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Development of a Fritless Packed Column for Capillary Electrochromatography-Mass Spectrometry

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Abstract

A novel procedure was developed for the fabrication of a fritless packed column for the coupling of capillary electrochromatography (CEC) to mass spectrometry (MS). The process involved the formation of internal tapers on two separate columns. Once the internal tapers are formed and the columns are packed, the untapered ends of each column were joined together by a commercially available connector. Several advantages of the fritless columns are described. First, the design used here eventually eliminates the need for any frits thus reducing the possibility of bubble formation seen with fritted packed columns. In addition, this is the first report in which the internal tapers are formed at both the inlet and outlet column ends making the fritless CEC-MS column more robust compared to only one report with externally tapered counterparts. Second, a comparison of internally tapered single frit packed CEC-MS (previously developed in our laboratory) column versus fritless CEC-MS column reported here shows that the latter provides better efficiency, suggesting no dead volume with equally good sensitivity and chiral resolution of ($\pm$)-aminoglutethimide. The fritless column procedure is universal and was used to prepare a series of columns with a variety of commercially available packing material (mixed mode strong cation exchange, SCX; mixed mode strong anion exchange, SAX; C-18) for the separation and MS detection of short chain non-chromophoric polar amines, long chain nonchromophoric anionic surfactant as well as oligomers of non-chromophoric non-ionic surfactants, respectively. The fritless columns showed good intra-day repeatability and inter-day reproducibility of retention times, chiral and achiral resolutions and peak areas. Very satisfactory column-to-column and operator-to-operator reproducibility was demonstrated.

Keywords

Novel Fritless column; Chiral CDMPC-SO₃; C₆/SCX packing; C₆/SAX packing; C-18 packing

1. Introduction

The coupling of packed column capillary electrochromatography (CEC) with mass spectrometry (MS) detection is one of the most recent hyphenated versions of capillary electrophoresis-MS [1–3]. Separations carried out with CEC combine the high efficiency of capillary electrophoresis (CE) with the excellent selectivity afforded by high performance

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liquid chromatography (HPLC) stationary phases. The use of MS detection has two major advantages over other detection systems like ultraviolet (UV) detection. The first is the high MS sensitivity that can be reached with the use of newer generations of mass spectrometers. In addition, MS provides molecular weight and structural information of the analyte from the formation of the molecular ion, daughter ions and the elucidation of fragmentation mechanisms. The CEC-MS system using various column designs and interfaces have been used for the analysis of a wide variety of compounds including pharmaceuticals [4–5], surfactants [6–8], proteins [9–10], and carcinogens [11].

Unlike CEC with UV detection, the use of packed columns with two frits is problematic when CEC is coupled to mass spectrometry (MS). The drawback consist of irreproducible retention times and even current breakdown, which occur due to the extensive bubble formation at the outlet end of the CEC column exposed to the nebulizing end of the MS instrument. This is because the packing material introduced into CEC capillaries is retained by sintering or frit formation. As mentioned above, this process can lead to bubble formation due to the change in electroosmotic flow (EOF) caused by differences in the physical and chemical nature of the sintered frit, especially the zeta potential () [12]. In CEC-UV, this problem can be overcome by pressurizing the inlet and outlet vials during the experiment. Unfortunately, this problem is exacerbated in CEC-MS, being the outlet end of the capillary directly exposed to the nebulization process inside the spray chamber of the MS, which is held at atmospheric pressure. Lord's group [13] worked to reduce some of the bubble formation by replacing the outlet retaining frit with an external taper, which maintains the packing material using the "keystone effect." Due to the extreme fragility and special equipment required to make external tapers, our group and others develop internally tapered capillaries [14–15]. These internally tapered capillaries were easier to fabricate due to simplified equipment requirements and the packed columns were more robust and stable due to the internal nature of the taper. This led to their recent applications for both achiral and chiral CEC-MS [16–19]. Although, using both external and internal tapered CEC-MS column, the potential for bubble formation due to the outlet frit is reduced, the possibility for problems caused by the inlet frit still remains.

Work is now ongoing to remove this inlet side frit in CEC-UV [19] and CEC-MS [20] and analysis. Her's group [20] recently reported in a brief communication on the fabrication CEC-MS capillaries with a single tapered end positioned on the inlet side and an untapered end on the outlet side. Using a negatively charged stationary phase, the electrophoretic mobility of the stationary phase pulls most of the packing material toward the inlet reservoir keeping it from moving towards the MS ionization source. However, according to the authors, with this design, some of the packing material near the outlet side of the CEC-MS column tends to get loose and migrate into the nebulizer, potentially clogging it. Therefore, to keep the particles from clogging the nebulizer an external taper was also formed at the outlet side of the capillary by these researchers. While this modification kept the particles from leaving the capillary, it did not always prevent the particles from moving towards the outlet end of the column due to the empty space between the tapered end and the packing material resulting in dead volume.

The research presented here is aimed to the development of a more universal and rugged fritless CEC-MS column using two internally tapered capillaries joined together by a commercially available New Objective PicoClear connector (Figure 1). This fritless column design overcomes the distinct disadvantages that are present in traditional two fritted capillary columns, externally tapered columns or one report on externally tapered fritless column discussed briefly in the recent literature [20]. As mentioned above, the fritless column design reported here is more universal making the design compatible with a variety of packing materials. The joined capillaries can be conveniently conditioned with mobile

phase on an external pump, followed by voltage conditioning in the CEC instrument in a similar fashion to conventionally packed fritted CEC columns. The robustness of separation ability of the fritless CEC-MS column are demonstrated with 90 back-to-back runs using a chiral stationary phase. Next, a central composite multivariate design was used to vary the inlet/outlet taper diameters to optimize R_s and S/N using enantiomers of ($\pm$)-aminoglutethimide (AG) as model test analyte. This was followed by packing the fritless columns with various commercially available mixed mode cation exchange (C_6/SCX), mixed-mode anion exchange (C_6/SAX) and reversed phase (C-18) packing materials for challenging separation and detection of non-chromophoric cationic amines as well as anionic and neutral surfactants, respectively under various CEC-MS conditions. **The intra-day and inter-day repeatability**, column-to-column reproducibility, as well as operator-to-operator skill for reproducibly packing fritless packed column was also found to be very satisfactory.

2. Experimental

2.1. Reagents and materials

The 3 μ m, 100 Å C_6/SCX (strong cation exchange), C_6/SAX (strong anion exchange) and C_{18} non-end capped silica packing material were purchased from Column Engineering, Inc (Ontario, California). The sulfated cellulose dimethylphenylcarbamate (CDMPC- SO_3) chiral stationary phase was synthesized in our lab according to procedures detailed previously [17]. The ($\pm$)-aminoglutethimide (AG), non-ionic surfactants Brij 30 and 56, monomethylamine (MMA) and dimethylamine (DMA) and 8-quinolinesulfonyl chloride were purchased from Sigma-Aldrich (Milwaukee, WI). The sodium alkyl sulfate salts, which include sodium n-hexyl sulfate (C_6-SO_4), sodium n-octyl sulfate (C_8-SO_4), sodium n-decyl sulfate ($C_{10}-SO_4$), sodium n-undecyl sulfate ($C_{11}-SO_4$), sodium n-dodecyl sulfate ($C_{12}-SO_4$), sodium n-tetradecyl sulfate ($C_{14}-SO_4$), sodium n-hexyldecyl sulfate ($C_{16}-SO_4$), sodium n-octadecyl sulfate ($C_{18}-SO_4$) were purchased from Lancaster Synthesis, Inc (Windham, NH). The HPLC grade organic solvents acetonitrile (ACN) and methanol (MeOH) were purchased from Fischer Scientific (Fair Lawn, NJ). Triethylamine (TEA) was purchased from Aldrich (99.5% Milwaukee, WI). The ammonium acetate (NH_4OAc) and ammonium formate (NH_4CO_2H) were obtained from Sigma (St. Louis, MO) as a 7.5 M solution and as a solid powder, respectively. All water used in the project was triply deionized using a Barnstead Nanopure II Water System (Barnstead International, Dubuque, IA).

2.2. Fritless CEC-MS Column Fabrication

Fused silica capillaries (O.D. 363 μ m $\times$ I.D. 75 μ m) obtained from Polymicro Technologies, Inc. (Phoenix, AZ) were used to construct the fritless CEC-MS capillaries. The process of internal taper formation and packing of the capillary have been outlined previously [15]. Briefly, two sections of capillary were cut to approximately 60 cm lengths. One end of each section was then tapered to the desired diameter (e.g., 15 μ m for inlet and 15 μ m for outlet after optimization) using a methane/oxygen micro-torch. Examples of these tapers can be seen in the Supplementary Information Figure S-1 (A–B). Using a 16 mg/mL slurry in ACN, the inlet portion of capillary was packed to 25 cm and the outlet portion was packed to 45 cm with the appropriate packing material. The capillaries were packed 10 cm beyond the length that was originally used in the experiments. This 10 cm of additional material increased the pressure at the points the columns would be cut ensuring that no particles would fall out of the 15-cm or 35-cm portion of the packed column. In addition, during packing the columns were left on the pump for over 2 hours after filling the capillary to also ensure tight packing of the stationary phase. The packed capillaries were cut to the appropriate lengths (15 cm for the optimized inlet side, 35 cm for the optimized outlet side shown in Figure 1), and were observed under magnification to ensure the untapered ends

had no cracks or irregularities, which could lead to leaking inside the connector. Next, the cut capillaries were joined together using a PicoClear Connector purchased from New Objective, Inc. (Woburn, MA), Supplementary Information Figure S-1 C and D. The union was observed under a microscope to ensure that no dead volume was left between the untapered halves of each capillary as shown in Figure S-1E. After successful conjunction, the capillary was conditioned on an HPLC pump with the running mobile phase, before voltage conditioning on the CEC instrument.

2.3. Single Fritted-Single Taper CEC-MS Column Fabrication

Fused silica capillaries (O.D. 363 μm $\times$ I.D. 75 μm) obtained from Polymicro Technologies, Inc. were also used to construct the single frit-single taper CEC-MS capillaries. The process of internal taper formation and slurry packing was the same as described in the previous paragraph. Once the capillary was packed to the desired length of 50 cm, an inlet frit was burned with a homemade frit burner and the excess empty column was cut away. The column was then conditioned on an HPLC pump with the running mobile phase, before voltage conditioning on the CEC instrument.

2.4. CEC-ESI-MS Instrumentation

The interface of the CE to MS was made possible by a G1603A CE-MS adapter kit and a G1607 CE-ESI-MS sprayer kit (both provided by Agilent Technologies, Palo Alto, CA). The sheath liquid was delivered to the ESI by an Agilent 1100 series HPLC pump equipped with a 1:100 splitter. All instrument controls and data analysis, including resolution calculations, were carried out using Agilent ChemStation and CE-MS add-on software (version B.04.02).

2.5. CEC-ESI-MS Conditions

Unless otherwise stated the following conditions were used to carry out the experiments. Based on our previous work [17], the chiral separation of ($\pm$)-AG was performed using a 70% ACN 5 mM $\text{NH}_4\text{CO}_2\text{H}$ pH 3.5 mobile phase using CDMPC- SO_3 packed column at 25 kV and 25°C. Analyte injection was done electrokinetically at 6 kV, 6 sec. The sheath liquid used was 90% MeOH 50 mM NH_4OAc at a flow rate of 5 $\mu\text{L}/\text{min}$. The spray chamber for the MS was set to a nebulizer pressure of 6 psi, drying gas flow rate of 5 L/min, and a drying gas temperature of 250°C. The enantiomers of AG were observed as the $[\text{M}+\text{H}]^+$ ion using select ion monitoring (SIM) at m/z of 233. The CZE-MS conditions for the separation of the underivatized methylamines were as follows: background electrolyte of 5 mM NH_4COOH at pH 3.0 with an applied voltage of 15 kV at 25°C. The CZE capillary was 65 cm (56.5 cm effective) $\times$ 50 μm i.d. The analytes were 0.5 mg/mL in 50/50 ACN/ H_2O injected at 10 mbar for 5 seconds. Taking into account our earlier work with other cationic compounds [7], the CEC-MS analysis of the methylamines were done using a mobile phase of 70% ACN, 15 mM NH_4OAc , 0.04% TEA pH 3.0 mobile phase with 15 kV applied at 25°C using mixed mode hydrophobic and cation exchange C_6/SCX packing material (attached to 3 μm , 100 Å silica particles). The methylamines injection was done electrokinetically at 10 kV, 10 s. The sheath liquid used was 70% MeOH 10 mM NH_4OAc with a flow rate of 7 $\mu\text{L}/\text{min}$. The spray chamber for the MS was set to a nebulizer pressure of 5 psi, drying gas flow rate of 5 L/min, and a drying gas temperature of 200°C. The underivatized MMA and DMA were observed as the $[\text{M}+\text{H}]^+$ ion using SIM at m/z of 32 and 46, respectively. On the other hand, the 8-quinolinesulfonyl derivatized forms of MMA and DMA, were seen at the respective m/z of 223 and 237. The separation of the alkyl sulfates was done using various ACN concentrations with 15 mM NH_4OAc , pH 7.5 mobile phase with 25 kV applied at 25°C using mixed mode hydrophobic and anion exchange C_6/SAX stationary phase (attached to 3 μm , 100 Å silica particles). Analyte injection was done electrokinetically at -5 kV, 5 s. The sheath liquid and spray chamber conditions were the same as that used for the cationic

analysis. The alkyl sulfates were observed as deprotonated molecular ions using a group SIM at m/z 181, 209, 237, 251, 265, 293, 321, and 349. The final experiments with the Brij 30 and 56 non-ionic surfactants were carried out using 80% ACN, 5 mM Tris, pH 8.0 mobile phase with electrokinetic injection at 10 kV, 10 s using a C_{18} stationary phase (attached to 3 μ m, 100 Å silica particles). The sheath liquid of 80% MeOH 1 mM NH_4COOH was delivered at 5 μ L/min. The MS spray chamber parameters were set to a nebulizer pressure of 5 psi, drying gas flow rate of 5 L/min, and a drying gas temperature of 200°C. The mobile phase conditions on nonchromophoric Brij series are based on our previous studies of chromophoric Triton series nonionic surfactants [8]. In order to observe as many different oligomers of the non-ionic surfactant, positive MS scans were carried from m/z 450–1150 for Brij 56, and m/z 350 – 800 for Brij 30. All analytes were prepared by dilution of stock solutions with water to reach the reported concentrations shown in the appropriate figure caption.

2.6. CEC-ESI-MS Instrumentation and conditions

All CEC-MS experiments were carried out using an Agilent capillary electrophoresis (Agilent Technologies, Palo Alto, CA) instrument hyphenated to an Agilent 1100 series single quadrupole mass spectrometer (MS) or hyphenated to a triple quadrupole mass spectrometer. Specific instrument settings and conditions are detailed in the Supplementary Information.

2.7. Multivariate data analysis

All multivariate experimental design data analysis and calculations were carried out using Design Expert 7 software (Stat-Ease, Inc, Minneapolis, MN). This included generation of response surface models (RSM), analysis of regression coefficients at 95% confidence interval and prediction of optimum conditions of the experimental design based on the criteria of best resolution, minimum overall run time, and higher signal-to-noise (S/N).

3. Results and discussion

3.1. Fritless and Single Frit Tapered Column Comparison

The first set of experiments were conducted to determine how the fritless column compares to the traditional single frit column. This comparison was done to test the robustness using the results of 90 consecutive runs for chiral separation of ($\pm$) aminoglutethimide (AG) on each type of column under the same CEC-MS conditions reported in our previous work [17]. Looking at Figure 2A and 2B, clearly the fritless column performs equally well to single frit column when comparing 90 runs on the two columns to determine the precision of S/N , efficiency (N) and resolution (R_s) under normal conditions. Note that the average N on the fritless column, 42,000 ($\pm 2,200$) plates/meter are slightly greater than values obtained for the single fritted column, 36,000 plates/meter ($\pm 2,000$). The slight improvement in N of the fritless column can most easily be explained by the absence of the inlet side frit in the fritless column, which would lead to less chance for bubble formation and disturbance of the flow through the capillary. In addition, the slightly higher N may be attributed to the higher permeability of the fritless column. Any disturbances in the current from bubble formation can lead to an unstable electroosmotic flow (EOF). On the other hand, destabilized EOF leads to the peaks being broader and less efficient because the EOF slows down and then speeds up causing the electromigration of the analytes to slow down and speed up as well.

The fritless column provides slightly faster analysis time while still maintaining baseline resolution of the ($\pm$)-AG. When comparing the reproducibility of the single fritted column versus the fritless column, the fritless column is shown to be just as stable and robust as the single fritted column with good replication of stable current (Figure 2C inset). The average

retention times ($t_{r_{avg}}$) for the two enantiomers of AG for 90 replicate injections using the fritless column have lower inter-day %RSD of 3.7 (Supplementary Information, Figure S-2), as compared to the %RSD of 5.5 for the single fritted column (Supplementary Information, Table S-1 column 1). The intraday repeatability (%RSD = 3.2 – 3.6, $n = 30$) of the $t_{r_{avg}}$ for the fritless column is also lower as compared to that of the single frit column (%RSD = 5.3 – 5.5, $n = 30$). When comparing the intra-day repeatability of the average peak areas, peak efficiency, signal-to-noise as well as resolution, the %RSD were very similar for both columns, (Supplementary Information, Table S-1 columns 2–5).

3.2. Multivariate optimization of inlet/outlet taper diameter

Once the feasibility of the fritless column had been established, multivariate optimization of the taper diameters of the fritless column was conducted to improve the R_s and S/N while still maintaining a homogenous tight packed bed. This was done using a central composite design (CCD). Initial studies in our laboratory established that the 20- μ m was the largest aperture for the outlet taper, which could be used without loss of particles from the capillary. In addition, the same work suggested the 10- μ m taper was shown to be the smallest taper diameter that could be used while still maintaining good flow through the inlet side of the column (data not shown). With the upper limit of inlet/outlet diameter set at 20 μ m, the lower diameter limit and the midpoint value were set at 10 μ m and 15 μ m, respectively (Table S-2, Supplementary Information). This resulted in 21 different combinations to explore the effects of inlet/outlet taper diameter on R_s , S/N and $t_{r_{avg}}$ allowing only a deviation of ± 0.1 μ m in the taper diameters. The results of these experiments are available in last three columns of Table S-2 of the Supplementary Information. Using the five repeated mean value runs (experiments 2, 9, 14, 17, and 20) the reproducibility of the model was determined to be good with %RSD for S/N at 9%, R_s at 7%, and average t_R at 4%. Over the course of all 21 experiments the ranges for the different S/N values were 378 – 550, 3.1 – 4.7 for the R_s and 15.3 – 21.0 min for the average t_R .

Figure 3, A–B shows the response surface plots (i.e., effects of the taper diameters) on R_s and S/N . Looking along the outlet diameter axis a very small change is seen in R_s as the diameter increases (Figure 3A). In contrast, a major shift can be seen in R_s along the inlet diameter axis. As the inlet diameter becomes smaller, R_s increases. This leads to the best chiral R_s being observed at a large outlet diameter and a small inlet diameter. This arrangement makes sense, because a smaller inlet taper would decrease the amount of sampled loaded onto the column. This in turn would lead to smaller peak widths and better efficiency. The improved efficiency could improve the R_s value.

The effect of the taper diameter on average S/N had very interesting trends (Figure 3B). The average S/N for the AG enantiomers, has no appreciable effect when looking at the axis for the outlet taper diameter. However, the inlet diameter, had a large effect as shown in the steep slope of the inlet diameter axis in the RSM plot. The average S/N increases as the inlet taper diameter becomes smaller. Initially this seemed counter to expectations, because the smaller the diameter the less sample would be expected to be introduced, thus lowering the signal and by extension the average S/N . Upon looking at the individual RSM plots for signal and noise data (Figure S-3, Supplementary Information) it can be seen that the signal does decrease only slightly with the shrinking of the taper, but the noise drops more rapidly than the decrease in signal. Therefore, the S/N ratio actually increases because the denominator is decreasing more rapidly than the numerator. No ready explanation is available at this time on this trend. Once the various combinations of inlet and outlet taper experiments were completed, the optimum diameters for each end of the columns could be predicted by the statistical DOE software. Because resolution does depend on retention time, the software was set to predict the optimum inlet and outlet taper diameter within the constraints of minimizing retention time of the second AG enantiomer while maximizing R_s

and average S/N . The software predicted that with an inlet taper diameter of 11 μm and an outlet taper diameter of 20 μm , the retention time of the last enantiomer of AG would be 16.6 minutes with a R_s of 4.3 and average S/N of 500. Two fritless columns were packed with the predicted taper dimensions for the inlet and outlet and joined to test the predicted optimum values. As shown in the inset caption (Figure 3C), the predicted optimum results match closely those of the experimental results of a t_R of 16.7 min of the last eluting AG enantiomer, R_s of 4.0 and average S/N at 550.

3.3. CEC-MS of short chain non-chromophoric amines

Once the inlet and outlet taper diameters were optimized, the first of the experiments showing the applicability of the fritless CEC-MS system was done using a C_6/SCX packing material. This packing material (3 μm , 100 $\text{\AA}$), which essentially possess mixed mode hydrophobic and cation exchange mechanism was used in an attempt to separate and detect non-chromophoric monomethylamines (MMA) and dimethylamines (DMA) present in dried lobster blood samples. Derby's lab has shown that spiny lobsters emit blood-borne alarm cues through their hemolymph that causes avoidance and suppression of feeding in other nearby lobsters [21]. It is theorized that these alarm cues may be based on simple amines present in the lobster blood. In addition, these amines have importance in tumor etiology [22]. Work has been done previously utilizing HPLC-UV [22–24], CEC-MS [25] and CZE-MS [26] for the separation of low molecular weight amines similar to the ones examined here. Our group has also carried out the analysis of these low molecular weight amines in CZE-MS mode. The results in CZE-MS were disappointing with poor efficiency and irreproducible run times (Figure S-4, Supplementary Information).

As shown in Figure 4A–C, the separation of underivatized MMA and DMA using on-line CEC-MS is not only challenging but their detection also poses a significant problem. For example, when looking at the total ion chromatogram (TIC) (Figure 4A) no peaks of MMA and DMA were observed above the background noise. However, the off-line direct infusion MS spectra showed the protonated molecular ion of each (data not shown). Thus, extract ion chromatograms (EIC) were obtained from the TIC at m/z 32 for MMA and 46 for DMA, respectively. These EIC are shown in Figure 4B and 4C, respectively. Unfortunately, the MMA and DMA peaks are not only broad and tailed in CEC-MS, but are very difficult to resolve due to similar electrophoretic mobility, which results in lower resolution and selectivity. In addition, due to the low m/z of the analytes, the S/N of the underivatized amines remains very weak for these two compounds.

To improve separation, selectivity, and MS detection, both amines were derivatized with 8-quinolinesulfonyl chloride to their 8-quinolinesulfonyl amide analogs, according to a procedure reported in the literature for HPLC-UV [23]. Interestingly, when these methylamines were derivatized (Figure S-5, Supplementary Information) significantly enhanced detectability in ESI-MS was observed. Furthermore, derivatization of MMA and DMA showed improved separation selectivity due to stronger retention via dual hydrophobic and cation exchange mechanism on C_6/SCX fritless column. Thus, using a group SIM of m/z 223 for derivatized MMA and 237 for the derivatized DMA, very good separation and high abundance could be obtained in CEC-MS/MS as illustrated in Figure 4D. As shown in Figure 4E, good LOD (244 ng/mL for MMA derivative; 122 ng/mL for DMA derivative) was obtained with CEC-MS/MS when using the precursor ions of 223 m/z and 237 m/z for derivatized MMA and DMA, respectively, and the product ion of 128 m/z for both in MRM mode even when spiked into aqueous samples of lobster hemolymph. In the range between 0.5 mg/mL and 122 ng/mL for DMA and 0.5 mg/mL and 244 ng/mL for MMA, the calibration curve showed good linearity with R^2 values of 0.9984 and 0.9961 for derivatized MMA and DMA, respectively (Figure 5). This application clearly suggests the potential of the fritless CEC-MS/MS system for difficult separations, quantitation and

enhanced detection of very small molecular weight compounds, which is possible with very little modification or difficulty.

3.4. CEC-MS of long chain non-chromophoric anionic surfactants

Alkyl sulfates are used extensively as cleaning agents, emulsifiers, solubilizers and stabilizers [27]. The wide spread use of these agents has led to extensive work being done for quality control and environmental contamination analysis using thin layer chromatography [28–29], gas chromatography [30], capillary electrophoresis [27,31] and liquid chromatography [32].

As noted before [20], other tapered fritless columns in CEC-MS have been restricted to using only negatively charged stationary phases to retain the packing material. A mixed-mode (C_6/SAX) material was packed and used in the fritless CEC-MS format for the separation and detection of non-chromophoric anionic alkyl sulfates with different chain lengths. The unique combination of C_6/SAX material and non-chromophoric nature of these analytes makes them, especially suitable to their analysis with the packed column CEC-MS system.

The alkyl sulfates with different chain length ($C_6-C_{18}SO_4^-$) were screened using different concentration of ACN as the mobile phase organic modifier to achieve baseline separation without excessive run time and good selectivity. As illustrated in Figures 6A–B, above 85% (v/v) ACN leads to a reduction in run time but the peak shapes for several long chain alkyl sulfates become distorted and often irreproducible. This is most likely due to the surfactant molecules of different chain length forming mixed aggregates at larger % (v/v) ACN [33]. The reduction in ACN to 85% and 80%, as shown in Figure 6C and 6D, respectively, improves resolution and provided reproducible peak shapes for all alkyl sulfates but increases the overall run time of the separation unnecessarily. When looking at the selectivity illustrated in the inset plot in Figure 6, the general trend is toward an increase in selectivity with the reduction in the amount of ACN in the run buffer. The mobile phase containing 85% (v/v) ACN (Figure 6C) was chosen to be the best compromise between the total run time vs. resolution and selectivity of the separation. In addition, column-to-column and operator-to-operator reproducibility skills were tested with the separation of C_6-SO_4 , C_8-SO_4 and $C_{10}-SO_4$ using C_6/SAX stationary phase examining retention time, peak area, peak width and efficiency. The chromatogram shown in Figure 7 and the data tabulated in Table 1 demonstrates that the fritless column is stable and robust with respect to reproducibility and repeatability of retention time, peak area, peak width and peak efficiency. Future work in this area will look further at optimizing the other parameters of the separation such as pH, electrolyte concentration and applied voltage.

3.5. CEC-MS for separations of non-chromophoric-nonionic surfactants

The last class of compound studied involves the use of non-chromophoric, non-ionic Brij series surfactants namely Brij 30 and Brij 56 with C_{12} and C_{16} chain length containing $n = 4$ and $n = 10$ average degree of ethoxylation (see Figure 8 inset). The separation of Brij 30 and Brij 56 is challenging with CE-UV detection due to the lack of electrophoretic mobility and lack of UV absorbing moiety. **It must be acknowledged that CEC analysis (without gradient) may not be ideal for the study of Brij series surfactants. There is potential that new ultrahigh pressure liquid chromatography-MS may be better than CEC.** However, due to previous work in our group showing the separation of the chromophoric non-ionic Triton X-series of surfactants [8,34], analysis of non-chromophoric Brij series surfactants with CEC-MS seems a good example of the fritless columns separation capabilities. The mobile phase (80/20 ACN/ H_2O , 5 mM Tris pH 8.0) from this previous work [8] was used as a starting point for the CEC-ESI-MS of two

of the Brij series of surfactants. The stationary phase used to pack the column was a simple uncapped C_{18} packing material. Traditionally, Tris buffers are not used with mass spectrometric analysis, but no significant signal suppression of Brij series peaks was observed (even in scan mode) when the buffers were used in small enough concentrations (less than 10 mM) in packed column CEC-MS operating at nL/min flow rates.

The CEC-MS of oligomers for the Brij 30 surfactant is shown in Figure 8A. As mentioned earlier, Brij 30 is a twelve-carbon surfactant that has an average degree of ethoxylation of four, but as shown this is not the only oligomer present in the sample. Multiple peaks are shown for the Brij 30 because each peak is associated with separate oligomer with C_{12} hydrocarbon chain length. The elution order of these oligomers on the C_{18} stationary phase is based primarily on increasing hydrophobicity. The range of retention times of the different ethoxylated forms varies from $[M+H]^+$ $m/z = 363$ for the $n=4$ oligomer (peak 1) to $[M-C_2H_5O_2+2H]^+ = 611$ m/z (peak 16) for $n = 11$. Between peaks 1–16, there are seven peaks (4–6, 8, 10, 12, and 14) with degrees of ethoxylation $n = 7$ to 13, corresponding to $[M+NH_4]^+$ ions accounting for the most abundant peaks. The next most prevalent adduct ions observed are the $[M-C_2H_5O_2+2H]^+$ ions with $n = 6$ to 11 (peaks 7, 9, 11, 13, 15 and 16). The $[M+H]^+$ species accounts for the least number of peaks for degrees of ethoxylation $n = 1$ to 3 (peaks 1–3). Because $[M+NH_4]^+$ are the most abundant adduct ions, the group SIM was used to determine all possible $[M+NH_4]^+$ ions for each of the degrees of ethoxylation from 4 to 13 and shown as inset to Figure 8A. The abundance of the $[M+NH_4]^+$ ion increases as the degree of ethoxylation increases up to $n = 8$ and then begins to decrease as n approaches a value of 13. The high abundance of $[M+NH_4]^+$ adducts with moderate degrees of ethoxylation ion is most likely due to the specific formulation present in the commercial analyte sample.

Using the same mobile phase and stationary phase conditions, we profiled the CEC-MS of Brij 56 surfactant. Brij 56 is more hydrophobic (i.e., sixteen-carbon chain with an average degree of ethoxylation of 10). As expected, the elution times are longer for the separated oligomers. Note that the distribution of the oligomers shown in Figure 8B, is different from that of the Brij 30 surfactant (Figure 8A). With Brij 56, the retention times range from the elution of $n = 5$ oligomer (peak 1 observed as $[M+H]^+$ ion at m/z 463) all the way to $n=16$ oligomer (peak 12 observed as $[M+NH_4]^+$ ion at 965 m/z). Similarly, to profile seen in Brij 30, the most abundant ion is the $[M+NH_4]^+$. The degrees of ethoxylation for the $[M+NH_4]^+$ ions range from $n = 7$ to 16 (i.e., peaks 3–16). There are only two peaks at which the $[M+H]^+$ ion is the most dominant (peaks 1 and 2). One significant difference between the Brij 30 and Brij 56 is the absence of any $[M-C_2H_5O_2+2H]^+$ ions for the later nonionic surfactant in the entire chromatogram. The inset to Figure 8B shows the sensitive detection in group SIM for the $[M+NH_4]^+$. The degrees of ethoxylation represented here range from $n = 5$ –13 with $n = 8$ being the most abundant ammonium adduct. Again, the same pattern of first increasing and then diminishing abundance of the eluted oligomers were seen as with the Brij 30 surfactants.

4. Conclusions

Presented here is a novel design for a fritless capillary column for the hyphenation of CEC to MS. First, the robustness and reproducibility of the column are compared to the traditional single fritted column is clear. The fritless system shows improved efficiency (due to its reduction in the formation of bubbles based on the lack of any fritted material in the capillary) with similar R_s and S/N compared to a single fritted packed column. Moreover, under the same conditions the fritless column shows its robustness and durability with higher reproducibility of retention time and very similar peak area compared to the single frit column. From these initial experiments, using the CCD multivariate analysis, the best $S/$

N and *R*s with the shortest possible retention time were used as criteria to optimize the inlet and outlet taper diameters for the chiral separation of (±)-AG.

The column design presented is universal and can be used to pack a variety of stationary phases for separation of various classes of non-chromophoric cationic, anionic and nonionic compounds. For example, short chain aliphatic cationic amines (MMA and DMA) were separated in less than 18 min with C₆/SCX fritless column with LOD as low as 122 ng/mL in lobster blood spike with MMA and DMA. On the other hand, using a C₆/SAX stationary phase, the fritless CEC-MS column was also used to separate a series of aliphatic anionic surfactants (C₆-C₁₈SO₄⁻) under 30 minutes with baseline resolution. Nonionic, non-chromophoric surfactants that are UV transparent could be successfully separated with CEC-MS with the fritless column. In particular, The Brij 30 and Brij 56 non-chromophoric nonionic surfactants with C₁₂ and C₁₆ alkyl chain were separated into their different oligomers with degree of ethoxylation *n* = 4–13 and *n* = 5–16, respectively, using a C₁₈ fritless column with high resolution in scan mode. Sensitivity of Brij series compounds could be further improved using group SIM.

Currently, work is being conducted to develop hybrid or segmented stationary phase using the fritless CEC-MS column with two different types of packing materials. For example, ongoing work involve analyzing commercially available surfactant solutions that contain mixtures of nonionic and cationic surfactants or nonionic surfactant with anionic surfactants in various commercial formulations. This is expected to be accomplished using the fritless CEC-MS column packed with a combination of C₁₈/SCX or C₁₈/SAX fritless columns to improve selectivity with high sensitivity in MS/MS mode.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Shamsi SA, Miller BE. Electrophoresis. 2004; 25:3927. [PubMed: 15597424]
2. Aturki Z, D'Orazio G, Rocco A, Bortolotti F, Gottardo R, Tagliaro F, Fanali S. Electrophoresis. 2010; 31:1256. [PubMed: 20209571]
3. Bragg, W.; Shamsi, SA. Encyclopedia of Analytical Chemistry. Meyers, RA., editor. John Wiley; Chichester: 2010. published online
4. Cahours X, Cherkaoui S, Rozing G, Veuthey JL. Electrophoresis. 2002; 23:2320. [PubMed: 12210238]
5. Lynen F, Buica A, deVilliers A, Crouch A, Sandra P. J Sep Sci. 2005; 28:1539.
6. Norton D, Zheng J, Danielson ND, Shamsi SA. Anal Chem. 2005; 77:6874. [PubMed: 16255585]
7. Norton D, Rizvi SAA, Shamsi SA. Electrophoresis. 2006; 27:4273. [PubMed: 17075924]
8. Norton D, Shamsi SA. Anal Chem. 2007; 79:9459. [PubMed: 18001007]
9. Liang Z, Zhang L, Duan J, Yan C, Zhang W, Zhang Y. Electrophoresis. 2005; 26:1398. [PubMed: 15765474]
10. Kele Z, Ferenc G, Klement E, Toth GK, Janaky T. Rapid Commun Mass Spectrom. 2005; 19:881. [PubMed: 15724233]
11. Zheng J, Shamsi SA. Anal Chem. 2006; 78:6921. [PubMed: 17007515]

12. Carney RA, Robson MM, Bartle KD, Myers P. *J High Resolut Chromatogr.* 1999; 22:29.
13. Lord GA, Gordon DB, Myers P, King BW. *J Chromatogr A.* 1997; 768:9.
14. Choudhary G, Horvath C, Banks JF. *J Chromatogr A.* 1998; 828:469. [PubMed: 9916325]
15. Zheng J, Norton D, Shamsi SA. *Anal Chem.* 2006; 78:1323. [PubMed: 16478129]
16. Bragg W, Norton D, Shamsi SA. *J Chromatogr B.* 2008; 875:304.
17. Zheng J, Bragg W, Hou J, Lin N, Chandrasekaran S, Shamsi SA. *J Chromatogr A.* 2009; 1216:857. [PubMed: 19108837]
18. Zheng J, Norton D, Shamsi SA. *Electrophoresis.* 2006; 27:2139. [PubMed: 16645981]
19. Mayer M, Rapp E, Marck C, Bruin GJM. *Electrophoresis.* 1999; 20:43. [PubMed: 10065957]
20. Chen CJ, Chang CH, Her GR. *J Chromatogr A.* 2007; 1159:22. [PubMed: 17574559]
21. Shabani S, Kamio M, Derby CD. *J Exp Biol.* 2008; 211:2600. [PubMed: 18689413]
22. Lin JK, Lee YJ, Chang HW. *Food Chem Toxicol.* 1983; 21:143. [PubMed: 6682076]
23. Saleh MI, Pok FW. *J Chromatogr A.* 1997; 763:173.
24. Djozan, Dj; Faraj-Zadeh, MA. *J High Resolut Chromatogr.* 1996; 19:633.
25. Santos B, Simonet BM, Ríos A, Valcárcel M. *Electrophoresis.* 2004; 25:3231. [PubMed: 15472961]
26. Santos B, Simonet BM, Ríos A, Valcárcel M. *Electrophoresis.* 2004; 25:3427. [PubMed: 15490449]
27. Shamsi SA, Danielson ND. *Anal Chem.* 1995; 67:4210.
28. Armstrong DW, Stine GY. *J Liq Chromatogr.* 1983; 6:23.
29. Mohammad A, Khatoon S. *Proc Indian Natl Sci Acad A Phys Sci.* 2010; 80:35.
30. Sandra P, David FJ. *High Resolut Chromatogr.* 1990; 13:414.
31. Heinig K, Vogt C, Werner G. *J Capillary Electrophor.* 1996; 3:261. [PubMed: 9384732]
32. Pan N, Pietrzyk DJ. *J Chromatogr A.* 1995; 706:327.
33. Seifar RM, Kraak JC, ThKok W. *Anal Chem.* 1997; 69:2772.
34. Norton D, Shamsi SA. *Electrophoresis.* 2004; 25:586. [PubMed: 14981685]

Highlights

- Novel fritless CEC-MS column made by joining 2 tapered columns with a union
- Fritless column has similar retention but better efficiency compared to single frit single tapered column.
- Column has excellent operator-to-operator and column-to-column repeatability.
- Column can be packed with chiral and achiral (anionic, cationic and non-ionic) stationary phases.

Figure 1. Schematic of fritless column with the New Objective PicoClear connector. The elastomeric insert of the connector ensures that the two capillaries do not grind together when joined. The arrows, at the bottom, represent the lengths of the inlet packed portion (15 cm) and outlet packed portion (35 cm) of the fritless column (not to scale).

Figure 2.

Comparison of chiral separation of ($\pm$)-aminoglutethimide. A.) Fritless column with two tapers. B.) A single frit capillary with one outlet taper. The $\pm$ values represent the standard deviation of 92 consecutive runs. C.) Current profiles for runs 1, 42, and 92 for the chiral separation the fritless CEC-MS column. Inset table shows the statistical analysis of the average current from all 92 runs (where the current of the column remain unaffected) was sampled every 0.6 seconds. See experimental section for conditions.

Figure 3.

Response surface models for the optimization of the inlet and outlet taper diameters, using ($\pm$)-AG enantioseparation. A.) Response surface model showing change in resolution as a function of inlet and outlet taper diameters. B.) Response surface model showing change in S/N as a function of inlet and outlet taper diameters. C.) The S/N , t_r , and R_s predicted to be obtained using the optimum inlet diameter (11 μm) and outlet diameter (20 μm) as compared to actual experimental values recorded when these optimum diameters are used. See Experimental Section for conditions.

Figure 4.

CEC-MS of short chain amines. (A) total ion chromatogram (TIC) of underivatized monomethylamine (MMA) and dimethylamine (DMA) at 31.1 $\mu\text{g/mL}$ and 45.1 $\mu\text{g/mL}$, respectively with CEC-MS, (B) extract ion chromatogram (EIC) of underivatized MMA at m/z 32, (C) Extract ion chromatograms of underivatized dimethylamine at m/z 46, (D) CEC-MS/MS chromatogram for the derivatized MMA and DMA at 0.5 mg/mL concentration (E) CEC-MS/MS chromatogram for the derivatized MMA and DMA at 122 ng/mL at lowest limit of detection for derivatized DMA spiked in aqueous lobster hemolymph sample. For (D) and (E), the precursor ions of m/z 223 (derivatized MMA) and m/z 237 (derivatized DMA) and product ions of m/z 128 were used for both MMA and DMA. Each amine is at injected at a concentration of 1 mM. See experimental section for detailed conditions.

Figure 5. Calibration curves for the limit-of-detection study of the derivatized methylamine (●) and dimethylamine (▲). Inset R^2 values are the squares of the correlation coefficient for the linear regression for each set of data, representing the linearity of the calibration curve.

Figure 6. Separation of alkyl sulfates with various chain lengths at different concentration of ACN in the mobile phase: (A) 95% ACN, (B) 90% ACN, (C) 85% ACN, (D) 80% ACN using fritless C₆/SAX column. Inset bar plot compares selectivity for each adjacent pair of peaks at various % (v/v) ACN. For other conditions see Experimental Section

Figure 7.

Representative chromatograms of C_6 - C_{10} - SO_4 for column-to-column and operator-to-operator reproducibility study. Conditions: Mobile Phase: 85/15% ACN/ H_2O , 15 mM NH_4OAc , pH 7.5; Column: 75 μm i.d. $\times$ 50 cm (15 cm injection side and 35 cm outlet side packed with C_6/SAX silica); -25 kV at 25°C; Injection: -2 kV, 2 sec; Analytes: 1.0 mg/mL in 50/50 ACN/ H_2O ; Sheath Liquid: 70/30% MeOH/ H_2O , 10 mM NH_4OAc at a flow rate of 7 $\mu L/min$. Spray Chamber: Nebulizer Pressure 5 psi, Drying Gas Flow Rate 5 L/min, Drying Gas Temperature 200°C, Fragmentor Voltage 90V, Capillary Voltage -3500V; Negative Group SIM at = 181, 209, and 237 m/z, corresponding to $[M-H]^-$

Figure 8.

(A.) Separation of Brij 30 non-ionic surfactant using fritless CEC-MS column packed with C_{18} stationary phase. Peaks **1**: $n = 4$ $[M+H]^+ = 363$ m/z, **2**: $n = 5$ $[M+H]^+ = 407$ m/z, **3**: $n = 6$ $[M+H]^+ = 451$ m/z, **4**: $n = 7$ $[M+NH_4]^+ = 512$ m/z, **5**: $n = 8$ $[M+NH_4]^+ = 556$ m/z, **6**: $n = 9$ $[M+NH_4]^+ = 600$ m/z, **7**: $n = 6$ $[M-C_2H_5O_2+2H]^+ = 391$ m/z, **8**: $n = 10$ $[M+NH_4]^+ = 644$ m/z, **9**: $n = 7$ $[M-C_2H_5O_2+2H]^+ = 435$ m/z, **10**: $n = 11$ $[M+NH_4]^+ = 688$ m/z, **11**: $n = 8$ $[M-C_2H_5O_2+2H]^+ = 479$ m/z, **12**: $n = 12$ $[M+NH_4]^+ = 732$ m/z, **13**: $n = 9$ $[M-C_2H_5O_2+2H]^+ = 523$ m/z, **14**: $n = 13$ $[M+NH_4]^+ = 776$ m/z, **15**: $n = 10$ $[M-C_2H_5O_2+2H]^+ = 567$ m/z, **16**: $n = 11$ $[M-C_2H_5O_2+2H]^+ = 611$ m/z; Inset is the group SIM for the $[M+NH_4]^+$ ions obtained from the scan of Brij 30 with peaks labeled with degrees of ethoxylation shown for first, highest and last peak. (B.) Separation of Brij 56 non-ionic surfactant. Peaks **1**: $n = 5$ $[M+H]^+ = 463$ m/z, **2**: $n = 6$ $[M+H]^+ = 507$ m/z, **3**: $n = 7$ $[M+NH_4]^+ = 569$ m/z, **4**: $n = 8$ $[M+NH_4]^+ = 613$ m/z, **5**: $n = 9$ $[M+NH_4]^+ = 657$ m/z, **6**: $n = 10$ $[M+NH_4]^+ = 701$ m/z, **7**: $n = 11$ $[M+NH_4]^+ = 745$ m/z, **8**: $n = 12$ $[M+NH_4]^+ = 789$ m/z, **9**: $n = 13$ $[M+NH_4]^+ = 833$ m/z, **10**: $n = 14$ $[M+NH_4]^+ = 877$ m/z, **11**: $n = 15$ $[M+NH_4]^+ = 921$ m/z, **12**: $n = 16$ $[M+NH_4]^+ = 965$ m/z. Inset is the group SIM for the $[M+NH_4]^+$ ions obtained from the scan of Brij 56 with peaks labeled with degrees of ethoxylation shown for first, highest and last peak. See Experimental section for all other conditions.

Intra- and Intercolumn reproducibility of the fritless CEC-MS column between two operators comparing average retention time (tr_{avg}), peak area (A_{avg}), peak width (PW_{avg}) and efficiency (N_{avg}) of three alkyl amines with C₆/SAX stationary phase

Table 1

Operator 1									
	tr_{avg} %RSD	A_{avg} %RSD	PW_{avg} %RSD	N_{avg} %RSD					
<i>Intracolumn</i>									
	C ₆ -SO ₄ C ₈ -SO ₄ C ₁₀ -SO ₄	C ₆ -SO ₄ C ₈ -SO ₄ C ₁₀ -SO ₄	C ₆ -SO ₄ C ₈ -SO ₄ C ₁₀ -SO ₄	C ₆ -SO ₄ C ₈ -SO ₄ C ₁₀ -SO ₄					
Column 1 n = 15	2.7 2.6 2.3	4.8 5.2 6.2	4.6 5.3 5.5	5.9 4.8 5.7					
Column 2 n = 15	3.9 4.2 3.6	4.6 4.1 5.9	6.1 6.0 5.5	4.6 3.7 5.9					
Column 3 n = 15	3.5 3.3 2.9	5.0 4.2 6.4	5.7 6.8 6.1	4.6 3.6 5.8					
<i>Intercolumn</i>									
n=45	3.4 3.4 2.9	4.7 4.4 6.0	5.3 5.9 5.6	5.1 4.0 8.1					
Operator 2									
	tr_{avg} %RSD	A_{avg} %RSD	PW_{avg} %RSD	N_{avg} %RSD					
<i>Intracolumn</i>									
	C ₆ -SO ₄ C ₈ -SO ₄ C ₁₀ -SO ₄	C ₆ -SO ₄ C ₈ -SO ₄ C ₁₀ -SO ₄	C ₆ -SO ₄ C ₈ -SO ₄ C ₁₀ -SO ₄	C ₆ -SO ₄ C ₈ -SO ₄ C ₁₀ -SO ₄					
Column 1 n = 15	4.4 4.4 4.5	5.8 3.2 3.4	2.2 1.9 4.4	6.1 6.1 6.1					
Column 2 n = 15	5.1 4.8 4.9	5.0 5.8 6.2	4.8 6.0 2.7	4.4 5.5 5.2					
Column 3 n = 15	2.9 2.4 2.2	4.3 3.9 4.0	4.4 3.5 3.1	3.2 7.2 3.2					
<i>Intercolumn</i>									
n=45	4.9 5.3 5.3	5.8 6.2 4.8	3.9 6.1 3.8	8.1 6.7 7.0					
<i>Interoperator</i>									
n = 90	13 14 13	6.7 8.9 7.2	11 12 9.2	5.8 7.6 8.1					