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RESEARCH ARTICLE

A passive membrane system for on-line mass spectrometry reagent addition

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Rationale: Post-separation addition of chemical modifiers in liquid chromatography–mass spectrometry is widely used for improving ionization sensitivity and selectivity. This is typically accomplished using a post-column T-junction, which can result in sample dilution and imperfect mixing. We present a passive semi-permeable hollow fiber membrane approach for the addition of chemical modifiers that avoids these issues.

Methods: Model compounds were directly infused by flow injection to an electrospray ionization triple quadrupole mass spectrometer after passing through a polydimethylsiloxane hollow fiber membrane. Ionization enhancement reagents were introduced into the flowing stream by membrane permeation from aqueous solutions. Ionization enhancement from volatile acids and bases in positive and negative electrospray ionization was evaluated to assess the feasibility of this approach.

Results: The membrane-based apparatus resulted in relative ionization enhancement factors of up to 14×, depending upon the analyte, reagent, and ionization mode used. Ionization enhancement signal stability is reasonable (relative standard deviation of 5–7%) for extended periods from the same reagent solution, and minimal analyte dilution is observed. A proof-of-concept demonstration of the chromatographic "trifluoroacetic acid fix" strategy is presented.

Conclusions: An on-line mass spectrometry ionization reagent addition method with potential post-chromatography reagent addition applications was developed using a hollow fiber polydimethylsiloxane membrane. This approach offers a promising alternative to traditional methods requiring additional hardware such as pumps and T-junctions that can result in sample dilution and imperfect reagent mixing.

1 | INTRODUCTION

The post-separation infusion of derivatizing reagents and chemical modifiers in liquid chromatography–mass spectrometry (LC/MS) workflows has been widely used to enhance ionization without altering chromatographic performance. This is done because optimal separation solvent systems can be quite different from those needed for efficient ionization.[1,2] Reagents are frequently introduced to the LC eluent via a T-junction, infusing reagent via a second pumping system. Other post-column addition techniques (still utilizing secondary liquid handling pumps) include the use of reaction coils[3] and triaxial electrospray probes.[4] Although analyte ionization is

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enhanced, post-column addition methods generally result in analyte dilution. Additional issues may also arise from imperfect mixing between the sample flow and the added reagent at the T-junction due to laminar flow effects, leading to higher background noise and/or reduced sensitivity.[5,6] Furthermore, the additional pumps and hardware required for reagent introduction complicate the instrumental setup and can require significant reagent volumes.[7]

Post-column reagent addition is largely used for the introduction of reagents such as volatile acids/bases, derivatizing reagents, fluorescent dyes, and photoionization reagents.[8–11] Most MS modifying reagents share the same purpose: to improve ionization efficiency and/or increase analyte specificity. For example, the post-column addition of aprotic organic solvents and long-chain alcohols to a LC/MS workflow has been demonstrated to enhance the signals of methylphosphonic acids in negative ion mode,[9] and the post-separation addition of halobenzenes and anisoles with atmospheric pressure photoionization (APPI) significantly improves charge exchange ionization for polycyclic aromatic hydrocarbons by LC/MS.[10] Volatile organic solvents such as ethyl acetate, acetone, methanol, and 1,4-dioxane added post-separation can increase the rate of evaporation, lowering the electrospray droplet surface tension and/or altering analyte distributions within the droplets themselves, resulting in increased ionization efficiencies.[9,12] Other methods for reagent addition include direct addition to the sample or addition to the mobile phase in LC, capillary electrophoresis, and other separation methods.[13] Although useful in certain cases, these techniques occur pre-separation, leading to the possibility of compromised or less efficient chromatographic separation, as well as the need for additional sample preparation steps.[14,15]

A potential alternative to existing reagent addition strategies is the use of a flow-through membrane-based system. Membrane-facilitated eluate/eluent modifications have been employed for ion chromatography for decades, using electrodialysis cells to adjust pH for optimal separation as well as to reduce background for electrochemical detection.[16,17] In our group, we have been utilizing membrane introduction MS (MIMS) as an effective sampling technique for direct MS analysis of analytes in complex matrices.[18–25] The choice of membrane material can be tailored towards specific analyses. Semi-permeable polydimethylsiloxane (PDMS) membranes, for example, allow for the selective permeation of small, neutral analytes while rejecting larger molecules (such as proteins), ions, and particulate matter.[18] Mass transport through a membrane is driven by the concentration gradient across the membrane and the diffusivity of the permeant as described by Fick's law.[19,26,27] A typical MIMS experiment utilizes an acceptor phase (liquid solvent or gas) that is continuously flowed over one side of the membrane, and a donor phase (sample) that is exposed to the other membrane surface. Hollow fiber membranes (HFMs) are frequently used, as they are simpler to couple with flowing liquids and can have smaller dead volumes than sheet-membrane-based flow cells. When acceptor phase is flowed through the interior of a HFM lumen, analytes permeating through the membrane from the donor phase are directly transferred to a mass spectrometer for detection, frequently utilizing tandem MS (MS/MS). This approach provides for a convenient on-line sample clean-up/preparation enabling simple workflows for trace-level, on-line, targeted analyte measurements.[27]

Membrane-based ionization reagent addition has been employed by our group for gas-phase MIMS measurements utilizing chemical ionization. Davey et al utilized a MIMS system employing a second membrane system to introduce chemical ionization (CI) reagents to a flowing helium acceptor prior to analyte sampling.[28] This allowed the on-line addition of gas-phase CI reagent, and the flexible utilization of CI reagent ions in a quadrupole ion trap without significant hardware modifications. This work, and the electrodialysis approaches used in ion chromatography, inspired us to develop the membrane-based liquid ionization reagent addition strategy for MS, with potential applications in LC/MS and other separation approaches. This strategy involves the on-line addition of volatile weak acids and bases via a passive membrane flow-through interface to enhance electrospray ionization (ESI) efficiency, while also mitigating significant dilution, imperfect mixing, and the requirement of large reagent volumes, all of which can limit the effectiveness of other post-separation ionization reagent addition techniques.

2 | EXPERIMENTAL

2.1 | Reagents

Reagent-grade formic acid (88%) and propionic acid (99%) were obtained from Sigma-Aldrich (Oakville, ON, Canada) and ACS-grade ammonium hydroxide (28–30%) was obtained from VWR International (Mississauga, ON, Canada). HPLC-grade methanol was purchased from Fisher Scientific (Boston, MA, USA). HPLC-grade trifluoroacetic acid (TFA; 99.9%) was obtained from Baker Analyzed (Phillipsburg, NJ, USA). Deionized water (18 MΩ cm) was produced on site (Facility Scale Reverse Osmosis/Ion Exchange Water Purification System, Applied Membranes Inc., Vista, CA, USA). Analytical reference standards used as model compounds included: nicotine, gemfibrozil, azoxystrobin, acetaminophen, 4-methylumbelliferone, phenacetin, phosmet, eicosapentaenoic acid, and chloramphenicol. Acetaminophen analytical standard was purchased from Cerilliant (Round Rock, TX, USA), eicosapentaenoic acid was purchased from Cayman Chemical (Ann Arbor, MI, USA), and the remaining compounds were purchased from Sigma-Aldrich (Oakville, ON, Canada).

2.2 | Preparation of stock solutions and samples

Standard stock solutions for each model analyte were prepared gravimetrically in 20.0 mL of methanol at 500 μg/mL. Combined standards were prepared volumetrically from the individual stock solutions in 40.0 mL of methanol at concentrations ranging from 1.25 to 25 ng/mL (compound dependent). TFA suppression studies used an acceptor phase of methanol with 0.1% TFA. Ionization enhancing

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reagent (i.e. donor phase) solutions of formic acid, propionic acid, and ammonia were prepared volumetrically in 40.0 mL of deionized water. Formic acid concentrations of 0.01%, 0.1%, 1.0%, 3.0%, 5.0%, and 10.0% (v/v) were tested for their protonation-enhancing abilities in positive ion ESI and ammonia concentrations of 0.01%, 0.1%, 1.0%, 3.0%, and 5.0% (v/v) were tested for their deprotonation-enhancing abilities in negative ion ESI.

2.3 | Mass spectrometry

A triple quadrupole mass spectrometer (TSQ Fortis™, Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated electrospray ion source (HESI™, Thermo Fisher Scientific) was used for all experiments. UHP-grade nitrogen and argon gas (Praxair, Nanaimo, BC, Canada) were used as the sheath gas and collision gas (2.0 mTorr), respectively. Direct infusion of 200 ng/mL standards at 5 µL/min to the electrospray ion source (bypassing the six-port valve) was used to optimize MS/MS parameters. MS/MS conditions are outlined in Table 1 with a 10 ms dwell time per MS/MS transition. Additional MS parameters are given in Table S1.

2.4 | Reagent addition membrane system

A schematic diagram of the experimental system used for these studies is given in Figure 1. A syringe pump (Fusion 100, Chemyx Inc., Stafford, TX, USA) equipped with a 10 mL gastight syringe (Hamilton Corporation, Reno, NV, USA) was used to deliver methanol liquid acceptor phase at 50 µL/min for all experiments. The six-port divert valve of the mass spectrometer system was utilized with a 20 µL sample loop to introduce sample aliquots of combined standards by flow injection to the flowing acceptor. A "J-probe" style HFM probe (described elsewhere[21,29]) was employed for reagent addition, utilizing a 2.2 cm length of PDMS HFM (Silastic® tubing, Dow Corning, Midland, MI, USA) with a wall thickness of 0.17 mm (0.30 mm inner diameter,

TABLE 1 Optimized mass spectrometer operating conditions for target analytes

Compound	MS/MS transition (*m/z*)	Ion polarity	Spray voltage (V)	Tube lens (V)	Collision energy (eV)	In-source fragmentation (V)
Gemfibrozil	249.1 → 121.1	Negative	3500	59	14.06	9.8
	251.1 → 129.2	Positive	3800	107	10.64	16.3
Acetaminophen	150.0 → 107.1	Negative	3500	89	16.96	24.5
	152.1 → 110.1	Positive	3800	93	16.33	31.0
4-Methylumbelliferone	175.0 → 133.1	Negative	3500	81	21.18	22.9
	177.1 → 77.1	Positive	3800	102	34.95	32.7
Chloramphenicol	321.0 → 257.1	Negative	3500	97	10.39	27.8
Eicosapentaenoic acid	301.1 → 257.2	Negative	3500	111	11.02	19.6
Phosmet	318.1 → 160.1	Positive	3800	111	15.61	21.2
Azoxystrobin	404.1 → 372.1	Positive	3800	123	14.56	18.0
Phenacetin	180.1 → 110.0	Positive	3800	100	20.88	29.4
Nicotine	163.2 → 130.1	Positive	4000	82	21.34	14.7

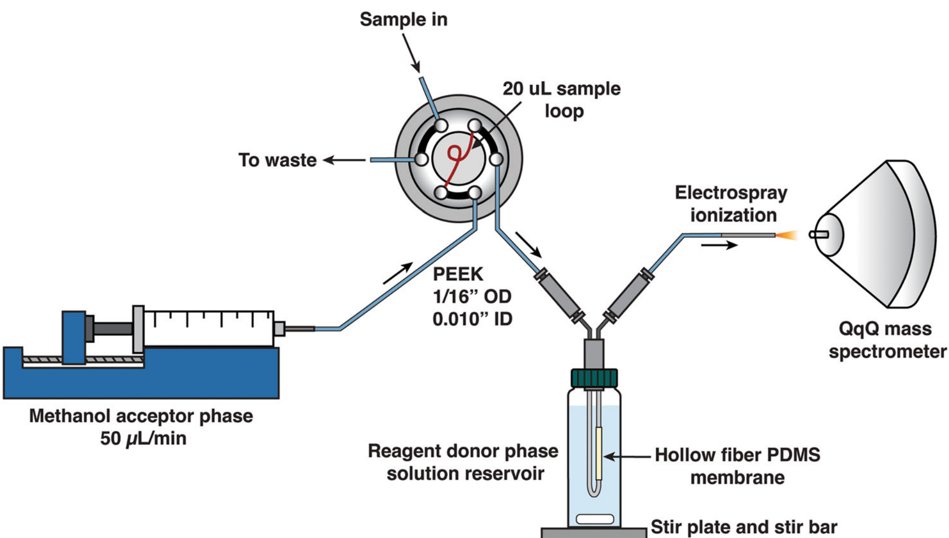

FIGURE 1 Experimental schematic illustrating the ionization reagent addition membrane system. Note: not to scale [Color figure can be viewed at wileyonlinelibrary.com]

0.64 mm outer diameter, internal volume 155 μL) for all experiments. Poly(ether ether ketone) (PEEK) tubing (1/16″ outer diameter, 0.010″ inner diameter; Chromatographic Specialties, Brockville, ON, Canada) was used for all liquid transfer lines. The reagent addition (donor phase) vial was magnetically stirred at 500 rpm.

2.5 | Data analysis

Analyte signals were integrated using the vendor-provided software for the mass spectrometer (FreeStyle™ version 1.7, Thermo Fisher Scientific, San Jose, CA, USA). All measurements were conducted in triplicate with uncertainties reported as the standard deviation. Relative signal enhancements (RSEs) were calculated by dividing the signal obtained in the presence of an aqueous ionization-enhancing reagent by the signal obtained using reagent-free deionized water.

3 | RESULTS AND DISCUSSION

In LC/MS, post-separation ionization reagent addition methods are often used, but can have a number of disadvantages. Larger solvent and reagent volumes are often required for continuous addition, increasing cost and waste disposal requirements, and hardware such as a pump(s), reaction loop(s) and/or mixers are necessary, complicating the analysis workflow and further increasing cost.[7] Additional complications arising from the use of traditional post-separation reagent addition methods include sample dilution and the possibility of imperfect reagent mixing due to laminar flow effects in T-junctions. The latter can result in increased detector noise, reducing the analytical sensitivity.[7] By using a passive membrane reagent addition system, many of the complications and disadvantages observed with T-junctions can potentially be avoided. Since only a small amount of the reagent permeates through the membrane, significantly less reagent is consumed and minimal sample dilution occurs, improving analytical sensitivity while reducing reagent requirements.[30] To this end, we examined the potential effectiveness of a passive PDMS HFM flow-through system for on-line ESI-MS ionization reagent addition, evaluating its performance with flow injection studies. This included investigating the effect of reagent concentration upon ionization enhancement, the extent to which the modifier system dilutes the analyte flow, the stability of reagent addition performance over an extended period, and a proof-of-concept demonstration of the "TFA fix" strategy, a post-column addition method used in LC/MS.[31,32]

3.1 | Ionization enhancement by addition of volatile acid or base

The application of volatile weak acids and bases as modifying reagents for ionization enhancement in ESI-MS is well documented in the literature.[33,34] The extent to which a modifying reagent increases signal intensity depends on the relative pH and pK_a values of the reagent and analyte, respectively. Volatile weak acids such as formic, acetic, and propionic acids are common additives utilized to increase positive ion formation in ESI.[34,35] If the pH of acid solutions is lower than the pK_a of the target analyte (or its conjugate acid), analyte protonation results in increased ionization efficiency. To explore protonation ionization enhancement with the reagent addition membrane system, a series of aqueous formic acid reagent donor phase solutions were evaluated. Due to the small size and relative volatility of formic acid, its neutral form rapidly permeates the PDMS membrane from the reagent donor phase and mixes with the methanol acceptor flow. A cocktail suite of six positively ionizing analytes that form $[M + H]^+$ ions (gemfibrozil, phosmet, azoxystrobin, 4-methylumbelliferone, phenacetin, and acetaminophen) was injected in 20 μL aliquots to the flowing methanol acceptor via a six-port valve (Figure 1), evaluating each acid concentration for its effect upon ionization enhancement by assessing the RSE observed with and without the formic acid in the reagent donor phase. The pK_a values of the target analytes examined are given in Table 2.

It was observed that the optimal formic acid concentration in the reagent donor phase varied from compound to compound, with RSE factors ranging from approximately one (no enhancement) to two (Table 2, Figure S1). Gemfibrozil demonstrated the greatest RSE (1.8×) using the 1.0% (v/v) formic acid donor phase. Phosmet, azoxystrobin, 4-methylumbelliferone, and phenacetin displayed modest RSE (Table 2) using a formic acid reagent donor phase of 10.0% (v/v). Acetaminophen had essentially no RSE improvement, regardless of formic acid concentration.

In negative ion ESI, volatile weak bases such as ammonia are frequently used as additives to increase ionization efficiency of negatively ionizing species via deprotonation.[33,34] Similar to proton donation by weak acids, weak bases will accept a proton from acidic analytes. This results in an increase in the abundance of $[M - H]^-$ ions formed in ESI. A series of aqueous reagent donor phases containing varying concentrations of ammonia were used to evaluate the deprotonating effects of weak bases upon a suite of five analytes (eicosapentaenoic acid, chloramphenicol, acetaminophen, 4-methylumbelliferone, and gemfibrozil) using negative mode ESI.

Overall, it was found that greater RSE was observed using ammonia in the reagent donor phase in negative ion mode ESI than using formic acid in positive ion mode (Table 2). Of the compounds studied, gemfibrozil had the greatest RSE (14×) using a 1.0% ammonia reagent donor phase (Figure 2, Table 2). Acetaminophen and eicosapentaenoic acid had significant RSE values of 13.5× and 4.2×, respectively, both with a 5.0% ammonia donor phase. RSEs for chloramphenicol and 4-methylumbelliferone were modest (1.6×) with 1.0% ammonia reagent donor phase (Table 2). The larger signal enhancements observed for gemfibrozil and acetaminophen indicate that significant deprotonation of these compounds occurs over the other target analytes tested in the presence of ammonia. Gemfibrozil and acetaminophen both have hydroxyl groups that can be deprotonated, explaining the greater RSE values. Eicosapentaenoic acid also had a greater observed RSE than other compounds in the suite: carboxylic acid deprotonation is expected. It is also possible that the compounds with greater RSE values are more surface-active and

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TABLE 2 Summary of analyte RSEs observed by addition of acid and base ionization-enhancement modifiers with on-line reagent addition membrane system

Analyte	[Analyte] (ng/mL)	Ion	pK_a [a]	Modifier	Optimal [modifier] (% v/v)	RSE [b]
Gemfibrozil	20.0	$[M - H]^-$	4.42	Ammonia	1.0	14.0
Acetaminophen	12.5	$[M - H]^-$	9.38	Ammonia	5.0	13.5
Eicosapentaenoic acid	25.0	$[M - H]^-$	4.82	Ammonia	5.0	4.2
Chloramphenicol	20.0	$[M - H]^-$	8.69	Ammonia	1.0	1.6
4-methylumbelliferone	12.5	$[M - H]^-$	7.8	Ammonia	1.0	1.6
Gemfibrozil	20.0	$[M + H]^+$	4.42	Formic acid	1.0	1.8
Phosmet	17.5	$[M + H]^+$	N/A	Formic acid	10.0	1.6
Phenacetin	5.0	$[M + H]^+$	14.98	Formic acid	10.0	1.5
Azoxystrobin	5.0	$[M + H]^+$	1.94	Formic acid	10.0	1.4
4-methylumbelliferone	12.5	$[M + H]^+$	7.8	Formic acid	10.0	1.4
Acetaminophen	12.5	$[M + H]^+$	9.38	Formic acid	3.0	1.1

[a] PubChem.[36]
[b] RSE = (signal area with modifier)/(signal area without modifier).

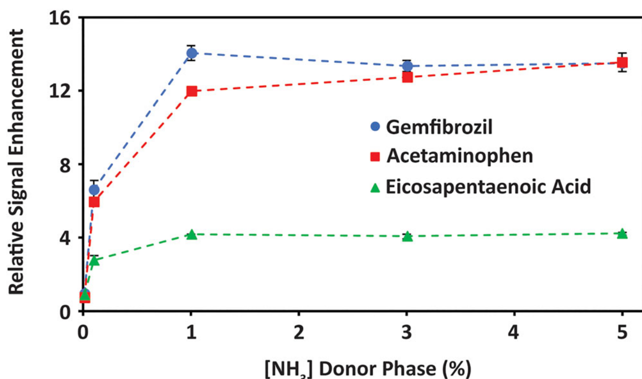

FIGURE 2 RSEs for 20.0 ng/mL gemfibrozil (blue), 12.5 ng/mL acetaminophen (red), and 25.0 ng/mL eicosapentaenoic acid (green) with increasing ammonia concentration in the aqueous ionization reagent donor phase using negative ion mode ESI-MS/MS. Error bars represent the standard deviation of three measurements [Color figure can be viewed at wileyonlinelibrary.com]

therefore are more likely to be released from the charged ESI droplets during the electrospray process.[12,37] Although not evaluated in this study, possible reduced surface activity for these analytes might also explain why minimal signal enhancement was observed for chloramphenicol and 4-methylumbelliferone in the presence of ammonia.

The observed RSEs of the compounds with the addition of weak acids and bases via the passive membrane reagent addition strategy demonstrate its potential utility to enhance ESI ionization efficiency. While the reagent permeability concentration dependence across the membrane was not assessed, depending upon the desired application(s), required modifier concentrations would be expected to be analyte dependent. Reagent donor phase concentrations beyond their maximal RSE typically demonstrate no further signal enhancements, and for the concentrations examined, no significant signal suppression. Optimal donor phase reagent concentrations are dependent upon several operational parameters (i.e. membrane geometry, acceptor phase composition, flow rates) and are meant to demonstrate feasibility rather than suggest global optima. The flow injection studies presented model conditions that would be encountered in isocratic LC separations. With the high (and similar) solubilities of small molecules such as ammonia and formic acid in water and methanol,[38] for gradient separation scenarios (either reversed or normal phase), providing reagent donor phase concentrations slightly beyond the maxima should yield effective ionization enhancements.

An additional approach to adjusting the optimal levels of ionization reagent would be to modify the membrane geometry utilized (i.e. thickness, diameter, length). While we chose a readily available PDMS HFM substrate for this work, the use of thinner and longer membranes with MIMS is an effective strategy to increase analyte permeation,[19,21,27] or in this case, increase reagent permeation. We caution the reader that the use of longer and/or larger-diameter membranes may yield impaired application when used with LC separations because of any associated dead volume increases. For higher liquid flow rates, increased dilution of the modifying reagent would be expected, and would require reassessment of the optimal reagent donor phase concentrations required. Although not examined in this study, this membrane-based system could also be used for the addition of other signal-enhancing reagents (e.g. haloaromatics for APPI), provided the desired reagents can permeate the membrane substrate utilized.

3.2 | Dilution and stability studies

The use of traditional post-separation reagent addition methods often results in dilution of the sample flow, thereby lowering analyte concentrations, reducing the ultimate sensitivity possible. However, when using a semi-permeable PDMS membrane, polar solvents

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(e.g. water) do not permeate to a large extent, and minimal sample dilution from the reagent addition donor phase occurs.[30] The extent to which the membrane apparatus possibly dilutes the sample flow was evaluated by comparing analyte signals for flow injections of 20.0 ng/mL gemfibrozil using air, methanol, and deionized water as reagent donor phase solvents in the absence of any ionization-enhancing reagents. The results from this experiment are summarized in Figure 3. A slight decrease in signal intensity was observed when the methanol donor phase vial was replaced by the deionized water donor phase. This could be caused by minor dilution of the acceptor phase flow from trace permeation of water. Alternatively, trace amounts of water permeating the membrane could also cause changes in ESI efficiency. In either case, the effect is minor with signal intensities *ca* 12% lower when using the deionized water donor phase compared to those when using methanol. When the methanol donor phase solvent matches the methanol acceptor phase, significant analyte dilution effects would not be expected because of the lack of a methanol concentration gradient across the membrane, consistent with the signals observed when the reagent addition membrane was in room air (Figure 3). Depending upon analyte specific physicochemical properties (i.e. hydrophobicity), analyte back-permeation from the sample stream into the reagent donor phase could potentially alter analyte signal levels. This bias, if it exists for a given analyte, could be compensated for with appropriate analytical calibrations. This study suggests that the water used as a solvent for the reagent addition donor phase does not permeate the membrane into the acceptor phase in appreciable amounts and has minimal effect upon signal intensities.

A potential disadvantage of a passive membrane reagent addition system is that the amount of the volatile acid or base reagent in the donor phase may deplete over time, affecting the extent of ionization enhancement during a lengthy measurement. Depletion could potentially occur via evaporation of a volatile ionization reagent, or from extended mass transport out of the reagent donor phase via permeation across the membrane. To evaluate this, a continuous 5.5 h reagent addition experiment was conducted to evaluate potential ionization reagent depletion via membrane permeation from a 1.0% aqueous ammonia donor phase (40 mL donor phase volume). While ammonia permeation occurs rapidly, the membrane interface was immersed in the donor phase and allowed to equilibrate *ca* 1.3 h to ensure full equilibration and steady-state reagent introduction levels (Figure 4). The signal intensities of gemfibrozil, acetaminophen, and eicosapentaenoic acid flow injections added to the continuously flowing acceptor were monitored at periodic intervals over the course of the experiment (Figure 4). To minimize any loss of volatile ammonia vapor, the reagent donor phase and PDMS reagent addition membrane were housed in a Teflon™-faced septum-sealed vial (Figure 1) for the duration of the experiment.

As demonstrated by this study, analyte signals remained relatively stable for the continuous addition of reagent during an extended measurement period, suggesting that the ammonia modifier in the donor phase is not being significantly depleted by mass transport across the passive reagent addition membrane. During membrane equilibration, only modest signal increases are observed, and for the three analytes examined, reasonable relative standard deviations (RSDs; 5–7%) were observed for seven flow injections measured over a post-equilibration period of *ca* 4 h. These modest signal variations may be due to subtle reagent permeability variations or minor ESI efficiency variations over the course of the experiment. This demonstrates the efficacy of a membrane-based system for reagent addition in analytical workflows, allowing for longer measurement times without needing to replenish the reagent donor phase.

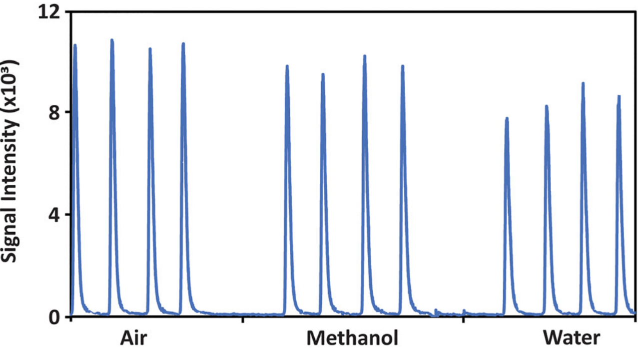

FIGURE 3 Evaluation of reagent donor phases upon signal intensity for replicate 20 μL flow injections of a 20.0 ng/mL gemfibrozil sample using negative ion mode ES-MS/MS [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | TFA fix demonstration

Mobile phase additives such as TFA and heptafluorobutyric acid (HFBA) are commonly used in the LC/MS analysis of peptides[32] and

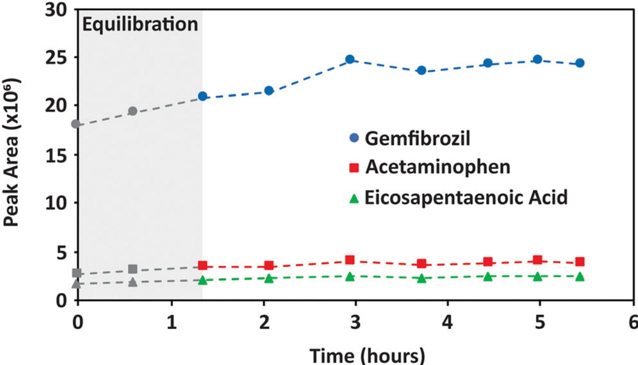

FIGURE 4 Evaluation of any potential depletion of ionization reagent from the donor phase (1.0% ammonia in deionized water) during 5.5 h of continuous reagent addition via passive membrane introduction. After *ca* 1.3 h of membrane equilibration with the reagent donor phase (gray area), seven periodic 20 μL flow injections of a cocktail suite containing 20.0 ng/mL gemfibrozil (7% RSD), 12.5 ng/mL acetaminophen (6% RSD), and 25.0 ng/mL eicosapentaenoic acid (5% RSD) were injected in the continuously flowing liquid acceptor delivered directly to the ESI source, monitoring the signal areas by negative ion mode MS/MS [Color figure can be viewed at wileyonlinelibrary.com]

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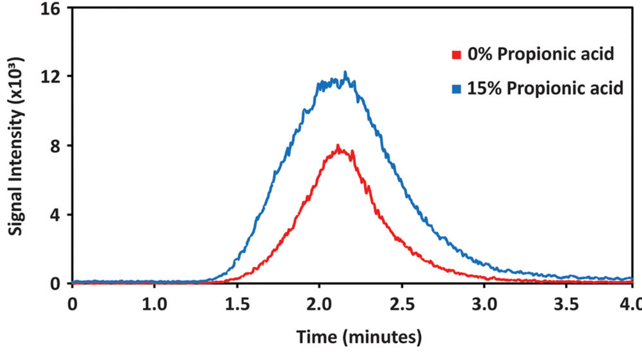

FIGURE 5 Signal chronograms for 20 μL flow injection aliquots of 5 ng/mL nicotine in continuously flowing 0.1% (v/v) TFA acceptor with a 15% aqueous propionic acid donor phase compared to that obtained with only deionized water in the ionization reagent donor phase reservoir [Color figure can be viewed at wileyonlinelibrary.com]

small, basic molecules[39] to improve chromatographic resolution. Improved chromatographic performance is achieved because TFA or HFBA forms an ion pair with the analyte, increasing retention times and improving peak resolution. Although sharper chromatographic peaks are produced, ionization efficiency with ESI is compromised due to the formation of the neutral ion pair.[35] Analyte signals can be recovered using a method called the TFA fix.[31,32] In this method, first demonstrated by Apffel et al, a weak acid such as propionic acid is introduced post-separation using a T-junction to mitigate ionization suppression caused by the stronger acid mobile phase additives.[31] Propionic acid acts to protonate the TFA and/or HFBA anions, thus freeing positively charged $[M + H]^+$ analyte ions from the ion pair.[39]

To demonstrate this application of the passive membrane reagent addition system, a simplified proof-of-concept demonstration of the TFA fix approach was conducted.[31] In this study, nicotine was used as the model compound and propionic acid as the signal recovery reagent. The apparatus for this experiment is the same as that described in Figure 1, where 5 ng/mL nicotine in methanol flow injections were introduced in a flowing methanolic TFA (0.1% v/v) acceptor. Different concentrations of aqueous propionic acid (0.5%, 5.0%, and 15% v/v) were initially evaluated as candidate reagent donor phase solutions to determine which gave the best signal recovery, with 15% propionic acid yielding the best results. Figure 5 illustrates the results of an experiment utilizing passive membrane reagent introduction to mitigate TFA-induced ionization suppression. The nicotine signal areas obtained using 15% aqueous propionic acid as the reagent donor phase increased by 1.7× relative to that observed without propionic acid. Although this is a proof-of-concept demonstration, it suggests that the use of a passive membrane for on-line MS reagent addition has potentially broader applications for post-separation ionization reagent addition.

4 | CONCLUSIONS

A passive PDMS membrane reagent addition system is demonstrated as a simple and convenient alternative to traditional MS reagent addition techniques, such as T-junctions. The presented system has demonstrated signal enhancements of up to 14×. The reproducibility, stability, and negligible sample dilution of the membrane system have been evaluated. The passive membrane system provided reproducible ionization enhancement for up to 5.5 h of continuous reagent addition without needing to replenish the reagent donor phase. We suggest this approach has potentially broad applicability for simplified reagent addition in flowing liquids being introduced to ESI and other MS ionization approaches. This method also has the potential for utilization in other applications outside those demonstrated here, for example, the addition of iodoacetic acid derivatization reagents for improved stability of thiol-containing compounds,[11] or for the post-separation addition of haloaromatic photoionization dopants in APPI LC/MS.[10] Future work will be aimed at demonstrating the use of the membrane system for post-column addition LC/MS experiments and additional studies to further improve signal enhancements and optimize the apparatus.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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