Published in final edited form as:

Anal Chem. 2015 March 3; 87(5): 2702–2709. doi:10.1021/ac503903j.

Electrophoretic Extraction of Low Molecular Weight Cationic Analytes from Sodium Dodecyl Sulfate Containing Sample Matrices for Their Direct Electrospray Ionization Mass Spectrometry

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Abstract

While the use of sodium dodecyl sulfate (SDS) in separation buffers allows efficient analysis of complex mixtures, its presence in the sample matrix is known to severely interfere with the mass-spectrometric characterization of analyte molecules. In this article, we report a microfluidic device that addresses this analytical challenge by enabling inline electrospray ionization mass spectrometry (ESI-MS) of low molecular weight cationic samples prepared in SDS containing matrices. The functionality of this device relies on the continuous extraction of analyte molecules into an SDS-free solvent stream based on the free-flow zone electrophoresis (FFZE) technique prior to their ESI-MS analysis. The reported extraction was accomplished in our current work in a glass channel with microelectrodes fabricated along its sidewalls to realize the desired electric field. Our experiments show that a key challenge to successfully operating such a device is to suppress the electroosmotically driven fluid circulations generated in its extraction channel that otherwise tend to vigorously mix the liquid streams flowing through this duct. A new coating medium, *N*-(2-triethoxysilylpropyl) formamide, recently demonstrated by our laboratory to nearly eliminate electroosmotic flow in glass microchannels was employed to address this issue. Applying this surface modifier, we were able to efficiently extract two different peptides, human angiotensin I and MRFA, individually from an SDS containing matrix using the FFZE method and detect them at concentrations down to 3.7 and 6.3 $\mu\text{g/mL}$, respectively, in samples containing as much as 10 mM SDS. Notice that in addition to greatly reducing the amount of SDS entering the MS instrument, the reported approach allows rapid solvent exchange for facilitating efficient analyte ionization desired in ESI-MS analysis.

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ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.

The use of sodium dodecyl sulfate (SDS) as an additive in separation buffers is an established practice today that has allowed significant advances in many areas of biological, chemical and environmental sciences.¹ Perhaps the most notable application among these is the utility of this surfactant in protein sizing assays using the SDS-PAGE technique.² In this gel-electrophoretic method, SDS has been shown to bind to proteins and polypeptides transforming them into rod-like molecules with approximately the same charge-to-mass ratio. As a result, the electrophoretic migration rates through a cross-linked matrix for these complexes have been observed to be dictated by their molecular weight, nearly independent of their secondary and/or tertiary structures. Over the past two decades, the significance of SDS in separation sciences has grown even further with the emergence of micellar capillary electrophoresis (MCE) as a versatile liquid phase analysis technique.³ The MCE method relies on the differential partitioning of the sample between a micellar pseudostationary phase (PSP) and the surrounding buffer (mobile phase) to yield high resolution separations of both neutral and charged species. In MCE analysis, SDS is the most commonly used PSP because of its high stability, low Krafft point and high solubilizing power.⁴

While the use of SDS in PAGE and MCE techniques enables several assays of high significance, this surfactant is known to interfere with the direct integration of these separation methods to mass spectrometry (MS) based downstream analyses. This is because any such coupling automatically introduces SDS into the mass-spectrometer which suppresses analyte ionization and contaminates the ion source in the instrument. The exact mechanism for this signal suppression is complex and more details on the topic may be obtained from the relevant literature.^{5–8} Nevertheless, such signal suppression presents a severe analytical challenge to performing ESI-MS analysis of MCE/PAGE samples. Unfortunately, researchers still rely on blotting protein bands onto a membrane followed by washing the membrane with solvents like acetone or methanol to get rid of the SDS molecules for analyzing PAGE specimens using a mass spectrometer.⁹ The manual sample handling procedures in this approach limits the throughput of the assay and often compromises the detection sensitivity of the analytes because of sample loss. Integration of MS techniques to MCE separations on the other hand, is not as restrictive as a result of successful development of several approaches for the purpose. Among these, partial-filling of MCE columns with SDS^{10–12} or anodically migrating the surfactant away from the electrospray ionization interface¹³ have been demonstrated to be particularly promising strategies. Unfortunately, the partial-filling and anodic migration techniques are reported to reduce the effective separation length and number of theoretical plates in the system, respectively, in addition to complicating the assay itself.^{9–13} Novel MS compatible PSPs

that offer high selectivity for the MCE process have been also developed to allow the integration of this separation method to mass spectrometers.^{14–22} Such PSPs, however, tend to be effective only for a select range of samples and therefore cannot altogether replace the use of SDS in MCE applications. Similarly, it may be possible to adopt microdialysis approaches for coupling MCE to MS instruments that were originally developed for reducing the amount of background contaminants in samples prior to their ESI-MS analysis.²³ The need for long dialysis columns (~20 cm) in these systems, however, can significantly deteriorate the separation efficiency of MCE assays curtailing the benefits of the overall design. Of course, SDS cleanup of MCE samples can be also performed offline using commercially available spin columns, for example, Pierce detergent removal spin column (Thermo Scientific). Such an approach though besides rendering the assay labor intensive, is particularly unsuitable for analyzing small sample sizes or tightly separated analyte bands eluting from a column. In this situation, there is a clear need for developing methodologies for inline removal of SDS from separation buffers in order to realize high throughput analyses of PAGE and certain MCE samples using mass spectrometry based techniques.

Free-flow zone electrophoresis (FFZE) offers a power approach for continuous fractionation of charged species based on their electrophoretic mobilities in the system.^{24,25} This technique is commonly practiced by continuously introducing a sample mixture into the separation chamber along with a cocurrent buffer flow using pressure-drive.²⁶ The liquid streams are subsequently subjected to a transverse electric field as they travel through the assay compartment to deflect the constituent analytes based on their electrophoretic mobilities. In this situation, analyte zones that exit the separation chamber at different lateral positions are formed enabling the desired fractionation. The FFZE technique has been successfully applied to a wide variety of samples ranging from small metabolites to large biological cells because of its high throughput and reliance on relatively gentle operating conditions.^{27,28} More recently, the FFZE assay has been integrated to other analytical procedures on the microfluidic platform significantly improving its ability to analyze complex mixtures.^{29–31} The miniaturization of FFZE separations to micrometer sized compartments has further enhanced their performance by reducing Joule heating effects, allowing the injection of narrow sample streams as well as minimizing hydrodynamic dispersion of the analyte zones.^{32–34}

In this Article, we report the application of a microchip based FFZE method for extracting low molecular weight cationic analytes from an SDS containing matrix into an ESI-compatible solvent stream for their inline ESI-MS analysis. The reported extraction process was accomplished in our current work in a glass channel with microelectrodes fabricated along its sidewalls to realize the desired electric field. While such a microchip device may be conceptually simple to operate, our experiments show that a key challenge to applying it for extracting cationic analytes from an SDS containing matrix with high efficiencies is to suppress the electroosmotically driven fluid circulations generated in its analysis channel. These circulations, if unsuppressed, have the tendency to vigorously mix the liquid streams flowing through the extraction channel both homogenizing and dispersing their contents.^{31,35–37} In our present research, this issue was resolved using a new coating

medium, *N*-(2-triethoxysilylpropyl) formamide, that has been recently shown by our laboratory to diminish EOF in glass microchannels by over 5 orders of magnitude practically eliminating the unwanted mixing observed in our FFZE device.³¹ Such minimization of fluid mixing then enabled us to efficiently extract cationic analytes from an SDS containing matrix before analyzing them with a mass spectrometer via the ESI interface. Applying this approach, we were able to detect two different peptides, human angiotensin I and MRFA, at concentrations down to 3.7 and 6.3 µg/mL, respectively, in samples containing as much as 10 mM SDS. This corresponds to an improvement in the limit of detection for the peptides by at least a factor of 270 and 159, respectively, which otherwise were undetectable using the ESI-MS method in the original sample even when present at levels as large as 1 mg/mL.

EXPERIMENTAL PROCEDURE

For fabricating the microfluidic devices employed in this work (see Figure 1), bottom substrates and cover plates made from borosilicate glass were purchased from Telic Company (Valencia, CA). The purchased cover plates and the bottom substrates came with a thin layer of chromium and photoresist laid down on one of their surfaces to enable the photo-patterning process. Custom designed photomasks created through Fineline Imaging Inc. (Colorado Springs, CO) were used to pattern the desired channel and electrode layouts onto the bottom substrate and cover plate, respectively, using standard photolithographic methods.^{38,39} The length and the width of the FFZE channel in our device were 3.5 cm and 600 µm, respectively, while the electrode patterns in it were chosen to be either 0.5 or 1 mm wide. The channel segments leading to and exiting from the FFZE conduit were exactly half as wide as the latter in all our designs. After completion of the photopatterning process, the photoresist layer was cured in microposit developer MF-319 (Rohm and Haas) and the chromium layer removed along the channel network with a chromium etchant (Transene Inc.). The fluidic ducts on the bottom substrate and electrode patterns on the cover plate were then etched to depths of 40 µm and 400 nm, respectively, using buffered oxide etchant (Transene Inc.). A 130 nm thick layer of chromium was later deposited within the electrode patterns followed by a 40 nm thick layer of gold using a dual metal evaporator system (Energy Beam Sciences, Inc.). The protective photoresist and chromium layers were subsequently removed from the bottom substrate and cover plate using the MF-319 and chromium etchant solutions, respectively. Finally, the two glass plates were aligned and bonded using sodium silicate as an adhesive layer based on procedures described in the literature.⁴⁰ Access holes were drilled on the bottom substrate at appropriate locations prior to this bonding process, however, using a powder blaster system to enable electrical contact with the microfabricated electrodes as well as introduction of liquid streams into the fluidic network. The separation voltage across the FFZE channel was applied by filling the relevant access holes with an electrically conductive paste (Chemtronics, catalog no. CW7100) and then immersing copper wires in it connected to the leads of an in-house built 12 V DC power source.

The reported microchip was prepared for an experiment by treating the entire fluidic network with 1 M NaOH for an hour followed by sequentially rinsing it with water, methanol, and acetone for 10 min each. The microchannels were then derivatized with a solution of *N*-(2-triethoxysilylpropyl) formamide for 2 h at room temperature to suppress the

electroosmotic flow in the system.^{31,41,42} This coating material was prepared by mixing stoichiometric amounts of commercially available 3-(aminopropyl)triethoxysilane (Sigma-Aldrich, catalog no. A3648) with ethyl formate (Sigma-Aldrich, catalog no. 112682) and reacting the two chemicals under ambient conditions for 48 h. These chemicals were mixed as obtained from Sigma-Aldrich without using any additional solvent. In the absence of this chemical modification, strong electroosmotically driven fluid circulations were observed in the FFZE channel which vigorously mixed the liquid streams flowing through it preventing any electrophoretic fractionation. Upon application of this coating material, minimal mixing of the sample and ESI-compatible solvent was observed in our device enabling the electrophoretic migration of the cationic peptide molecules from the former to the latter stream leading to the desired extraction process. The samples were prepared by incubating the peptide in the relevant SDS containing matrix at 25 °C for 1 h. The addition of SDS to our peptide samples was observed to modify their pH values from 7.0 (no SDS) to ~6.7 (10 mM SDS). Syringe pumps were used for introducing the solvent and sample streams into our FFZE device and the extracted cationic analyte was detected by directly electrospraying the stream exiting terminal 3 in Figure 1 into a mass-spectrometer (MS). The MS instrument (LCQ Classic, Finnigan) was interfaced to our microchip by connecting a commercial ESI nozzle (New Objective, Inc.) to port 3 through a 10 cm long-75 μ m i.d. glass capillary and an appropriate flow adaptor (IDEX Corporation). This integration however increased the hydrodynamic resistance of the flow path to the electrospray interface, which in turn modified the splitting of the fluid stream exiting the separation channel toward ports 3 and 4. This splitting ratio was controlled in our experiments by appending again a 75 μ m i.d. capillary of an appropriate length to port 4 using a flow adaptor identical to that employed at the ESI nozzle port. The electrospray was realized in all our experiments by applying 3 kV across the ESI nozzle and MS instrument.

In our analysis, the signal from the human angiotensin I species in any sample was taken to be the total ion current recorded by the MS instrument for an m/z range of 1296.6 ± 0.5 averaged over 40 scans minus the corresponding value for a blank solution. The noise level on the other hand, was calculated as three times the standard deviation in the reported signal for the same set of scans. The scans used in computing the signal and noise in our assays were collected continuously in time by the MS instrument over a period of about 60 s after the signal had reached a steady state value. Also notice that the position along the FFZE channel where the extraction of the peptide from the sample was completed in our device varied with the operating conditions. This position, for example, moved in the upstream direction with an increase in the voltage drop across the electrodes or a reduction in the pressure-driven flow velocity in the FFZE channel. The completion of the peptide extraction process was established in our present work by monitoring the total ion current measured by the mass spectrometer for the human angiotensin I peak. In general, if this current did not rise with an increase in the lateral electric field or a decrease in the pressure-driven flow velocity, the extraction process was assumed to be complete prior to splitting of the liquid stream flowing through the FFZE chamber.

RESULTS AND DISCUSSION

Effect of SDS on Analyte Detection

Although SDS and other anionic surfactants are known to severely interfere with the mass spectrometric detection of cationic analytes, there is only a handful of scientific publications documenting this effect in quantitative terms even for standard samples.^{20,43} In this situation, it becomes necessary for us to perform such a study in order to accurately assess the performance of our FFZE device toward enabling direct ESI-MS analysis of cationic analytes prepared in SDS matrices. To this end, human angiotensin I samples prepared in a solvent containing 25% methanol (v/v) in deionized water and various amounts of SDS were analyzed using the ESI-MS technique. For these experiments, the sample was directly electrosprayed into the mass spectrometer at 3 $\mu\text{L}/\text{min}$ from a 250 μL glass syringe (Hamilton Company USA) using an ESI nozzle. In Figure 2a, we have included some representative mass spectra from these measurements which show a suppression of the human angiotensin I peak with increase in SDS concentration in the sample. Our experiments show that for a sample containing 50 $\mu\text{g}/\text{mL}$ of human angiotensin I, the analyte peak was nearly unaffected by SDS when this surfactant was present at concentrations below 3 μM . Conversely, the signal from the cationic peptide present at the same concentration was observed to be below the noise level when the amount of SDS in the sample was raised above 0.5 mM. Interestingly, the mass spectra for samples with large amounts of SDS are seen to be dominated by peaks which correspond to the sodium adduct of different aggregates of the SDS molecule. This observation actually is in conflict with electrical conductivity measurements previously reported on aqueous SDS solutions which suggest the monomeric form of the surfactant to be the dominant species in such matrices when this reagent is present at levels below its critical micelle concentration.⁴⁴ In Figure 2b, we have presented the signal-to-noise ratio (S/N) for the angiotensin I peak as a function of the peptide concentration in our samples containing different amounts of SDS. The figure shows a linear relationship between these two quantities for the chosen analyte concentrations with the slope of the curve decreasing for larger amounts of SDS in the matrix. The interfering effect of this surfactant was further assessed by estimating the limit of detection (LOD) for human angiotensin I in our samples containing different amounts of SDS. This LOD value was defined in the current analysis as the peptide concentration at which $S/N = 1$ as determined by the curves shown in Figure 2b. Our data shows (see Figure 2c) that the LOD for the chosen peptide was nearly unaffected by the presence of SDS in the sample when the concentration of this surfactant was below 3 μM . However, as the SDS amount in the matrix was raised to 0.3 mM, the limit of detection for the chosen peptide species increased from 1.2 $\mu\text{g}/\text{mL}$ (LOD in the absence of SDS) to 41.4 $\mu\text{g}/\text{mL}$. In fact, for SDS levels above its critical micelle concentration value (~ 8.3 mM at 25 $^{\circ}\text{C}$),⁴⁴ we were unable to detect human angiotensin I in our samples even when this peptide was present at concentrations as large as 1 mg/mL.

Establishment of Peptide Extraction Conditions

Having demonstrated the adverse effects of SDS on the detection of human angiotensin I using the ESI-MS method, we proceeded to explore the possibility of extracting this peptide molecule into an SDS-free solvent stream using the FFZE device shown in Figure 1 in order

to improve its detectability. To this end, samples containing known amounts of the chosen peptide prepared in an SDS containing matrix was introduced cocurrently with an SDS-free ESI-compatible solvent (25% methanol (v/v) in deionized water) into the device after coating its channel walls with *N*-(2-triethoxysilylpropyl) formamide. The electrophoretic extraction was enabled in our experiments by applying a voltage between 1 and 5 V across the width of the analysis channel. In Figure 3a, we have included representative mass spectra from these experiments that demonstrate the dramatic improvement observed in the detectability of the human angiotensin I species based on our approach. This improvement in the detectability of the peptide molecules occurs due to their electrophoretic migration from the SDS containing sample into the ESI-compatible solvent stream. For sample matrices containing SDS below its critical micelle concentration (~8.3 mM at 25 °C), such migration is anticipated as the electrophoretic force acting on the cationic peptide and the anionic surfactant molecules is likely to easily overcome the interaction between these two species. Interestingly, our experiments show that the reported extraction process is also effective for samples with SDS levels as high as 10 mM. Under these conditions, the SDS micelle-peptide complex is speculated to fall apart because of a reduction in the concentration of free SDS molecules in the sample stream during the FFZE process on account of their electrophoretic migration toward the high voltage electrode. This decoupling frees up the peptide species allowing it to then migrate into the SDS-free solvent stream based on its cationic nature. We are currently designing a detailed study for understanding the true mechanism for this peptide extraction process in order to further improve the performance of our FFZE assays. We anticipate the reported assay to be also applicable to extraction of neutral and anionic analytes from sample matrices containing SDS. However, the algebraic difference between the electrophoretic mobility of the analyte and surfactant molecules in that case is expected to be smaller than that in the current situation. As a result, a greater resolving power would be desirable in such assays making them somewhat more challenging to realize. Interestingly, suppression of ESI-MS signal for anionic and neutral analytes by SDS is not as severe, and may therefore yield reasonable *S/N* values for moderate separation between the two species. It must be pointed out that because the electric field in our device focuses the anionic SDS molecules around the high voltage electrode, the concentration of this surfactant in our ESI-compatible solvent stream can be expected to be significantly lower than that predicted based on the diffusive migration of this species across the channel width enabling the clean extractions depicted in Figure 3(a). The data shown in this figure correspond to a sample containing 15 µg/mL of the peptide and 3 mM SDS prepared in the 25% (v/v) methanol-water solvent with the SDS-free solvent flowing at a rate that was 5 times larger than the sample. The dilution of the sample stream as it flowed from the inlet of the FFZE channel to the ESI nozzle was minimized in our experiments by ensuring that the liquid fraction entering port 3 was equal to the relative sample flow rate (ratio of the sample flow rate to the total liquid flow rate) in the system. To control such splitting of the liquid stream between ports 3 and 4, the length of the capillary connected to the waste nozzle was adjusted to tune its hydrodynamic resistance.

Having established the feasibility of our approach to enhancing mass-spectrometry based detection of cationic analytes in SDS containing matrices, we proceeded with experiments that would allow us to characterize the performance of the reported microfluidic device

under different operating conditions. In Figure 3b, we have presented data from such a study depicting the effect of the FFZE voltage on our ability to extract human angiotensin I from an SDS containing matrix. The signal recovery plotted on the y-axis in this figure corresponds to the ratio of S/N values yielded by our FFZE assays to that obtained by electrospraying an SDS-free peptide sample of identical concentration, that is, 15 $\mu\text{g/mL}$, directly into the mass spectrometer. The values marked against the curves in the figure here represent the total liquid flow rate through the analysis channel with the relative sample flow rate set to 1/6. The figure shows an increase in signal recovery with the extraction voltage (ϕ) as expected, that levels off as ϕ approaches 5 V for the 6 $\mu\text{L/min}$ case. For higher liquid flow rates, the residence time for the peptide species in the analysis channel was reduced causing such leveling off to occur at larger values of ϕ . The figure further shows that under the chosen operating conditions, our device was able to recover only about 38% of the signal-to-noise ratio corresponding to the SDS-free 15 $\mu\text{g/mL}$ peptide sample. Careful analysis revealed that a majority of this loss in the S/N value was due to a higher noise level in the total ion current for the human angiotensin I peak. Although higher current intensities were also observed in these recordings for the sample and the blank solutions, the difference between these two quantities (signal) was measured to be similar to that recorded for the SDS-free sample. While the reason behind these observations has not been confirmed, we suspect the larger current intensity and noise level in our FFZE assays to be arising from the unwanted migration of other sample constituents (e.g., sodium ions) from the sample to the SDS-free solvent stream. Nevertheless, no sodium adduct of the SDS aggregates were observed in the mass spectra from these assays for samples containing the surfactant at concentrations below 3 mM. Such SDS peaks only became noticeable for higher levels of the surfactant eventually dominating the mass spectrum for SDS concentrations greater than 10 mM. Moreover, operating at a relative sample flow rate of 1/6 and a total liquid flow rate of 6 $\mu\text{L/min}$ implied a flow of 1 $\mu\text{L/min}$ at the ESI nozzle, which was suboptimal for our assays. Previous experiments had shown that the S/N value for human angiotensin I was reduced by a factor of ~ 1.5 when a flow rate of 1 $\mu\text{L/min}$ was used at the ESI interface as opposed to an optimum choice of 3 $\mu\text{L/min}$ that was employed in the direct electrospray experiments described in Figure 2. A value of 1 $\mu\text{L/min}$ was also determined to be the minimum flow rate needed at the ESI nozzle to realize a stable electrospray in our setup. In addition, minor sample loss into port 4 because of broadening of the peptide stream during the FFZE process may have also contributed to the reduced signal recovery in our assays.

It must be pointed out that the maximum applicable FFZE voltage (ϕ_{max}) before electrochemical gas generation at the microfabricated electrodes started affecting the fluid flow profile was observed to be about 5 V in our system for samples containing SDS at concentrations ≤ 3 mM when the relative sample flow rate was set to 1/6. For higher SDS levels or smaller relative sample flow rates, this voltage dropped substantially due to an increase in the overall electrical conductivity of the stream flowing through the analysis channel. For example, ϕ_{max} was seen to decrease to 4.5 V as the SDS amount in the sample was raised to 10 mM maintaining the relative sample flow rate at 1/6, which turned out to be the most concentrated SDS containing matrix we could electrophoretically extract the peptide from using our FFZE device. For samples with even higher concentrations of the surfactant, the allowable FFZE voltage reduced sharply incapacitating our microfluidic unit

from accomplishing any meaningful extraction of the human angiotensin I species. Finally, we looked into the effect of the relative sample flow rate on the performance of our device maintaining the total liquid flow rate through the analysis channel at 6 $\mu\text{L}/\text{min}$ to hold the same extraction time for our assays (~ 8.4 s). In Figure 3c, the results from this study have been included which show an improvement in signal recovery for large values of ϕ with reduction in the relative sample flow rate. This trend, however, is counterintuitive as the migration distance to the ESI solvent stream for the peptide molecules reduces with an increase in the relative sample flow rate. The reported observation nevertheless may be explained based on the fact that as the relative sample flow rate was increased in these experiments, a higher noise level in the total ion current measurement was noticed again likely due to an increased migration of the unwanted matrix ions to the ESI-compatible solvent stream. Interestingly, the dependence of the signal recovery on the relative sample flow rate is reversed for small values of ϕ as the S/N value yielded under these conditions is limited by the sample loss into port 4 due to incomplete extraction of the peptide species into the SDS-free solvent stream. Although the figure suggests that higher signal recovery may be possible at an extraction voltage of 5 V for relative sample flow rates even lower than 1/6, these conditions correspond to a liquid flow rate below 1 $\mu\text{L}/\text{min}$ through port 3 which was unsuitable for the ESI process.

Improvement in Analyte Detectability using the FFZE Device

On the basis of the findings reported in the previous section, the improvement in the detectability of human angiotensin I in SDS samples was quantitated by performing our assays setting the relative sample flow rate at 1/6, $\phi = \phi_{\text{max}}$ and total liquid flow rate to 6 $\mu\text{L}/\text{min}$. Figure 4a shows that the reported FFZE device preserves the linear relationship between the recorded S/N value and human angiotensin I concentration rendering our assays simple to quantitate. Moreover, the slopes of these lines are seen to decrease with an increase in the amount of SDS in the sample as expected because of a larger noise level in the total ion current measurements. However, this decrease is prominent only at significantly larger values of SDS as compared to that seen in the case of the unprocessed samples (see Figure 2b). For example, while SDS levels below 0.01 mM were not observed to substantially deteriorate the signal-to-noise ratio for the unprocessed specimens, the corresponding value for the samples processed with the reported FFZE device was about 0.3 mM. Unsurprisingly, the signal recovery by our microchip unit was seen to deteriorate for specimens with larger amounts of SDS. In the case of a 15 $\mu\text{g}/\text{mL}$ angiotensin sample prepared in a matrix containing 3 mM SDS, Figure 3c shows such recovery to be only about 38% which increases to a value over 60% for a 5 $\mu\text{g}/\text{mL}$ peptide sample containing vanishing amounts of the surfactant (see Figure 4b). This loss in S/N value also translated into an increase in the limit of detection for the peptide species upon FFZE processing relative to the SDS-free case. Figure 4a shows that as the SDS level in the sample is varied between 0.3 mM and 10 mM, the LOD for the processed specimen increased from 1.5 to 3.7 $\mu\text{g}/\text{mL}$. Notice that the LOD values for the SDS-free sample were recorded to be 1.33 and 1.27 $\mu\text{g}/\text{mL}$ for our FFZE assays and the direct electrospray experiments, respectively. Finally, in Figure 4b we have compared the S/N value for a 5 $\mu\text{g}/\text{mL}$ peptide sample containing different amounts of SDS as yielded by our FFZE device and that by directly electrospraying the specimen into the mass spectrometer. The figure highlights the ability of

our microchip unit to handle significantly higher levels of SDS in a sample matrix. As a comparison, the reported device was seen to yield the same signal-to-noise ratio (~ 2) for a specimen containing 2.5 mM SDS as that measured for an unprocessed sample having 0.025 mM of the surfactant.

To establish the generality of our approach for extracting cationic analytes prepared in SDS containing matrices, we have also applied it to detecting another peptide, MRFA, having a molecular weight of 523.6 Da. The results from these assays have been included in the Support Information and were obtained setting the total liquid flow rate at 6 $\mu\text{L}/\text{min}$, the relative sample flow rate at 1/6 and $\phi = \phi_{\text{max}}$. Again, a significant improvement in the ESI-MS based detection of MRFA ($\text{pI} \approx 11$) was observed in our experiments for samples processed with the reported FFZE device. Interestingly, the signal recovery for this peptide was found to be similar to that for human angiotensin I ($\text{pI} \approx 8$) in spite of its greater electrophoretic mobility under the operating conditions. It must be pointed out that while the reported microfluidic device has been presented as an isolated platform for extracting cationic analytes from SDS containing matrices, it can be readily integrated downstream of an MCE column or an MCE/PAGE microchannel. The latter approach can be particularly attractive when realized on a monolithic footprint as it would likely minimize the band broadening introduced at the interface of the separation and the SDS cleanup units. While the current work does not focus on any such integration, it lays down a strong foundation for realizing this ultimate objective by successfully applying the FFZE method toward extracting cationic analytes from SDS containing matrices.

CONCLUSIONS

To summarize, we have demonstrated large improvements in the mass spectrometric detection of two model cationic analytes prepared in an SDS containing matrix using a microchip based free-flow zone electrophoresis device. The reported improvement was realized through electrophoretic extraction of the cationic analytes into an SDS-free ESI-compatible solvent stream in a continuous fashion and subsequently electrospraying this liquid into a mass spectrometer. Applying this approach, we were able to detect human angiotensin I and MRFA at concentrations down to 3.7 and 6.3 $\mu\text{g}/\text{mL}$, respectively, in samples containing as much as 10 mM SDS. This corresponded to an improvement in the limit of detection for the peptides by at least a factor of 270 and 159, respectively, under the chosen conditions, which otherwise were undetectable using the ESI-MS method in the original sample even when present at levels as large as 1 mg/mL . With the reported device, however, we were unable to realize any meaningful extractions using samples containing over 10 mM of SDS as the maximum applicable FFZE voltage in our system dropped sharply for such specimens because of electrochemical gas generation at the microelectrodes. It is important to point out that this restriction on the SDS concentration, although may be undesirable, is not as debilitating as it may seem. Several MCE separations of practical interest, for example, have been realized with buffers containing this surfactant at 10 mM concentration or lower levels.^{45–47} Moreover, it is possible to apply the reported approach toward analyzing liquid samples prepared in matrices with higher SDS concentrations by simply diluting the specimens to bring down the surfactant level in them below the 10 mM mark. Although this strategy would somewhat compromise the

detectability of the analytes, the overall detection limit for the assay is expected to be still substantially better than that realized without employing any SDS cleanup procedures. Nevertheless, we are currently remodeling our device to allow its operation using electrodes placed outside of the extraction channel in order to realize larger electric fields for the FFZE process. In addition to enabling our microfluidic system to extract low mobility analytes, for example, proteins, or species with mobilities similar to that of SDS, e.g., anionic peptides, such remodeling will permit the direct usage of higher ionic strength samples (including those containing SDS at concentrations greater than 10 mM) and MS compatible solvents in our assays expanding their applicability to analyzing more diverse specimens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This research work was supported by the Defense Threat Reduction Agency (DTRA) through contract HDTRA1-09-C-0013. D.D. also acknowledges funds from the National Science Foundation (DBI 0964211) and the National Institutes of Health (1R15AG045755-01A1) for completing some of the experiments included in this manuscript.

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Figure 1.

(a) Image of the FFZE device used in our current work to electrophoretically extract human angiotensin I and MRFA from an SDS containing matrix. Notice that the channel layout in this image has been outlined artificially for clarity. (b) Schematic of the FFZE device depicted above.

Figure 2.

(a) Mass spectra for 50 $\mu\text{g/mL}$ human angiotensin I samples containing different amounts of SDS. (b) Recorded signal-to-noise ratios as a function of human angiotensin I concentration in samples containing different amounts of SDS. (c) Measured variation in the limit of detection (LOD) for the human angiotensin I species with increase in SDS concentration in the sample matrix.

Figure 3.

(a) Mass spectra for a 15 $\mu\text{g/mL}$ human angiotensin I sample containing 3 mM SDS before and after processing it with the reported FFZE device. An extraction voltage of 5 V was used for generating the top spectrum while the middle one was recorded by electrospraying the original sample directly into the MS instrument. The bottom spectrum corresponds to a direct electrospray measurement of the 15 $\mu\text{g/mL}$ human angiotensin I sample containing no SDS. A total liquid flow rate of 6 $\mu\text{L/min}$ and a relative sample flow rate of 1/6 were chosen for these experiments. (b) Effect of the FFZE voltage on the peptide extraction process for various total liquid flow rates through the analysis channel (indicated by the number associated with each curve). The flow rate for the sample was set to 1/6th of the

total liquid flow in these experiments. (c) Effect of the FFZE voltage on the peptide extraction process for various relative sample flow rates (indicated by the number associated with each curve) with the total liquid flow rate set to 6 $\mu\text{L}/\text{min}$. The signal recovery plotted on the y-axis in panels b and c here corresponds to the ratio of S/N values yielded by our FFZE assays to that obtained by electrospraying an SDS-free peptide sample of identical concentration, that is, 15 $\mu\text{g}/\text{mL}$, directly into the mass spectrometer.

Figure 4.

(a) Response curves for human angiotensin I samples containing different amounts of SDS after processing them using the reported FFZE device. (b) Signal-to-noise ratios recorded for 5 $\mu\text{g/mL}$ human angiotensin I samples containing different amounts of SDS before and after processing with the reported FFZE device. The data included in both these figures were obtained by setting the relative sample flow rate to $1/6$, $\phi = \phi_{\text{max}}$, and the total liquid flow rate through the extraction channel to 6 $\mu\text{L/min}$.