Published in final edited form as:

Anal Chem. 2018 January 02; 90(1): 737–744. doi:10.1021/acs.analchem.7b02986.

A Customizable Flow Injection System for Automated, High Throughput, and Time Sensitive Ion Mobility Spectrometry and Mass Spectrometry Measurements

Daniel J. Orton, Malak M. Tfaily, Ronald J. Moore, Brian L. LaMarche, Xueyun Zheng^{iD}, Thomas L. Fillmore, Rosalie K. Chu, Karl K. Weitz, Matthew E. Monroe, Ryan T. Kelly, Richard D. Smith^{iD}, and Erin S. Baker^{*,iD}

Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99352, United States

Abstract

To better understand disease conditions and environmental perturbations, multiomic studies combining proteomic, lipidomic, and metabolomic analyses are vastly increasing in popularity. In a multiomic study, a single sample is typically extracted in multiple ways, and various analyses are performed using different instruments, most often based upon mass spectrometry (MS). Thus, one sample becomes many measurements, making high throughput and reproducible evaluations a necessity. One way to address the numerous samples and varying instrumental conditions is to utilize a flow injection analysis (FIA) system for rapid sample injections. While some FIA systems have been created to address these challenges, many have limitations such as costly consumables, low pressure capabilities, limited pressure monitoring, and fixed flow rates. To address these limitations, we created an automated, customizable FIA system capable of operating at a range of flow rates (~50 nL/min to 500 μ L/min) to accommodate both low- and high-flow MS ionization sources. This system also functions at varying analytical throughputs from 24 to 1200 samples per day to enable different MS analysis approaches. Applications ranging from native protein analyses to molecular library construction were performed using the FIA system, and results showed a highly robust and reproducible platform capable of providing consistent performance over many days without carryover, as long as washing buffers specific to each molecular analysis were utilized.

Graphical abstract

*Corresponding Author Mailing Address: 902 Battelle Boulevard, P.O. Box 999, MSIN K8-98, Richland, WA 99352, United States; Phone: 509-371-6219; erin.baker@pnnl.gov (E.S.B.).

ORCID

Xueyun Zheng: 0000-0001-9782-4521

Richard D. Smith: 0000-0002-2381-2349

Erin S. Baker: 0000-0001-5246-2213

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b02986.

A detailed description of the Accelerated nFlow FIA configuration including a figure and text on the specific components (PDF)

The authors declare no competing financial interest.

As the desire for more mass spectrometry (MS)-based analyses continues to increase due to the growing importance of screening studies, multiomic approaches, and faster computational capabilities requiring thousands of biological and environmental replicates,¹ so does the need for better automated sample injection methods. Automated sample injection relieves the manual process of injecting thousands of samples by hand, enables highly reproducible measurements, and allows continuous, unattended sample analyses. These characteristics are all necessary for high throughput and high quality MS studies. Flow injection analyses (FIA) have provided an important solution for the automation of sample injections over the last 40 years.^{2–6} The first FIA system was introduced in 1975, providing a highly versatile technique where a well-characterized analyte band was moved through a flowing solvent stream to the instrument of choice.⁷ Over the last 20 years, FIA systems have been created using lab-on-a-chip⁸ and lab-on-a-valve^{9,10} microflow setups to reduce sample size needs and buffer consumption. While both lab-on-a-chip and lab-on-a-valve systems have enabled important studies and show great promise for the future,^{11–15} each has caveats.^{16–18} For example, lab-on-a-chip technology uses very small sample and buffer volumes, but requires microfabrication and integrated microfluidic pumping, which are more challenging to couple with MS than its lab-on-a-valve counterpart. Furthermore, highly complex samples can easily plug and ruin a chip, escalating analysis costs. Lab-on-a-valve systems were initially designed to reduce reagent-based assays to the microliter or submicroliter level.¹⁸ While they are easily coupled with MS,¹⁹ some systems have costly consumables (e.g., nonreusable electrospray ionization (ESI) emitters) to address sample carryover and spray stability, increasing the cost of large-scale studies. Other noted limitations of lab-on-a-valve systems are that the flow rates are usually fixed or only slightly adjustable, and throughput is constrained by the time it takes to load a sample and dispose of the used consumables.² Additionally, high pressures and instantaneous feedback to alert of a malfunction (e.g., clogging) are also not always available in FIA systems, and in many cases, the expense and limited versatility of FIA automated systems force laboratories to perform manual sample injections.

Manually injecting samples is not without challenges either. Manual injections usually involve a syringe, syringe pump, and collection of fittings to deliver the sample to the MS source. Due to the increased speed and sensitivity of modern mass spectrometers, acquisition times ranging from seconds to minutes are common. Therefore, the time required to clean the syringe and lines during manual injections, along with loading the subsequent sample,

generally exceeds the MS analysis time. Other complications with manual injections are the large amounts of sample needed to fill the transfer line, making them incompatible with limited sample volume studies. Viscous samples can also cause hairline fractures in glass syringes since typical borosilicate syringes are only capable of withstanding approximately 1000 psi before failure, and this pressure can easily be reached when large volumes of viscous sample fill the transfer line. Detecting these hairline fractures can also be extremely difficult at low flow rates, often causing losses to precious samples. Furthermore, when the liquid flow is interrupted during manual injections to clean the syringe or load washing solvents, the small sample volume at the end of the spray tip near the heated MS inlet can rapidly evaporate and precipitate sample components, resulting in spray tip instability and clogging. These challenges illustrate that new ways of automating the sample injection process and creating a feedback loop are desperately needed.

We have developed a new sample injection system based on lab-on-a-valve technology that contains feedback-specific software to alleviate many of the challenges inherent in both manual injection processes and current FIA systems. This system provides the ability to operate over a wide range of flow rates and inject small sample volumes bracketed with buffer solutions, so no sample is sacrificed, carryover is eliminated, and plugging in the transfer line and emitter is reduced. In this system, up to six sample trays are stored in a cooled environment (4 °C). Both operation and feedback are automated so samples can run continuously and unattended over extended periods of time to fully utilize the duty cycle of expensive mass spectrometers. In addition, our system is controlled by software capable of performing different methods, so acquisition times from 1 min to hours can be used for different sample types or MS data acquisition strategies. The software also monitors the pump pressure to evaluate when clogging is occurring, and runs are stopped whenever the pressure is outside the defined maximum or minimum values. Finally, flow rates from ~50 nL/min to 500 μ L/min are possible with this system to fit the conditions of low-flow nanoESI sources and high-flow sources such as atmospheric pressure chemical ionization (APCI) or ESI. Here, we describe the designed FIA system and its robustness, carryover, and associated costs. Its application to evaluating native proteins, determining protein/ligand binding dissociation constants, constructing molecular libraries, and studying samples prone to decomposition over short time periods, are also detailed.

MATERIALS AND METHODS

FIA System

To create an automated FIA system with adjustable flow rates from nL/min to μ L/min, while having the ability to inject up to 1200 samples/day, we designed a flexible platform that could be configured in three ways. In all three setups, a PAL Autosampler (CTC Analytics AG), Cheminert six port injection valve (Valco Instruments Co. Inc.), and narrow-bore fused silica capillary transfer line (Polymicro Technologies) were utilized. For the high-flow configuration, which we termed μ Flow FIA (Figure 1A), the PAL was operated at a flow rate from 5 to 500 μ L/min as defined by the user. Fluidic connections from the valve to the source were made using silica capillaries having internal diameters of 20 to 50 μ m. Smaller inner diameters were usually used to minimize Taylor dispersion and lateral dispersion, and

higher values were only used to avoid excessive backpressures or fouling from highly viscous samples. The injection valve was positioned as close to the mass spectrometer source as possible (~0.5 m in our case) and electrically isolated from the motor by a PEEK collar. The autosampler wash solvents were varied according to application for maintained sample integrity and reduced carryover, and a Labjack U12 data acquisition (DAQ) board was used to send a contact closure signal to the mass spectrometer. Finally, the autosampler, valve, and contact closure were controlled by the in-house built LCMSNet software, which is freely available for download at <https://github.com/PNNL-Comp-Mass-Spec/LCMSNet>.

To perform the low-flow measurements at different analysis speeds, we designed two configurations, where both required the addition of a 1200 nanoLC pump (Agilent Technologies). The first configuration, named nFlow FIA, operated from ~50 nL/min to 1 μ L/min and injected a sample every 3 to 60 min (Figure 1B). The nanoLC pump was utilized to transport the sample and wash the transfer line between injections. In many cases, we utilized a buffer consisting of (95:5) (v/v) water:acetonitrile with 0.1% formic acid (Fisher)), but this could be changed based on the analysis needs (especially for native protein studies). To provide sufficient back pressure to reach the 50 bar minimum required by the nanoLC pump, a short packed capillary LC column was placed between the pump and the valve. The column used in our study was 12 cm long $\times$ 360 μ m o.d. $\times$ 75 μ m i.d. and prepared in-house by slurry packing 3 μ m Jupiter C₁₈ (Phenomenex, Torrance, CA) into fused silica capillary (Polymicro Technologies Inc., Phoenix, AZ) with a 1 cm sol-gel frit at the end for media retention. However, any short column should suffice for this task. The sample loop size and LC flow rates were adjusted according to the conditions required by the samples, but in most cases we utilized either a 3 or 5 μ L sample loop, and its length was adjusted using the equation $V = \pi r^2 h$, if longer connections were needed. In the equation, V is the volume of the sample amount in mL, r^2 is the radius of capillary in cm, and h is the capillary length in cm.

The second low-flow configuration was termed Accelerated nFlow FIA and again operated from ~50 nL/min to 1 μ L/min. This setup, however, required two valves and a nanoLC pump so that a sample could be injected every minute, allowing up to 1200 sample analyses per day (Figure 1C). Two sample loops were utilized to connect the two valves, allowing sample injection and washing to be alternated and enabling the high throughput needed for this system. A detailed schematic for the Accelerated nFlow FIA is shown in the Supporting Information since it is the most complicated of the three configurations. In the Accelerated nFlow FIA, again the valves, autosampler, and pump were controlled by the LCMSNet software, enabling instant feedback to the user. All three setups were tested with standards and various applications to evaluate their performance. The outcome of these measurements is illustrated in the Results and Discussion.

Materials and Sample Preparation

FIA Performance Testing Studies—To evaluate the performance of the FIA configurations, an ESI low-concentration tune mix from Agilent Technologies (Santa Clara, CA) and a ten peptide mixture were utilized. The ten peptide mix contained des-Pro-Ala bradykinin, fibrinopeptide A, Tyr C peptide, human osteocalcin fragment 7–19, syntide 2,

diazepam binding inhibitor standard, porcine dynorphin A fragment 1–13, (D-Ala-6) luteinizing hormone releasing hormone (LHRH), bradykinin fragment 1–7, porcine renin substrate, and bradykinin, all of which were individually purchased from Sigma-Aldrich. Each peptide was dissolved in water to 10 μM , and all were combined and diluted with (49.75:49.75:0.50) (v/v/v) water:methanol:acetic acid to a concentration of 100 nM per peptide. The Agilent tune mix was also diluted at a ratio of (44.25:5.00:0.75) (v/v/v) acetonitrile: tune mix:water. Each sample was alternately injected into the FIA platform to examine the reproducibility and carryover of the different configurations.

K_D Study—Human carbonic anhydrase I was purchased from Sigma-Aldrich (St. Louis, MO), filtered using a 10K spin filter (EMD Millipore), buffer exchanged into 200 μM ammonium acetate to reduce residual sodium contamination, and brought to a final concentration of 10 μM . Benzenesulfonamide, ethoxzolamide, acetazolamide, and 4-carboxybenzenesulfonamide were purchased from Sigma-Aldrich (St. Louis MO) and mixed with the carbonic anhydrase in multiple ratios to determine the binding efficiency of each. Each mixture was analyzed with a home-built ion mobility spectrometry (IMS)/MS instrument and data were collected from m/z 200 to 14 000. To calculate the K_D of the protein/ligand complexes studied, the following equation was utilized, where $I(P \cdot L)$ is the intensity of the protein/ligand complex, $I(P)$ is the intensity of the protein alone, $[P]_0$ is the initial protein concentration, and $[L]_0$ is the initial ligand concentration.²⁰

$$\frac{I(P \cdot L)}{I(P)} = \frac{1}{2} \left(-1 - \frac{[P]_0 - [L]_0}{K_D} + \sqrt{4 \frac{[L]_0}{K_D} + \left(\frac{[L]_0 - [P]_0}{K_D} - 1 \right)^2} \right)$$

Protein pH Study—For the pH experiments, a gammaproteobacterial protein from *E. coli* with the NESG id ER309 (Swiss-Prot id YEJL_ECOLI/P0AD24) was used. For the study, a stock solution of 50 μM ER309 in 200 mM ammonium acetate was created. To perform the pH experiments, the stock solution was diluted to 5 μM with 200 mM ammonium acetate that was pH adjusted using acetic acid or ammonium hydroxide to reach the desired pH values of 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12. Each sample was analyzed with the home-built IMS/MS instrument, and data were collected from m/z 100 to 3200 to understand how the protein charge state distribution and collisional cross section changed with pH.

Soil Organic Matter Study—Peat soil samples were collected from northern Minnesota at a depth of 75 cm as described elsewhere.^{21,22} The water-extractable fraction (referred to here as dissolved organic matter, DOM) was prepared in triplicate by adding 3 mL of water (18 M Ω ionic purity) to 300 mg of bulk soil and shaking for 2 h on an Eppendorf Thermomixer in 2 mL capped glass vials. The samples were then removed from the shaker and left to stand before spinning down and removing the supernatant to halt the extraction. The supernatant from each replicate was then split into six aliquots and each aliquot was stored in five separate vials, at -20°C , so freeze thaw cycles could be minimized. A vial from each of the aliquots was then analyzed by Fourier transform-ion cyclotron resonance (FT-ICR) MS on T_0 , T_1 , T_2 , T_3 , and T_{30} days to monitor changes in organic matter composition with time. The ion accumulation time was varied to account for differences in

carbon concentration between samples, and the extraction efficiency was estimated to be approximately 15%.²¹

IMS/MS Instrumentation and Data Analysis

The IMS/MS studies were executed with two different drift tube IMS (DTIMS)/MS platforms. The first was an in-house home-built DTIMS/MS instrument that coupled a 1 m IMS drift tube with an Agilent 6224 TOF MS upgraded to a 1.5 m flight tube (providing MS resolution of ~25 000),²³ and the second was the Agilent 6560 IM-QTOF MS platform (Agilent Technologies).^{24–26} The Agilent 6560 was outfitted with the commercial gas kit (Alternate Gas Kit, Agilent) and a precision flow controller (640B, MKS Instruments) to allow for real-time pressure adjustment based on absolute readings of the drift tube pressure using a capacitance manometer (CDG 500, Agilent). For the DTIMS measurements on both instruments, ions were passed through an inlet capillary, focused by a high-pressure ion funnel, and accumulated in an ion funnel trap. Ions were then pulsed into the drift tubes filled with ~3.95 Torr of nitrogen gas, where they traveled under the influence of a weak electric field (10 to 20 V/cm). Ions exiting the drift tube were refocused by a rear ion funnel prior to TOF or QTOF MS detection, and their arrival times were recorded.

FT-ICR MS Data Acquisition and Analysis

Ultrahigh resolution MS characterization was carried out using a 12 T FT-ICR MS. Samples were directly injected into the mass spectrometer, and the ion accumulation time was optimized for all samples. A standard Bruker ESI source was used to generate negatively charged molecular ions, and experimental conditions were as follows: needle voltage, +4.4 kV; Q1 set to m/z 50; and the heated resistively coated glass capillary was maintained at 180 °C. A total of 96 individual scans were averaged for each sample, and internal calibration was performed using an organic matter homologous series separated by 14 Da (–CH₂ groups). The mass measurement accuracy was less than 1 ppm for singly charged ions across a broad m/z range (m/z 100 to 900), and the mass resolution was ~350 K at m/z 339. Data Analysis software (BrukerDaltonics version 4.2) was used to convert raw spectra to a list of m/z values (“features”) by applying the FTMS peak picker at a signal-to-noise ratio (S/N) threshold of 7 and absolute intensity threshold of 100. Chemical formulas were then assigned using an in-house built software following the Compound Identification Algorithm (CIA), described in ref 27 and modified in ref 28. Chemical formulas were assigned based on the following criteria: $S/N > 7$, mass measurement error <1 ppm, and taking into consideration the presence of C, H, O, N, S, and P, while excluding other elements. To interpret the resulting data set, the chemical character of all data points for each sample spectrum was evaluated with van Krevelen diagrams on the basis of their molar H:C ratios (y -axis) and molar O:C ratios (x -axis).²⁹ van Krevelen diagrams were further used to visualize and compare the average properties of organic matter and enable identification of the major biochemical classes (i.e., lipids, proteins, lignin, carbohydrates, and condensed aromatics) of the compounds present in the samples.

RESULTS AND DISCUSSION

FIA Performance Testing Studies

There is a great need to speed up current MS-based measurements and provide more data to fully evaluate biological and environmental systems. To meet this desire, we developed an automated FIA platform capable of operating from low- to high-flow rates (50 nL/min to 500 μ L/min), interfacing with multiple instrument sources, and varying analysis times for long low-concentration sample evaluations or high throughput library building and screening studies. These desired capabilities resulted in three distinct FIA configurations termed μ Flow, nFlow, and Accelerated nFlow. Specifically, the μ Flow setup allowed high-flow injections from 5 to 500 μ L/min, while the nFlow setup operated from $\sim$ 50 nL/min to 1 μ L/min and delivered a sample every 3 to 60 min, and the Accelerated nFlow FIA allowed low-flow analyses but with high throughput (1 min/sample) for the analysis of up to 1200 samples per day. The specific arrangement for each configuration is illustrated in the Materials and Methods section.

To test the capabilities of the three FIA configurations with respect to reproducibility and carryover, two different samples (Agilent tune mix and a 10 peptide mixture) were alternately injected using each setup. In the μ Flow setup, 200 μ L of each sample was injected for 4 min (flow rate of 40 μ L/min), and the sample loop and transfer line were washed following the defined analysis period with the PAL injection system (Figure 2A). Carryover was analyzed in each sample's spectra, and none was observed as long as washing buffers specific to each molecular analysis were utilized. This observation was further accredited to the small inner diameter (i.d.) fused silica tubing transfer line and thorough washing of the small surface area contacted by the sample. The narrow-bore tubing also greatly minimized the impact of Taylor dispersion and lateral diffusion on the sample plug as noted by the sharp rise and fall of the sample peaks. Reproducibility was assessed by monitoring the peak intensities for m/z 622 and 922 in the Agilent tune mix and the relative standard deviation (RSD) for each at 1, 2, 6, 12, 24, 36, and 48 h time periods. Evaluation of all time periods showed peak intensity RSDs less than 2%, illustrating the robustness of the system. The carryover and reproducibility were also accessed for the nFlow (Figure 2B) and Accelerated nFlow setups (Figure 2C), and similar results were noted. For the nFlow FIA analyses, 5 μ L of each sample was injected into the buffer-filled transfer line at a flow rate of 200 nL/min and analyses were performed for 7 min prior to washing the transfer line with the PAL. Sharp peaks, no carryover, and RSDs less than 2% were again observed for each sample. In the final Accelerated nFlow setup, 5 μ L of each sample was injected at a flow rate of 200 nL/min, and sample-to-sample throughput was set to 1 min per sample. Utilization of the two-valve setup allowed washing to be performed in one sample loop, while the other loop injected sample, resulting in only short time periods where buffer was infused to the mass spectrometer. As with the other configurations, sharp peaks were observed for the sample plugs, RSDs less than 2% were noted, and no carryover was detected. Furthermore, all configurations utilized the LCMSNet control software, which employs an error response system. Thus, if a pump overpressurizes due to a plugged line or if the injection syringe fails, all analyses were halted, preserving precious samples and providing further assurance

that the system can operate unattended overnight without great risk to small sample volumes.

Since the performance of all FIA setups was robust, applications requiring rapid injections or those difficult to perform with manual injections were selected to test the different configurations. These applications ranged from native protein studies that easily clog transfer lines and ESI emitters to screening applications that require high throughput. The different applications performed on the designed FIA system are illustrated below.

Protein/Ligand Binding Studies

The study of protein/ligand dissociation constants (K_D) is an ideal application for the FIA systems as it requires proteins to be sprayed in native conditions. In manual analysis of native proteins, two main challenges are often observed due to the greater viscosity of solutions. First, syringes often acquire hairline fractures along the body due to overpressurization from the viscous samples, thereby resulting in inconsistent flow rates and sample loss. Second, when the flow is interrupted between each analysis, the ESI emitters often plug. Both of these challenges can be addressed by the FIA system in conjunction with the IMS/MS instrument, which provides a powerful tool for probing the stability of native protein structures before and after noncovalent ligand binding.^{30,31} In this K_D study, the IMS/MS and the FIA system were utilized to screen several different drugs with human carbonic anhydrase I and each sample was analyzed in triplicate. This specific protein and set of ligands were selected for study as they have been well characterized using ESI, surface plasmon resonance (SPR), and isothermal titration calorimetry (ITC)²⁰, and thus provide a well-characterized system for performance evaluation. In our study, we found the K_D values for human carbonic anhydrase I with ethoxzolamide, acetazolamide, benzenesulfonamide, and 4-carboxybenzenesulfonamide were 0.0017, 0.78, 3.9, and 9.0 μM (Figure 3). These values are in strong agreement with those determined elsewhere,²⁰ which were 0.005, 0.619, 2.1, and 4.6 μM for the same protein/ligand mixtures. These complexes were analyzed for 5 min each using the nFlow FIA setup. Thus, the required 63 protein/ligand analyses with 63 blanks in between each (further ensuring sample carryover was avoided) took place in ~10 h and were performed overnight, making normally difficult and tedious K_D studies much easier.

Creating Libraries and Screening Samples

Creating libraries from standards is another important application for FIA. In these studies, standards are usually analyzed 24 h a day and in triplicate to assess standard deviations for the properties of interest. Both the μFlow and nFlow setup were recently utilized to analyze the DTIMS collision cross sections (CCS) for over 500 metabolites and xenobiotics.³² The μFlow setup was essential for the CCS values acquired from the APCI and APPI sources, while the nFlow was used for the nanoESI values. Each standard was run in triplicate in both positive and negative polarity. Thus, the study resulted in over 3000 discrete injections in addition to the molecules that did not ionize well and were omitted from the database. Without the use of the FIA, the creation of this database would have been impractical.

The ability to screen for molecular changes in various buffer conditions is also an important application requiring many sample runs. While these studies are important in all omic analyses, they are especially essential in determining conserved protein domains of unknown function, which is a necessary step toward understanding the function of cellular components. This structural knowledge offers promise for mapping conserved residues, characterizing surface characteristics such as possible binding pockets or electrostatic features, and identifying proteins having homologous tertiary structure, but lacking sequence similarity. Since IMS/MS allows the rapid analysis of protein structural changes under different conditions,^{30,31,33} it can be extremely useful for screening proteins prior to extended NMR analyses that require high concentrations. However, an automated injection system such as our FIA platform is required to perform these high throughput analyses. Therefore, we analyzed a gammaproteobacterial protein from *E. coli* with the NESG id ER309 using the nFlow FIA system in combination with IMS/MS. ER309 was analyzed at ten different pH values, though only three are illustrated in Figure 4 (pH = 3, 7, and 10) for clarity. At pH 3, only monomers were present in the solution, and there was no evidence of dimers. However, dimers were observed at neutral pH and exceeded the monomer concentration at higher pH values. Because previous publications have indicated that proteins (and other biological molecules) unfold in the gas phase at higher charge states due to the additional electrostatic repulsion,^{34,35} we focused on the lowest charge state of each complex since it is considered closest to that observed in solution (or by NMR). At all pH values, the lowest charge state monomers existed as multiple partially unfolded structures spanning a range of cross sections. NMR chemical shifts also confirmed that the low pH state corresponded to an unfolded structure. The lowest charge state dimer however existed primarily in a single conformation in the IMS/MS experiments for pH 7 and 10 and did not seem to change structures. The information from IMS/MS was very useful for quickly characterizing the protein oligomerization states and interactions at multiple pH values. Thus, by combining IMS/MS with the FIA system, multiple pH values could be quickly analyzed to better understand structural properties.

Unstable Sample Analyses

Sample stability is also a significant concern for many applications. Since the FIA allows rapid sample analyses, the concern that samples may degrade prior to their evaluation can be addressed, particularly when the Accelerated nFlow FIA setup is employed. To illustrate this, we tested the hypothesis that the molecular composition of dissolved organic matter (DOM) extracted from peat soils would change over prolonged storage times. In this study, individual aliquots of DOM from six replicate soil samples were examined. Each replicate was split into five vials and stored at -20°C to minimize freeze thaw cycles. A vial for each replicate was then analyzed on day 0, 1, 2, 3, and 30 with the FT-ICR MS to determine sample stability. Through use of the molecular formulas observed and the relative intensity of each feature, the PCA clearly separated T_0 , T_1 , T_2 , and T_3 from T_{30} along axis 1 (Figure 5A), and PC2 separated T_0 and T_{30} from T_1 , T_2 , and T_3 . This analysis revealed differences in the DOM molecular composition with increasing storage time. Next, we utilized variable importance in projection³⁶ to identify the molecular formulas that changed during storage. In the van Krevelen diagram, compounds in the lipid-like region, condensed aromatic region, and tannin-like region disappeared after 30 days of storage due to degradation, oxidation,

and/or saturation of double bonds (Figure 5B). Conversely, compounds in the amino acid region appeared after 30 days of storage due to degradation of proteins. These data indicate it is best not to freeze these samples for more than a week; otherwise, the DOM composition will change significantly due to degradation. Thus, rapid analyses, such as those possible with the FIA system, are needed so deviations due to storage time are not included in the measurements.

CONCLUSIONS

A user-configurable FIA platform was developed to enable sample delivery at different flow rates and throughputs, allowing sample injection to be customized for different instrumental MS data acquisition strategies and study demands. These desired capabilities resulted in three distinct FIA configurations termed μ Flow, nFlow, and Accelerated nFlow. Specifically, the μ Flow setup allowed high-flow injections from 5 to 500 μ L/min, the nFlow setup operated from ~50 nL/min to 1 μ L/min and delivered a sample every 3 to 60 min, and the Accelerated nFlow FIA allowed low-flow analyses but with high throughput (1 min/sample), enabling up to 1200 injections per day. Each setup was shown to be robust as dispersion and carryover were negligible for all configurations, and consistent performance was achieved over many days as assessed by peak intensity RSDs being less than 2% for over 48 h of operation. The FIA platform also required limited consumables as the ESI emitters were reusable, and the transfer lines and valves needed little maintenance. These characteristics greatly reduced the materials costs and user intervention, making it an integral component in lab operations. When the FIA platform was utilized in native protein analyses such as K_D studies, the customizable platform provided reliable results that matched literature values. The FIA system also enabled the analysis of samples that degrade rapidly such as dissolved organic matter and enabled screening studies by analyzing up to 1200 samples/day. This adjustable platform fits a current need in research studies as sample demands continue to increase due to the need to evaluate more biological and environmental replicates, use smaller sample volumes, or perform more omic studies per sample. Therefore, platforms such as this FIA system will aid in more efficient utilization of expensive MS instruments and enable large-scale analyses in a timely manner before sample decomposition occurs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Portions of this research were supported by grants from the National Institute of Environmental Health Sciences of the NIH (R01 ES022190), National Institute of General Medical Sciences (P41 GM103493), NIH (P42 ES027704), and the Laboratory Directed Research and Development Program at Pacific Northwest National Laboratory. This research utilized capabilities developed by the Pan-omics program (funded by the U.S. Department of Energy Office of Biological and Environmental Research Genome Sciences Program). This work was performed in the W. R. Wiley Environmental Molecular Sciences Laboratory (EMSL), a DOE national scientific user facility at the Pacific Northwest National Laboratory (PNNL). PNNL is operated by Battelle for the DOE under contract DE-AC05-76RL0 1830.

References

1. Jansson JK, Baker ES. *Nat. Microbiol.* 2016; 1:16049. [PubMed: 27572648]
2. Schug KA, Fan H. *LCGC N. Am.* 2012; 30:26–33.
3. Henion J. *LCGC N. Am.* 2009; 27:900–915.
4. Trojanowicz M, Kolacinska K. *Analyst.* 2016; 141:2085–2139. [PubMed: 26906258]
5. Olsen S, Pessenda LCR, Ruzicka J, Hansen EH. *Analyst.* 1983; 108:905–917.
6. Choi JW, Oh KW, Thomas JH, Heineman WR, Halsall HB, Nevin JH, Helmicki AJ, Henderson HT, Ahn CH. *Lab Chip.* 2002; 2:27–30. [PubMed: 15100857]
7. Ruzicka J, Hansen EH. *Anal. Chim. Acta.* 1975; 78:145–157.
8. Vilkner T, Janasek D, Manz A. *Anal. Chem.* 2004; 76:3373–3385. [PubMed: 15193114]
9. Hansen EH. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* 2005; 40:1507–1524.
10. Ogata Y, Scampavia L, Carter TL, Fan E, Turecek F. *Anal. Biochem.* 2004; 331:161–168. [PubMed: 15246009]
11. Folch A. *In Vitro Cell Dev-An.* 2012; 48:14–14.
12. Whitesides GM. *Nature.* 2006; 442:368–373. [PubMed: 16871203]
13. Sackmann EK, Fulton AL, Beebe DJ. *Nature.* 2014; 507:181–189. [PubMed: 24622198]
14. Luque de Castro MD, Ruiz-Jimenez J, Perez-Serradilla JA. *TrAC, Trends Anal. Chem.* 2008; 27:118–126.
15. Avivar J, Ferrer L, Casas M, Cerda V. *Talanta.* 2011; 84:1221–1227. [PubMed: 21641430]
16. Temiz Y, Lovchik RD, Kaigala GV, Delamarche E. *Microelectron. Eng.* 2015; 132:156–175.
17. Wikswo JP, Block FE, Cliffl DE, Goodwin CR, Marasco CC, Markov DA, McLean DL, McLean JA, McKenzie JR, Reiserer RS, Samson PC, Schaffer DK, Seale KT, Sherrod SD. *IEEE Trans. Biomed. Eng.* 2013; 60(3):682–690. [PubMed: 23380852]
18. Ruzicka J. *Analyst.* 2000; 125:1053–1060.
19. Wang JH, Hansen EH. *TrAC, Trends Anal. Chem.* 2003; 22:225–231.
20. Jecklin MC, Schauer S, Dumelin CE, Zenobi RJ. *Mol. Recognit.* 2009; 22:319–329.
21. Tfaily MM, Chu RK, Tolic N, Roscioli KM, Anderton CR, Pasa-Tolic L, Robinson EW, Hess NJ. *Anal. Chem.* 2015; 87:5206–5215. [PubMed: 25884232]
22. Tfaily MM, Chu RK, Toyoda J, Tolic N, Robinson EW, Pasa-Tolic L, Hess NJ. *Anal. Chim. Acta.* 2017; 972:54–61. [PubMed: 28495096]
23. Baker ES, Clowers BH, Li FM, Tang K, Tolmachev AV, Prior DC, Belov ME, Smith RD. *J. Am. Soc. Mass Spectrom.* 2007; 18:1176–1187. [PubMed: 17512752]
24. Ibrahim YM, Baker ES, Danielson WF, Norheim RV, Prior DC, Anderson GA, Belov ME, Smith RD. *Int. J. Mass Spectrom.* 2015; 377:655–662. [PubMed: 26185483]
25. May JC, Goodwin CR, Lareau NM, Leaptrot KL, Morris CB, Kurulugama RT, Mordehai A, Klein C, Barry W, Darland E, Overney G, Imatani K, Stafford GC, Fjeldsted JC, McLean JA. *Anal. Chem.* 2014; 86:2107–2116. [PubMed: 24446877]
26. May JC, Dodds JN, Kurulugama RT, Stafford GC, Fjeldsted JC, McLean JA. *Analyst.* 2015; 140:6824–6833. [PubMed: 26191544]
27. Kujawinski EB, Behn MD. *Anal. Chem.* 2006; 78:4363–4373. [PubMed: 16808443]
28. Minor EC, Steinbring CJ, Longnecker K, Kujawinski EB. *Org. Geochem.* 2012; 43:1–11.
29. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. *Science.* 1989; 246:64–71. [PubMed: 2675315]
30. Hyung SJ, Robinson CV, Ruotolo BT. *Chem. Biol.* 2009; 16:382–90. [PubMed: 19389624]
31. Niu S, Rabuck JN, Ruotolo BT. *Curr. Opin. Chem. Biol.* 2013; 17:809–817. [PubMed: 23856053]
32. Zheng X, Aly NA, Zhou Y, Dupuis KT, Bilbao A, Paurus V, Orton DJ, Wilson R, Payne SH, Smith RD, Baker ES. *Chemical Science.* 2017; 8:7724–7736.
33. Baker ES, Burnum-Johnson KE, Ibrahim YM, Orton DJ, Monroe ME, Kelly RT, Moore RJ, Zhang X, Theberge R, Costello CE, Smith RD. *Proteomics.* 2015; 15:2766–2776. [PubMed: 26046661]

34. Benesch JL, Ruotolo BT, Simmons DA, Robinson CV. Chem. Rev. 2007; 107:3544–3567. [PubMed: 17649985]
35. Baker ES, Dupuis NF, Bowers MT. J. Phys. Chem. B. 2009; 113:1722–1727. [PubMed: 19193169]
36. Eriksson, L., Johansson, E., Kettaneh-Wold, N., Trygg, J., Wikström, C., Wold, S. Multi- and Megavariate Data Analysis Part I: Basic Principles and Applications. Umetrics; Cheshire, U.K: 2006.

Figure 1.

Schematic diagram of the three distinct FIA configurations, all of which utilize a PAL autosampler (light blue), six port injection valve (gray), and narrow-bore transfer lines. (A) The simplest configuration is the μ Flow FIA system, which only consists of a single valve for automated sample injection into high-flow sources. The two low-flow configurations however require a nanoLC pump (dark blue) and either (B) one valve for the nFlow FIA system or (C) two valves for the Accelerated nFlow FIA to increase throughput. The injection port, waste, transfer line, and sample loops are labeled in the diagram to show their location on each configuration. A more detailed schematic is shown in the Supporting Information for the Accelerated nFlow FIA, illustrating specific lengths and components, since it is the most complicated system.

Figure 2.

Reproducibility and carryover were analyzed for each FIA setup using two different samples (Sample 1 = Agilent tune mix (red) and Sample 2 = ten peptide mixture at 100 nM each (blue)). The results are shown for the (A) μ Flow, (B) nFlow, and (C) Accelerated nFlow configurations. All configurations had sharp peaks for each sample plug illustrating that diffusion and carryover were not occurring.

Figure 3.

Analysis of the dissociation constants of human carbonic anhydrase I with ethoxzolamide, acetazolamide, benzenesulfonamide, and 4-carboxybenzenesulfonamide. All analyses were performed in triplicate with error shown for each value. The resulting K_D values are in close agreement with ref 20.

Figure 4.
pH analyses for the gammaproteobacterial protein from *E. coli* with the NESG id ER309.
The nested IMS/MS spectra from ER309 illustrate that as pH is increased, dimerization also increases. Monomers in the figure are noted as M, and dimers are noted as D with the charge states following.

Figure 5.

Sample stability over time is problematic for dissolved organic matter. (A) The ordination PCA plot for all the detected molecular formulas and normalized signal intensities of the soil samples illustrates that if each sample is analyzed repeatedly throughout the month, changes occur causing the samples not to group together and cluster by day instead. (B) The van Krevelen diagram also summarizes the different classes of compounds that changed upon storage.