
In this paper, we present our development of a MEMS chip with an overhanging polymer microcapillary 2.5 mm in length and with a 5 μm*10 μm orifice size at the tip. The fabricated chips have been successfully interfaced with a mass spectrometer (MS) to validate electrospray ionization (ESI) for biochemical analysis. The prediction of a reduction in Taylor cone size has also been observed with real time ESI fluid visualization from our chip. Built-in micro particle filters and centimeter long serpentine microchannels were fabricated on the chip with a low temperature process by using the Parylene polymer as a structural material, aluminum and photoresist as sacrificial layers, and bromine trifluoride (BrF/sub 3/) gas phase etching for final microcapillary releasing. The use of an overhanging polymer structure adds a new a level of mechanical robustness that was never achievable with other thin films. Functionality of our device was proven by consistent detection of myoglobin in a 200 nM solution at a flow rate of 35 nL/min and a voltage potential of 1.5 kV. This MS interface chip represents vital and significant improvements in MEMS process technology and MS functionality with respect to the silicon nitride (Si/sub x/N/sub y/) ESI nozzles previously reported. (14 References).


Protein identification through peptide mass mapping by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a standard technique, used in many laboratories around the world. The traditional methodology often includes long incubations (6-24 h) and extensive manual steps. In an effort to address this, an integrated microanalytical platform has been developed for automated identification of proteins. The silicon micromachined analytical tools, i.e., the microchip immobilized enzyme reactor (mu-chip IMER), the piezoelectric microdispenser, and the high-density nanovial target plates, are the cornerstones in the system. The mu-chip IMER provides on-line enzymatic digestion of protein samples (1 μL) within 1-3 min, and the microdispenser enables subsequent on-line picoliter sample preparation in a high-density format. Interfaced to automated MALDI-TOF MS, these tools compose a highly efficient platform that can analyze 100 protein samples in 3.5 h. Kinetic studies on the microreactors are reported as well as the operation of this microanalytical platform for protein identification, wherein lysozyme, myoglobin, ribonuclease A, and cytochrome c have been identified with a high sequence coverage (50-100%).


We describe an integrated analytical system consisting of a microfluidics device micromachined using photolithography/etching technology, a panel of computer-controlled high-voltage relays, and an electrospray ionization tandem mass spectrometer. Movement of solvents and samples on the device and off the device to the mass spectrometer was achieved by directed electroosmotic pumping induced by the activation of a suitable constellation of high-voltage relays. The system was used for the sequential automated analysis of protein digests. We demonstrate low femtomole per microliter sensitivity of detection and compatibility of the system with the automated analysis of
proteins separated by two-dimensional gel electrophoresis.


Analytical biochemistry, in particular the analysis of regulatory proteins that control biological systems and pathways, is dependent on methods of ever-increasing sensitivity. Capillary electrophoresis (CE) has long been recognized as an ultrasensitive analytical technique. In spite of the high sensitivity, CE has not penetrated protein discovery research as a standard analytical method. In this review article we summarize recent technical developments which have significantly enhanced CE as a tool for the analysis of trace amounts of proteins. Specifically, we review recent advances in the development and application of capillary electrophoresis-mass spectrometry (CE-MS) and on-line analyte concentration techniques, and introduce the emerging field of microfluidics as a front end to mass spectrometry (MS).


Microfabrication technology offers the opportunity to construct microfluidic modules which are designed to perform specific, dedicated functions. Here we report the construction of a microfabricated device for the generation and delivery by electroosmotic pumping of solvent gradients at nanoliter per minute flow rates. The device consists of three solvent reservoirs and channels which were etched in glass. Solvent gradients and solvent flows were generated by computer controlled differential electroosmotic pumping of aqueous and organic phase, respectively, from the solvent reservoirs. The device was integrated into an analytical system consisting of the solvent gradient delivery module, a reverse phase microcolumn and an electrospray ionization ion trap mass spectrometer (MS). The system was used for the analysis at high sensitivity of peptides and peptide mixtures generated by proteolytic digestion of proteins. We have measured an absolute limit of detection as low as 1 fmol and a concentration limit of detection at the 100 amol/microL level. The system was also successfully used for the identification of proteins separated by 1D and 2D gel electrophoresis. This was achieved by gradient frontal analysis of the peptide mixture generated by proteolysis of the respective proteins, and the automated generation and interpretation of collision-induced dissociation spectra.


An integrated platform is presented for rapid and sensitive protein identification by on-line protein digestion and analysis of digested proteins using electrospray ionization mass spectrometry or transient capillary isotachophoresis/capillary zone electrophoresis with mass spectrometry detection. A miniaturized membrane reactor is constructed by fabricating the microfluidic channels on a poly(dimethylsiloxane) substrate and coupling the microfluidics to a poly(vinylidene fluoride) porous membrane with the adsorbed trypsin. On the basis of the large surface area-to-volume ratio of porous membrane media, adsorbed trypsin onto the poly(vinylidene fluoride) membrane is employed for
achieving ultrahigh catalytic turnover. The extent of protein digestion in a miniaturized membrane reactor can be directly controlled by the residence time of protein analytes inside the trypsin-adsorbed membrane, the reaction temperature, and the protein concentration. The resulting peptide mixtures can either be directly analyzed using electrospray ionization mass spectrometry or further concentrated and resolved by electrophoretic separations prior to the mass spectrometric analysis. This microfluidic system enables rapid identification of proteins in minutes instead of hours, consumes very little sample (nanogram or less), and provides on-line interface with upstream protein separation schemes for the analysis of complex protein mixtures such as cell lysates.


For this work, two different plastic microfluidic devices are designed and fabricated for applications in high-throughput residue analysis of food contaminants and drug screening of small-molecule libraries. Microfluidic networks on copolyester and poly(dimethylsiloxane) substrates are fabricated by silicon template imprinting and capillary molding techniques. The first device is developed to perform affinity capture, concentration, and direct identification of targeted compounds using electrospray ionization mass spectrometry. Poly(vinylidene fluoride) membranes sandwiched between the imprinted copolyester microchannels in an integrated platform provide continuous affinity dialysis and concentration of a reaction mixture containing aflatoxin B1 antibody and aflatoxins. The second microfluidic device is composed of microchannels on the poly(dimethylsiloxane) substrates. The device is designed to perform miniaturized ultrafiltration of affinity complexes of phenobarbital antibody and barbiturates, including the sequential loading, washing, and dissociation steps. These microfabricated devices not only significantly reduce dead volume and sample consumption but also increase the detection sensitivity by at least 1-2 orders of magnitude over those reported previously. Improvements in detection sensitivity are attributed to analyte preconcentration during the affinity purification step, limited analyte dilution in the microdialysis junction, minimal sample loss, and the amenability of ESI-MS to nanoscale sample flow rates.


A novel microfabricated multichannel emitter for electrospray ionization mass spectrometry (ESI-MS) was implemented with polydimethylsiloxane (PDMS) using a soft lithography technique. The emitters are formed as electrospray tips along a thin membrane on the edge of the device with channels of 100 microm x 30 microm dimensions. The electrospray performance of the PDMS emitters for a single channel device and a four channel device interfaced with a time-of-flight mass spectrometer was evaluated for detecting the molecular weight of reference peptides (angiotensin I and bradykinin). The emitters were durable at the flow rate of 1-20 microL min(-1) for more than 30 h of continuous electrospray with limit of detection of 1 microM (S/N 18). This microfabrication method for a PDMS multichannel emitter as an integral part of a microfluidic device will facilitate development of more complex microfluidic analysis
A multichannel electrospray ionization (ESI) emitter was fabricated as part of a poly(dimethylsiloxane) (PDMS) microfluidic device using a three-layer photoresist process which also produces a self-alignment system to make a bonding between the top and bottom PDMS parts. The prototype device (2 cm high X 5 cm wide X 5 cm long) had 16-channels (30 mum wide X 50 mum deep) with emitters of 1 mm length and 60degree point angle. The PDMS emitter tips enabled interfacing the device to ESI-mass spectrometry; a stable electrospray from the tips was performed with limits of detection under 1 muM for reference peptides (adrenocorticotropic hormone fragment 1-17, angiotensin I and III).


Silicon microtechnology has been used to develop a microstructure toolbox in order to enable high accuracy protein identification. During the last 2 years we developed and applied monocrystalline silicon structures and established new automated protein analysis platforms. The development of a high throughput protein platform is presented where fully automated protein identifications are performed. It includes the reduction and alkylation of the protein sample in a standard 96- or 384-well plate format prior to injection of 1 microl samples into the continuous flow based microtechnology platform. The processed sample is transferred to a microchip nanovial array target using piezoelectric microdispensing. Identification is made by MALDI-TOF MS and a database search. After the initial sample reduction and alkylation period of 50 min the platform can digest and process protein samples at a speed of 100 samples in 210 min. An optional configuration of the platform, operating the dispenser in the 'static mode', enables on-target enrichment of low abundant proteins and peptides e.g. from 2DE samples. This makes detection at the low attomole level possible.


An emerging field for the analysis of biological systems is the study of the complete protein complement of the genome, the 'proteome'. There are several complementary tools available for proteome analysis including 2D protein electrophoresis and mass spectrometry. Emerging technologies for proteome analysis include spotted-array-based methods and microfluidic devices. Taken together, these technologies provide a wealth of information that is useful in discovery-based science. However, there are some key limitations of these approaches and new technology is required to be able to fully integrate proteomic information with information obtained about DNA sequence, mRNA profiles and metabolite concentrations into effective models of biological systems.

[References: 42]


The separation of biological mixtures in open micro-channels using electrophoresis with rapid and simple coupling to mass spectrometry is introduced. Rapid open-access channel electrophoresis employs microchannels that are manufactured on microchips. Separation is performed in the open channels, and the chips are transferred to a matrix-assisted laser desorption/ionization (MALDI) source after the solvent is evaporated. The matrix (2,5-dihydroxybenzoic acid) is placed in the solution with the run buffer before the separation of the analyte components. After separation, the solvent is evaporated and the microchip is ready for MALDI-MS analysis. The microchip is placed directly into a specially designed ion source of an external source Fourier transform mass spectrometry instrument. Separation of simple mixtures containing oligosaccharides and peptides is shown.


Desorption mass spectrometry has undergone significant improvements since the original experiments were performed more than 90 years ago. The most dramatic change occurred in the early 1980s with the introduction of an organic matrix to transfer energy to the analyte. This reduces ion fragmentation but also introduces background ions from the matrix. Here, the authors describe a matrix-free strategy for biomolecular mass spectrometry based on pulsed-laser desorption-ionization from a porous silicon surface. Their method uses porous silicon to trap analytes deposited on the surface, and laser irradiation to vaporize and ionize them. They show that the method works at femtomole and attomole levels of analyte, and induces little or no fragmentation, in contrast to what is typically observed with other such approaches. The ability to perform these measurements without a matrix also makes it more amenable to small-molecule analysis. Chemical and structural modification of the porous silicon has enabled optimization of the ionization characteristics of the surface. The authors' technique offers good sensitivity as well as compatibility with silicon-based microfluidics and microchip technologies. (30 References).


Here we report on the development of a proteomic platform utilizing a piezoelectric flow-through dispensing unit made from silicon microstructures. The use of a novel surface coating, where matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI MS) targets were uniformly precoated with a thin film of matrix/nitrocellulose, made the sample preparation straightforward and enabled the enrichment and analysis of proteins at low levels in proteomics samples. We demonstrate this by analyzing excised spots in a biological sample originating from a human fetal fibroblast cell line that was subjected to 2D gel-electrophoresis. Furthermore, a sample deposition rate below 30 Hz results in an increased analyte density on the dispensed sample spot, rendering signal amplification. In general, the sensitivity for proteins and peptides can be enhanced 10-50 times compared to traditional MALDI sample preparation techniques. (17 References).

A miniaturised-SYNthesis and Total Analysis System (mu SYNTAS) was used for the solution-phase synthesis and on-line analysis (TOF-MS) of Ugi multicomponent reaction (MCR) products. This approach provides an unusually high degree of control of the MCR and delivers detailed, novel information on reaction intermediates in real-time. Specifically, the Ugi 4 component condensation (4CC) involving the reaction of an amine, acid, aldehyde and isocyanide species was performed at room temperature in a controllable fashion. Furthermore, observation of the nitrilium intermediate, cyclohexyl(2-piperidin-1-ylethyldyne)ammonium chloride, is presented for the first time.


Matrix-assisted laser desorption ionization mass spectrometry (MALDI MS) has been applied to increase the informational output from DNA sequence analysis. It has been used to analyze DNA by hybridization with microarrays of gel-immobilized oligonucleotides extended with stacked 5mers. In model experiments, a 28 nt long DNA fragment was hybridized with 10 immobilized, overlapping 8mers. Then, in a second round of hybridization DNA-8mer duplexes were hybridized with a mixture of 10 5mers. The stability of the 5mer complex with DNA was increased to raise the melting temperature of the duplex by 10-15 degrees C as a result of stacking interaction with 8mers. Contiguous 13 bp duplexes containing an internal break were formed. MALDI MS identified one or, in some cases, two 5mers contiguously stacked to each DNA-8mer duplex formed on the microchip. Incorporating a mass label into 5mers optimized MALDI MS monitoring. This procedure enabled us to reconstitute the sequence of a model DNA fragment and identify polymorphic nucleotides. The application of MALDI MS identification of contiguously stacked 5mers to increase the length of DNA for sequence analysis is discussed.


There is a need to develop reliable portable analytical systems for biomonitoring lead (Pb) in noninvasively collected saliva samples. In addition, appropriate pharmacokinetic analyses are used to quantitate systemic dosimetry based on the saliva Pb concentrations. A portable microfluidics/electrochemical device was developed for the rapid analysis of Pb based on square wave anodic stripping voltammetry, in which a saliva sample flows over an electrode surface, Pb2+ is chemically reduced and accumulated, and the electric potential of the electrode scanned. The system demonstrates a good linear response over a broad Pb concentration range (1-2000 ppb). To evaluate the relationship between saliva and blood Pb, rats were treated with single oral doses ranging from 20 to 500 mg Pb/kg of body weight, and 24 hours later were administered pilocarpine, a muscarinic agonist to induce salivation. To correlate saliva levels with internal dose, blood and saliva were collected and quantitated for Pb by
inductively coupled plasma-mass spectrometry (ICP-MS) and by the microanalytical system. The quantitation with the microanalytical system was slightly less (approximately 75-85%) than with ICP-MS; however, the response was linear, with concentration suggesting that it can be used for the quantitation of salivary Pb. To facilitate modeling, a physiologically based pharmacokinetic (PBPK) model for Pb was modified to incorporate a salivary gland compartment. The model was capable of predicting blood and saliva Pb concentration based on a limited data set. These results are encouraging, suggesting that once fully developed the microanalytical system coupled with PBPK modeling can be used as important tools for real-time biomonitoring of Pb for both occupational and environmental exposures.


A microfluidic device is described in which an electrospray interface to a mass spectrometer is integrated with a capillary electrophoresis channel, an injector and a protein digestion bed on a monolithic substrate. A large channel, 800 microm wide, 150 microm deep and 15 mm long, was created to act as a reactor bed for trypsin immobilized on 40-60 microm diameter beads. Separation was performed in channels etched 10 microm deep, 30 microm wide and about 45 mm long, feeding into a capillary, attached to the chip with a low dead volume coupling, that was 30 mm in length, with a 50 microm i.d. and 180 microm o.d. Sample was pumped through the reactor bed at flow rates between 0.5 and 60 microL/min. The application of this device for rapid digestion, separation and identification of proteins is demonstrated for melittin, cytochrome c and bovine serum albumin (BSA). The rate and efficiency of digestion was related to the flow rate of the substrate solution through the reactor bed. A flow rate of 1 or 0.5 microL/min was found adequate for complete consumption of cytochrome c or BSA, corresponding to a digestion time of 3-6 min at room temperature. Coverage of the amino acid sequence ranged from 92% for cytochrome c to 71% for BSA, with some missed cleavages observed. Melittin was consumed within 5 s. In contrast, a similar extent of digestion of melittin in a cuvet took 10-15 min. The kinetic limitations associated with the rapid digestion of low picomole levels of substrate were minimized using an integrated digestion bed with hydrodynamic flow to provide an increased ratio of trypsin to sample. This chip design thus provides a convenient platform for automated sample processing in proteomics applications. Copyright 2000 John Wiley & Sons, Ltd.


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vaporize and ionize them. We show that the method works at femtomole and attomole levels of analyte, and induces little or no fragmentation, in contrast to what is typically observed with other such approaches. The ability to perform these measurements without a matrix also makes it more amenable to small-molecule analysis. Chemical and structural modification of the porous silicon has enabled optimization of the ionization characteristics of the surface. Our technique offers good sensitivity as well as compatibility with silicon-based microfluidics and microchip technologies.


A novel microfabricated device for isoelectric focusing (IEF) incorporating an optimized electrospray ionization (ESI) tip was constructed on polycarbonate plates using laser micromachining. The IEF microchip incorporated a separation channel (50 micro x 30 micro x 16 cm), three fluid connectors, and two buffer reservoirs. Electrical potentials used for IEF focusing and electrospray were applied through platinum electrodes placed in the buffer reservoirs, which were isolated from the separation channel by porous membranes. Direct ESI-mass spectrometry (MS) using electrosprays produced directly from a sharp emitter "tip" on the microchip was evaluated. The results indicated that this design can produce a stable electrospray and that performance was further improved and made more flexible with the assistance of a sheath gas and sheath liquid. Error analysis of the spectral data showed that the standard deviation in signal intensity for an analyte peak was less than approximately 5% over 3 h. The production of stable electrosprays directly from microchip IEF device represents a step towards easily fabricated microanalytical devices. Microchannel IEF separations of protein mixtures were demonstrated for uncoated polycarbonate microchips. Direct microchannel IEF-ESI-MS was demonstrated using the microfabricated chip with an ion-trap mass spectrometer for characterization of protein mixtures.


Microfabricated multiple-channel glass chips were successfully interfaced to an electrospray ionization mass spectrometer (ESI-MS). The microchip device was fabricated by standard photolithographic, wet chemical etching, and thermal bonding procedures. A high voltage was applied individually from each buffer reservoir for spraying sample sequentially from each channel. With the sampling orifice of the MS grounded, it was found that a liquid flow of 100-200 nL/min was necessary to maintain a stable electrospray. The detection limit of the microchip MS experiment for myoglobin was found to be lower than 6 x 10^(-8) M. Samples in 75% methanol were successfully analyzed with good sensitivity, as were aqueous samples. The parallel multiple-channel microchip system allowed ESI-MS analysis of different samples of standard peptides and proteins in one chip.


In continuation of our work to develop an integrated multichannel microchip interfaced to
electrospray mass spectrometry (ESI-MS), this paper demonstrates one of several applications of this approach in monitoring tryptic digestion products. The multichannel microchip allowed integration of sample preparation onto the microchip to facilitate the analysis process. Melittin was selected as a model oligopeptide because it possesses a cluster of four adjacent basic residues which enable probing the site specificity of trypsin as a function of digest times. Reactions were performed on-chip in different wells for specific time periods and then analyzed by infusion from the microchip by ESI-MS, using leucine enkephalin as internal standard. The rate of formation and disappearance of the molecular ion and individual fragments was followed for a melittin to trypsin concentration ratio of 300:1. The results indicate the potential of integrating enzymatic reactions with multichannel microchip ESI-MS for automated optimization of reaction condition while consuming only small amounts of sample.


The comprehensive analysis of biological systems requires a combination of genomic and proteomic efforts. The large-scale application of current genomic technologies provides complete genomic DNA sequences, sequence tags for expressed genes (EST's), and quantitative profiles of expressed genes at the mRNA level. In contrast, protein analytical technology lacks the sensitivity and the sample throughput for the systematic analysis of all the proteins expressed by a tissue or cell. The sensitivity of protein analysis technology is primarily limited by the loss of analytes, due to adsorption to surfaces, and sample contamination during handling. Here, the authors summarize their work on the development and use of microfabricate fluidic systems for the manipulation of minute amounts of peptides and delivery to an electrospray ionization tandem mass spectrometer. New data are also presented that further demonstrate the potential of these novel approaches. Specifically, the authors describe the use of microfabricated devices as modules to deliver femtomole amounts of protein digests to the mass spectrometer for protein identification. The authors also describe the use of a microfabricated module for the generation of solvent gradients at nl/min flow rates for gradient chromatography-tandem mass spectrometry. The use of microfabricated fluidic systems reduces the risk of sample contamination and sample loss due to adsorption to wetted surfaces. The ability to assemble dedicated modular systems and to operate them automatically makes the use of microfabricated systems attractive for the sensitive and large-scale analysis of proteins. (34 References).