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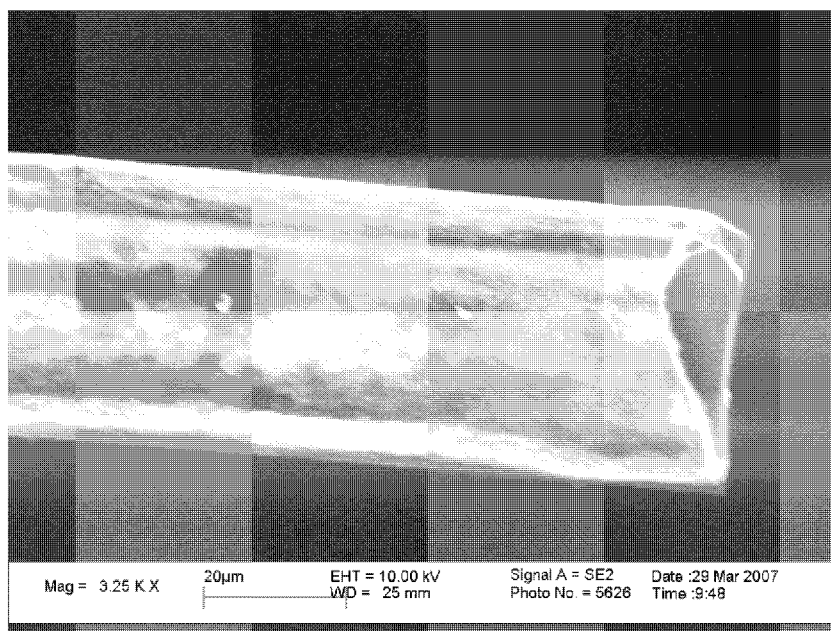
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(54) Title: INTERFACING LOW-FLOW SEPARATION TECHNIQUES

Figure 1



(57) Abstract: A capillary column, and method for forming a capillary column, in which the capillary column comprises at least one porous segment at a terminus of the capillary column, wherein the at least one porous segment is formed by exposing the segment to one or more of a solution of acid, base, and a mechanical tool.

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5 **INTERFACING LOW-FLOW SEPARATION TECHNIQUES**

CROSS REFERENCE TO RELATED APPLICATIONS

 This application claims the benefit of U.S. Provisional Application Serial No. 60/852,800 filed October 18, 2006, which is incorporated by reference herein.

10 **BACKGROUND**

 Analysis of a large number of complex samples at low quantity and limited sample volume requires an analytical technique with fast analysis time, high specificity, high sensitivity, and high resolution. To achieve high resolution and high sensitivity under electrospray ionization (ESI) technique, separation techniques that use narrower
15 columns/capillaries with low flow rates, such as nano-liquid chromatography (nano-LC) and capillary electrophoresis (CE), have become wide spread. For example, in high performance liquid chromatography-mass spectrometry (HPLC-MS), 75- μm -i.d. reverse phase columns with flow rates of ~ 250 nL/min (nano-LC) have become the column of choice for proteomics study. In nano-liquid chromatography-mass spectrometry (nLC-MS), as long as the flow rates
20 in the analytical capillaries are >100 nL/min, electrical connection to the end of these capillaries for electrospray ionization is usually achieved through a "zero dead volume" union/Tee.

 In conventional capillary electrophoresis mass spectrometry (CE-MS), where a 50 or 75- μm -i.d. capillary is usually used, sheath-flow interfaces that provide voltage to the
25 capillary outlet through addition of conductive liquid are utilized. To achieve higher resolution and more sensitivity, even narrower columns with lower flow rates such as nLC-MS using column diameter of less than 75- μm , or alternatively CE-MS with narrower capillaries (<30 - μm -i.d.) with flow rates in the low nL/min are gaining popularity [1]. At these low flow rates however, conventional interfacing techniques, such as attaching a nano-
30 spray tip to the analytical capillary using a union, are no longer useful because of the dead volume that they introduce in the interface region.

 Over the years, three general techniques have been developed to address the need for interfacing narrow capillaries with low flow rates to MS via ESI: sheath-flow, sheathless, and split-flow interfaces [2-11]. Sheath-flow techniques bear several disadvantages: (1) dilution
35 of the analyte by the sheath liquid; (2) competition for available charge between the species present in the sheath-flow and the analyte in the ESI process (Gale and Smith 1993); and (3) effects on separation, solubility, or molecular conformation which vary according to sheath

5 liquid composition (Thompson et al. 1993, Foret et al. 1994, Smith et al. 1991). Therefore, in recent years, sheathless and split-flow interfaces have become more popular for interfacing low flow rates capillaries to MS because of their higher sensitivity of detection, which results from the absence of a sheath liquid to dilute the capillary effluent.

In split-flow techniques, a small portion of the capillary flow is diverted outside of the capillary through a small hole near the capillary outlet [12]. However, when applied to
10 capillaries with i.d. <30- μ m, controlling the split ratio using mechanical tools was difficult. This disadvantage was eliminated with a porous junction design [2], in which an electrical connection to the CE capillary outlet was achieved by making a small section of the capillary near the outlet porous.² After sharpening the capillary outlet tip, the porous junction was
15 inserted into the existing ESI needle (or sheath metal tubing) filled with a conductive solution (background electrolyte-BGE). Application of high voltage to the sheath metal containing BGE causes oxidation (in positive mode) or reduction (in negative mode) of water (if aqueous solution was used as the BGE). Ion-transport through the porous junction closes the CE circuit and provides voltage for ESI. In this design it is ion and not liquid transport
20 through the porous section that provides electrical connection to the capillary outlet.

The use of ion transport through a porous section of a capillary for closing the electrical circuit has been employed before including: (1) a nanospray tip attached to the CE capillary outlet using polysulphone microdialysis tubing [13], (2) a liquid junction through a porous segment around the entire circumference of the capillary near the outlet [14], and (3)
25 through a porous glass joint [15]. However, the major disadvantage of attaching a nanospray tip to the capillary outlet using polysulphone microdialysis tubing is that because the capillary inner diameter is usually smaller than the wall thickness, there is a relatively large dead volume where the two capillaries are joined. The draw back of employing a liquid junction for making the electrical connection is that since the entire circumference of the
30 capillary was etched till porous, the porous section of the capillary is very weak and requires a liquid junction to hold the two segments of the capillary (before and after the porous section) together.

Making a section of a capillary porous has also been used in CE for other purposes than CE-to-MS interfacing. For example, porous capillary at the inlet end was recently used
35 for the on-line concentration of proteins and peptides in capillary electrophoresis in which a short length along the capillary (around the entire circumference of the capillary) was etched

5 with HF [16]. In CE with electrochemical detection, porous CE capillaries have been used to isolate the electrochemical detector from the CE electrical field [17-21]. In our previously reported porous junction design [2], because only a very small section of the circumference of the capillary is made porous, the capillary maintains its integrity. In addition, the inner wall of the capillary remains intact and therefore, no dead volume was introduced into the
10 capillary. Moreover, since no liquid is added to the capillary outlet, the porous junction design provided high sensitivity. However, there were two disadvantages with the porous junction design. Because mechanical tools are used to make the well to produce porous junction, fabrication of the interface on a reproducible and large scale is impractical. In addition, the capillary outlet has to be sharpened in a separate process. Furthermore, lack of
15 an automated, robust, and reproducible method for interfacing narrow capillary with low flow rates to MS has, for example, prevented CE-MS to become a wide-spread separation technique.

SUMMARY

The present disclosure provides, among other things, approaches for interfacing low
20 flow separation techniques, such as capillary electrophoresis and nano HPLC to mass spectrometry via electrospray ionization [22]. More particularly, it concerns capillary columns having a porous segment, or porous tip, useful for interfacing low flow separation techniques, as well as methods and systems using such capillary columns.

The design of the porous tip of the present disclosure has several advantages over
25 previous designs. First, production may be automated and the mass produced in a reproducible manner. Another advantage is that several capillary outlets may be etched at about the same time in ~30 min (for 150- μm -o.d. and 20 μm -i.d. capillaries). Additionally, the interface may be used for both nano-LC-MS and CE-MS. Furthermore, the etching process sharpens the tip and makes the capillary porous in one step, thus eliminating the
30 separate tip sharpening process of previous designs. Finally, because of the long length of the porous section (~1 inch), if the outlet tip is blocked or damaged, a small section (~1 mm) of it may be cut or etched away using HF without any loss of the interface performance, thus extending its useful life. Because a longer section of the porous tip (~0.5-1 inch) has the same wall thickness (Figure 1) removing a small section (~1 mm each time for a total of say ~1
35 cm) of it does not affect its performance. In addition to these advantages, the inner wall of the capillary remains intact and there is no dead volume associated with the porous tip design.

5 This ensures the best resolution from the CE separation whereas most designs have dead
volume which leads to peak broadening. Moreover, because the actual metal/liquid contact
occurs outside of the capillary, bubble formation due to redox reactions of water at the high
voltage electrode does not affect separation or MS performance. Because the tip of the porous
tip design is glass, there is no reason for the porous tip to increase the risk of arcing, which
10 could reduce capillary durability. The performance of this interface is demonstrated by the
analyses of amino acids, peptide, and protein mixtures.

While the present disclosure is susceptible to various modifications and alternative
forms, specific example embodiments have been shown in the drawings and are herein
described in more detail. It should be understood, however, that the description of specific
15 example embodiments is not intended to limit the invention to the particular forms disclosed,
but on the contrary, this disclosure is to cover all modifications and equivalents as illustrated,
in part, by the appended claims.

DRAWINGS

Some specific example embodiments of the disclosure may be understood by
20 referring, in part, to the following description and the accompanying drawings.

Figure 1 is an electron micrograph of a porous tip capillary using scanning electron
microscopy.

Figure 2 is a graph of the average etching rate of 20- μm -i.d. capillaries, etched in
fresh 49% HF solution.

25 Figure 3 is a schematic of the porous tip.

Figure 4 (top) is a base peak chromatogram of the peptide standard (110 cm long
capillary): 1) Oxidized-YGGFM, 2) YGGFM, 3) YGGFL, 4) VYV, 5) DRVYIHPF and
(bottom) a mass spectrum of peak 1 in Figure 4 (top).

Figure 5 (top) is a base peak electropherogram of 17 amino acid standard and
30 (bottom) a mass spectrum of the peak representing glutamine-crown ether complex.

Figure 6 is a base peak acetropherogram of the 6 protein standard (top): 1) Protein
fragment peak, 8,297MW, 2) Hem-A- β -subunit, 3) Protein fragment peak, 8,562MW, 4)
Trypsinogen, 5) Carbonic anhydrase, 6) Hem-A α -subunit, and 7) Mass spectrum of
cytochrome c (bottom).

35 Figure 7 is a base peak electropherogram of 6 protein digest containing: hemoglobin
A (MW64,000), trypsinogen (MW 24,000), carbonic anhydrase (MW 29,000), cytochrome c

5 (MW 12,360), α -casein (MW 23,000), and β -casein (MW 24,000) (top). Bottom graph shows the mass spectrum of the peak marked with *.

Figure 8 is a run-to-run, day-to-day, and long-term (capillary-to-capillary) reproducibility of the analysis of the peptide standard using a BGE containing 33 nM PB (Panel A) or 33 nM PE (Panel B) and using one point calibration.

10 Figure 9 is an electropherogram of a (CE-MS-MS) [6], in which the digest of a 6-protein mixture was injected every 10 min followed by CE-MS-MS analysis using dynamic exclusion.

Figure 10 (top), is a base peak nano-LC-MS chromatogram of the enolase digest using 75- μ m-inner diameter (i.d.) LC column with a flow rate of 1 μ m/min and (bottom) is a mass spectrum of peak # 3 of the top panel.

Figure 11 is the molecular structures of polyE_323 (PE) and polybrene (PB).

Figure 12 is a comparison of 33 nM PB in 0.1% acetic acid (A) and 33 nM PE in 0.1% acetic acid (B) as the self-coating BGE for the CE-MS analysis of the 6 intact protein mixture containing: (1) β -lactoglobulin (bovine), (2) myoglobin (horse), (3) hemoglobin- α (human), (4) hemoglobin- β (human), (5) lysozyme (chicken), and (6) cytochrome c (horse). The insets show the average (over one minute) background mass spectra of Panel A and B.

Figure 13 is the average background mass spectra (from 2-3 minutes) of the electropherograms from Figure 3 using (A) 0.1% acetic acid BGE in an uncoated capillary, (B) 0.1% acetic acid in a PB pre-coated capillary, (C) 660 fmol PB additive in 0.1% acetic acid in an uncoated capillary, (D) 0.1% acetic acid in a PE pre-coated capillary. (E) 660 fmol PE additive in 0.1% acetic acid in an uncoated capillary.

Figure 14 is a comparison of the CE-MS analyses of the stock solution of the six protein digest using the 33 nM PB (Panel A1) and PE (Panel B1), and the 10X diluted protein digest (Panels A2 and B2). The insets show the ion electropherograms of m/z 737 (protonated KEFGVER from rat phosphorylase b) for these Panels and the separation efficiencies of this peak under the 4 different experimental conditions.

While the present disclosure is susceptible to various modifications and alternative forms, specific example embodiments have been shown in the figures and are herein described in more detail. It should be understood, however, that the description of specific example embodiments is not intended to limit the invention to the particular forms disclosed,

5 but on the contrary, this disclosure is to cover all modifications and equivalents as illustrated, in part, by the appended claims.

DESCRIPTION

The present disclosure provides, among other things, approaches for interfacing low flow separation techniques, such as capillary electrophoresis and nano HPLC to mass spectrometry via electrospray ionization. More particularly, it concerns capillary columns having a porous segment, or porous tip, useful for interfacing low flow separation techniques, as well as methods and systems using such capillary columns.

The present disclosure also provides capillary columns comprising at least one porous segment at a terminus of the capillary column, wherein the at least one porous segment is formed by exposing the segment to one or more of a solution of acid, base, and a mechanical tool. Such capillary columns (having a porous tip) may be used to interface low flow separation techniques by providing an electrical connection to the capillary outlet. The capillary columns of the present disclosure may be used to in, for example, separation techniques that use electron spray ionization (ESI), capillary electrophoresis, high performance liquid chromatography (HPLC), nano-LC, and mass spectrometry (MS). Additionally, the capillary columns of the present disclosure may be used as a nanospray tip which may be filled with analyte for long infusion. More than one porous segment may be installed on a capillary. For example, a second porous segment may be made at an inlet for sample concentration, or another one in the center to dynamically change the voltage in the capillary, as shown by our previous publication [23].

The present disclosure also provides methods for forming a porous segment, or tip, comprising providing a capillary column comprising a wall and a segment at a terminus of the capillary column and creating a porous segment at the terminus of the capillary column. Generally, the porous section is created by etching the tip using an acid or base solution or mechanical tools until it is porous. When using an acid or base solution, etching time may be dependent upon the concentration of the acid or base solution. For example, higher concentrations correspond to lower etching times and vice versa. In some instances, the capillary column wall comprises a polymer coating such as polyimide, which may be at least partially removed before etching the tip.

35 In one embodiment, the capillary is made by removing ~1 inch polyimide coating of the capillary outlet and etching it by immersing it into a solution of 49% HF until it is porous.

5 For a given capillary o.d. and i.d., the etching time is constant, so long as a fresh HF solution is used each time. For example, the etching time is ~30 min for a capillary with 150- μm -o.d. and 20- μm -i.d. In some instances, only a portion of the tip may be etched. The methods of the present disclosure may be used to produce several capillary at the same time. The etching process also sharpens the tip of the capillary outlet for stable electrospray ionization. The
10 electrical connection is achieved simply by inserting the capillary outlet containing the porous tip into the existing ESI needle (sheath metal) and filling the needle with a conductive solution (background electrolyte-BGE).

The ESI needle may be made of any conductive metal, such as stainless steel, platinum, gold, etc., or it may be a non-conductive tube with a metal connection such that
15 voltage is applied to the BGE. Suitable examples of BGE include, but are not limited to, water, acid, or base solution, such as 0.1% acetic or formic acid or ammonium acetate solution. pH and concentration of BGE can affect EOF, however, pressure assisted CE can be used to modify BGE flow rate in the CE capillary without any significant loss of separation efficiency. Additives may also be included in the BGE, such as polybrene or Poly 323.
20 Electrochemical (redox) reactions at the sheath metal and transfer of small ions through the porous tip into the capillary provide the electrical connection for the ESI and for the CE outlet electrode. The design is suitable for interfacing all capillary sizes with a wide range of flow rates to MS via ESI, however, it is especially useful for interfacing narrow (30- μm < i.d.) capillaries and low flow rates (<100 nL/min) such as those used in nano-liquid
25 chromatography-mass spectrometry (nLC-MS) or capillary electrophoresis (CE-MS). CE flow rates for CE-MS are usually in the range of ~1-100 nL/min. Spray stability depends on the inner diameter and wall thickness of the CE-outlet/ESI tip. Lower flow rates require narrower capillaries and sharper tips. For example, ~10- μm -i.d. CE capillaries are used for flow rates below ~10 nL/min, etc.

30 According to certain embodiments, the present disclosure provides systems comprising a capillary column comprising a wall and at least one porous segment at a terminus of the capillary column, wherein the at least one porous segment is formed by exposing the segment to one or more of a solution of acid, base, and a mechanical tool; and one or more instruments chosen from an electrophoresis instrument, a high performance
35 liquid chromatography instrument, and a mass spectrometry instrument, wherein the porous segment serves as the interface between the capillary column and the instrument.

5 According to certain embodiments, the present disclosure provides methods for chemically analyzing a chemical or biological sample comprising providing a capillary column having a wall and at least one porous segment at a terminus of the capillary column; providing a mass spectrometer in operable relation to the capillary column; interfacing the mass spectrometer with the at least one porous segment at a terminus of the capillary column;
10 injecting a mixture containing the chemical or biological sample into the capillary column; transporting at least a portion of the mixture to the mass spectrometer; and analyzing the portion with the mass spectrometer to identify the composition of the chemical or biological sample in the portion. Yet another application of the porous tip is its use in conjunction with Matrix Assisted Laser Desorption Ionization (MALDI). In this case the outlet of the capillary
15 is grounded through the porous tip, and CE eluents are deposited onto a MALDI plate for MS analysis.

To facilitate a better understanding of the present invention, the following examples of specific embodiments are given. In no way should the following examples be read to limit or define the entire scope of the invention.

20

EXAMPLES

Materials & Equipment

High performance liquid chromatography (HPLC) grade water and 49% HF are commercially available from Fischer Scientific, Pittsburgh, PA. All other chemicals used are commercially available from Sigma Chemical Co., St. Louis, MO. The Sigma HPLC peptide
25 standard contains five peptides: GY (MW238), VYV (MW 379), YGGFL (leucine-enkephalin, MW 555), YGGFM (methionine-enkephalin, MW 573), and DRVYIHPF (angiotensin II, MW 1046). The contents of the vial (0.5 mg of each peptide) was dissolved in 1 mL of HPLC-grade water (Fischer). A 5x dilution of a 17-amino acid standard (2.5 μ moles/mL in 0.1 N HCl, except L-cystine at 1.25 μ moles/mL) was used, in the
30 amino acid standard test. The protein standard contained a 10x dilution of the following proteins: hemoglobin A (MW 64,000), trypsinogen (MW 24,000), carbonic anhydrase (MW 29,000), and cytochrome c (MW 12,360). Each was tested under a 10x dilution. The protein digest contained: hemoglobin A (MW 64,000), trypsinogen (MW 24,000), carbonic anhydrase (MW 29,000), cytochrome c (MW 12,360), α -casein (MW 23,000), and β -casein
35 (MW 24,000). They were digested according to the method published by Takada [24].

5 A P/ACE system MDQ CE instrument (Beckman-Coulter Instruments, Fullerton, CA) was used in conjunction with a Finnigan LCQ MS (Finnigan, San Jose, CA). The mass spectrometer was scanned in the mass/charge (m/z) range of 375-600 for the analysis of the peptide standard, 515-700 for the AAI18-C-6-TCA complex, 700-2000 for the protein standard, and 500-1500 for the complex protein digest.

10 For the analyses of the peptide mixture, the protein digest, and the protein mixture, the CE inlet electrode was -30kV (reverse polarity mode), and the ESI voltage of was ~1.4 kV. A solution of 0.1% polybrene in 0.1% acetic acid is used as the background electrolyte. For the analysis of the amino acid mixtures, the CE inlet electrode was maintained at -20kV. A solution of 15mM 18-crown-6-tetracarboxylic acid (in 1M formic acid) was used as the
15 complexation reagent to enhance the sensitivity of detection of amino acids [25].

Different 20- μ m- i.d. and 150- μ m- o.d, fused-silica capillaries (Polymicro Technologies, Phoenix, AZ), varying in length from 60cm-120cm, were used throughout the experiment. The outlet of the capillary was made porous and sharp using 49% HF as N_2 gas was passed through it to minimize inner wall etching. The HF on the capillary wall was
20 neutralized by a saturated solution of ammonium bicarbonate by successively immersing the outlet (etched section) of the capillary first into the ammonium bicarbonate solution and then into distilled water (both under the hood). The capillaries were then removed from under the hood and rinsed with distilled water off the tap. Nitrile gloves were worn during the process and disposed after each use.

25 **Fabrication of Porous Tip Design**

In a first reduction to practice of the present invention, two capillaries were made porous at the same time. The fabrication procedure was as follows. Before etching the capillary outlets by HF solution, ~1 inch of the polyimide coating of the capillary outlets was removed using a flame. Other methods of removing polyimide coating, such as using sulfuric
30 acid, also may be used. The exposed fused silica sections of the capillaries were then immersed into a Teflon container containing fresh HF solution. Nitrogen gas was introduced into the capillaries to prevent etching of the capillary inner walls during the etching process. The Teflon container was positioned inside a well-ventilated hood. For each capillary o.d. and i.d, the etching time was experimentally determined by first measuring the etching rate of
35 the fused-silica in fresh HF solution. This was achieved by measuring capillary o.d. after successive etching time intervals (every ~5 min), and testing the capillary outlet for porosity.

5 The porosity was checked by installing the capillary tip into an electrospray apparatus on the bench and testing the porous tip for EST. If the 0.1% solution of acetic acid exiting the capillary sprayed at less than 2 kV, the tip was porous. It was found that the fused silica material etched at an average rate of $\sim 1.8 \mu\text{m}/\text{min}$ (Figure 2). The capillaries become porous when the thickness of the wall of the capillary approached $\sim 5 \mu\text{m}$. For example, a nominal
10 $150\text{-}\mu\text{m}\text{-o.d.}, 20\text{-}\mu\text{m}\text{-i.d.}$ capillary becomes porous in ~ 28.5 minutes in 49% fresh HF solution [$\sim 150 - 20$ (for polyimide coating) = $\sim 130 - 20$ (for i.d.) = $\sim 110 / 2 = 55 =$ wall thickness]. Because of its short etching time, and its good thermal conductivity, $150\text{-}\mu\text{m}\text{-o.d.}$ are the capillary of choice for CE-MS analysis, it was used in this study. However, other capillary outer and inner diameters also are utilized. For example, a $360\text{-}\mu\text{m}\text{-o.d.}$ capillaries provide
15 better mechanical durability, however, ~ 82 minutes of etching time is required for a $30\text{-}\mu\text{m}\text{-i.d.}$ capillary. Figure 3 shows the overall schematic of a porous tip design.

Performance Results

Based on previous CE-MS studies, the Sigma HPLC peptide mixture is a suitable standard for testing the overall CE-ESI-MS performance. Baseline separation of the
20 components of the test mixture is indicative of adequate performance of the capillary for the analysis of different mixtures. Figure 4 shows the base peak electropherogram of the peptide standard. In the analysis of peptide standard, GY was not detected in the scanned mass range, angiotensin II was seen as a doubly charged compound (m/z 524), and methionine-enkephalin was seen in the oxidized state (m/z 590). As shown, the full width at
25 half maximum (FWHM) of most peptides are ~ 5 s.

To test the utility of the porous tip for the analysis of amino acids, in which a 1 M solution of formic acid is the main ingredient of the BGE, CE-MS of an amino acid standard containing 17 amino acids was analyzed. Figure 5 shows the base peak electropherogram of the 17 amino acid standards. Cysteine was the only amino acid not observed in the
30 chromatogram, with methionine and proline co-migrating. Cysteine usually comes off as a dimer, which when complexed with 18-C-6 falls out of the scanned mass range. As shown, baseline separation of amino acids was observed, indicating the viability of porous tip for the analysis of small molecules such as amino acids [25].

The performance of the porous tip design for the analysis of protein mixtures and
35 digest of protein mixtures, the mixture of intact proteins, and the peptide mixture obtained from tryptic digestion of the 6-protein mixture was analyzed. Figure 6 shows the base peak

5 electropherogram obtained from the 5 protein mixture injection, as well as good peak separation for the known compounds, while observing minimal peak broadening. Figure 7 shows the base peak electropherogram obtained from the tryptic digest of the 6 protein mixture. To increase throughput, a 60-cm-long capillary was used, which resulted in the analysis time of ~12 min and ~8 min for the protein mixture and the digest of the protein
10 mixture, respectively. Almost complete baseline separation was achieved in both cases.

The porous tip is very robust and may last for weeks (Figure 8), and is reproducible. For example, Figure 9 shows an electropherogram of a (CE-MS-MS) [6], in which the digest of a 6-protein mixture was injected every 10 min followed by CE-MS-MS analysis using dynamic exclusion. Recently (CE-MS-MS)ⁿ has been introduced for high sequence coverage
15 proteomics [26]. The porous tip may allow for ease of interfacing CE to MS.

Application of Porous Tip to nLC-MS

To demonstrate the utility of the porous tip for interfacing higher flow separation technique such as nano-LC to MS, a 60-cm-long, 20- μm -i.d. capillary with a porous tip was connected to a nano-LC C₁₈ column using a Peek micro-union. Inserts of Figure 3 show two
20 ESI plumes at a flow rate of 250 nL/min (small plume), and a flow rate of 1 μL /min. As shown, a uniform spray was observed in both cases without any pneumatic assistance. A sharp tip, good electrical connections, and minimal bubble formation are the primary reasons for the uniformity of the electrospray plume under porous tip design. Another advantage of the porous tip is that the inlet and outlet inner diameters are the same, which reduces the
25 chance of tip clogging, since any particulate that enters the porous tip capillary will exit from the other end of the capillary. In contrast, the use of a nano-spray interface using pulled tips can trap particulates with diameters larger than the tip opening because their tip is usually drawn to less than 10 μm -i.d. Since only the tip is sharp, tip clogging results in the loss of the spray tip and the analysis. Because the porous tip has similar o.d. and i.d. for ~1 inch, any
30 damage to the tip may be repaired by cutting ~ 1 mm of the tip.

The application of the porous tip for protein identification is demonstrated in Figure 10, in which digested yeast enolase was analyzed using nLC in conjunction with FTICR MS. Figure 10 shows the base peak chromatogram of the enolase digest using 75- μm -column with a flow rate of 1 μm /min. Since the dual-nozzle, dual-sprayer was used in this experiment,
35 there were several reference peaks in every spectrum [27, 28]. These reference peaks were used for internal calibration of each spectrum resulting in a mass accuracy of less than 1 ppm

5 in most peptide m/z. For example, Figure 10 (bottom), shows the mass spectrum of peak # 3 of Figure 10 (top), in which the mass spectrum includes the m/z of one peptide bracketed with two reference peaks [29]. The accurate masses of the top ten intense peptide peaks are listed in Table 1 below.

TABLE 1. Measured vs. calculated masses for peaks of Figure 3.

10

Peak #	m/z Measured	MH+ Calculated	Delta ppm
1	814.5040	814.5038	0.21
2	1159.6120	1159.6111	0.74
3	1286.7120	1286.7109	0.88
4	1288.7110	1288.7113	-0.20
5	1412.8240	1412.8225	1.0
6	1416.7220	1416.7222	-0.15
7	1578.8030	1578.8015	0.93
8	1755.9470	1755.9493	-1.3
9	1840.9250	1840.9227	1.2
10	1872.9680	1872.9682	-0.11

Background Electrolyte-BGE

To simplify CE-MS operation, a BGE containing a polymer additive may be introduced that allows the analysis of peptides and protein mixtures in underivatized fused-silica capillaries without any pretreatment, thereby increasing throughput. Examples of suitable BGEs include Polybrene (PB) and PolyE (PE).

Polybrene (PB) and PolyE 323 (PE) are cationic polymers that are able to non-covalently attach to the negatively charged wall of fused-silica capillary. The excess positive charges on the newly created surface generate a stable anodic EOF that is independent of pH within the pH range of 4–8. The molecular structure of both PB and PE are shown in Figure 11. The structure of PE is purposely constructed to contain mixed bonding characteristics. For example, the nitrogen atoms in the backbone of PE are separated by three atoms length whereas nitrogen atoms in PB are separated by six carbon atoms. The length of the spacer arm between the nitrogen atoms in the backbone can affect the polymer's flexibility, and hydrophobicity. Also, hydroxyl groups in PE increase polymer immobilization on the capillary wall by hydrogen-bonding [30-32]. PB and PE are very effective in reversing the charge on the capillary wall, which prevents peptide and protein attachment to the capillary wall, thereby significantly improving separation efficiency (Figure 12). To be useful in

5 CE/ESI-MS, the suppression effect of the additive reagent on analytes of interest must be insignificant. PE and PB have such characteristics, i.e., they do not produce significant background signal (Figure 13) and do not significantly suppress the signals of the proteins and peptides under electrospray ionization (Figure 14), thereby allowing them to be used as an additive to common BGEs that are used for CE-MS analysis of peptide and protein
10 mixtures. In addition, because the fused-silica capillary inner wall is continuously coated with the polymer additive, migration irreproducibility, due to the degradation of the capillary inner wall coating, under CE-MS is minimized. High sensitivity of detection, migration reproducibility, and ease of fabrication allow CE-MS analyses that require long analysis time, such as (CE-MS/MS)_n, to be performed with ease. Recently we have shown the utility
15 of these background electrolytes for the analysis of complex protein digests and intact proteins [32].

Polybrene was purchased from Sigma Chemical Co. (St. Louis, MO). PolyE 323 polymer was prepared according to a reported procedure [30]. Briefly, a stock solution of PolyE 323 polymer was synthesized by combining 0.1 mol of 1,2-bis(3-
20 aminopropylamino)ethane with 20 mL of water and 0.1 mol of epichlorohydrine. This mixture was then stirred for 48 h, after which an additional 100 mL of water was added before storage at 8°C without further purification. A stock solution of polybrene (hexadimethrine bromide, average MW = 15,000) was made by mixing 5 mg of polybrene in 100 mL of HPLC grade water (3.3 μM). Aliquots of this solution were diluted with 0.1%
25 acetic acid to final concentrations of 33, 66, 330, and 660 nM.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contain certain errors necessarily resulting from the standard deviation found in their
30 respective testing measurements.

Therefore, the present invention is well adapted to attain the ends and advantages mentioned as well as those that are inherent therein. While numerous changes may be made by those skilled in the art, such changes are encompassed within the spirit of this invention as illustrated, in part, by the appended claims.

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5

CLAIMS

What is claimed is:

1. A method for forming a porous segment comprising:
providing a capillary column comprising a wall and a segment at a terminus of
the capillary column; and
10 creating a porous segment at the terminus of the capillary column.
2. The method of claim 1, wherein the wall comprises a coating, and further
comprising the step of removing a portion of the coating prior to the step of creating a porous
segment.
3. The method of claim 1, wherein the porous segment is created by exposing the
15 segment to one or more of a solution of acid, base, and a mechanical tool.
4. A capillary column comprising:
at least one porous segment at a terminus of the capillary column, wherein the
at least one porous segment is formed by exposing the segment to one or more of a solution
of acid, base, and a mechanical tool.
- 20 5. The capillary column of claim 4, wherein the capillary column comprises a
wall comprising a polymer coating.
6. The capillary column of claim 4, wherein the capillary column comprises a
wall comprising a polymer coating, and wherein at least a portion of the polymer coating is
removed prior to exposing the segment to a solution of acid or base, or a mechanical tool.
- 25 7. The capillary column of claim 4, further comprising a conductive solution
8. The capillary column of claim 4, further comprising a conductive solution,
wherein the conductive solution comprises one or more cationic polymers.
9. The capillary column of claim 4, further comprising a conductive solution,
wherein the conductive solution comprises Polybrene or PolyE 323 or both.
- 30 10. A system comprising:
a capillary column comprising a wall and at least one porous segment at a
terminus of the capillary column, wherein the at least one porous segment is formed by
exposing the segment to one or more of a solution of acid, base, and a mechanical tool; and
one or more instruments chosen from an electrophoresis instrument, a high
35 performance liquid chromatography instrument, and a mass spectrometry instrument, wherein
the porous segment serves as the interface between the capillary column and the instrument.

- 5 11. A method for chemically analyzing a chemical or biological sample comprising:
- a capillary column comprising a wall and at least one porous segment at a terminus of the capillary column, wherein the at least one porous segment is formed by exposing the segment to one or more of a solution of acid, base, and a mechanical tool;
- 10 providing a mass spectrometer in operable relation to the capillary column;
- interfacing the mass spectrometer with the at least one porous segment at a terminus of the capillary column;
- injecting a mixture containing the chemical or biological sample into the capillary column;
- 15 transporting at least a portion of the mixture to the mass spectrometer; and
- analyzing the portion with the mass spectrometer to identify the composition of the chemical or biological sample in the portion.
12. The method of claim 11, further comprising:
- preconditioning the capillary column with a buffer solution prior to the step of
- 20 injecting the mixture.
13. The method of claim 11, further comprising:
- providing a metal sheath;
- inserting the capillary column having a wall and at least one porous segment at a terminus of the capillary column into the metal sheath or a container containing conductive
- 25 solution to which voltage is applied prior to the step of interfacing the mass spectrometer with the at least one porous segment at a terminus of the capillary column; and
- filling the metal sheath with a conductive solution.
14. A method for depositing a capillary electrophoresis eluent on a Matrix Assisted Laser Desorption Ionization plate comprising:
- 30 providing a capillary electrophoresis eluent disposed within a capillary column, the capillary column comprising a wall and at least one porous segment at a terminus of the capillary column, wherein the at least one porous segment is formed by exposing the segment to one or more of a solution of acid, base, and a mechanical tool;
- grounding the capillary column through the porous tip; and
- 35 depositing the capillary electrophoresis eluent onto a Matrix Assisted Laser Desorption Ionization plate.

DRAWINGS

Figure 1

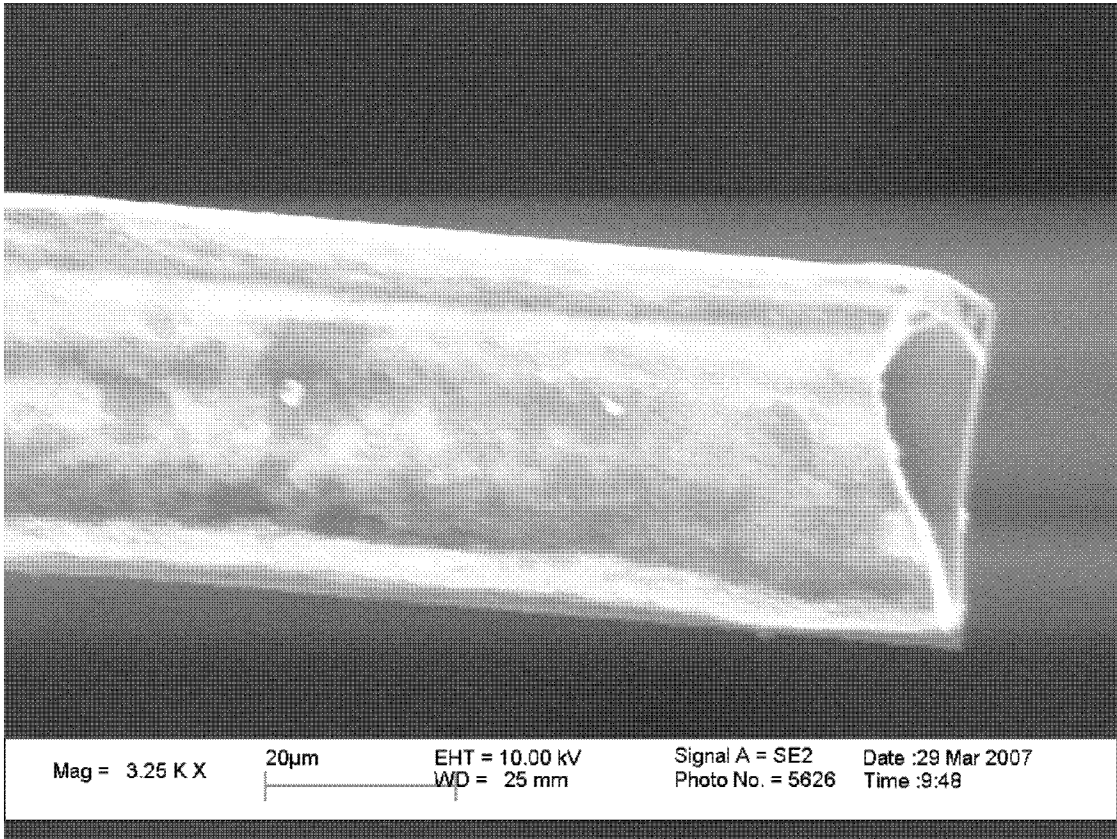


Figure 2

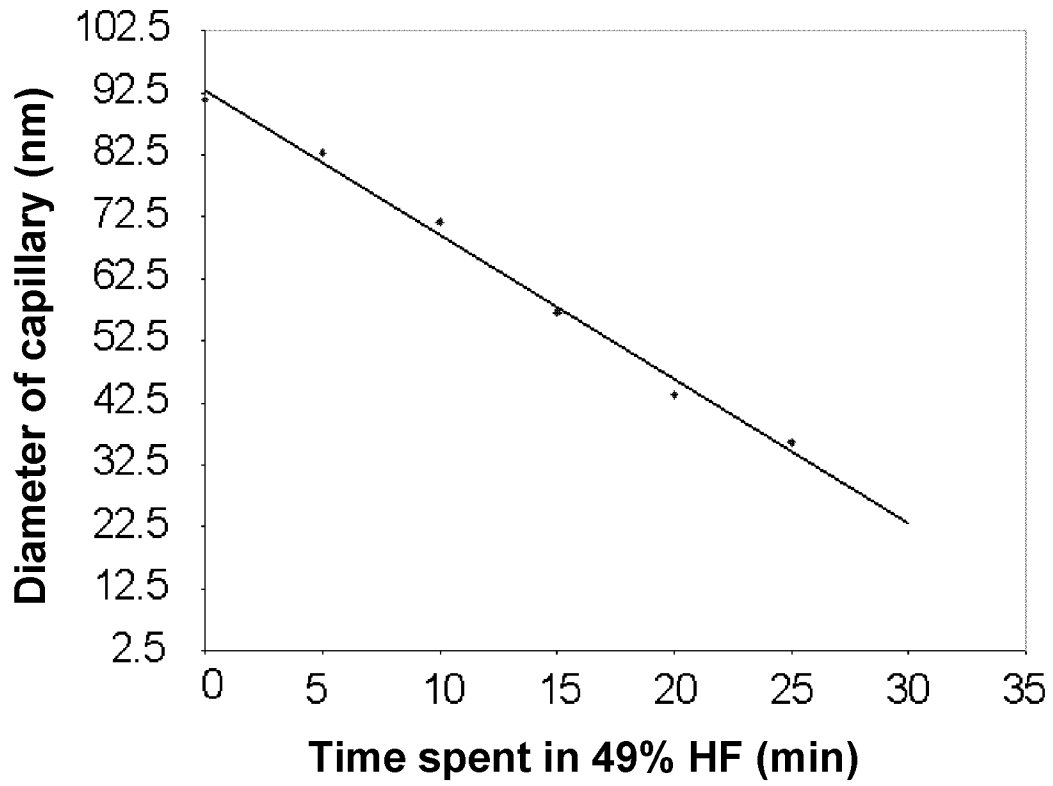
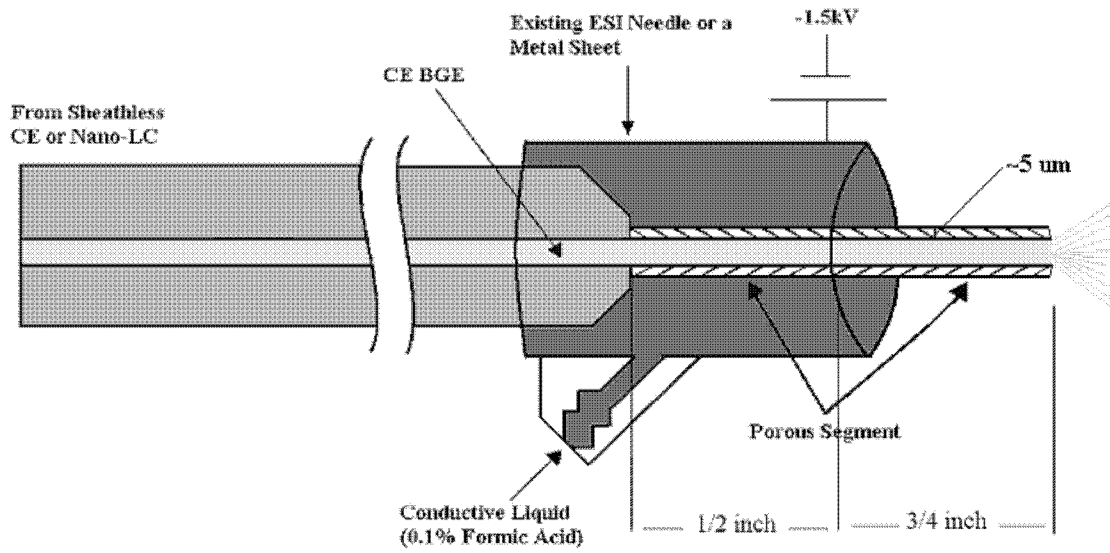
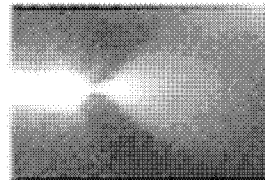


Figure 3



250 nL/min flow
(magnified ~100x)



1000 nL/min flow
(magnified ~50x)

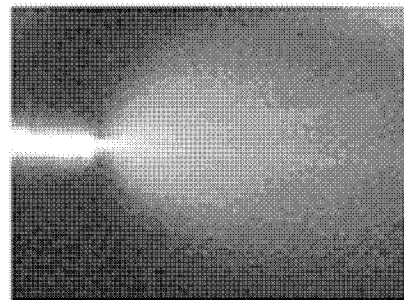


Figure 4

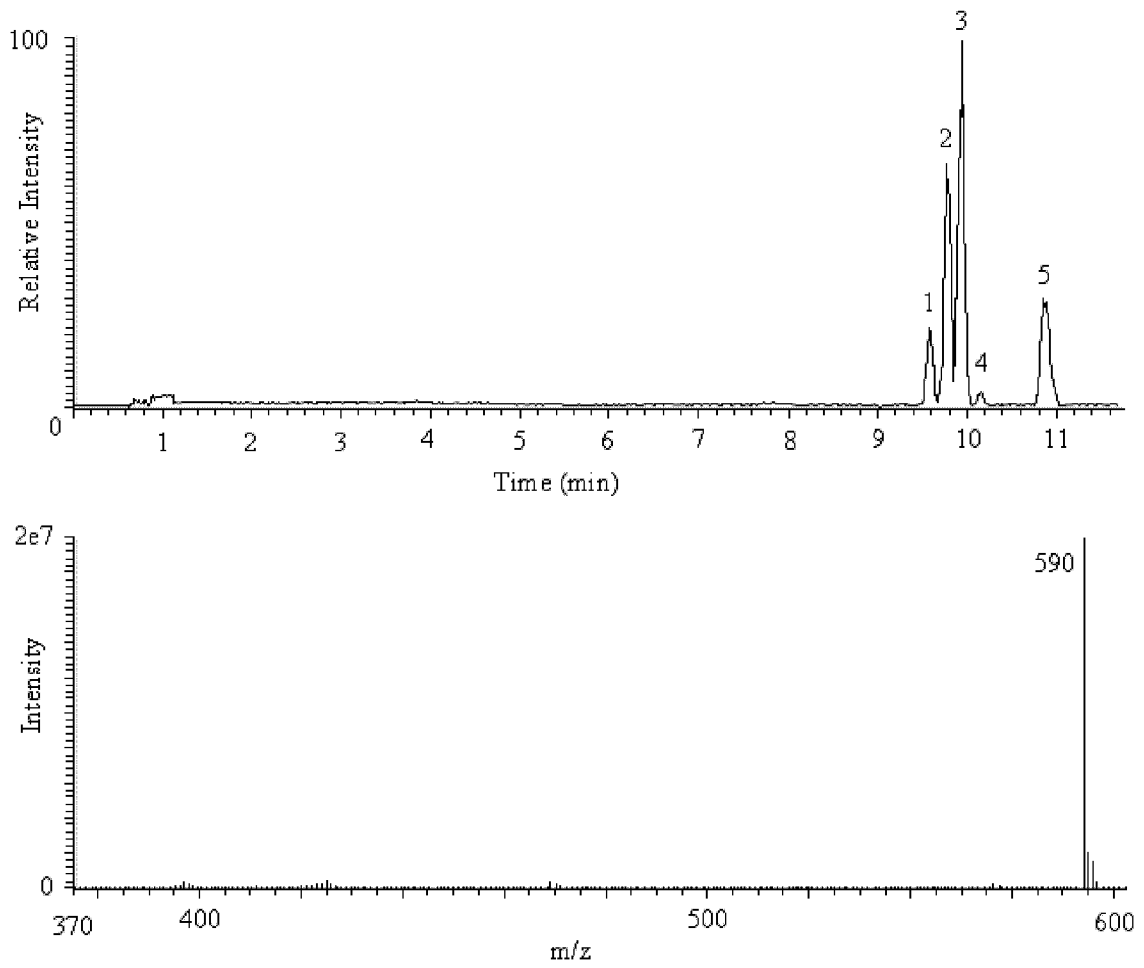


Figure 5

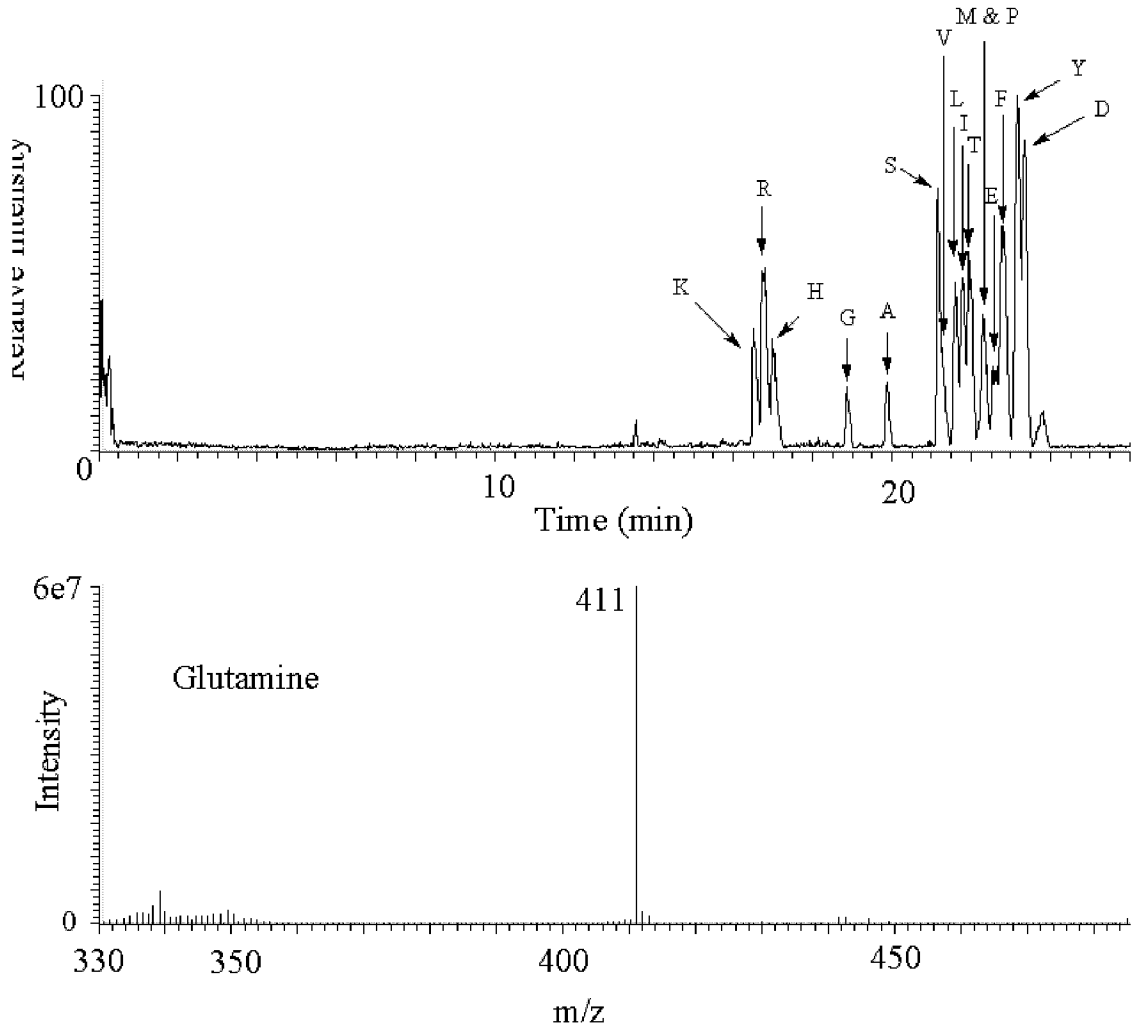


Figure 6

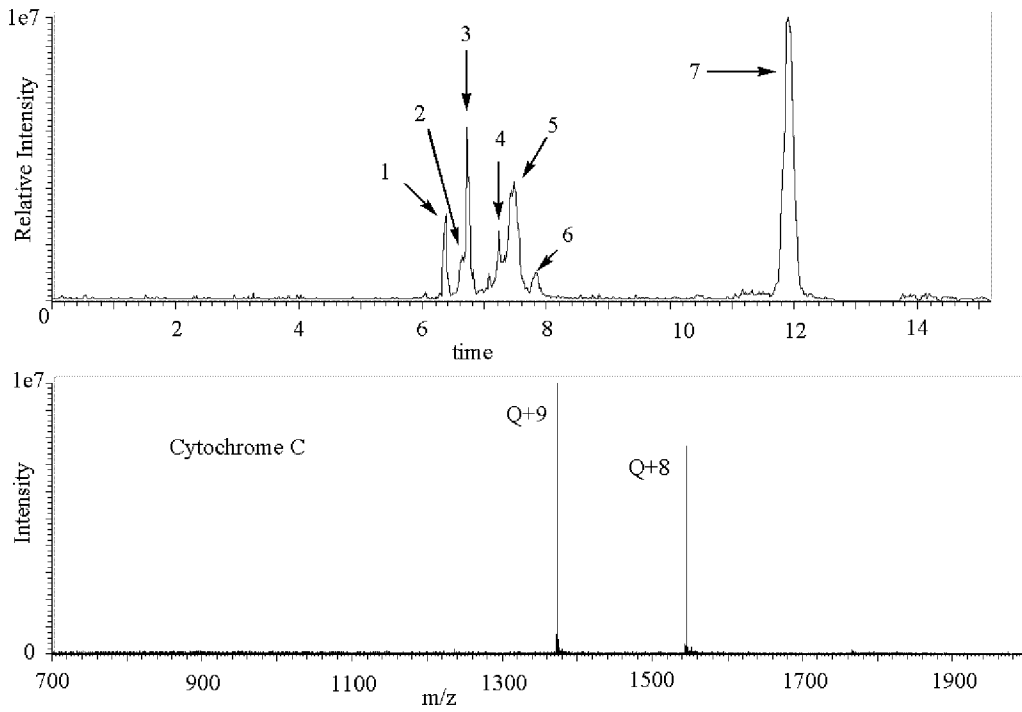


Figure 7

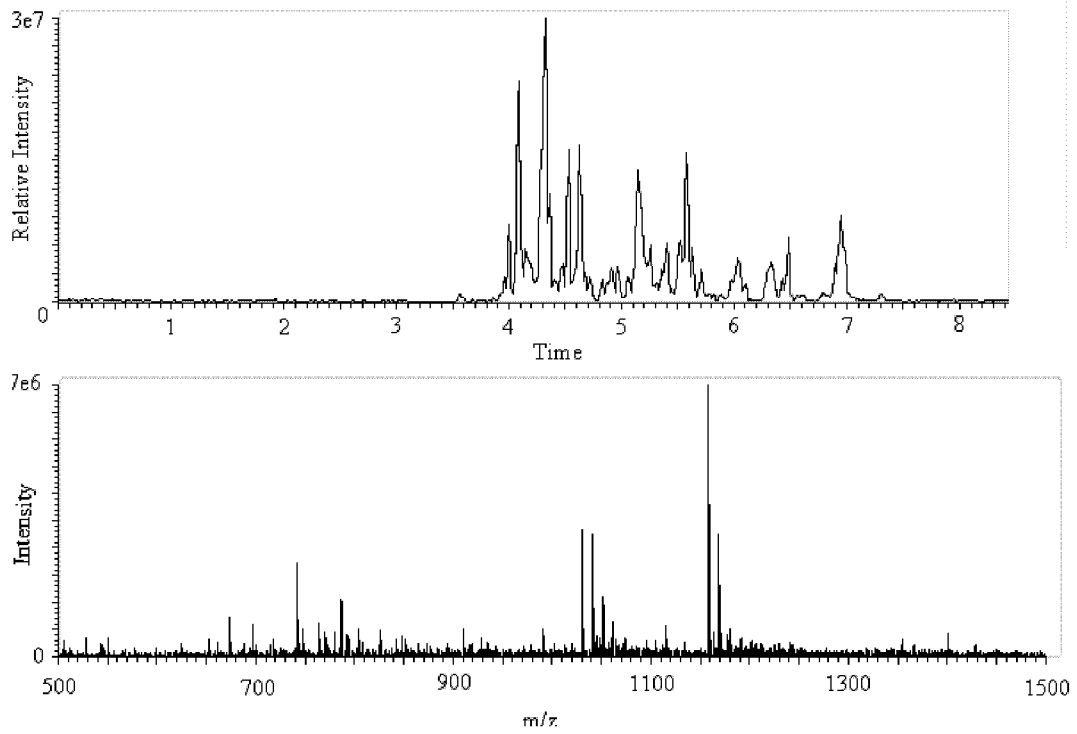


Figure 8

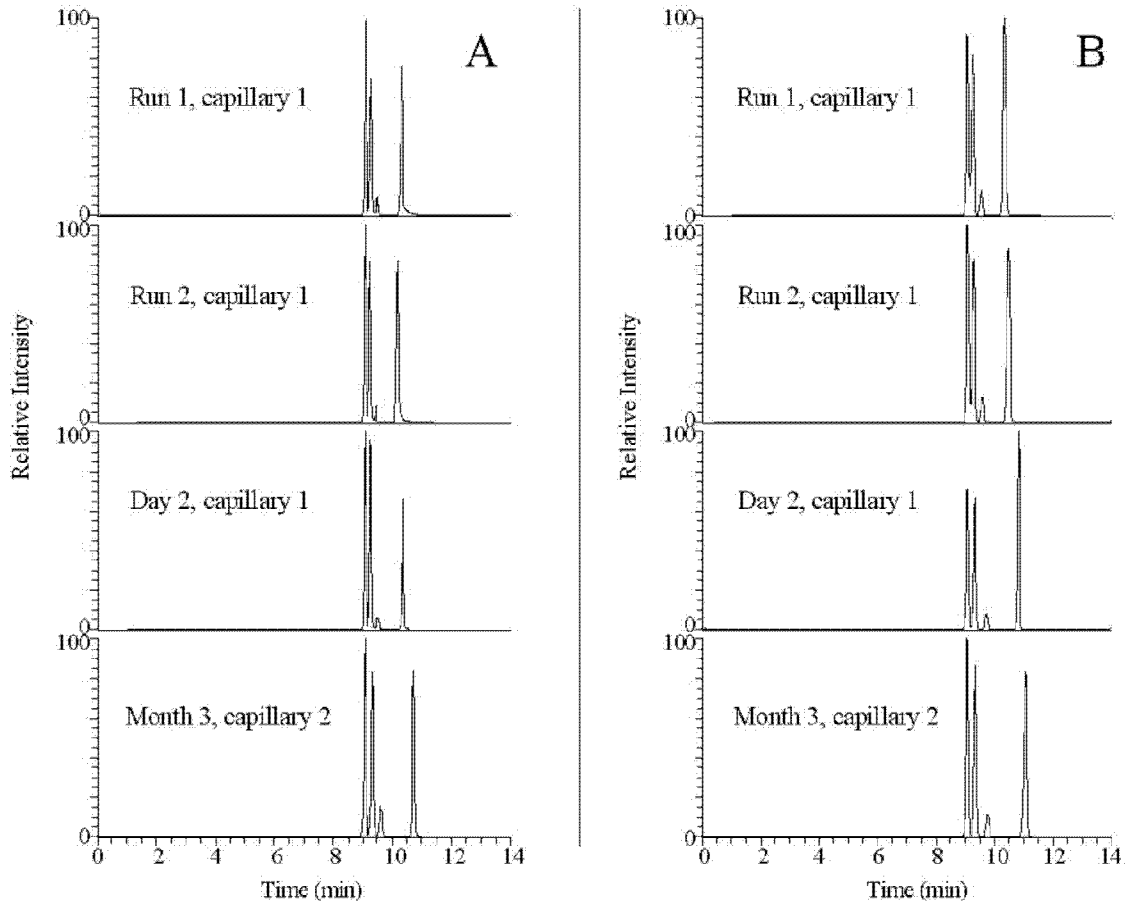


Figure 9

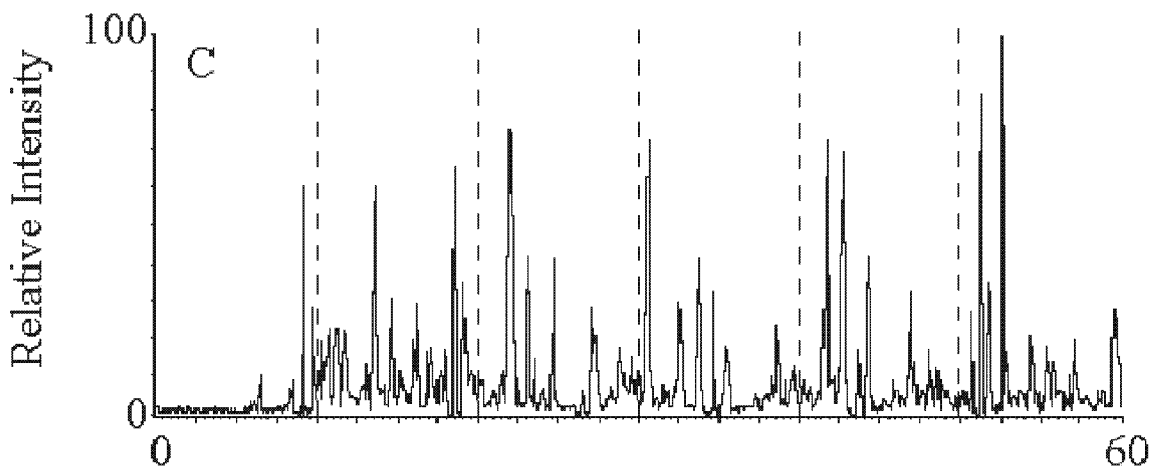


Figure 10

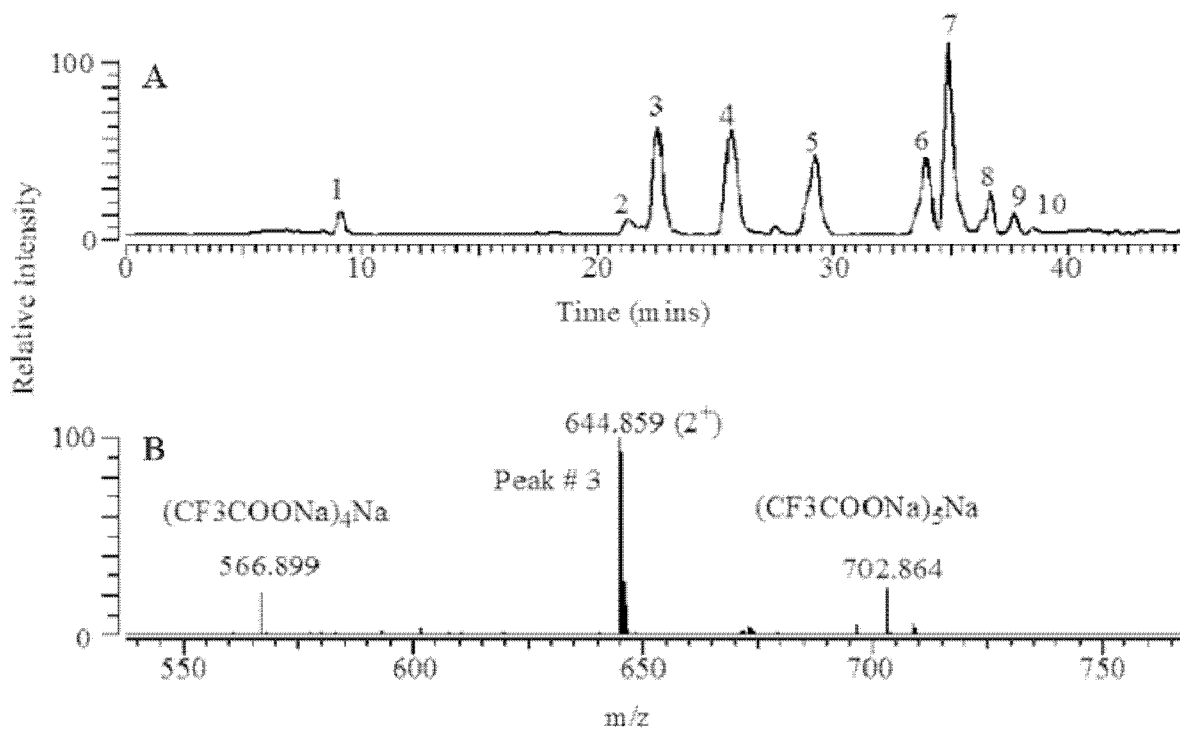
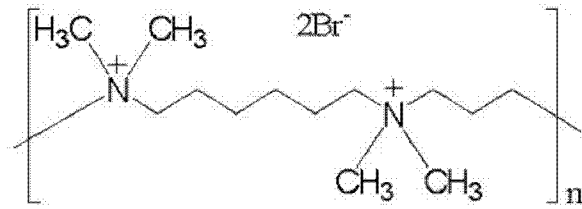


Figure 11

Polybrene



PolyE_323

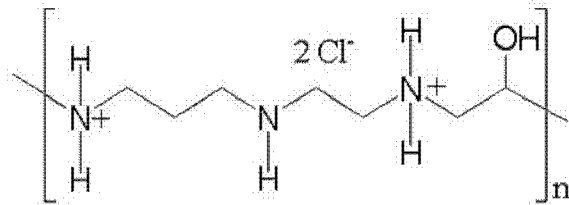


Figure 12

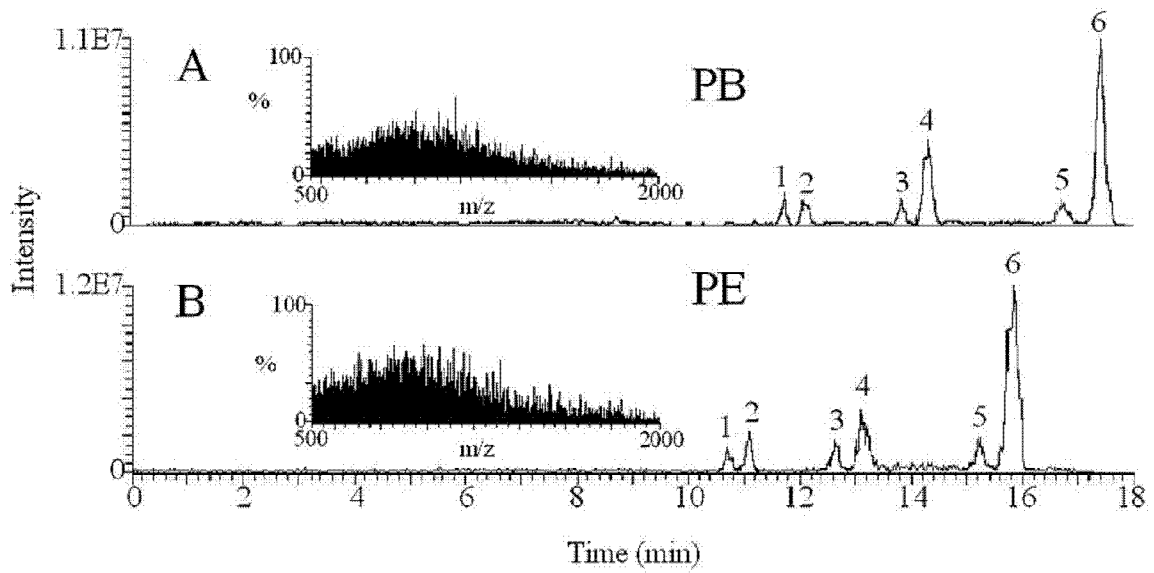


Figure 13

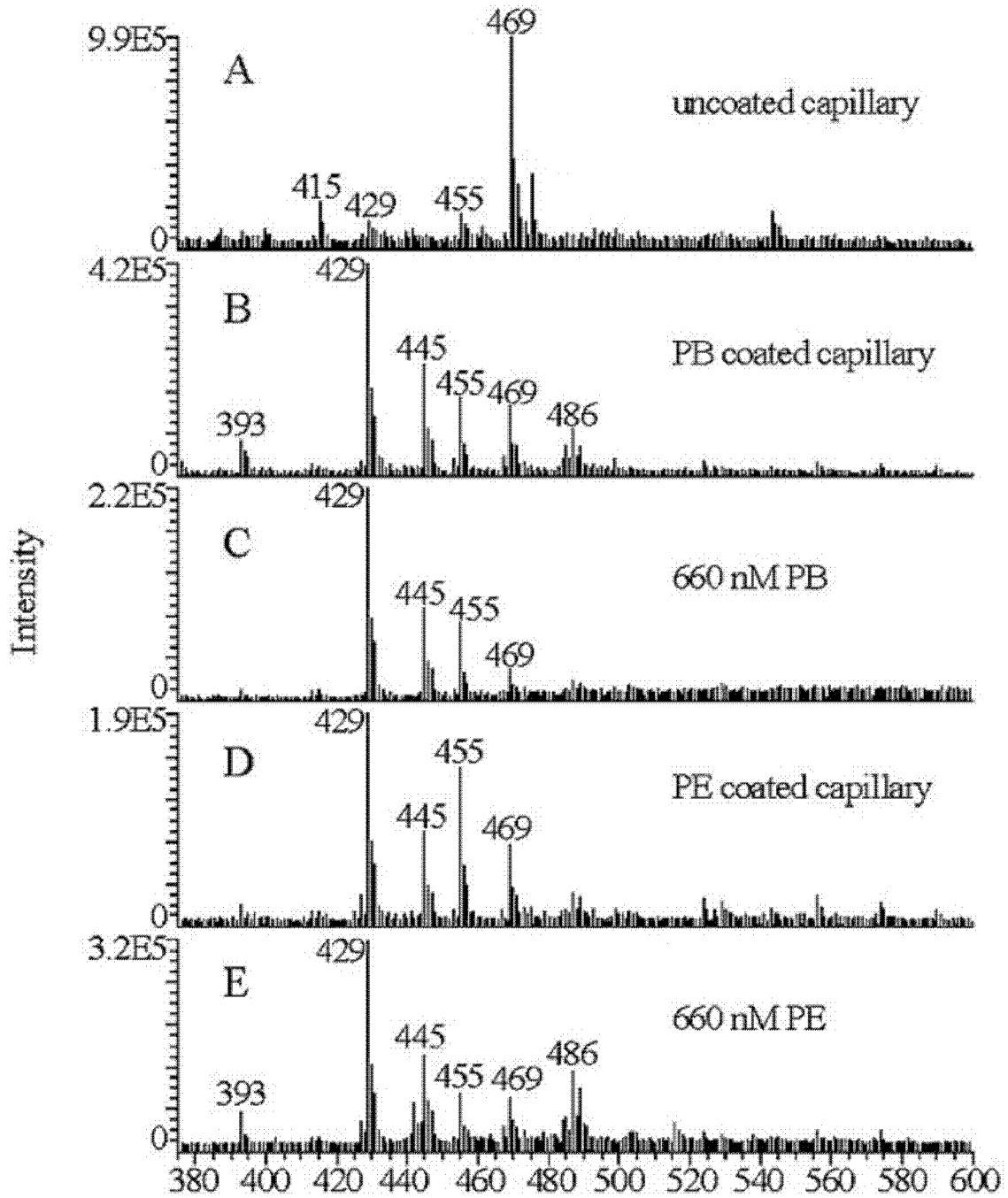
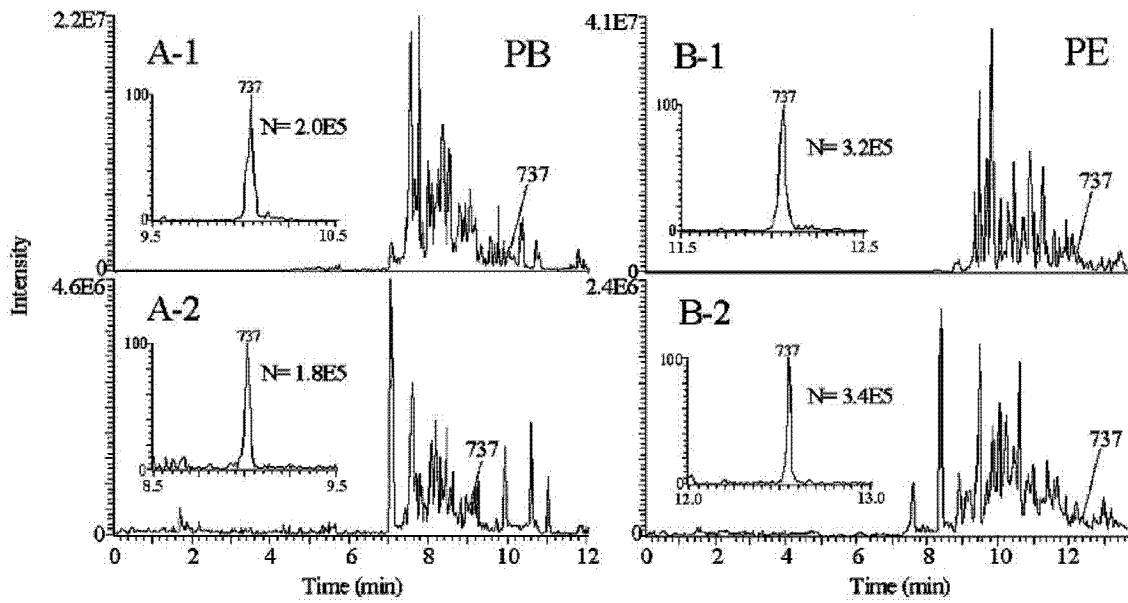


Figure 14



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/51003

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - G01N 30/02 (2008.04)
 USPC - 436/161
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 USPC: 436/161

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 Electronic Databases Searched: USPTO WEST (PGPUB, EPAB, JPAB, USPT), Google patent, Google Scholar. Search Terms Used: porous segment, polybrene, terminus, transp\$, eluent, metal sheath, ionization plate, mass spectrometer, chemical or biological sample, matrix assisted laser, capillary column, acid and amine or base

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,462,646 A (Shieh) 31 October 1995 (31.10.1995) entire document especially figure 1; col 2, ln 56-67; col 8, ln 09-17; col 9, ln 31-42; col 9, ln 45-49; col 3, ln 07-12 ; col 8, ln 09-34; col 10, ln 32-48	1-14
Y	US 6,159,353 A (West et al.) 12 December 2000 (12.12.2000) entire document especially figure 9; col 7, ln 36-62;	1-14
Y	US 4,988,870 A (Diehl) 29 January 1991 (21.01.1991) entire documen especially col 1, ln 48 to col 2, ln 9	11-13
Y	US 6,175,112 B1 (Karger et al.) 16 January 2001 (16.01.12001) entire documen especially col 2, ln 55 to col 3, ln 08	14

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 16 April 2008 (16.04.2008)	Date of mailing of the international search report 16 MAY 2008
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Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774
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