3 point median filter to remove spikes due to particulates and then convoluted with a Gaussian function with a 100 ms standard deviation.

A nonlinear least squares routine was used to fit a Gaussian function to the peak

$$\text{signal}(\text{time}) = \text{Amplitude} * e^{-0.5 * (\text{time} - t_0)^2 / \text{sigma}^2} + \text{offset}$$

where t_0 is the peak center, sigma is the peak standard deviation, and offset is the background signal. The separation efficiency of the filtered data was 400,000 theoretical plates.

Noise was determined from the standard deviation of the baseline for the first 1.33 minutes of the electropherogram. The signal-to-noise ratio was 275, which results in a concentration detection limit (3σ) of 1.2 pM and a mass detection limit of 50 yoctomoles (30 copies) injected. While offering simpler construction, easier assembly, the performance of the cuvette is in agreement with that of the earlier versions, which generally produce detection limit of < 100 yoctomoles.^{7,14,21–22}

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Figure 1.

(A) The square quartz flow chamber with top and bottom tube. (B) The assembled sheath flow cuvette with cross and tee TEFZEL adapters. (C) The schematic drawing of the assembled cuvette showing the different components. Cp - Capillary, SL – Sheath Liquid Inlet, CO – Closed Outlet, F – Ferrule, CTT – Cuvette Top Tube, CC – Cuvette Chamber, CBT – Cuvette Bottom Chamber, EG – Electrical Ground, W –Waste.

Figure 2. Photograph of the new sheath flow cuvette. A high-optical quality square-wall detection chamber has tubes fused to each end that are held by a TEFZEL cross (top) and tee (bottom). A ground connection for the cuvette is provided by the red wire threaded through one arm of the cross.

Figure 3.

Capillary electrophoresis of a 47 pL injection of a 100 pM solution of 5 –carboxyl teramethylrhodamine in 10 mM aqueous borate buffer. The insert shows a close-up of the peak. The data were collected at 50 Hz. The signal was passed through a three point median filter and then convoluted with a Gaussian function with a 100-ms standard deviation. The noise was estimated as the standard deviation of the first 4,000 data points (1.33 min). The peak was fit with a Gaussian function using a nonlinear least square routine. The concentration detection limit (3σ) was 1.2 pM and the mass detection limit was 50 yoctomoles (30 copies). The separation efficiency for the filtered electropherogram was 400,000 theoretical plates.