Anal. Chem. **2009,** *81,* 10201–10207

Microfluidic Analysis of Complex Samples with Minimal Sample Preparation Using Gradient Elution Moving Boundary Electrophoresis

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Sample-in answer-out analytical tools remain the goal of much lab on a chip research, but miniaturized methods capable of examining minimally prepared samples have proven elusive. Complex samples, including whole milk, various types of dirt and leaves, coal fly ash, and blood serum, were analyzed quantitatively for dissolved potassium, calcium, sodium, magnesium, lithium, and melamine using gradient elution moving boundary electrophoresis (GEMBE) and contactless conductivity detection with the single preparatory step of dilution or suspension in sample buffer. GEMBE is a simple, robust analytical technique, well-suited to microfluidic analysis of complex samples containing material, such as particulates or proteins, that would confound the majority of other microfluidic techniques. GEMBE utilizes electrophoretic flow to drive electrically charged analytes into a microfluidic channel or capillary for detection, while opposing electro-osmotic and variable pressure-driven flows prevent the remainder of the sample from entering the channel. Contactless conductivity detection further simplifies device construction and operation, positioning GEMBE for inexpensive and facile multiplexed implementation outside laboratory settings.

Many microfluidic analytical procedures, particularly separations, can be described as highly evolved but often require extensive off-chip sample preparation. Samples are generally complex mixtures containing material, such as particulates, cells, proteins, or carbohydrates, that must be removed via filtration, centrifugation, or other procedures before introduction on-chip. Benchtop sample preparation typically requires significantly longer times and substantially larger fluid volumes than needed for the subsequent microfluidic analysis, limiting the overall functionality and impact of microfluidic tools for biochemical and chemical analysis of complex samples. Consequently, the full advantage of microfluidic techniques has yet to be realized, for example, for the creation of field-portable tools operable without a specialized skill set to analyze small sample volumes. Integrating sample preparation on-chip has therefore been increasingly identified as critical to the realization of more comprehensive lab on a chip devices for widespread use.[1]

The prevailing approach to actualizing fluidic analysis of complex samples on-chip has been the serial integration of individual, miniaturized unit operations. Each functional unit accomplishes one step of the desired analysis, including filtration, extraction, preconcentration, separation, detection, and so on. Their concerted operation bestows increased functionality to the composite device, for example enabling integrated sample preparation.[2−4] While work in this regard has expanded the realm of analytical problems addressable by microfluidic technology, structures built in this way are often unwieldy to fabricate or operate reliably and too complicated for facile parallelization.

An alternative tactic is to move away from preparatory sample handling altogether. This translates into a need to address the presence of particulates and other contaminating material in samples that can confound microfluidic analysis either by removing the offending materials from the detection region, perhaps through clever fluid handling on-chip, or by choosing a detection scheme that is insensitive to their presence. A recent review by Crevillen et al. provides discussion and relevant experimental studies in the literature toward this goal.[1] For example, Vrouwe et al. analyzed lithium in blood plasma using moving boundary

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10.1021/ac902075c CCC: $40.75
Published on Web 11/10/2009

electrophoresis in a microfluidic device to direct the desired analytes into another channel for capillary zone electrophoresis with no off-chip sample preparation beyond obtaining plasma from whole blood.[5] In another study, Vrouwe et al. measured alkali metal cations in whole blood without sample preparation using microfluidic capillary electrophoresis.[6] Only plasma was injected into the channel for capillary electrophoresis, because blood cells, with their slower electrophoretic mobilities compared to the analytes of interest, did not travel far enough into the device during loading to become injected into the separation channel. However, this approach resulted in fouled chips that were discarded after each analysis and was later abandoned in favor of incorporating a filter between the whole blood sample and microfluidic channels.[7] Zimmerman et al.[8] and Gervais and Delamarche[9] used blood serum processed off-chip from whole blood to detect C-reactive protein in a capillary microfluidic system that had been prepatterned with antibodies for fluorescence detection. Garcia and Henry examined renal function markers in unfiltered urine diluted with buffer using microfluidic capillary electrophoresis and pulsed amperometric detection.[10] These examples toward microfluidic analyses of minimally prepared samples only begin to tackle the problem of contaminants in complex samples: Vrouwe et al. moved to filtration, but filters are prone to clogging; the devices of Zimmerman et al. and Gervais and Delamarche required prepatterning; and, urine can contain cells or other material that might clog the device of Garcia and Henry. The advantage of the work presented here over previous studies lies in the ability of GEMBE to interrogate a wide variety of complex samples for similar analytes in rapid succession, without laborious or time-consuming device or sample preparation or device cleaning between separations.

GEMBE is an analysis technique that uses a capillary (or microchannel) between a few millimeters and several centimeters in length spanning the distance between a reservoir for sample and a reservoir for run buffer (Figure 1a). A constant electrical voltage is applied to drive electrically charged analytes from the sample reservoir into the capillary for detection, while opposing bulk flow is simultaneously directed from the run buffer reservoir toward the sample reservoir. This bulk counterflow is a combination of electro-osmotic flow (EOF) and controlled, variable pressure-driven flow that is decreased gradually over a separation experiment.[11,12] An analyte enters the capillary and is detected when its electrophoretic velocity overcomes the bulk counterflow. GEMBE has previously been reported for the multiplexed, high-throughput analysis of dye molecules, amino acids, DNA molecules, and immunoassay products,[11] in combination with isotachophoresis,[13] and for monitoring enzyme activity.[14]

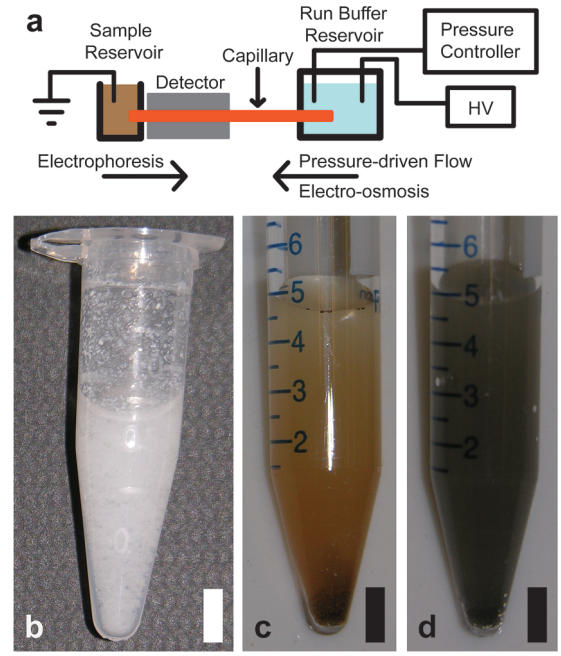

Figure 1. (a) Schematic of the experimental setup for the analysis of complex samples using GEMBE with contactless conductivity detection (not to scale). Bulk flow from electro-osmosis and a gradually decreasing pressure-driven flow prevents contamination of the capillary. An electrically charged analyte electrophoreses under a constant high voltage (HV) and enters the capillary for detection when its electrophoretic velocity overcomes the bulk counterflow. Photographs of typical (b) milk, (c) dirt, and (d) coal fly ash sample solutions. Scale bars are 5 mm high in panel b and 7 mm high in panels c and d.

The work described here extends the applicability of GEMBE to the analysis of minimally prepared, complex samples containing particulates and proteins. No sample preparation (beyond suspension or dilution in buffer) is needed, because these sample constituents are prevented by the bulk counterflow from entering the microfluidic portion of the apparatus. Only the analytes of interest, transported by electrophoresis, enter the capillary, while the remainder of the sample is simply pipetted from the sample reservoir postanalysis and replaced with the next sample for separation. Contactless conductivity detection also avoids the difficulty of optical detection systems and potential complications arising from direct contact between detection electrodes and fluid for straightforward interpretation of the separation data.

EXPERIMENTAL SECTION

Chemicals and Reagents. Whole milk (4% milkfat, Giant Food) was purchased from a local grocery store. Dirt was collected directly into a vial from under several oak trees on the Gaithersburg campus of the National Institute of Standards and Technology. Standard reference materials (SRM) of estuarine sediment (SRM 1646a), coal fly ash (SRM 1633b), tomato leaves (SRM 1573a), peach leaves (SRM 1547), and citrus leaves (SRM 1572) were obtained from the National Institute of Standards and Technology along with their certificates of analysis. The use of

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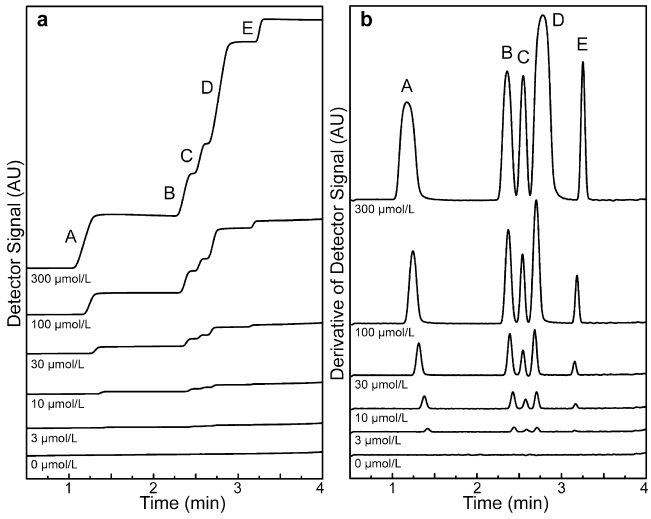

Figure 2. (a) Representative scans showing stepwise increases in detector signal for 0, 3, 10, 30, 100, and 300 μmol/L of (A) potassium chloride, (B) calcium chloride, (C) sodium chloride, (D) magnesium chloride, and (E) lithium chloride added to sample buffer. (b) Peaks were obtained from the data in panel a as described in the text by differentiation with respect to time. These data were taken at an applied voltage of 400 V/cm and applied pressure starting at 20 kPa and decreasing 100 Pa/s. Scans are shifted vertically for clarity.

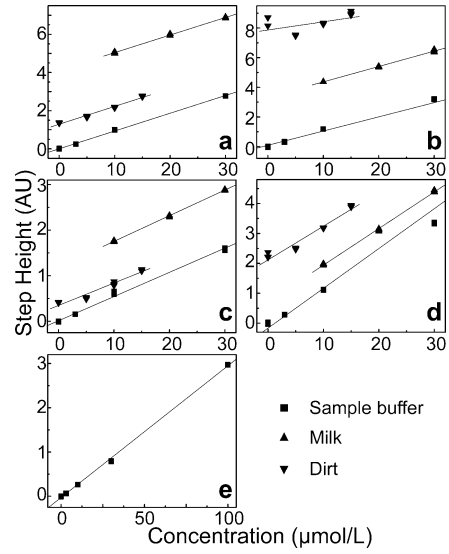

Figure 3. Detected step heights plotted versus added concentration of (a) potassium chloride, (b) calcium chloride, (c) sodium chloride, (d) magnesium chloride, and (e) lithium chloride for sample buffer (■), milk (▲), and dirt (▼) samples. Lines are linear fits to each data set for concentrations ≤100 μmol/L. R^2 values for the fits are 1.0, with the exception of dirt in panel b, which has an R^2 value of 0.6. Separations were performed in triplicate, and every experiment of each triplicate set is plotted with a separate symbol with uncertainty smaller than the data symbol. These data were obtained at an applied voltage of 400 V/cm and applied pressure starting between 20 and 55 kPa and decreasing 100 Pa/s.

SRMs provided independent analyses of total elemental content against which to compare analyte concentrations measured using GEMBE. Bovine calf serum was purchased from HyClone Laboratories, Inc.

Sample buffer consisted of 12 mmol/kg L-histidine (Fluka) and 50 mmol/kg acetic acid (Sigma) in 18 MΩ cm water with a measured pH 4.4. Run buffer was identical to the sample buffer with the addition of approximately 58 μmol/kg didodecyldimethylammonium bromide (DDAB, Aldrich).

Stock solutions of potassium chloride (Mallinckrodt), calcium chloride (Fisher Scientific), sodium chloride (Mallinckrodt), magnesium chloride (Fluka), and lithium chloride (Mallinckrodt) were prepared at 1 mol/L in 18 MΩ cm water prior to further dilution using sample buffer and eventual addition to the sample buffer, milk, and dirt for the data shown in Figures 2 and 3. A stock solution of melamine (Aldrich) was prepared directly in sample buffer at 10 mmol/L and diluted further with sample buffer for the data presented in Figure 5. A stock solution of 10 mmol/L lithium chloride in sample buffer was used for dilution into bovine calf serum for the data shown in Figure 5.

Whenever possible, glass containers and instruments were avoided in favor of plastic vessels to minimize cationic contamination.

Apparatus and Instrumentation. The experimental apparatus is shown schematically in Figure 1a. Reservoirs for sample buffer and run buffer were machined from polyoxymethylene (Delrin, DuPont) and polysulfone, respectively. A 5.5 cm long fused silica capillary (Polymicro Technologies) with approximate outer and inner diameters of 363.5 and 13.5 μm, respectively, was inserted though holes drilled into the sides of the reservoirs such that the capillary protruded approximately 1 mm into the sample reservoir and 5 mm into the run buffer reservoir. Double-sided adhesive tape was affixed between the run buffer reservoir and a high pressure fitting (Upchurch) to hold the capillary securely in place. For analyte detection, the capillary was threaded through a TraceDec contactless conductivity detector. The detection point was approximately 15 mm from the capillary inlet in the sample reservoir. Detector settings were the following: frequency, 2× high; voltage, 0 dB; gain, 200%; offset, 14; filter, slow; and data acquisition rate, 19.8 Hz. Constant dc voltage (PS350, Stanford Research Systems) was applied during experiments via high purity platinum wires inserted into the reservoirs. A precision pressure controller (Series 600, Mensor), backed by pressurized helium, controlled the pressure inside the sealed run buffer reservoir. Data were recorded using vendor supplied detector software (TraceDec Monitor 0.07a). Custom LabView software controlled and monitored the pressure controller and high voltage source. The loosely sealed sample reservoir was at ambient pressure. The apparatus was contained inside an enclosure to minimize the effects of temperature fluctuations due to stray air currents on the detector signal.

Experimental Procedures. A new capillary was filled by driving run buffer from the run buffer reservoir through the capillary using pressure. As soon as a bead of fluid was visible on the opposite end of the capillary inserted into the sample reservoir, the sample reservoir was filled with sample buffer. Before initial use, run buffer was flushed through the capillary for several minutes to form a coating of DDAB on the capillary surface. The run buffer was replaced at the start of each day of experimentation with 2 mL of fresh run buffer. Prior to analysis of a new type of sample, the sample reservoir was rinsed three times with 18 MΩ cm water, rinsed once with the new sample solution, and filled with 200 μL of fresh sample solution for analysis. The sample was replaced between replicate separations. Analyte step/peaks were identified by performing separations of samples comprised of individual analytes prepared in sample buffer. Analysis of blank

sample buffer after each sample indicated that contamination of the system by the samples was below the limit of detection (LOD) of the apparatus. The apparatus was stored by replacing the fluid in the sample reservoir with 18 MΩ cm water and reducing the pressure to between 2 and 5 kPa.

Separations were effected by holding the pressure on the run buffer reservoir at a high constant pressure between 25 and 60 kPa for 6 s. The high voltage was switched on, while the pressure was reduced to the starting pressure for that separation, and held for 10 s. The pressure was subsequently decreased by 100 Pa/s until enough time had elapsed to allow the analytes of interest to elute through the capillary. The capillary was then flushed at high pressure, typically approximately 5 kPa larger than the pressure applied at the start of the separation, for at least 10 s. The high voltage was switched off, and the system was held in this configuration for at least 1 min before the start of the next separation.

DDAB is a dynamic surfactant coating[15] that served to reverse EOF in the capillary, so that both EOF and hydrodynamic flow opposed the electrophoretic motion of the cations analyzed. DDAB was chosen, because it is not contaminated with the analytes of interest and was reported to provide a stable, reversed EOF for conventional capillary electrophoresis applications.[15] For the experiments reported here, however, this coating proved unstable over a period of days, as evidenced by a slow shift of analyte elution to higher pressures as EOF in the capillary tended to zero. Rinsing the capillary with 0.1 mol/L sodium hydroxide and recoating with DDAB was therefore necessary once over the course of these experiments.

Data Analysis. The detector signal showed steps (Figure 2a), which can be analyzed directly or converted to peaks (Figure 2b) with no loss of information. Data were processed for visualization purposes only using a 51 point Savitsky−Golay derivative to transform the raw detector signal to peaks (Origin, OriginLab).

Quantitative data analysis was performed using Mathematica (Wolfram Research). Data were binned into approximately 0.2 s increments for derivation and semiautomated peak finding. The original data, unbinned and undifferentiated, was then fit to an error function and a linear offset to account for background signal over a span of time containing the step of interest and approximately one step width on either side of the step. Potassium, lithium, and melamine steps were fit individually, while calcium, sodium, and magnesium steps were fit simultaneously to the sum of three error functions and a linear offset.

RESULTS AND DISCUSSION

Sample Buffer. Sample buffer alone and with known concentrations of the analytes (excluding melamine) were used to characterize the experimental apparatus, optimize the separation parameters, and construct calibration curves. Triplicate measurements were taken of sample buffer with various concentrations (3, 10, 30, 100, and 300 μmol/L) of each of the inorganic cations potassium, calcium, sodium, magnesium, and lithium. Figure 2a shows representative steps in the detector signal corresponding to each of the analytes, and Figure 2b gives the peaks calculated by taking the time derivative of the data in Figure 2a. These data can then be analyzed using the methods commonly employed for interpreting peaks in conventional electropherograms.

The detector response (step height versus concentration) was determined to be effectively linear up to an analyte concentration of 100 μmol/kg. Plots of detector signal step height versus concentration of added analyte were fitted to lines for sample buffer with $\leq$100 μmol/L of each additional analyte (Figure 3a−e). LOD values (calculated as the concentration corresponding to the step height at zero added concentration plus three times the mean of the standard deviations of replicate measurements at each added concentration) are collected in Table 1 and are approximately twice the LOD values reported for conventional capillary electrophoresis using a similar detector.[16]

Whole Milk. Milk was chosen to demonstrate the ability of GEMBE to quantitatively determine the cationic content of food products containing particulates. Data from a representative separation of whole milk is shown in Figure 4a. Whole milk was diluted 1000× in sample buffer. Upon addition to the acidic buffer, the milk proteins and fat coagulated into irregular particles (Figure 1b). No effort was made to avoid pipetting these particles into the sample reservoir. A standard addition method was used to assess any matrix effects and provide quantitative measurement of the cation content of the milk. Triplicate measurements were taken at 10, 20, and 30 μmol/L of each analyte (except lithium and melamine) added to the sample solution (Figure 3a−d). Results are summarized in Table 1. Apparent recoveries[17] were calculated as the ratio of the slope measured for the standard addition curve for the milk to the slope measured for the calibration curve with sample buffer. Typical apparent recoveries were within 3 standard deviations of 100% (with the exception of magnesium) indicating minimal interference with the diluted milk. Measured cation concentrations were comparable to those reported in recent studies using conventional capillary electrophoresis methods for milk analysis.[18]

Dirt, Estuarine Sediment, and Coal Fly Ash. Dirt, estuarine sediment, and coal fly ash were examined to ascertain whether GEMBE would be able to measure the cationic content of samples that are themselves collections of particulates of widely varying sizes. Figure 4b shows data from a representative separation of dirt, which was suspended in sample buffer at a concentration of 5 mg/mL. Figure 1c shows a photograph of a typical prepared dirt sample. A standard addition method was used for quantitative analyte determination. Triplicate measurements were taken at 0, 5, 10, and 15 μmol/L of each analyte (except lithium and melamine) added to the sample solution. Each dirt sample solution was mixed using a vortex mixer for approximately 5 s and placed upright in a holder. A timer was started, and sample was pipetted out near the top of the sample solution at approximately 2.5, 6, and 10 min without disturbing the solution as the sample sedimented. The data showed no significant trend as the sedimentation proceeded, indicating that sedimentation had no observable effect on the reproducibility of these measurements.

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Table 1. Summary of Results Obtained for Cationic Analytes Measured in Complex Samples Using GEMBE with Contactless Conductivity Detection

	K	Ca	Na	Mg	Li
		Sample Buffer			
LOD (μmol/L)	0.22	0.31	0.67	0.27	0.39
RSD[a] (%)	0.45	0.49	2.40	0.71	0.44
		Milk (Diluted 1000×)			
C (μmol/L)	44.7 ± 0.9	32.1 ± 0.9	21.1 ± 0.4	5.9 ± 0.4	
recovery[b] (%)	99 ± 2	109 ± 3	106 ± 2	91 ± 2	
RSD[a] (%)	0.85	0.64	0.86	1.39	
		Dirt (5.0 mg/mL)			
C (μmol/L)	13.8 ± 0.7	142 ± 65 (81.1 ± 2.7)[d]	7.1 ± 0.8	18.8 ± 1.5	
recovery[b] (%)	100 ± 4.3	58 ± 27	93 ± 6	84 ± 6	
RSD[a] (%)	0.90	1.61	3.32	1.55	
		Estuarine Sediment (0.28 mg/mL)			
C (μmol/L)	3.6 ± 0.3	20.6 ± 1.3	54.4 ± 0.9	10.3 ± 1.0	
recovery[c] (%)	5.8 ± 0.8	56.8 ± 7.1	60.3 ± 6.3	23.0 ± 3.3	
RSD[a] (%)	8.07	5.65	1.43	8.71	
		Coal Fly Ash (18.8 mg/mL for K, 0.095 mg/mL for Ca, Na, Mg)			
C (μmol/L)	26.4 ± 0.5	49.4 ± 0.7	9.8 ± 1.1	12.3 ± 0.9	
recovery[c] (%)	0.28 ± 0.01	138.1 ± 6.5	118.0 ± 13.6	65.3 ± 5.1	
RSD[a] (%)	1.88	0.16	9.99	5.51	
		Tomato Leaves (0.060 mg/mL)			
C (μmol/L)	41.4 ± 0.2	60.3 ± 1.0	1.0 ± 0.6	22.7 ± 0.7	
recovery[b] (%)	99.9 ± 10.2	79.8 ± 8.2	282 ± 172	76.6 ± 8.6	
RSD[a] (%)	0.08	1.13	39.3	1.25	
		Peach Leaves (0.070 mg/mL)			
C (μmol/L)	40.2 ± 0.5	7.9 ± 2.2	*e*	13.5 ± 0.7	
recovery[c] (%)	92.4 ± 9.4	29.0 ± 8.6		108.5 ± 12.4	
RSD[a] (%)	1.31	22.6		1.79	
		Citrus Leaves (0.065 mg/mL)			
C (μmol/L)	28.9 ± 0.3	13.2 ± 0.5	*e*	14.3 ± 0.7	
recovery[c] (%)	95.5 ± 10.1	25.9 ± 2.9		92.2 ± 11.3	
RSD[a] (%)	0.75	1.44		0.72	

[a] Relative standard deviation. [b] Apparent recovery[17] calculated as the ratio of the slope measured for the standard addition curve to the slope measured for the calibration curve with sample buffer. [c] Apparent recovery[17] calculated as the ratio of the concentration measured using GEMBE to the expected concentration calculated from the SRM certificate and assuming complete dissolution of the analytes. [d] Value calculated using the calibration curve obtained with sample buffer. [e] Value below the LOD.

Experimental results for dirt are listed in Table 1. Potassium, calcium, sodium, and magnesium were present in the dirt at measurable concentrations. Apparent recoveries[17] were calculated as the ratio of the slope measured for the standard addition curve for dirt to the slope measured for the calibration curve with sample buffer. Standard errors for the measured concentrations and apparent recoveries were greater than those for milk, which is likely due to the greater heterogeneity of the dirt samples relative to the milk samples; dirt samples were prepared with separate, approximately 25 mg dirt samples weighed for each added analyte concentration, while milk samples were mixed from a common stock solution of diluted milk.

Representative data from separations of estuarine sediment and coal fly ash are shown in Figure 4c,d. Estuarine sediment was suspended in sample buffer at a concentration of 0.28 mg/mL, while coal fly ash was prepared in sample buffer at concentrations of 18.8 mg/mL (to measure potassium) and 0.095 mg/mL (to measure calcium, sodium, and magnesium). Triplicate measurements were made on the samples directly. Results are given in Table 1, with concentrations determined using the mean step heights and the calibration curve measured for the sample buffer. Apparent recoveries were calculated as the ratio of the concentration measured using GEMBE and the expected concentration calculated from the SRM certificate values for potassium, calcium, sodium, and magnesium, assuming complete dissolution of these elements into the sample buffer.

The SRM certificate values report the total elemental composition, whereas GEMBE detects only the portion of the analytes dissolved in the sample buffer. Comparison between the measured values and the SRM certificates shows that GEMBE detected approximately $^1/_2$ of the potassium and calcium, $^2/_3$ of the sodium, and $^1/_4$ of the magnesium that constitute the estuarine sediment and approximately all of the calcium and sodium and $^2/_3$ of the magnesium content of the coal fly ash. Almost none of the expected potassium was detected in the coal fly ash. These discrepancies between the GEMBE results and the SRM certificate values suggest that much of the potassium, calcium, sodium, and magnesium in the estuarine sediment and coal fly ash samples remained undissolved or otherwise unavailable for detection using GEMBE. However, the measurement of dissolved analytes is sufficient for numerous applications beyond those demonstrated here, for example to determine the nutrients available in soil for plants, to examine pollution in drainage water,[16] or to identify chemical signatures of explosive detonation.[19]

Leaves. Tomato leaves, peach leaves, and citrus leaves were analyzed to show that GEMBE is also capable of analyzing cations in a complex matrix of biological material. The leaves were

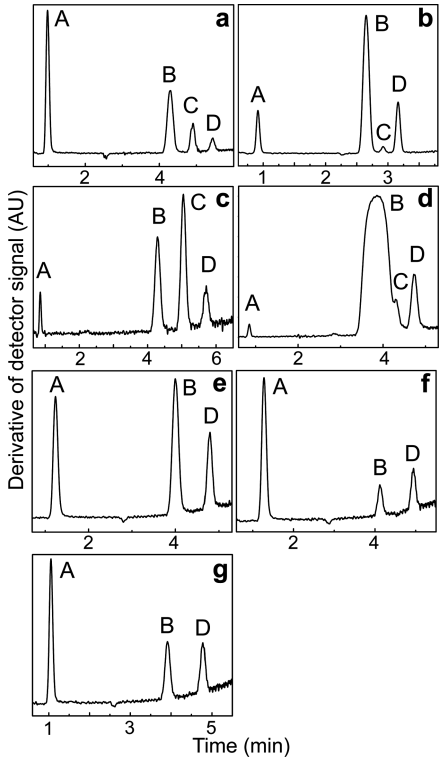

Figure 4. Representative time derivatives of detector signals plotted versus time from (a) milk, (b) dirt, (c) estuarine sediment, (d) coal fly ash, (e) tomato leaf, (f) peach leaf, and (g) citrus leaf samples show clear peaks from (A) potassium, (B) calcium, (C) sodium, and (D) magnesium. These data were obtained at an applied voltage of 400 V/cm and applied pressure starting between 47 and 57 kPa and decreasing 100 Pa/s.

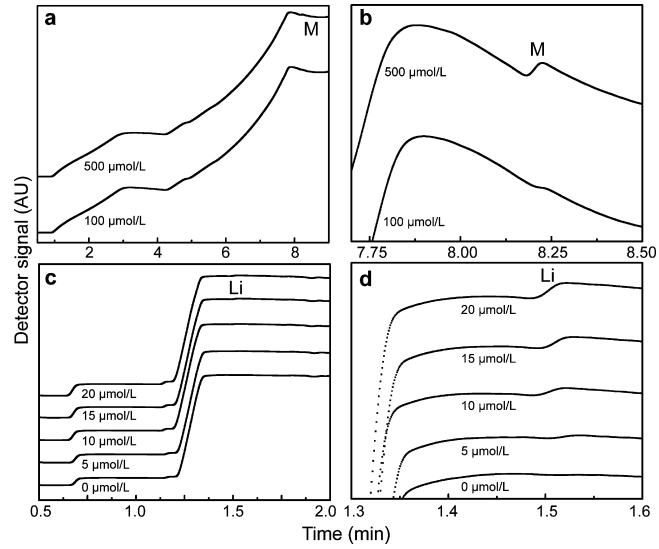

Figure 5. Detection of small quantites of analytes against large backgrounds using GEMBE with contactless conductivity detection. (a) Representative scans showing a stepwise increase in detector signal plotted versus time for whole milk diluted 10× in sample buffer containing 100 and 500 μmol/L melamine added (M). (b) Enlarged region of the plot in plot a shows the melamine step (M) clearly against the background with increasing step height for increasing concentration added. Analysis time can be reduced to under 3 min by decreasing the starting pressure, without impacting the resolution of the melamine step. (c) Representative scans showing stepwise increases in detector signal plotted versus time for bovine calf serum diluted 100× in sample buffer with 0, 5, 10, 15, and 20 μmol/L lithium chloride added (Li). (d) Enlarged region of the plot in plot c shows the lithium step (Li) clearly against the background with increasing step height for increasing concentration added. Data have been shifted vertically for clarity. These data were taken at an applied voltage of 400 V/cm and applied pressure starting at 55 kPa (milk) and 10 kPa (blood serum) and decreasing 100 Pa/s.

suspended in sample buffer by mixing with a vortex mixer at 0.060 mg/mL (tomato), 0.070 mg/mL (peach), and 0.065 mg/mL (citrus), and analyzed directly in triplicate for potassium, calcium, sodium, and magnesium. Sodium content for the peach and citrus leaves was below the LOD, as expected from the certificates of analysis. Figure 4e−g shows representative data from these separations. Sample vials were simply inverted once to resuspend leaf fragments prior to pipetting new sample solution into the sample reservoir. Table 1 summarizes the results. Apparent recoveries were calculated as the ratio of the concentration measured using GEMBE and the expected concentration calculated from the SRM certificate, along with the assumption of complete dissolution of these elements into the sample buffer. Comparison to the SRM certificates reveals that approximately all of the potassium and magnesium and approximately $^1/_3$ (peach and citrus) or $^2/_3$ (tomato) of the calcium present in the samples was dissolved and measurable using GEMBE.

Trace Analysis in Complex Samples with Large Backgrounds. The ability to detect the presence of a small amount of an analyte against a much larger background removes the need for additional sample preparation to reduce that background. This capability simplifies analysis and is advantageous for numerous applications, including food safety, health care, and biochemical warfare agent detection. As an example, measurements were made using GEMBE to analyze 10× diluted whole milk with 100 μmol/L (triplicate measurements) and 500 μmol/L (single measurement) of melamine added (Figure 5a,b). The melamine step was well resolved, despite the gross overloading and consequent distortion of the calcium, sodium, and magnesium steps. The LOD estimated for melamine was 16 μmol/L in the diluted sample, corresponding to a melamine concentration of 160 μmol/L, or approximately 20 mg/kg, in the undiluted milk. This LOD corresponds to a concentration that is approximately 1 order of magnitude larger than that currently deemed safe by the United States Food and Drug Administration and the Commission of the European Communities.[20,21] As a further example, GEMBE was used to measure lithium in bovine calf serum (Figure 5c,d). Blood serum was diluted 100× with sample buffer and measured in quintuplicate with 0, 5, 10, 15, and 20 μmol/L of lithium chloride added to the sample solution. The sodium step was slightly overloaded, while the lithium step was clearly resolved with an LOD of 1.5 μmol/L. This value corresponds to an LOD for lithium in undiluted serum of 150 μmol/L, well below the typical therapeutic range.[7]

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These results establish GEMBE as a viable means of analyzing complex samples with minimal sample preparation for small concentrations of analytes against large backgrounds. The cationic background is observed directly in the detector signal, while anionic and uncharged components of the sample solution never enter the capillary for detection. Experimental conditions were not optimized to detect melamine and lithium in these complex matrices, and LOD values may be reduced by optimizing GEMBE specifically for the detection of melamine in milk or lithium in blood serum, rather than using the general experimental method reported here.

CONCLUSIONS

GEMBE is a quantitative, robust technology for the sample-in answer-out analysis of minimally prepared, complex samples, as demonstrated here by the analysis of potassium, calcium, sodium, magnesium, lithium, and melamine in various types of dirt and leaves, coal fly ash, milk, and blood serum. The broad range of samples that can be interrogated using GEMBE include those that can be suspended or dissolved in a buffer, so that the analytes become available in the fluid for transport through the capillary for detection. The complexity of the samples that have been demonstrated was generally limited not by shortcomings intrinsic to the GEMBE technique (only analytes present in the sample buffer, not particulates, enter the microfluidic portion of the apparatus) but by the sensitivity of the conductivity detector (samples needed to be dilute enough to reach analyte concentrations within the linear range of the detector response).

Contactless conductivity detection was chosen in anticipation of eventual parallelization and operation of the experimental apparatus outside laboratory settings. Although the present implementation employs a 5.5 cm capillary and contactless conductivity detector, nothing about these results precludes the use of shorter capillaries (for reduced footprint and improved LOD values) and channel current detection that are arguably more amenable to massive parallelization and field-portability as described by Ross and Kralj[14] and Ross and Romantseva.[22] System integration into a portable package may benefit from further miniaturization of the experimental fluidic components and would require significant size reduction of the pressure controller and detector.

As with conductivity detection in general, LOD values achievable using GEMBE with contactless conductivity detection are larger than with fluorescence detection methods. LOD values reported here could be improved by optimizing the performance of the pressure controller to deliver a more continuous pressure gradient and increased temperature stability of the experimental apparatus. GEMBE is also easily coupled to fluorescence detection,[11,13] and the results presented here suggest that GEMBE may be a valuable tool for fluorescence analysis of complex samples with minimal sample preparation and reduced LOD values.

Because of its ability to easily and rapidly interrogate minimally prepared, complex samples, GEMBE shows enormous promise for diverse applications, including monitoring chemical contamination in food and water supplies, measuring nutrients in soil, regulating environmental pollutants, detecting biochemical warfare agents, and for biomedical diagnostics.

ACKNOWLEDGMENT

This work was performed while E.A.S. held a National Research Council Research Associateship Award. Certain commercial equipment, instruments, or materials are identified to adequately specify the experimental procedure. Such identification implies neither recommendation or endorsement by the National Institute of Standards and Technology nor that the materials or equipment identified are necessarily the best available for the purpose.

Received for review September 15, 2009. Accepted October 26, 2009.

AC902075C

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