Glass nebulizer interface for capillary electrophoresis–Fourier transform infrared spectrometry

Jessica L. Jarman, Richard A. Todebush[1], James A. de Haseth*

Department of Chemistry, University of Georgia, Athens, GA 30602-2556, USA

Abstract

A capillary electrophoresis system has been successfully interfaced to a Fourier transform infrared spectrometer. The design of the interface is a custom-designed glass microconcentric nebulizer. Typical deposit characteristics include reproducible circular deposits of uniform thickness that lack any splatter as found in earlier designs. Interface performance is demonstrated in that there is no loss of electrical current during operation and spectra of analytes can be readily produced. Furthermore, it has been shown that the interface maintains the plug flow characteristic of capillary electrophoresis.
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1. Introduction

Interfaces between spectrometry and chromatographic techniques have yielded highly reliable and informative analytical methods. Regrettably, the potential of infrared spectrometry as an interface method is still being realized. Infrared spectrometry, especially conventional Fourier transform infrared (FT-IR) spectrometry, is a rapid method of analysis that may reveal unequivocal structural information about analyte molecules. The typical spectrum collected has a high signal-to-noise ratio and can be collected in approximately 50–200 ms on most commercial instruments. Additionally, FT-IR spectrometry is highly sensitive and detection limits have been reported in the picogram range [1]. Attempts to interface FT-IR spectrometry and chromatographic techniques include gas chromatography (GC) [2–11], liquid chromatography (LC) [12–16], and supercritical fluid chromatography (SFC) [17–19]. GC–FT-IR spectrometric techniques have been available for well over 30 years while LC–FT-IR methods were developed within the last 20 years, and SFC–FT-IR has been commercialized in the last few years. Capillary electrophoresis (CE), however, has only recently been successfully interfaced to FT-IR spectrometry [20].

Capillary electrophoresis (CE) is a separations technique that uses an electrical current to separate charged species. A typical CE apparatus includes two buffer reservoirs, a high voltage supply, a fused-silica capillary, and a detector. The high voltage is applied across the column, which causes both electrophoretic and electroosmotic flow to occur within the column. Analyte molecules are carried along in the electroosmotic flow of the electrolyte ''buffer'' and elute according to their charge-to-mass ratio. The analytes separate due to different electrophoretic

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*Corresponding author. Tel.: +1-706-542-1968; fax: +1-706-542-9907.

E-mail address: dehaseth@uga.edu (J.A. de Haseth).

[1]Current address: Unilever Home & Personal Care – NA, 3100 Golf Road, Rolling Meadows, IL 60008, USA.

mobilities in a given buffer system. Separations are carried out quite rapidly and require only nanoliters of sample. The resolution from the electrophoretic properties of the system is very high and separations of complex mixtures, such as fragments from protein digests, are common practice [12,21–23]. Temperature can be widely varied which allows for a large number of applications, and separations are carried out at near ambient pressure, which adds to the ease of the analyses. Typically, detection is performed on column with UV–Vis or laser-induced fluorescence (LIF) detectors, but neither of these methods provides structural information and analyte identification is accomplished only with migration time comparisons. Several successful CE–mass spectrometry (MS) interfaces [24–26] have been developed in an effort to obtain more analyte information, but MS is sample-destructive and is therefore not suited for samples that require further analysis after the CE separation. Furthermore, the strength of mass spectrometric analysis lies in the identification of homologues but does not readily lend itself to the identification of isomers. Due to the high degree of structural information found in analyte infrared spectra, an ideal detection method for CE is FT-IR spectrometry. This information, combined with the high resolving power of CE, creates a highly sensitive and informative analysis technique that is performed quickly and allows sample recovery for further analyses.

Successful CE interfaces have three main requirements [27]. First and foremost, a stable electrical contact must be maintained throughout the course of the analyses. Interfaces meet this requirement with a variety of methods that include sheath liquids [28], coupled columns [27,29,30], pierced or cracked columns [24], wires that surround or are inserted into the column [31], and metal coatings [32]. The interface described in this paper is a metal coating interface in which a thin layer of a conductive paint is used to coat the end of the column and is connected to electrical ground. The capillary is inserted into a glass nebulizer to complete the interface. The second main requirement is that the extremely low flow-rates of CE systems (~100 nl/min) are adapted to the higher flow requirements of the sample nebulization system. Some interfaces use ''make-up'' liquids [27], to account for the extra volume needed for a nebulizer, while others choose

to accept the consequential losses in resolution and increased migration times in lieu of a volume equalizing system. This method of approach leads to the third and final concern. Laminar flow is induced when the flow-rate of the CE system is not matched in some way to the flow-rate of the nebulization system. Currently, there are no commercially available nebulizers designed to operate at typical CE flow-rates [29]. During sample nebulization and introduction into the interface, the flow profile inside the column should remain plug-like. Laminar flow in a CE column, while it still allows for rapid separation, decreases the high resolving power of the technique and leads to migration time shifts, peak broadening, and loss of resolution. Some methods of laminar flow prevention include those that allow the nebulizer to pull through a separate liquid that never enters the capillary column [27], the application of a negative pressure to the inlet vial to counteract the pull of the aspiration system [33], and minimization of the nebulization gas or liquid flow [24].

The development of a CE–FT-IR spectrometric interface involves two additional concerns. Capillary electrophoresis uses electrolytic solutions, commonly referred to as CE buffers, to conduct electrical current and facilitate analyte separation. Many of these electrolytes exhibit high absorbances in the IR region and may mask any analyte identification information. Even spectral subtraction cannot completely remove the masking bands, and solvents that do not rapidly evaporate can become trapped in the sample matrix. Therefore, a successful interface must employ either an electrolytic solution which is not IR active, or must include a solvent and buffer elimination step. Secondly, because the sample is deposited directly onto an infrared transparent window, there must be a method of sample containment. CE–MS interfaces require complete sample introduction into a closed chamber during which ionization and desolvation steps are accomplished. To accomplish this, a complete sample aerosol is necessary. In a CE–FT-IR interface, the nebulization must produce deposits of neat analyte in a confined area. Furthermore, the deposit should be of uniform thickness and not ''splattered''. The concentration of as much analyte as possible into a single deposit allows for highly sensitive IR analyses. Deposits should therefore be contained in as small an area as possible and should not show splatter patterns.

Previously, we reported the first successful CE–FT-IR interface with a metal nebulizer used in the interface design [20]. This design successfully addressed many difficulties typically encountered in a CE interface while it also overcame obstacles specific to CE–FT-IR interfaces: constant electrical contact, absence of buffer interferences in the IR spectra, and sample containment to maximize spectral sensitivity. The newer improvement on this design is described herein and uses a custom designed microconcentric glass nebulizer to accomplish sample deposition onto calcium fluoride or zinc selenide IR crystals for off-line spectral collection with an FT-IR microscope.

2. Experimental

2.1. Chemical reagents

The following electrolytic solutions were used in separate experiments: ammonium acetate (NH_4Ac, Fisher Scientific, Atlanta, GA, USA); sodium borate ($Na_2B_4O_7 \cdot 10H_2O$, Sigma, St. Louis, MO, USA); and sodium hydroxide (NaOH, Fisher Scientific). All were used as received.

Electrolyte solution concentrations varied from 1.0 to 5.0×10^{-2} M depending on experimental parameters. Higher electrolyte concentrations were used in an attempt to decrease separation time and obtain larger currents. All solutions were prepared with 18.0 MΩ deionized water purified with a Barnstead Nano Ultrapure water system and had a pH of 8.5.

The following samples were used to test the stability and separation ability of the interface: caffeine and salicylic acid (Baker, Phillipsburg, NJ, USA), and *N*-acetylglucosamine (GlcNAc) (Sigma). Solutions of each compound were made with 18 MΩ water to final concentrations of 5.0×10^{-3} M.

The samples were deposited onto 25-mm diameter×6-mm thick zinc selenide (ZnSe) or calcium fluoride (CaF_2) windows (Spectral Systems, Hopewell Junction, NY, USA).

2.2. Capillary electrophoresis and FT-IR spectrometric microscope

Two capillary electrophoresis systems were used to separate samples of interest and discern the success of the interface. A Beckman P/ACE 5000 CE instrument (Beckman, Fullerton, CA, USA) equipped with a P/ACE UV absorbance filter detector was used in addition to an Agilent ^{3D}CE instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a UV–Vis diode array detector. The system was controlled with ^{3D}CE Chemstation software. Results presented herein are from the Agilent CE system unless otherwise noted. CE–FT-IR spectra were collected with a Perkin-Elmer Spectrum 2000 FT-IR spectrometer coupled with a Perkin-Elmer *i*-series FT-IR microscope with an adjustable limiting aperture (Perkin-Elmer). The microscope detector was a liquid nitrogen cooled mercury–cadmium–telluride (MCT) detector (Perkin-Elmer). The aperture allows for the beam size to be set from 10 to 400 µm in diameter to allow for variations in sample size. The spectra were collected with PEImage software. Spectral manipulation such as digital subtraction, baseline correction, and normalization, were calculated with the use of GRAMS/32 AI v.6.00 software (Thermo Galactic, Salem, NH, USA).

Uncoated fused-silica capillaries (Agilent Technologies, Wilmington, DE, and Polymicro Technologies, Phoenix, AZ, USA), with an outer diameter of 360 µm and inner diameters of 50 and 75 µm were used. Capillary length varied from 40 to 120 cm and the distance from the inlet to the UV–Vis detector was 22 cm for extended capillaries. This length could be varied somewhat, but it was found to be unnecessary for the investigations described here. Analyses were carried out at 25 °C with an applied voltage of 25–30 kV and simultaneous UV–Vis detection at 195 nm as a back-up for the IR detection method. Injection was accomplished by applying 50 mbar of pressure to the sample vial for 5 s.

2.3. Glass interface

The glass interface, shown in Fig. 1, was designed with the use of a female tee (Upchurch Scientific, Oak Harbor, WA, USA) and a standard sidearm fitting (J.E. Meinhard, Santa Ana, CA, USA). The last 20 cm of the extended fused-silica capillary was coated with silver paint (Fullam, Latham, NY, USA) and threaded through a metal clamp to maintain electrical contact. The capillary was then passed through the tee and fitting and inserted into the capillary of a custom-designed microconcentric glass
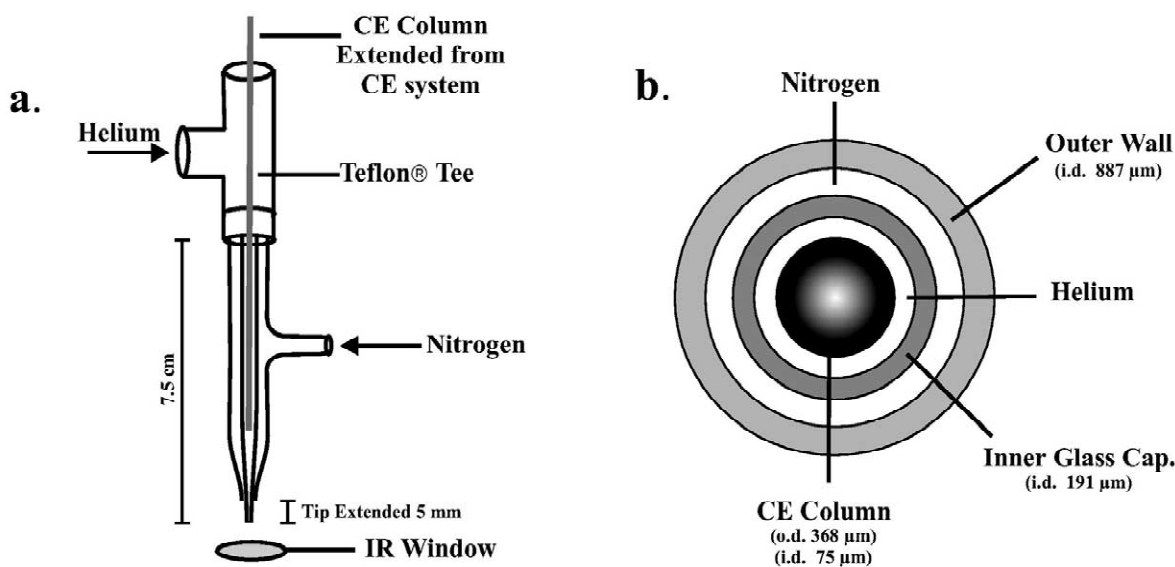
Fig. 1. (a) A schematic representation of the interface with the PTFE tee and microconcentric glass nebulizer. Helium and nitrogen ports are open to the capillary and shell, respectively. The resulting aerosol is deposited directly onto a calcium fluoride or zinc selenide IR window. (b) Cross-section of interface tip showing the layers of capillary, helium, nitrogen, and glass.

nebulizer (J.E. Meinhard). A helium flow was introduced through the tee while a nitrogen flow was introduced through the outer shell of the nebulizer. The tip of the nebulizer capillary extended 5 mm past the outer shell and the inner diameter of the glass capillary was large enough to accommodate the painted silica column when inserted just to the narrowing point. At the narrowest point, the tip is 191 μm I.D. These dimensions are variable to accommodate a variety of systems and produce somewhat different results with respect to deposit shape and size. All gas flows were controlled manually with the aid of metered needle valves. Deposits were visualized and photographed with an ausJENA stereomicroscope (Jenavert, Germany) and a Hitachi digital camera (Hitachi, Denshi America, Woodbury, NY, USA).

3. Results and discussion

The interface design described above was chosen to satisfy the requirements discussed earlier. The use of a nebulizer with an extended tip of extremely small inner diameter allows the flow-rates of the nebulizer and the capillary electrophoretic system to be more closely matched. Additionally, the aerosol is more easily contained. The helium introduced between the column and the nebulizer capillary aids in solvent evaporation and nebulization while the outer flow of nitrogen is used to help contain the deposit in a small area. This apparatus allows suitable deposits to be made in as little as 1 s, although for very dilute samples it is more advantageous to deposit multiple layers to increase spectral sensitivity. Therefore, the width of the analyte peak can determine the optimal deposition time. Detection with the diode array detector was performed simultaneously with interface operation due to the off-line characteristics of the system and to verify true analyte deposition. The new glass interface was compared with the previously described metal interface through examination of the deposit characteristics of both apparatuses. Deposits from the metal interface exhibited a significant amount of splatter patterns and interference patterns, which indicate regions of variable thickness. The glass nebulizer exhibited neither of these undesirable artifacts, thus, the glass interface is an improved design.

A typical deposit from the glass nebulizer, shown in Fig. 2 and obtained on the Beckman instrument, is round in shape and has a diameter between 200 and
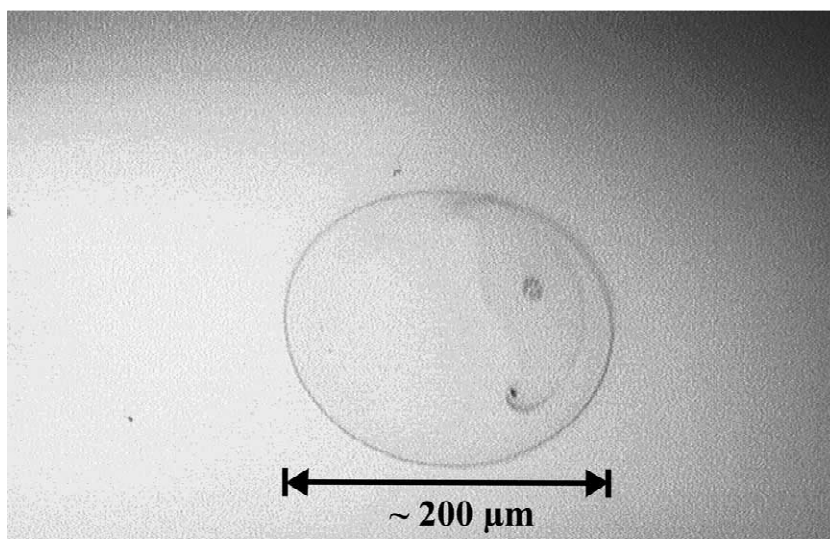
Fig. 2. A typical deposit from the CE–FT-IR spectrometric interface of 5 m*M* caffeine in 50 m*M* sodium borate buffer is shown. (The deposit is digitally enhanced to show its clear boundary). Deposits are of relatively uniform thickness and no splatter droplets are evident which indicates the concentration of all the analyte is confined to a small volume.

400 μm. The deposit is from an aqueous solution of caffeine and sodium borate electrolyte (5 and 50 m*M*, respectively) that has been allowed to dry as completely as possible to obtain a deposit of the solute and electrolyte mixture. In this case, the nonvolatile electrolyte molecules cannot be eliminated from the analyte matrix. Through visualization under an optical microscope, rings of variable thickness are not evident and there is even distribution of analyte molecules instead of a ring of high concentration on the outer edge. For the purposes of visualization, the deposit image has been digitally enhanced hence there is an artificial ring around the deposit. The original deposit was completely transparent and did not display any evidence of uneven thickness. It should also be noted that all the analyte has been concentrated into a single deposit, which is optimal for high spectral sensitivity.

To examine the thickness characteristics of a typical interface deposit further, a second deposit of an aqueous solution of sodium borate buffer was characterized using the FT-IR spectrometric microscope. After setting an aperture of 30 μm on the microscope, sequential spectra were collected for the center of the deposit, a 300$\times$400-μm area. The maximum characteristic absorbance was then re-corded and plotted in relation to the area of the deposit that was sampled. The resulting contour map, shown in Fig. 3, reveals a relatively uniform deposit thickness with sufficient absorbance to achieve a high quality infrared spectrum. Additionally, the
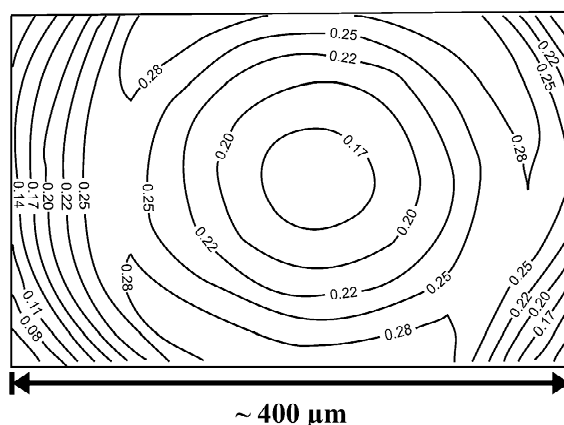

Fig. 3. Contour map of a sodium borate deposit with absorbance indicated on each contour line. Deposit is revealed to be only slightly variable in thickness and more than sufficient for high quality spectral analysis. Circular thickness regions indicate even deposition onto the infrared transparent window as opposed to weighted or skewed deposition as can be caused by uneven air flow or interface set up.

slight gradient in thickness follows a circular pattern, which indicates that the liquid is evenly deposited onto the window's surface. This deposit is larger than normally created as the larger size was required to map the deposit accurately.

Several properties of the successful CE–FT-IR spectrometric interface were characterized. The primary concern was that stable electrical contact was well maintained throughout the entire course of a separation. Coating interfaces in general have had limited success with electrical contact due to the fragile nature of the metal coating when columns are threaded through tight fittings or clamps. Our interface, however, has demonstrated remarkable durability. In order to verify the stable contact, the electrical current through the column is monitored throughout a normal analysis. Any instability in the current is revealed as sharp dips or spikes in the recorded trace. Fig. 4 shows a typical electropherogram obtained during the separation of a 50:50 (v/v) mixture of caffeine and salicylic acid. The current trace has been overlaid on the electropherogram. This current trace, monitored throughout the entire separation, does not exhibit any dips or spikes. The conclusion can then be made that stable electrical contact is maintained throughout the course of the CE analysis.

Adaptation of low CE flow-rates has posed a problem for several other interface designs, which includes earlier attempts at CE–FT-IR interfaces as well many currently used CE–MS interfaces. The
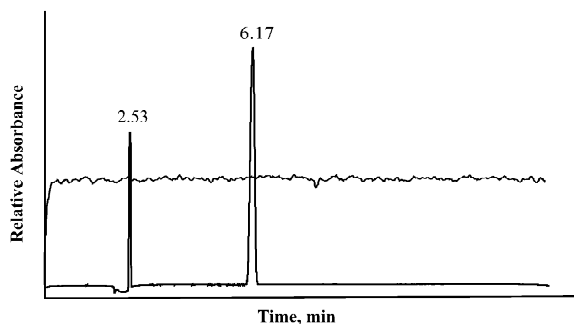

Fig. 4. CE–FT-IR separation of 5 mM caffeine and 5 mM salicylic acid in 50 mM borate buffer with 15 kV at 25 °C with a bare fused-silica column (103.5 cm×75 μm I.D.). The stable current trace overlaid on the electropherogram indicates constant electrical contact was maintained throughout the course of the separation.

usual result is a compromise between mismatched flow-rates and a loss of resolution. Because the nebulizer design used in this interface was created to accommodate lower flow-rates, and because the CE system used is capable of using external pressure to drive the electroosmotic flow at a slightly faster rate, we are able to match the flow-rate of the CE system more closely to that of the nebulizer. During the CE–FT-IR spectrometric analyses, an external pressure of 2 bar was applied to the inlet buffer vial. This pressure ensured that the liquid maintained contact with the silver paint that coats the outlet of the column. This pressure, however, is minimal and does not appear to alter the flow characteristics of the CE system.

A commonly encountered obstacle in capillary electrophoretic interfaces is alteration of the plug flow by the nebulization of the eluent. This distortion creates laminar flow inside the column. In order to determine the existence of laminar flow inside the capillary column, studies as described by Schaumlöffel and Prange [27], were carried out. By subjecting the interface to these conditions, any suction from the nebulization process and its effect on the liquid inside the column are clearly evident. When favorable results are obtained, the conclusion can be drawn that the interface nebulization process does not have an adverse effect on the typical electrophoretic plug flow in CE separations. Therefore, the following studies were completed on the glass nebulizer CE–FT-IR interface:

(1) The electric current of 30 μA at an applied voltage of 15 kV was measured again after the capillary had been left open to air at the inlet for 1 h while the nebulizer was operating. The presence of current indicates that air, which disrupts the current and breaks electrical contact, was not drawn into the capillary during 1 h of nebulization.

(2) The capillary was flushed with water and placed in the inlet vial containing a 5 mM solution of caffeine in 50 mM borate buffer. The absence of analyte peaks or a solvent front after 1 h of continuous nebulization indicates that the solution was not drawn into the capillary as a result of the nebulization gas flow.

Additionally, the nebulizer did not emit any droplets or aerosols during either study.

In mass spectral interfaces, there are additional concerns due to the sample introduction into an evacuated chamber; that is, lower sample chamber pressures increase the possibility of introduction of laminar flow profiles in the CE system. The CE–FT-IR spectrometric interface, however, is operated at ambient pressure and temperature with a nebulizer designed to handle lower liquid flow-rates. Therefore, with careful gas flow control, we are able to maintain the plug flow profile, which is a main advantage of CE.

It was determined that the deposits were of uniform thickness, as evidenced by the lack of ridges in the central area of the deposit and by the uniform appearance when visualized under a microscope. Because interference patterns are not visible, we can conclude that the deposits at the thickest point are still thinner than the wavelength of visible radiation. This average thickness, if the entire analyte volume is collected in a single deposit, can be calculated by likening the deposit to a cylinder. For a typical injection of 20 ng and a deposit size of 300 μm diameter, the thickness can be calculated as approximately 400 nm.

Finally, one major obstacle in the development of a successful CE–FT-IR interface is the interference of electrolyte absorbance bands in the analyte spectra. To determine the feasibility of alternative buffers

that are not as electrolytic, but are more volatile, two solutions of N-acetylglucosamine (GlcNAc), 5 mM in 50 mM borate and 50 mM ammonium acetate, were analyzed with the interface on the Beckman instrument. Spectra of the pure borate electrolyte solution and the pure compound were also collected. The results, shown in Fig. 5, reveal the spectral improvements gained with a more volatile buffer. Spectra of a deposit of pure ammonium acetate could not be collected as it is too volatile to leave a deposit.

4. Conclusion

The interface as described proved to be successful in a number of areas. Three main requirements of CE interfaces were satisfied which indicates that the system functions as a working interface. The apparatus has proven successful on two separate capillary electrophoresis systems, which attests to its flexibility and sturdy design.

Future projects are aimed at improvement of the interface design and further characterization its analytical properties. Although the interface is currently designed for off-line FT-IR analyses, an on-line design is in progress, and precise metering valves will be added to obtain a more accurate measure of actual helium and nitrogen gas flows. In order to address the problem of solvent absorption in the IR region, the interface was designed to accomplish solvent elimination concurrent with eluent nebulization. This step requires a relatively volatile buffer, which is a problem in most capillary electrophoretic analyses. More specifically, the electrolyte itself must be relatively volatile. Typical CE analyses involve aqueous electrolyte solutions. Water is easily removed from the sample deposit through simple evaporation. Any remaining electrolyte molecules, however, are much more problematic and can be trapped in the sample matrix so that buffer removal is not always possible and spectral identification may be significantly impaired. The difficulties with weaker (more volatile) electrolytes arise when high volatility buffers are incapable of carrying the current needed for reliable, reproducible separations. Common CE electrolytes are sodium borate and sodium phosphate but more volatile alternatives
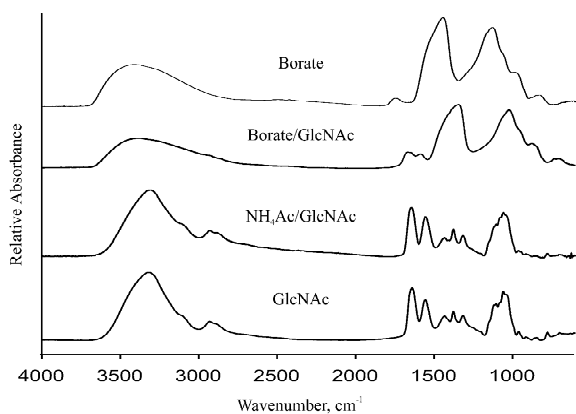

Fig. 5. CE–FT-IR spectra of pure borate buffer, N-acetylglucosamine (GlcNAc) in 50 mM borate and 50 mM ammonium acetate buffers, and of the pure monosaccharide. Borate absorbance bands almost completely mask the characteristic analyte bands, but the analyte spectrum is clearly evident when the volatile ammonium acetate buffer is used.

currently under investigation include ammonium phosphate, ammonium formate, and ammonium acetate.

Acknowledgements

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