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A Dual-Column Solid Phase Extraction Strategy for Online Collection and Preparation of Continuously Flowing Effluent Streams for Mass Spectrometry

Jeffrey R. Enders^{1,2,3}, Christina C. Marasco^{3,4}, John P. Wikswo^{3,4,5,6}, and John A. McLean^{1,2,3}

¹Department of Chemistry, Vanderbilt University, Station B 351822, Nashville, Tennessee 37235

²Vanderbilt Institute of Chemical Biology, Vanderbilt University, 896 Preston Building, Nashville, Tennessee 37232

³Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University, Station B 351807, Nashville, Tennessee 37235

⁴Department of Biomedical Engineering, Vanderbilt University, Station B 351631, Nashville, Tennessee 37235

⁵Department of Physics and Astronomy, Vanderbilt University, Station B 351807, Nashville, Tennessee 37235

⁶Department of Molecular Physiology and Biophysics, Vanderbilt University, 702 Light Hall, Nashville, Tennessee 37232

Abstract

Current desalination techniques for mass spectrometry-based protocols are problematic for performing temporal response studies where increased temporal resolution requires small samples and faster sampling frequencies, which greatly increases the number of samples and sample preparation time. These challenges are pertinent to cellular dynamics experiments, where it is important to sample the biological system frequently and with as little sample waste as possible. To address these needs, we present a dual-column online solid phase extraction (SPE) approach capable of preconcentrating and preparing a constantly perfusing sample stream, with minimal to no sample loss. This strategy is evaluated for use in microfluidic bioreactor studies specifically aimed at characterizing suitable sample flow rates, temporal resolving power, and analyte concentrations. In this work we demonstrate that this strategy may be used for flow rates as low as 500 nL/min, with temporal resolving power on the order of 3 minutes, with analyte loadings ranging from fmol to pmol for metabolites. Under these conditions recoveries of *ca.* 80% are obtained even at fmol loadings.

Keywords

desalting; online desalting; online desalination; solid phase extraction; online SPE; online chromatography; mass spectrometry; cellular dynamics; microfluidics; cell culture

Introduction

With the rise of systems biology, biological research has experienced a shift of emphasis over the years from comprehensive measurements of the genome, transcriptome, and proteome to dynamic measurements of the metabolome. Methods such as DNA microarrays and two-hybrid screening, which have been critical for the advances in genomics and proteomics, are less useful for establishing the instantaneous metabolic state of an organism. Instead, techniques more capable of rapid analysis have come to the forefront of biological research due to the highly transient nature of the metabolome. Temporal integrity has become a vital component in these analyses, especially in fields such as cellular dynamics, where sufficiently frequent global metabolomics measurements can provide an understanding of internal metabolism kinetics.¹ A faster sampling frequency and a greater number of detectable analytes allow for a more comprehensive interpretation of the system. Analyses on these timescales and breadth are well suited for the fast, broad detection capabilities of mass spectrometry (MS).

Mass spectrometry, when paired with electrospray ionization (ESI), is capable of complex and dynamic liquid sample analysis, making it a unique fit for the measurement of dynamic living systems. However, the application of MS in these areas has been encumbered in the past by the need to prepare all samples to remove the abundant low-volatility inorganic salts essential to life. Salts, or electrolytes, are used by cells as a general means of maintaining a precise osmotic balance and resting transmembrane potentials. In addition, they are required by certain cell types for other functions, such as muscle contraction (*e.g.*, heartbeat), nerve signaling, and blood homeostasis. Electrolytes are found in varying concentrations in extracellular and intracellular fluids, and levels rise and fall through ingestion and perspiration.² To sustain cell viability in culture, cell culture medium contains these essential salts, typically at concentrations of 100–150 mM.^{3,4} These salts significantly interfere with the processes required to ionize and vaporize the sample prior to mass analysis. The suppression of ion signal in ESI involves charge competition mechanisms during ionization and decreased sensitivity due to a division of ion signal across multiple salt cluster species.^{5–7}

There are several techniques available to remove contaminant salts from biological samples in an offline, or quasi-offline fashion, including but not limited to solid phase extraction (SPE), liquid chromatography (LC), and capillary electrophoresis (CE). Collecting samples such as blood, lymph, serum, plasma, or cell culture medium effluent from a dynamic system (*e.g.*, living organism or live cell culture) and preparing the samples offline result in an accumulation of samples and a proportional increase in sample preparation time. Additionally, the collection of fractions in this manner results in averaging of dynamic responses with a temporal resolution proportional to the duration of each fraction collection. In order to properly sample dynamic fluctuations, the frequency with which these samples are collected must be no less than half the frequency of the highest fluctuation required by the Nyquist-Shannon sampling theorem.^{8,9} For example, if a biomolecular secretion is oscillating at a frequency on the order of 0.1 min^{-1} (as may be seen in glucose-stimulated insulin secretion¹⁰), each sample fraction collection must contain no more than 5 minutes of secreted material (assuming continuous collection and no sample loss) in order for the oscillation to be observable without aliasing.

Separation techniques such as LC and CE can perform sufficient online salt removal, but often at the cost of losing significant temporal resolution. Numerous online SPE methods exist in the patent and research literature;^{11–23} however, these methods can suffer from extensive sample loss, inadequate handling of contaminant streams, and/or poor time resolution. Additionally, many of these online techniques are used exclusively to process

fractionated samples, not continuous sample streams. Dynamic measurement requirements necessitate several modifications for such methods to be integrated into a rapid-sampling platform. These modifications require the apparatus to accept a constantly flowing sample stream, whereas most current online preparation methods utilize single or discrete injections for sample introduction (similar to an LC autosampler). Another modification includes a second column to continue to accept sample eluent while the first column is being prepared for analysis. This minimizes sample waste and therefore loss of valuable information and temporal resolution. A waste cycle needs to be incorporated so that contaminants (salt, etc.) are not directed towards the mass spectrometer, as this could cause fouling and plugging of nano-junctions and orifices, which is of particular relevance to ESI. Lastly, the apparatus would ideally be miniaturized in order to accept and prepare the output flow of various cell culture microfluidics, which could be as low as 50 to 500 nL/min.

With these considerations in mind, we designed and implemented a three-valve, dual-column online SPE apparatus that is able to (i) handle a continuously flowing sample stream (without directing to waste), (ii) direct contaminants to a separate waste port during washing, (iii) analyze flow rates on the order of 500 nL/min or less and, (iv) provide variable preconcentration durations to optimize temporal resolution based on analyte concentrations. In these studies we implemented our online dual-column SPE apparatus in conjunction with a poly(dimethylsiloxane) (PDMS) microfluidic bioreactor as proof-of-concept of this approach. Specifically, we have used a cell trapping microfluidic device known as a Multitrap Nanophysiometer (MTNP).^{24–27} This device is designed to trap and sustain cultures of adherent and non-adherent cells. We sequentially perfused solutions of varying biomolecular identities through the microfluidic bioreactor to simulate the dynamic online output of a cellular culture for temporally coherent preparation of this continuous bioreactor effluent stream for ESI-MS.

Experimental

Desalting Instrumentation

SPE columns are made of 360 μm OD/100 μm ID fused silica tubing and are packed with 3 μm , 300 Å, C₁₈ phase Jupiter Bulk Packing (Phenomenex, Torrance, CA) using a PIP-500 Pressure Injection System (New Objective, Woburn, MA). Packing was performed at *ca.* 1000 psi, with column length ranging from 10–15 cm. Similar to typical chromatography specifications, these columns are replaced following *ca.* 200 injections, and are noted to retain consistent load and elution characteristics throughout. The Phenomenex Jupiter phase has been found to have an 80% recovery rate with a small molecule, hematin (Fig S1). Three 10-port Nanovolume UPLC Valves with 360 μm fittings, C72MH-4690ED (VICI Valco Instruments Co. Inc., Houston, TX), were used to implement the online desalting apparatus. Valve actuation was automated using VCOM software v1.1.01 (VICI Valco Instruments Co. Inc., Houston, TX). The aqueous solvent and both organic solvent lines, running at 500 nL/min, were supplied with an Eksigent Nanoflow Metering System (AB SCIEX, Framingham, MA). Additionally, 360 μm OD/50 μm ID PEEK tubing was used between all junctions and throughout the entire assembly except for the SPE columns.

Sample/Solvent Preparation

RPMI-1640 media (ATCC, Manassas, VA) was mixed with Val⁵-Angiotensin I and Val⁵-Angiotensin II (Sigma-Aldrich, St. Louis, MO) to make 5 $\mu\text{g/mL}$ standard solutions of each. Aqueous solvent (solvent A) is composed of 95% distilled deionized water (DDW, 18.2 MG cm) and 5% Chromasolv methanol (Sigma-Aldrich, St. Louis, MO) with 0.1% formic acid (Fisher Scientific, Pittsburgh, PA). Organic solvent (solvent B) is composed of 95%

Chromasolv methanol (Sigma-Aldrich, St. Louis, MO) and 5% DDW with 0.1% formic acid (Fisher Scientific, Pittsburgh, PA).

Microfluidic Bioreactor/Online SPE Apparatus

A MTNP microfluidic device designed to culture mammalian cells was used to simulate the biological microenvironment.^{24–27} This device was fabricated in-house out of PDMS. Briefly, SU-8 is used to produce a photo-activated polymerization resist. PDMS is then poured over the positive resist and baked to create a cured PDMS device. The device is placed on a glass coverslip prior to use. These devices were silanized with 2-[methoxy(polyethyleneoxy)propyl]-trimethoxysilane (Gelest, Morrisville, PA) to minimize analyte adhesion as demonstrated previously.²⁸ No cells were used in this proof-of-concept study. Online sample effluent from a dynamic biological system was simulated by using a PicoPlus syringe pump (Harvard Apparatus, Holliston, MA) to perfuse media solutions through the PDMS bioreactor.

Mass Spectrometry Analysis

All mass spectrometry analyses were conducted on a Synapt G2 (Waters Corp., Milford, MA) time-of-flight mass spectrometer (TOFMS) with a nano-electrospray ionization (nESI) source. Data were collected in continuous fashion to demonstrate the characteristics of the desalting apparatus.

Results and Discussion

Since the inception of the ESI technique,²⁹ salt mediated ion suppression effects have proven to be a formidable challenge, depending on the design of the experiment. A detailed examination of prior strategies for continuous flow stream online desalting showed that these techniques did not meet our requirements for dynamic cellular studies. To address these needs we propose the present dual-column solid phase extraction strategy. The following describes (i) the chief causes of the salt ion suppression effect, (ii) the metrics and design criteria for comparing allied desalting techniques, and (iii) the evaluation and validation of the dual-column solid phase extraction strategy.

Theory of Ion Suppression Effect

The electrospray process functions through a series of evaporative events and Coulombic-repulsion based explosions.^{30–32} Sample matrices (*i.e.*, salts/electrolytes and other non-volatile species) have long been known to have serious suppression effects on the electrospray ionization process and are often viewed as contaminants. Ion suppression causes poor quantitation conditions and is considered to be a limitation of ESI and nESI. An understanding of the mechanisms of charged droplet formation and fission is imperative to determining the causes of ion suppression. Previous studies have assessed ion suppression effects using fundamental approaches, and their findings support the idea that the most likely suppression mechanism involves reduced evaporation efficiency (Fig. 1a, b). The main cause of this reduced evaporation is thought to be several-fold:^{5,7,33–35}

- i. The non-volatile contaminants raise the boiling point of the droplet through alteration of the colligative properties of the solution.
- ii. Certain non-volatile contaminants (*e.g.*, detergents) congregate at the surface of the droplet due to their increased surface activity and compete with analyte molecules for these sites. This increase in non-volatile species at the surface of the droplet increases the surface tension and thereby decreases evaporation of solvent and analyte from the droplet.

- iii. Some contaminants, such as salts, readily crystallize and, in turn, encourage co-crystallization with analyte molecules, thereby locking them in the solid state and negating gas-phase mass spectrometric detection.
- iv. The sphere of hydration for strongly solvated ions (*e.g.*, Na⁺, K⁺, Li⁺) is large and restricts mobility of not only the solvated ion but also surrounding analyte ions in the droplet bulk solution. This constrained mobility prevents ions that may initially exist in the bulk from migrating to the surface of the droplet to be evaporated. In addition, this large sphere of hydration shields the droplet by presenting uncharged solvent molecules on the droplet surface to the surrounding electric field.

Only through near complete salt removal (Fig. 1b) prior to ionization can these unwanted suppression effects be avoided.

Metrics and Design Criteria

There are several techniques available to desalt time-course or time-correlated samples for mass spectrometry in an offline or online fashion (Table 1). In these types of analyses the general workflow follows the basic scheme and order of (i) sample/fraction collection (*i.e.*, from the organism or bioreactor), (ii) sample preparation (*i.e.*, desalination), and (iii) sample analysis. An important distinction is made here to differentiate offline from online experimental protocols. In this work, offline techniques are defined as those where sample/fraction collection, sample preparation, and sample analysis are performed in separate locations or on separate non-connected pieces of equipment. This is often accomplished by storing samples in discrete containers (*e.g.*, well plates or microcuvettes). Online techniques are those where these steps are performed on the same piece of equipment; all parts and processes are fluidly connected in series without human intervention occurring between steps. The techniques listed as “Continuous sample treatment” in Table 1 are those which are able to accept a continuous incoming flow stream, purge it of contaminants such as salts, and subsequently direct it to a detector. Online capillary electrophoresis and liquid chromatography are not exclusively used to prepare continuous samples streams; however, in principle they are capable of doing so, and there are examples of this type of implementation in the literature.^{36–41}

The most common offline techniques, such as SPE, dialysis, and CE, are generally straightforward methods to implement using well-established protocols. When used to desalt time-correlated samples offline, these types of techniques require that sequential fractions be collected in order to store and later prepare the samples elsewhere. The time-consuming sample preparation protocols which follow are often countered by collecting fewer fractions or pooling fractions. This, however, leads to lower sampling frequency and increased averaging of analyte levels, and precludes the observation of high-frequency perturbations. Additionally, offline sample preparation often involves multiple transfer steps that may contribute to sample losses.

Online methods of desalting, such as LC,^{36,37} online CE,^{38–44} and online single-column SPE,^{11–23} do not suffer as much from long preparation times since sample collection and sample detection is integrated with the sample preparation. They do, however, encounter challenges related to sample loss and sampling frequency. LC, CE, and SPE utilize one column/capillary to do all sample accumulation, processing, and eluting. This dictates the use of discrete samples, or in the case of a continuous sample stream, that the incoming sample stream be directed to waste while the single column is being prepared. This sample loss is detrimental to a complete temporal record (*i.e.*, between input and output streams) since the analyte and information inherent to this lost material are unrecoverable. In terms of continuous flow, packed phase column-based techniques (such as LC and SPE) and open capillary column techniques (such as CE) are generally able to accept a wide range of

collection flow rates. In these online techniques, analytes must physically stop for a period of time (whether it be on the LC or SPE column or in the CE capillary) in order to carry out the physical separation of salts from analytes of interest. While this does increase averaging of molecular signal and preparation duration, it also provides an opportunity to preconcentrate low abundant signals.

Microdialysis methods as described in the recent literature are a viable option for continuous online sample analyses.^{45–49} They have the best potential for high temporal integrity because it involves diffusional forces across a concentration gradient in an often constantly flowing solution stream. Ignoring the potential for loss of low molecular weight material through the dialysis membrane itself, online microdialysis does not require the incoming sample stream to be diverted to waste and therefore retains all incoming analyte material. Microdialysis, when paired with MS, may require post-dialysis solvent conditioning for optimal ESI performance and consequently analyte dilution. It should be noted that flow rate and back pressure need to be optimized for fundamentally different processes in both microdialysis and for ESI and that these values may be dissimilar depending on the arrangement that is used. Cell trapping microfluidics, such as the MTNP used in this work, function at flow rates on the order of 500 nL/min with minimal back pressure (conditions required for cell viability).

Dual-column online SPE, such as that presented here, has the potential to provide a method of desalting which provides favorable figures of merit for those outlined. The use of two columns negates sample loss by accepting the continuous sample stream on one column while the other column is being purged and eluted. The preparation time, while not as expedient as online microdialysis, is variable and can, in principle, achieve temporal resolution depending only on the number of columns utilized and the duration necessary to wash out salts given the particular column characteristics. The non-instantaneous nature of the preparation also provides the opportunity to preconcentrate the sample should it be necessary for the detection of low abundance analytes. Additionally, similar to most column-based methods, it is better suited for low collection/sample flow rates.

Method Validation

The valve arrangement presented here (Fig. 2a, b) uses a six-step cycle (Fig. 2c) which provides a constant loading condition, ensuring that all analyte material in the online sample effluent will be collected if desired. In addition, contaminants are rinsed from the SPE columns during a purge step which is directed to waste. Valve cycle steps can be scaled in time uniformly to yield a cycle which is better for detecting high frequency oscillations (short step durations) or one which is better for detecting very low abundance analytes (long step durations). Figure 2c outlines the valve switching scheme for online sample preparation. In step 1, the valve orientation is as pictured in Figure 2a, such that SPE column A is loaded with online sample effluent and SPE column B is desalting a sample plug and sending salts to waste. Valve 3 is switched such that SPE column A is receiving sample effluent and SPE column B is eluted and directed towards the MS for ion detection. Because this is the first cycle, column B has not actually been loaded with any analytical material at this time and so this elution is blank (as observed later). After this, valve 3 is switched so that SPE column B is equilibrated with aqueous solvent in preparation for its subsequent loading step. SPE column A continues to load sample throughout the equilibration of SPE column B. Next, valve 1 is switched such that the online sample effluent is directed to the newly equilibrated SPE column B. SPE column A is purged with water at this step, allowing for desalination of the sample collected on the column. Valve 2 is switched and SPE column A is eluted with organic solvent, while SPE column B is left in the loading position. Lastly, valve 2 is switched to equilibrate SPE column A and the SPE apparatus starts the entire cycle again. Note that the MS is analyzing a sample for only two of the six steps. Were Fig.

2 implemented in triplicate, it would be possible for six samples to be interleaved without sample loss or overlap, enabling analysis, for example, of three parallel bioreactors that could provide one control and two different experimental conditions near simultaneously.

Various standards were perfused through a MTNP microfluidic bioreactor and onto the dual-column online SPE apparatus to validate temporal response and reproducibility (Fig. 3a). Figures 3b and 3c depict the valve timing and resulting total ion chromatogram (TIC), respectively, for the online SPE apparatus. The valve cycle produces a pattern of two alternating sets of peaks (*i.e.*, every other peak originates from the same column). When the time-step durations are set equally, this yields a single time period of elution followed by two time periods of clean solvent, hence the rise and fall shape of the chromatogram. Importantly, the length of these time steps can be adjusted to shorter durations to emphasize temporal dynamics or longer durations to extend preconcentration steps and thereby aid in identifying lower abundance signals. In Figures 3 and 4 the time steps were set to one minute, yielding three minutes of load time, and one minute each for purging, eluting, and equilibrating. Using this timing scheme the limit of detection for a model metabolite (hematin) was observed to be 50 ng/mL or *ca.* 75 pg (118 fmol) of total material (see Table S1 for details).

In order to simulate dynamic cellular output, three syringe pumps were connected to the inlets of an MTNP microfluidic bioreactor device (Fig. 3a). The first pump delivered a solution containing 5 $\mu\text{g/mL}$ of Val⁵-Angiotensin I (Standard #1) in RPMI 1640 medium through the device and into the desalting apparatus for 15 minutes, followed by delivery by the second pump of RPMI 1640 medium (without any additives) for 15 minutes, and then the third pump delivered 5 $\mu\text{g/mL}$ of Val⁵-Angiotensin II (Standard #2) in RPMI 1640 medium for an additional 30 minutes. After this, RPMI 1640 medium was flowed for 30 minutes using the second pump, followed by clean deionized water for 80 minutes using the first pump, which originally delivered Angiotensin I (sequence shown in Fig. 4a).

In Figure 4b, the data show a total ion count increase for 30 minutes, at which point the ion signal reaches a steady state, and then decreases after the deionized water has been flowing for approximately 30 minutes. The TIC peaks displayed in Fig. 4b were integrated to determine the repeatability of these elution cycles. Importantly, only the peaks occurring from minute 27 to minute 120 were used, as this was considered the time over which the signal was in equilibrium. The peak areas were found to have a percent relative standard deviation of *ca.* 15% throughout these experiments. Note that intracolumn (only for column A or B) reproducibility and intercolumn (for both column A and B) reproducibility are 14.6 % ($n = 16$, $m = 2$) and 15.0 % ($n = 32$), respectively. Figure 4c shows the extracted ion chromatogram for Val⁵-Angiotensin I, m/z 642.40. These data show that although Val⁵-Angiotensin I was perfused at minute 0, it was not detected until the second cycle (minute 6), owing to the inherent blank first cycle (discussed above). Additionally, the signal for Val⁵-Angiotensin I was not greatly reduced until minute 25 and residual traces of m/z 642.40 were observed throughout the experiment. This hysteresis is typical when using C₁₈ phase SPE columns and can be mitigated by using more rigorous column conditioning methodologies.⁵⁰ Figure 4d shows an extracted ion chromatogram (XIC) for Val⁵-Angiotensin II, m/z 516.80. Similar to Val⁵-Angiotensin I, the signal for Val⁵-Angiotensin II appears at the second cycle and some hysteresis is observed. In Figure 4e, the XIC for glucose ([M+Na]⁺, m/z 203.01) is shown. Glucose was a unique identifier of cellular media in these experiments (*i.e.*, only present in the RPMI 1640 media). RPMI 1640 medium was present from 0–90 minutes in this time course; however, the ion signal for glucose was not detected until minute 27. These results suggest changes in column affinity as other cellular material began to bind and interact with the column. Glucose was also susceptible to hysteresis, as evidenced by the lingering signal even 60 minutes after RPMI 1640 medium

was discontinued. Alternatively, the hysteresis effect could be related to carbohydrate affinity for silica surfaces (*e.g.*, C₁₈ phase bead surface). Increasing the time-step duration (and thereby decreasing the resolution) allows one to better observe lower abundance analytes and mitigates the observed hysteresis. Decreased hysteresis is accomplished due to a longer elution step, which ensures that more material is removed from the SPE column every cycle. To further assess the repeatability of these elutions, the peaks resulting from glucose specifically (from minutes 27–120), were integrated and found to have percent relative standard deviations of *ca.* 20% for both intracolumn and intercolumn precision. Regardless, this apparatus exhibits satisfactory desalting capabilities, as shown in Figure 5. In the absence of any form of desalting, a stable electrospray was not able to be generated and therefore no ions were observed. With the use of the dual-column SPE apparatus, an abundant analyte signal was observed for both model peptides.

This apparatus demonstrates the capability to successfully analyze and ionize small peptide analytes within a continuous salt-rich sample stream using dual-column online SPE desalting techniques (Fig. 5). This capability is required when performing analyses on lab-on-a-chip/microfluidic bioreactor devices or other bioreactors where analyses need to be performed online in a temporal fashion and with small sample amounts. Measurements on this timescale were previously time-consuming due to the offline nature of most preparation work-flows. By keeping the sample online and preparing sample material using two SPE columns in parallel, sample loss is negated and sample preparation time is drastically reduced. Thus more frequent measurements and comprehensive data can be used to interpret biological systems.

Conclusions

We have described a strategy with a demonstrated ability to perform temporally resolved online desalination of a continuous sample stream at a resolution of three minutes with minimal loss of analyte and a lower limit of detection in the fmol to pmol range for small molecules. Under these conditions recoveries of *ca.* 80% are obtained even at fmol loadings. These experiments show that highly dynamic analyte fluctuations can be observed and non-volatile contaminants can be removed prior to downstream ESI-MS. Continuous online desalination provides a decrease in sample preparation time compared to fractionation (on the order of 10–30 minutes per sample). Importantly, we demonstrated the ability to observe analyte concentration changes in less than five minutes. This feature could prove useful for metabolic flux analyses and metabolic control analyses. This apparatus can also be used to prepare fractionated samples; however, the most beneficial application will be the study of the dynamic metabolic output of living systems or organisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

A depiction of the various effects of salt on electrospray analysis. (a) In a schematic representation of an electrospray droplet, solvated inorganic ions (*e.g.*, sodium, potassium) restrict movement of hydrophilic analytes towards the droplet exterior, where they would undergo evaporation. Surface-active contaminants preferentially occupy the droplet surface and also reduce the likelihood of analyte evaporation. Additionally, as the solvents evaporate, the salt concentrations increase and trap analyte in the solid phase (upper right inset region). All of these effects are thought to contribute to salt suppression in ESI. (b) An illustrative example showing the effect of salt concentration on a standard peptide, bradykinin (observed in the spectra in its $[M+2H]^{2+}$ form, m/z 531.12). Even at a salt concentration of 10 mM (compared to cellular medium salt concentrations of 100–150 mM), extensive signal suppression and increased baseline effects can be readily observed (adapted from reference 27).

Figure 2.

(a) The three-valve arrangement used in the online SPE experiments. All three valves were individually actuated by coordinated, automatic computer control. The legend indicates the valve orientation notation. (a) Simplified diagram showing 4- and 6-port valves. (b) Implementation of (a) using 10-port valves with unused ports being capped. (c) A chart showing the sequence of states for valves (listed in numerical order), SPE column A, SPE column B, and the detector (MS) through the six-step cycle.

Figure 3.

Experimental scheme with valve timing and sample elution for the online apparatus. (a) Scheme depicting how the various sample solutions were perfused through a MTNP microfluidic device and then into the desalting apparatus. This arrangement was chosen to simulate a dynamically varying continuous sample stream originating from a live cell bioreactor. (b) Chart illustrating the valve cycle. Importantly, the chart shows that the loading step is continuous and occurs on one column while the other column is purged of contaminants, eluted to remove analyte material, and then equilibrated to prepare for the next loading cycle. (c) An example total ion chromatogram (TIC) showing the typical peak and trough pattern produced from the cycle shown in (a). The peaks are labeled based on the column from which the analytes are eluted.

Figure 4.

A variable analyte concentration experiment, showing perfusion of different analytes (in RPMI 1640 medium) and H₂O through the MTNP, to characterize the online SPE desalting apparatus. (a) A chart showing the progression of solutions through the apparatus over the course of a 170-minute experiment. (b) The TIC for the experiment. (c–e) Extracted ion chromatograms for (c) Standard #1, Val⁵-Angiotensin I, m/z 642.40, (d) Standard #2, Val⁵-Angiotensin II, m/z 516.80, and (e) the $[M+Na]^+$ ion of glucose, m/z 203.01, in the RPMI common to the first four analytes. The asterisk (*) corresponds to a break in the data between minutes 86 and 88, which was the result of the data file reaching its maximum length and the initialization of a second file. The two files were combined based on their accurate time tags.

Figure 5. Demonstration of desalting capabilities of the dual-column SPE apparatus when used to assess an online dynamically fluctuating flow stream. (a) Combined mass spectrum from minute 45 to 48 showing the peptide DRVYVHPF (standard #2, Val⁵-Angiotensin II) at m/z 516, from desalted cell culture medium. (b) Inset of the mass spectrum for the region immediately surrounding the peptide DRVYVHPF. In a companion experiment without desalting, a stable spray could not be produced for this sample without first desalting it, thus no ions were observed and no spectrum was generated.

Table 1
Typical relative figures of merit for methods commonly used for the removal of salt from biological samples

Desalting techniques	Relative figure of merit				Representative references
	Acceptable flow rate	Sample loss ^a	Ability to pre-concentrate ^b	Commercially available	
<i>Continuous sample treatment (i.e., online)</i>					
Dual-column solid phase extraction	nL/min to mL/min	No	Yes	No	Present studies
Online microdialysis	nL/min to μ L/min	No	No	No	49,51,52
Single-column solid phase extraction	nL/min to mL/min	Yes	Yes	Yes	14,15,19,21–23
Capillary electrophoresis	nL/min to μ L/min	Yes	Yes	Yes	38–41
Liquid chromatography	μ L/min to mL/min	Yes	Yes	Yes	36,37
<i>Discrete sample treatment (i.e., offline)</i>					
Solid phase extraction	N/A	No	Yes	Yes	53
Microdialysis	N/A	No	No	Yes	47
Capillary electrophoresis	N/A	No	Yes	Yes	54,55

^aDistinguishes the prospect of this technique to direct the incoming flow stream to waste

^bSpecifies the capacity of the technique for allowing sample material to accrue in some manner