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Fabrication of calcium fluoride capillary electrophoresis microdevices for on-chip infrared detection

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Abstract

In this paper, we demonstrate microfluidic capillary electrophoresis (CE) devices made in CaF_2, for optical detection in a broad spectral range. We have designed methods for micromachining and enclosing capillaries in CaF_2. The utility of these microdevices has been shown through CE analysis of fluorescently labeled amino acids. We have also performed infrared spectroscopy for analyte identification in microfluidic CaF_2 channels. These CaF_2 microdevices open the door to microchip separations with optical detection in the ultraviolet, visible, and infrared spectral regions.

Keywords: Detection, electrophoresis; Chip technology; Instrumentation; Calcium fluoride; Amino acids

1. Introduction

Miniaturization of tools for chemical analysis offers significant advantages [1] in terms of speed, throughput, and reagent consumption. The benefits of decreased size in separation methods are illustrated best by the significant advances in microchip capillary electrophoresis (CE) technology during the past decade [2]. Laser-induced fluorescence (LIF) detection at visible wavelengths was used in the initial demonstration of the microchip CE concept [1], and this detection scheme continues to enjoy broad use at present [3]. LIF detection at ultraviolet (UV) wavelengths [4] or in the near infrared (IR) [5] is less commonly utilized, though detection in these regions of the spectrum offer distinct advantages in terms of chromophore availability for UV wavelengths, and reduced background for the near IR region.

Many different substrates have been used for construction of microfluidic electrophoresis systems, including glass [6–8], quartz [4], silicon [9] and polymeric materials [10–12]. Borosilicate glass is transparent from 350 to 2000 nm [13], but detection with devices composed of this material is not possible beyond the near IR or in the UV region of the spectrum. The use of quartz substrates for microchip CE enables UV detection [4], but useful IR, as well as the shorter UV wavelengths still remain inaccessible. Maximum versatility in optical detection methods for laboratory-on-a-chip systems would be achieved with substrate materials having the broadest wavelength transmission ranges. Microdevices composed of such materials would allow not only quantitative analysis by probing samples in one region of the spectrum (e.g. UV or visible), but also qualitative analyte identification by IR spectroscopy. CaF_2 is an ideal candidate material for such experiments because it has a transmission range of 170–7800 nm [14]. Indeed, a recent report demonstrated the successful coupling of an IR-transparent CaF_2 flow cell with a conventional fused silica capillary for end column Fourier-transform (FT) IR detection in CE [15]. However, the post-column coupling of a CaF_2 cell with a capillary column increases instrumental complexity and can contribute to band broadening. Ideally, IR detection should be performed on column, but this approach requires the construction of a separation system with suitable optical properties.

Here, we present the fabrication and use of microfluidic CE devices made from CaF_2 substrates. The application of these devices to chemical analysis was demonstrated by the separation of fluorescently labeled amino acids. Also, on-chip IR detection in microfluidic channels was accomplished for the first time, demonstrating the potential for separating, quantifying, and spectroscopically identifying analytes in a microfluidic platform.

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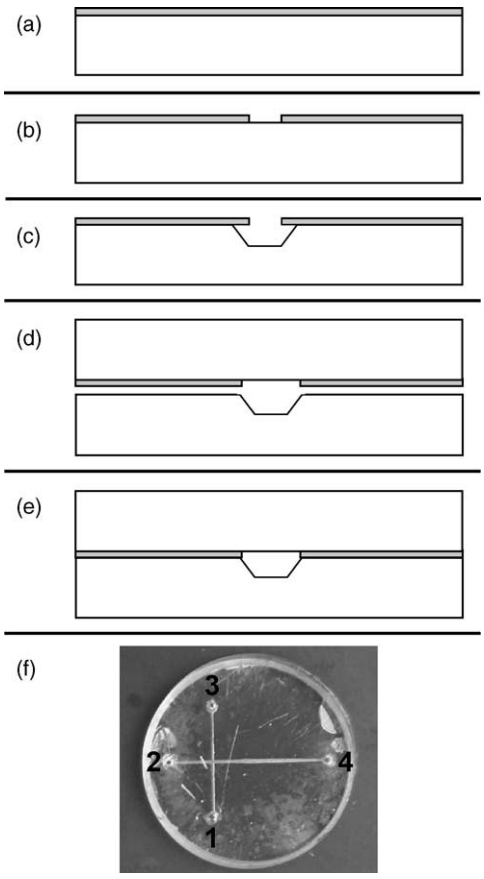

Fig. 1. Fabrication of CaF_2 microdevices for CE. (a) The CaF_2 substrate (white) is spin-coated with photoresist (gray). (b) Exposure and development of the photoresist provides the surface pattern on the CaF_2. (c) The substrate is etched in unmasked areas. (d) The etched CaF_2 piece is aligned with the patterned photoresist on another CaF_2 substrate, and the two pieces are brought into contact. (e) The aligned CaF_2 plates are clamped and heated to cause the photoresist to bond the substrates together. (f) Photograph of a bonded CaF_2 microchip. The substrate diameter is 1 in. Reservoirs are (1) injection, (2) buffer, (3) injection waste and (4) high voltage. The injection channel runs between reservoirs 1 and 3, while the separation channel connects reservoirs 2 and 4.

2. Experimental

2.1. Microfabrication

Fabrication of CaF_2 CE microdevices is depicted schematically in Fig. 1. Briefly, the microchips were made by photolithographic patterning, followed by chemical etching of CaF_2 substrates in a saturated aqueous $Fe(NH_4)(SO_4)_2$ solution. Each etched CaF_2 piece containing the channel design was then bonded to a second, photoresist-patterned CaF_2 substrate to form microcapillary structures.

Conventional photolithographic procedures were used to pattern the microchannel and cover plate, following previously reported work [16]. The microchip CE device pattern consisted of 100 μm wide channels with a simple cross-injector [6]. The injection channel was 1.5 cm long, while the separation channel was 2.0 cm long, and the injection channel intersected the separation channel 0.5 cm from reservoir 2 (Fig. 1f). 1.0 in. diameter, 0.12 in. thick CaF_2 substrates (Casix, Chatsworth, CA, USA) were spin coated with 1.1 μm of S1813 photoresist (Shipley, Marlborough, MA, USA) (Fig. 1a) (1 in. = 2.54 cm). Next, the microfluidic design was transferred to the photoresist, as illustrated in Fig. 1b, and the unpatterned side of each substrate was coated with acrylic fingernail polish to prevent backside etching. The exposed channel areas were etched in a saturated aqueous $Fe(NH_4)(SO_4)_2$ solution for 24 h at room temperature (Fig. 1c); the etchant solution was stirred using a PTFE-coated spin bar rotating at 100–120 rpm. After etching, the residual photoresist and fingernail polish were removed using acetone.

Access holes were drilled using a 0.021 in. carbide drill bit (Federal-Mogul, Chicago, IL, USA) to form each of the four buffer reservoirs in the etched CaF_2 substrate. A second CaF_2 piece was spin coated with Shipley S1813 photoresist and patterned like the first, but with a channel width of 200 μm, rather than 100 μm, as shown in the top half of Fig. 1d. This created an optically transparent window over the entire etched microchannel structure. The photoresist served as an adhesive to bond the two substrates together [15] once they were aligned under a microscope, clamped together, and placed in an oven at 135 °C for 30 min (Fig. 1e). Elevated temperature treatment was necessary to develop the full mechanical strength and chemical resistance of the Shipley S1813 photoresist. An Alpha-step 200 stylus profilometer (Tencor, Mountain View, CA, USA) scanned with 2 μm lateral resolution was used to measure the dimensions of the etched CaF_2 patterns.

2.2. Separation and detection of amino acids

Glycine, arginine, and phenylalanine were purchased from Sigma (St. Louis, MO, USA) and diluted in the run buffer, 30 mM borate, pH 9.0. The borate buffer was filtered using a 0.2 μm pore diameter filter (Pall, East Hills, NY, USA) prior to use. Each amino acid was labeled fluorescently by conjugating fluorescein 5-isothiocyanate (FITC) (Sigma) to the amine group [16,17]. All the FITC-tagged amino acids had a concentration of 300 μM in borate buffer after labeling.

Channels were filled by micropipetting 20 μl of run buffer on top of reservoirs 1, 2, and 3 and applying vacuum to reservoir 4 (see Fig. 1f for reservoir layout), after which reservoir 4 was covered with 20 μl of run buffer. One miroliter of sample was transferred into the bottom of reservoir 1 using a 25 μl syringe, and platinum electrodes were inserted into all buffer reservoirs to provide electrical contact. “Pinched” injection [7] for 10 s was used to load sample on the column prior to separation, and the injection volume was ~400 pl. During injection, reservoirs 1, 2, and 4 were grounded and reservoir 3 was maintained at +0.6 kV; for separation, reservoirs 1 and 3 were held at +0.6 kV, reservoir 2 was grounded, and reservoir 4 was maintained at +1.0 kV. The LIF detection system and the setup for data acquisition

have been described elsewhere [16]. The sampling rate for data collection in the software was chosen to be 5 Hz.

2.3. FT-IR spectrometer

A FT-IR microscope composed of a Nexus 670 FT-IR spectrometer (Nicolet, Madison, WI, USA) and an IR-Plan IR Microscope Accessory (Spectra Tech, Stamford, CT, USA) was used for on-column IR detection. The CaF_2 microdevice channels were filled with toluene (Fisher, Pittsburgh, PA, USA). To enable the IR beam to be focused correctly, the CaF_2 microdevice was placed in an external optical focusing unit, constructed in the laboratory, in a Perspex unit purged with dry air. The IR beam from an external optical port of the spectrometer was focused on the injection intersection region of the CaF_2 microdevice by means of an off-axis parabolic mirror, prior to impinging on a mercury cadmium telluride detector. Sixty-four scans were co-added for each spectrum, and the spectral resolution was 4 cm^{-1}. Reference measurements on toluene were obtained using 1 in. diameter CaF_2 windows with 100 μm spacing between the plates.

3. Results and discussion

We have fabricated CaF_2 microdevices for rapid biochemical analysis and flexible optical detection. To generate microfluidic structures in CaF_2, it was necessary to develop methods for etching this material. Although CaF_2 can be etched by laser-induced heating [18] or accelerated ion beams [19], these approaches require costly and sophisticated instrumentation. Thus, we explored methods for the wet etching of CaF_2 and found saturated aqueous $Fe(NH_4)(SO_4)_2$ to be a suitable choice. We also determined that the etch rate of the CaF_2 is dependent upon the stirring of the etchant solution. In an unstirred solution, the etch rate is ∼8 μm per day, while in a stirred solution the etch rate can be increased to as much as 18 μm per day. These etch rates are sufficient for fabricating microfluidic arrays in CaF_2. We believe the etching mechanism to involve the reaction of Fe^{3+} ions in solution with F^- ions in CaF_2 to form the coordination complex $[FeF_6]^{3-}$. Stirring the etchant solution also helped to prevent the precipitation of byproducts on the channel surface, which hindered the etching process.

The channel width designed in the photomask was 100 μm; however, we observed some undercutting of the photoresist protecting layer such that the width of the etched channels typically exceeded the photomask linewidth. We also observed that etchant stirring speeds of 100–120 rpm produced the narrowest channel features, typically having ∼200 μm top widths. Profilometry measurements indicated that the channels in the CaF_2 microdevices used for separation experiments had depths of ∼10 μm, top widths of ∼200 μm, and bottom widths of ∼100 μm.

Developing a reliable procedure for bonding two CaF_2 substrates together to form enclosed microcapillaries proved to be somewhat difficult, because of the crystallinity and high melting temperature of CaF_2. We tried to bond CaF_2 wafers using water, acid, ferric ammonium sulfate solution, or aqueous EDTA under a range of temperatures from 25 to 1100 °C. However, none of these methods produced adequate, water-stable bonding. Thus, we opted to use a 1.1 μm layer of Shipley S1813 photoresist to adhere the CaF_2 substrates together [15]. We found that this bonding approach provided sufficient mechanical strength, chemical resistance to aqueous buffer solutions, and stability in the presence of elevated voltages to be compatible with CE experiments. Another advantage of this method is that the bonding photoresist can be photolithographically patterned to leave an optically transparent window along the length of the etched channels. Finally, the thin photoresist layer appears to cause minimal band broadening in these devices, even though dif-

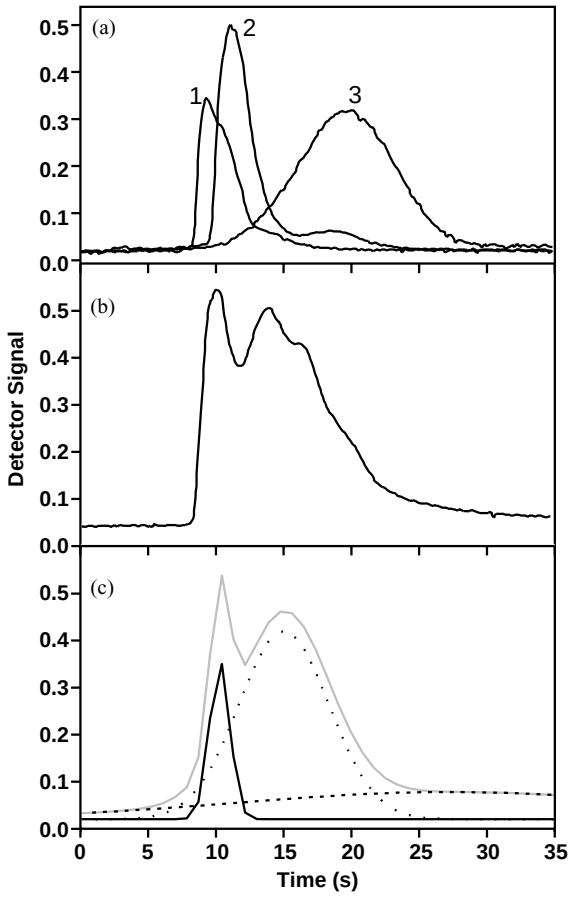

Fig. 2. Electropherograms of FITC-labeled amino acids separated using a CaF_2 microdevice. (a) Injection and detection of individual amino acids: (1) Gly, (2) Arg and (3) Phe. (b) Separation and detection of a mixture of FITC-labeled Gly, Arg, and Phe. (c) Peak fitting of the electropherogram in (b) shows three distinct components (solid, dotted and dashed black lines) with migration times similar to those in the individual runs in (a). The sum of the fit to the data is indicated by the gray line in (c). Potentials for injection and separation in all runs are described in Section 2.

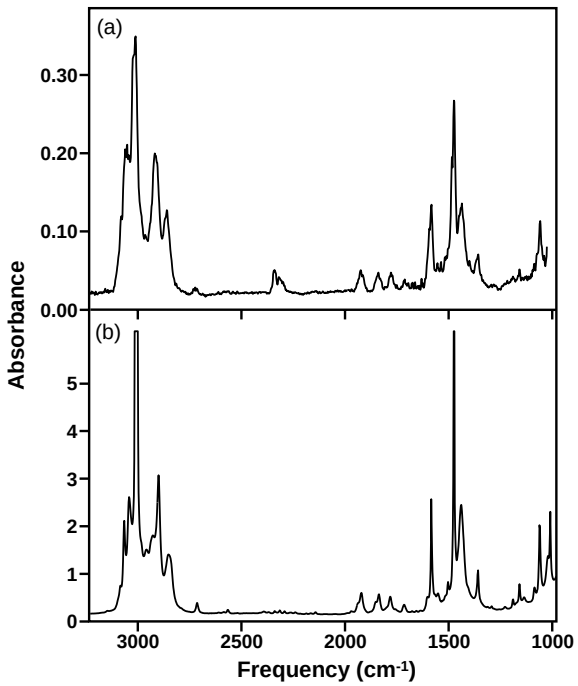

Fig. 3. FT-IR detection in CaF_2 microchannels. (a) FT-IR spectrum of toluene in the injection region of a microchannel in a CaF_2 microdevice. (b) FT-IR spectrum of toluene in a standard CaF_2 IR cell.

ferent materials defining a channel cross-section can create inhomogeneities in ζ-potential [20,21].

To demonstrate the utility of CaF_2 microchips for biological analysis, we performed CE separations on a mixture of fluorescently labeled amino acids. Separation and detection were performed as described in Section 2. Fig. 2a depicts the electropherograms of individual amino acids (glycine, arginine and phenylalanine) injected and detected in a CaF_2 microdevice. The electropherograms show that each amino acid eluted within 25 s, and the peaks were symmetrical, indicating minimal analyte adsorption on the channel surfaces. These results indicate the suitability of CaF_2 as a substrate for rapid electrophoretic analysis of biological samples. Fig. 2b shows a ∼30 s separation of a mixture of the same three FITC-labeled amino acids. Peak fitting was used to deconvolute the partially overlapping analyte bands in this separation, and Fig. 2c displays the results of peak fitting. Three individual components, corresponding to glycine, arginine, and phenylalanine are observed, based on comparison with migration times in Fig. 2a. Importantly, the sum of the component peaks closely approximates the experimental data in Fig. 2b.

FT-IR microscopy was used to test the possibility of using IR spectroscopy for on-chip analyte identification in CaF_2 microdevices. We filled the channels with toluene, water, and acetonitrile, respectively in separate experiments, and obtained IR absorbance spectra. Fig. 3a depicts the FT-IR absorbance spectrum of toluene in the injection area of a microchannel in a CaF_2 microdevice, while Fig. 3b shows an IR absorbance spectrum of toluene using standard CaF_2 windows. Comparison of the spectra in Fig. 3 confirms that toluene within the microchannels can be detected and identified readily, even though the absorbance in Fig. 3a is lower than in Fig. 3b because the optical path length was 10× shorter. Similar agreement between absorbance spectra in microchannels and the sample cell was observed for water and acetonitrile (data not shown). Moreover, the IR spectrum in Fig. 3a agrees well with the toluene reference IR spectrum in the NIST online database [22], indicating that CaF_2 microdevices are suitable for on-chip IR detection and analyte identification. In these experiments we set the resolution to $4\,cm^{-1}$ to obtain high-resolution IR spectra. However, for real time detection in CE or other separation methods, lower spectral resolution should provide sufficient detail for qualitative analysis, and the decreased optical measurement time should enable higher temporal resolution.

4. Conclusion

We have successfully developed methods for the design and fabrication of CaF_2 microfluidic devices for maximum flexibility in optical detection. CE of FITC-labeled amino acids has been performed, and the results indicate that CaF_2 provides a suitable platform for rapid biochemical separations. Moreover, FT-IR tests indicate that CaF_2 microfluidic devices are suitable for real time, on-column FT-IR identification of analytes in microchannels. These CaF_2 microchips should enable both quantitative and qualitative optical analyses in laboratory-on-a-chip systems.

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