



(43) International Publication Date
16 January 2014 (16.01.2014)

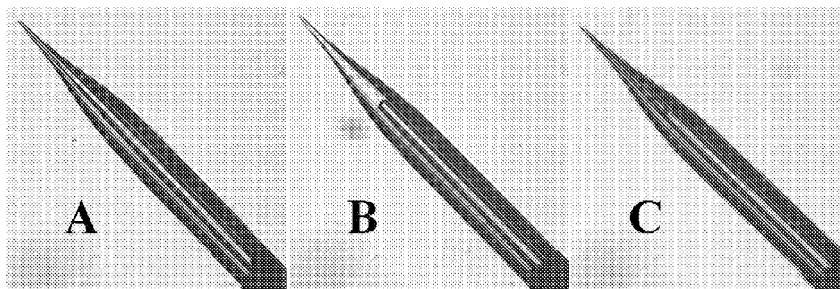
- (51) International Patent Classification:
B01D 15/08 (2006.01) *G01N 30/00* (2006.01)
- (21) International Application Number:
PCT/US2013/045892
- (22) International Filing Date:
14 June 2013 (14.06.2013)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/671,393 13 July 2012 (13.07.2012) US
- (71) Applicant: **BROWN UNIVERSITY** [—/US]; P.O. Box 1949, 47 George Street, Providence, Rhode Island 02912 (US).
- (72) Inventors: **BAZEMORE WALKER, Carthene Rolanda**; 79 Lauriston Street, Providence, Rhode Island 02912 (US).
ELLISOR, Michael Barrett; 2 King Philip Avenue, Barrington, Rhode Island 02806 (US).
- (74) Agent: **IWANICKI, John P.**; 28 State Street, Suite 1800, Boston, Massachusetts 02109 (US).

- (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: CAPILLARY COLUMN WITH FRITTED TIP



(57) Abstract: The present invention is directed to a capillary tube having an emitter tip with a polymerized porous frit therein and to methods of making such a capillary tube.



WO 2014/011356 A1

CAPILLARY COLUMN WITH FRITTED TIP

RELATED APPLICATION

This application claims priority from U.S. provisional patent application number 61/671,393, filed July 13, 2012, which is hereby incorporated herein by reference in its
5 entirety for all purposes.

FIELD

The present disclosure relates to the capillary columns and methods of making capillary columns.

BACKGROUND

Capillary columns are used in well-known techniques such as liquid chromatography and mass
10 spectrometry. Such capillary columns often include a tapered emitter tip. Capillary columns with tapered emitter tips are described in US 2010/0193683, US 6,190,559, US 5,997,746, Ficarro et al., *Anal. Chem.* 2009, 81, 3440-3447 and Gibson, *Mass Spectrometry Reviews*, 2009, 28, 918-936. However such capillary columns with emitter tips may be prone to clogging.

Accordingly, it is an object of the present disclosure to provide capillary columns with emitter tips
15 that are useful, for example, in liquid chromatography and mass spectrometry methods. Accordingly, it is a further object of the present disclosure to provide methods of making capillary columns with emitter tips that are useful, for example, in liquid chromatography and mass spectrometry methods.

These and other objects, features, and advantages described in the present disclosure will be
20 apparent to those skilled in the art from the following disclosure and description of exemplary embodiments.

SUMMARY

Embodiments of the present disclosure are directed to a method of making a tube with a frit in an emitter tip. The tube has an intermediate portion. The emitter tip has a channel and an orifice.
25 According to one aspect, the emitter tip is unitary with the tube. According to one aspect, the emitter tip is integral with the tube. According to one aspect, the emitter tip is an integrated emitter tip. The method includes placing a polymerizable liquid into the channel of the emitter tip of the tube and polymerizing the polymerizable liquid into a polymerized porous frit within the channel of the emitter tip of the tube.

According to one aspect, the polymerizable liquid is introduced into the tube through the emitter tip. According to one aspect, the emitter tip is a tapered emitter tip. According to one aspect, the polymerized porous frit extends from the orifice of the emitter tip into the emitter tip. According to one aspect, the polymerized porous frit extends a distance from the orifice of the emitter tip into the emitter tip. According to one aspect, the polymerized porous frit extends from the orifice of the emitter tip into the emitter tip and into the intermediate portion of the tube. According to one aspect, the polymerized porous frit occupies the channel within the emitter tip. According to one aspect, the polymerized porous frit substantially occupies the channel within the emitter tip. According to one aspect, the polymerized porous frit completely occupies the channel within the emitter tip.

According to one aspect, the polymerizable liquid is placed into the channel of the emitter tip of the tube by capillary action. According to one aspect, the polymerizable liquid is placed into the channel of the emitter tip of the tube by vacuum drawing the polymerizable liquid into the emitter tip. According to one aspect, the polymerizable liquid is placed into the channel of the emitter tip of the tube by pressure forcing the polymerizable liquid into the emitter tip. According to one aspect, the emitter tip of the tube is placed into the polymerizable liquid, the polymerizable liquid is placed into the emitter tip, the emitter tip is withdrawn from the polymerizable liquid, and the polymerizable liquid is polymerized into a polymerized porous frit within the emitter tip. According to one aspect, the polymerizable liquid is polymerized into a polymerized porous frit by uniformly heating the emitter tip. According to one aspect, the polymerizable liquid is polymerized into a polymerized porous frit by placing the emitter tip into a heating block and uniformly heating the emitter tip. According to one aspect, the polymerizable liquid is polymerized into a polymerized porous frit by placing the emitter tip into a hollow cylindrical heating block and uniformly heating the emitter tip.

According to one aspect, a tube is provided having a first end portion having a first orifice, an intermediate portion, and a second end portion having a second orifice, with the second end portion being an emitter tip, wherein a polymerized porous frit is within the emitter tip. According to one aspect, the emitter tip is a tapered emitter tip. According to one aspect, the polymerized porous frit extends from the second orifice into the emitter tip. According to one aspect, the polymerized porous frit extends a distance from the second orifice into the emitter tip. According to one aspect, the polymerized porous frit extends from the second orifice into the emitter tip and into the intermediate portion of the tube. According to one aspect, the polymerized porous frit occupies a channel within the emitter tip. According to one aspect, the polymerized porous frit substantially occupies a channel within the emitter tip. According to one aspect, the polymerized porous frit completely occupies a channel within the emitter tip.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a photograph of a tube having a tapered emitter tip. Figure 1B is a photograph of the tube having a tapered emitter tip of Figure 1A with a polymerizable silicate-based liquid therein. Figure 1C is a photograph of the tube having a tapered emitter tip of Figure 1B with a polymerized silicate-based porous frit within the emitter tip.

DETAILED DESCRIPTION OF CERTAIN EXEMPLARY EMBODIMENTS

Embodiments of the present disclosure are directed to a tube including an intermediate tube portion and a tip or end having a polymerized porous structure within the tip or end and through which media is intended to pass. A polymerized porous structure within the scope of the present disclosure may be referred to as a frit. The tube includes a first end portion including a first orifice into which media enters the tube. The tube includes a channel defined by the wall or walls of the tube and through which media flows within the tube. The channel defines an axis through the tube. The tube includes a second end portion including a second orifice through which media is to pass. The tube includes an intermediate tube portion which is intermediate to the first end portion and the second end portion. According to one aspect, the tube includes a channel extending from the first orifice to the second orifice and through which media flows from the first orifice to the second orifice. The channel may include structure therein which is useful in liquid chromatography or mass spectrometry methods. Such structure includes mixing devices or separating devices. The channel may include various packing materials. Exemplary packing materials may include those used in reversed phase chromatography, hydrophilic interaction chromatography, or size exclusion chromatography. Exemplary packing and/or separation materials known to those of skill in the art and readily identifiable based on the disclosure herein may include 1.8 micron - C18, 3 micron - C18, 3 micron - porous C18, monolithic silica C18, and monolithic packing materials.

The second end portion including an orifice through which media is to pass may be referred to herein as an emitter tip. An emitter tip is intended to include an end of a tube through which media is emitted. An emitter tip may be unitary with a tube. An emitter tip may be integral with a tube. Accordingly, a tube is provided having an emitter tip at one end of the tube. Media enters the tube through the first end portion having a first orifice and exits the tube through the second end portion having a second orifice or emitter tip. A channel extends through the tube. Accordingly, an emitter tip includes a wall of the tube defining a channel through the emitter tip to an orifice. According to one aspect, the emitter tip includes a polymerized porous structure within the emitter tip.

According to certain aspects of the present disclosure, exemplary tubes are those known to those of skill in the art and can be made of ceramic materials, glass, borosilicate glass, fused-silica, polyimide coated fused silica and aluminum coated fused-silica. Exemplary tubes can also be made of polymeric materials or fused silica-lined polymeric materials. Exemplary polymeric materials include fluoropolymers, such as ethylene tetrafluoroethylene (ETFE), fluorinated ethylene propylene (FEP) and polytetrafluoroethylene (PTFE). Additional exemplary polymeric materials include polyolefins such as high density linear polyethylene (HDPE), low density linear polyethylene (LDPE) and polypropylene. Additional exemplary polymeric materials include polyketones such as polyetheretherketone (PEEK) and silica-lined PEEK. Additional exemplary polymeric materials include acrylics such as polymethylmethacrylate (PMMA), polyamides, such as nylon 6, nylon 11 and nylon 12. Additional exemplary polymeric materials include polyimides.

Exemplary tubes and tips of the tubes may have desired configurations and parameters. Tubes may be unitary or monolithic structures or they may be integral structures to the extent that two or more tubes may be connected together with the walls forming a channel through the connected tubes. A configuration of a tube or a tip is referred to herein with respect to the cross-section of the tube or the tip. For example, tubes or tips described may have a round or circular cross-section, an oval cross-section, a square cross-section, a rectangular cross-section, a polygonal cross-section, such as pentagonal, hexagonal and the like. According to an additional embodiment, the tubes may have an irregular shape. According to an additional embodiment, the emitter tip may have a geometry or shape or cross-section different from that of the tube. Exemplary emitter tips include etched open tubular emitters (EOEs), pulled-tapered emitters (PTEs), and molded polymeric emitters (e.g. PST-4PP+LC nozzles) such as described in Reschke et al., *Journal of the American Society for Mass Spectrometry*, 2011, 22, 2115-2124 hereby incorporated by reference in its entirety for all purposes.

According to certain aspects, tubes have dimensions such as length, cross-sectional width, inner diameter, outer diameter etc. According to one aspect, tubes described herein may have any length suitable to a particular purpose. Exemplary lengths of tubes of the present disclosure include between about 5 mm and about 400 cm, between about 10mm and about 350 cm, between about 12 mm and about 300 cm, between about 30 mm and about 250 cm, between about 40 mm and about 200 cm, between about 50 mm and about 150 cm, between about 70 mm and about 100 cm, between about 10 mm and about 100 mm, between about 20 mm and about 90 mm, between about 30 mm and about 70 mm, between about 40 mm and about 60 mm, between about 1 cm and about 100 cm, between about 10 cm and about 90 cm, between about 20 cm and about 80 cm, between about 30 cm and about 70 cm, between about 40 cm and about 60 cm or any value or range in between whether overlapping or not. Exemplary inner diameters or inner cross-sectional widths of

tubes of the present disclosure include between about 1 μm and about 3.0 mm, between about 5 μm and about 2 mm, between about 10 μm and about 1.5 mm, between about 20 μm and about 1.0 mm, between about 30 μm and about 900 μm , between about 50 μm and about 750 μm , between about 75 μm and about 500 μm , between about 100 μm and about 400 μm , between about 150 μm and about 300 μm , between about 200 μm and about 250 μm and any value or range in between whether overlapping or not. Exemplary outer diameters and outer cross-sectional widths of tubes of the present disclosure include between about 150 μm and about 10 mm, between about 200 μm and about 7 mm, between about 300 μm and about 5 mm, between about 350 μm and about 3 mm, between about 400 μm and about 1 mm, between about 150 μm and about 600 μm , between about 200 μm and about 500 μm and between about 250 μm and about 400 μm and any value or range in between whether overlapping or not. It is to be understood that the ends of ranges described herein can be combined to create ranges, as well, without having to expressly provide all combinations of possible ranges herein. References disclosing exemplary packing and/or separation materials, tube lengths, inner diameters, outer diameters, and operating temperatures and pressures include Hyung et al., *Analyst*, 2011, 136, 2100-2105; Thakur et al., *Molecular & Cellular Proteomics*, 2011, 10, 1-9; Shen et al., *Analytical Chemistry*, 2001, 73, 1766-1775; Liu et al., *Journal of Chromatography A*, 2007, 1147, 30-36; Luo et al., *Analytical Chemistry*, 2005, 77, 5028-5035; Kim et al., *Bulletin of the Korean Chemical Society*, 2004, 25, 1833; Iwasaki et al., *Analytical Chemistry*, 2010, 82, 2616-2620; van de Meent, *Trends in Analytical Chemistry*, 2011, 30, 1809-1818 each of which is hereby incorporated by reference in its entirety for all purposes.

According to certain aspects of the present disclosure, the emitter tip of a tube may have a configuration or design known to those of skill in the art. Such exemplary designs are described in Choi et al., *Rapid Communications in Mass Spectrometry*, 2007, 21, 2101-2108; Gibson et al., *Mass Spectrometry Reviews*, 2009, 28, 918-936; Reschke et al., *Journal of the American Society for Mass Spectrometry*, 2011, 22, 2155-2124; Wang et al., *Journal of the American Society for Mass Spectrometry*, 2012, 23, 442-445 each of which is hereby incorporated by reference in its entirety for all purposes. The emitter tip may have an outer configuration and a channel configuration. The outer configuration of the emitter tip is referred to herein as the shape or configuration or orientation of the outer surface of the emitter tip. The outer surface of the tube including the first end portion, the intermediate portion and second end portion in general is smooth. The channel configuration of the emitter tip is referred to herein as the shape or configuration or orientation of the channel through the emitter tip defined by the wall of the tube and ending in an orifice. The surface of the channel in general is smooth.

Such emitter tip outer configurations include a straight configuration relative to the tube or a tapered configuration. A straight configuration is referred to as being in the same line as the

intermediate portion of the tube, i.e. the tube simply extends in a straight line to the orifice. A tapered configuration is referred to herein as a configuration that narrows in a uniform manner relative to an intermediate tube portion. A tapered configuration is referred to herein as a configuration that is angled relative to an intermediate tube portion. Accordingly, the outer configuration of the emitter tip may narrow relative to the tube. Accordingly, the outer configuration of the emitter tip may angle toward the axis of the channel through the tube. The channel configuration of the emitter tip includes a straight configuration or a tapered configuration. A straight configuration is referred to as being in the same line as the surface of the channel, i.e. the interior surface of the tube, in the intermediate portion of the tube, i.e. the surface of the channel simply extends in a straight line to the orifice. Accordingly, the channel configuration of the emitter tip, i.e. the interior surface of the tube, may narrow relative to the channel configuration of the intermediate portion of tube. Accordingly, the channel configuration of the emitter tip, i.e. the interior surface of the tube, may angle toward the axis of the channel through the tube.

According to certain aspects, the outer configuration of the emitter tip may be a straight configuration and the channel configuration of the emitter tip may be a straight configuration. According to certain aspects, the outer configuration of the emitter tip may be a tapered configuration and the channel configuration of the emitter tip may be a straight configuration. According to certain aspects, the outer configuration of the emitter tip may be a straight configuration and the channel configuration of the emitter tip may be a tapered configuration. According to certain aspects, the outer configuration of the emitter tip may be a tapered configuration and the channel configuration of the emitter tip may be a tapered configuration.

According to certain aspects, reference to a tapered emitter tip includes the outer configuration of the emitter tip being a tapered configuration and the channel configuration of the emitter tip being a tapered configuration. Where such tapered emitter tips are used, the end of the tip having the orifice has an inner diameter of between about 1 μm and about 200 μm , between about 5 μm and about 150 μm , between about 10 μm and about 100 μm , between about 20 μm and about 75 μm , between about 10 μm and about 50 μm , between about 10 μm and about 30 μm and any value or range in between whether overlapping or not.

According to certain aspects of the present disclosure, a tapered emitter tip may be made using methods known to those of skill in the art. One such method includes use of a laser-based pipet puller. One such pipette puller is referred to as P-2000 and is commercially available from Sutter Instruments, Novato, CA.

According to certain aspects of the present disclosure, the emitter tip includes a polymerized porous structure within the emitter tip. According to certain aspects, the polymerized porous structure is positioned proximate the orifice of the emitter tip. According to certain aspects, the polymerized porous structure is positioned within the emitter tip a distance away from the orifice of the emitter tip. According to certain aspects, the polymerized porous structure is positioned at the orifice of the emitter tip. According to certain aspects, the polymerized porous structure terminates at the orifice of the emitter tip. According to certain aspects, the polymerized porous structure terminates at the orifice of the emitter tip and does not extend beyond the emitter tip. According to certain aspects, the polymerized porous structure terminates at the orifice of the emitter tip and does not extend beyond the emitter tip outside of the tube. According to certain aspects, the polymerized porous structure extends from the orifice into the tube. According to certain aspects, the polymerized porous structure extends from the orifice into the emitter tip. According to certain aspects, the polymerized porous structure extends from a distance away from the orifice of the emitter tip and within the emitter tip. According to certain aspects, the polymerized porous structure extends from a distance away from the orifice of the emitter tip and within the tube. According to certain aspects, the polymerized porous structure extends from the orifice of the emitter tip within the emitter tip and into the intermediate portion of the tube. According to certain aspects, the polymerized porous structure extends from a distance away from the orifice of the emitter tip within the emitter tip and into the intermediate portion of the tube. According to certain aspects, the polymerized porous structure extends from the orifice of the emitter tip within the emitter tip and terminates at the intermediate portion of the tube. According to certain aspects, the polymerized porous structure extends from a distance away from the orifice of the emitter tip within the emitter tip and terminates at the intermediate portion of the tube.

According to certain aspects of the present disclosure, the polymerized porous structure is formed from a polymerizable composition which is placed within the emitter tip of the tube and then polymerized. The polymerizable composition is a liquid and generally includes a polymerizable compound, such as a monomer, such as an ethylenically unsaturated monomer, capable of being polymerized. The polymerizable material may also include other components known to those of skill in the art useful for making polymerized porous structures such as crosslinking agents, initiators, porogens and the like. An exemplary polymerizable composition includes a polymerizable silicate-based composition. Such a polymerizable silicate-based composition includes lithium silicate, tetramethylammonium silicate and formamide. Other exemplary polymerizable compounds include potassium silicate (Kasil-1), formamide, trimethylolpropane trimethacrylate (TRIM), 2,3-epoxypropyl methacrylate (GMA), ethylene dimethacrylate (EDMA), butyl-methacrylate (BMA), azobisisobutyronitrile (AIBN), 2-hydroxyethyl methacrylate, [2-

(methacryloyloxy)ethyl]trimethylammonium chloride, butyl acrylate, 1,3-butanediol diacrylate (BDDA), 3-(trimethoxysilyl)propyl methacrylate, vinylsulfonic acid (VSA), and 1,4-bis(acryloyl)piperazine (PDA). Exemplary initiators include α -methoxy- α -phenylacetophenone, methacryloxypropyltrimethoxysilane (γ -MAPS), benzoin methyl ether (BME), ammonium persulfate, and 2-hydroxy-2-methyl-phenyl-1-acetone (HMPA). Exemplary porogens include isooctane, toluene, hexane, methanol, 1-propanol, 1,4-butanediol, ethanol, acetonitrile, phosphate buffer, and dodecanol.

According to certain aspects, the polymerizable compositions described herein may be polymerized by use of heat, light, such as ultra violet light or other light of suitable wavelength or other means known to those of skill in the art based on the type of polymerizable composition utilized. Different formulations and methods can be used to produce frits of different porosities and properties. Exemplary materials and methods for producing polymers are described in Cortes et al., Journal of High Resolution Chromatography, 1987, 10, 446; Viklund et al., Chemistry of Materials, 1997, 463-471; Chen et al., Analytical Chemistry, 2000, 72, 1224-1227; Wang et al., Analytical Sciences, 2006, 22, 1099-1104; Yu et al., Analytical Chemistry, 2001, 73, 5088-5096; Ma et al., Analytical Sciences, 2007, 23, 371-374; Yu et al., Electrophoresis, 2000, 21, 120-127; Xie et al., Analytical Chemistry, 2007, 79, 1529-1535; Koerner et al., Analytical Chemistry, 2004, 76, 6456-6460; Rocco et al., Journal of Chromatography A, 2008, 1191, 263-267; Xu et al., Analytica Chimica Acta, 2011, 690, 86-93 each of which is hereby incorporated by reference in its entirety for all purposes.

According to a certain aspect, the polymerizable composition is placed into the emitter tip of the tube. According to a certain aspect, the polymerizable composition enters the tube through the orifice of the emitter tip. Methods of placing a liquid polymerizable composition into a tube include placing the emitter tip of the tube within a liquid polymerizable composition and allowing the liquid polymerizable composition to enter the emitter tip of the tube by capillary action. According to a certain aspect, the emitter tip of the tube is placed within a liquid polymerizable composition and pressure is used to force the liquid polymerizable composition into the emitter tip. According to a certain aspect, the emitter tip of the tube is placed within a liquid polymerizable composition and a vacuum is used to draw the liquid polymerizable composition into the emitter tip. Once the liquid polymerizable composition is within the emitter tip, the liquid polymerizable composition is polymerized to form the polymerized porous structure or frit.

According to one aspect of the present disclosure, the emitter tip with the liquid polymerizable composition is exposed to energy sufficient to cause the liquid polymerizable composition to polymerize. According to one aspect, the energy is heat energy, According to one aspect, the

energy is light energy. According to one aspect, the entire emitter tip or substantially the entire emitter tip is uniformly exposed to energy so as to uniformly polymerize the liquid polymerizable composition. According to one aspect, the entire emitter tip or substantially the entire emitter tip is uniformly exposed to energy so as to uniformly polymerize the liquid polymerizable composition by placing the emitter tip into an energy source such that the energy source supplies energy uniformly around the emitter tip. According to one aspect, the entire emitter tip or substantially the entire emitter tip is uniformly exposed to energy so as to uniformly polymerize the liquid polymerizable composition by placing the emitter tip into an energy source such that the energy source uniformly surrounds the emitter tip and such that the energy source supplies energy uniformly around the emitter tip. According to one aspect, a plurality of emitter tips may be processed simultaneously to polymerize a liquid polymerizable composition within the emitter tips by placing each of the emitter tips within the plurality into a corresponding energy source and polymerizing the liquid polymerizable composition within the emitter tips. In this manner, a batch process is provided whereby porous frits are produced within a plurality of emitter tips at substantially the same time. According to one aspect, a plurality of energy sources may be provided in an array format. According to one aspect, a plurality of energy sources may be provided as a manifold. Each energy source is configured to receive a corresponding emitter tip. An exemplary energy source includes a heat generating source with a compartment configured to receive an emitter tip and with the compartment uniformly surrounding the emitter. According to one aspect, heat is transferred from the energy source to the emitter tip in a manner to cause polymerization of the liquid polymerizable composition. According to one aspect, heat is transferred from the energy source uniformly to the emitter tip in a manner to cause polymerization of the liquid polymerizable composition. An exemplary energy source includes a light generating source with a compartment configured to receive an emitter tip and with the compartment uniformly surrounding the emitter. According to one aspect, light is transferred from the energy source to the emitter tip in a manner to cause polymerization of the liquid polymerizable composition. According to one aspect, light is transferred from the energy source uniformly to the emitter tip in a manner to cause polymerization of the liquid polymerizable composition. According to one aspect, a plurality of compartments is provided in an energy source manifold. A plurality of corresponding emitter tips having a liquid polymerizable composition therein is placed into corresponding compartments. Energy transfer from the compartments to the emitter tips causes polymerization of the liquid polymerizable composition and formation of a porous frit in the emitter tips.

According to one aspect, the polymerized porous structure or frit allows media to move through the frit and exit the orifice of the emitter tip. According to one aspect, the polymerized porous

structure may have pores having a pore size of between about 20 nm and about 20 μm , between about 50 nm and about 10 μm , between about 0.1 μm and about 5 μm , between about 0.15 μm and about 4 μm , between about 0.2 μm and about 1 μm and between about 0.5 μm and about 75 μm and all values and ranges in between whether expressly recited or not. According to one aspect, the polymerized porous structure of the present disclosure may include pores of varying pore sizes and pore lengths with the length of a given pore having a pore wall and being permeated along the pore wall with additional pores having an additional pore size smaller than the pore size. According to one aspect, the pores of the polymerized porous structure or frit are permeated with pores of smaller size. References that describe porous materials with varying porosity and pore size for use in an emitter tip include Chen et al., *Analytical Chemistry*, 2000, 72, 1224-1227; Ficarro et al., *Anal. Chem.* 2009, 81, 3440-3447 and Gibson et al., *Mass Spectrometry Reviews*, 2009, 28, 918-936 each of which are hereby incorporated by reference herein in its entirety for all purposes.

It is to be understood that the embodiments of the present invention which have been described are merely illustrative of some of the applications of the principles of the present invention. Numerous modifications may be made by those skilled in the art based upon the teachings presented herein without departing from the true spirit and scope of the invention. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference in their entirety for all purposes.

The following examples are set forth as being representative of the present invention. These examples are not to be construed as limiting the scope of the invention as these and other equivalent embodiments will be apparent in view of the present disclosure, figures, and accompanying claims.

EXAMPLES

The following examples are specific embodiments of the present disclosure but are not intended to limit it.

EXAMPLE I

Method of Making a Tube with Emitter Tip Having a Frit

Microcapillary analytical columns were constructed with an integrated emitter tip from fused silica capillary tubing (360 μm o.d. x 75 μm i.d.). A section (~2.5 cm) of the polyimide coating was removed with an ethanol burner ~5 cm from the end of a 30 cm piece of capillary tubing. An integrated emitter tip (< 2 μm diameter) was fabricated using a laser-based pipet puller (P-2000, Sutter Instruments, Novato, CA) (Fig. 1A).

The emitter tip was inverted and dipped into a silicate solution (62.5% Lithium silicate, 31.25% Tetramethylammonium silicate, 6.25% Formamide) which was allowed to migrate into the emitter tip via capillary action (Fig. 1B). The solution was then polymerized (Fig. 1C) with uniform radiant heat from a hollow cylindrical aluminum hot block heated to 375°C. The frit was then heated with a lighter for 2 sec and rinsed with 0.1% acetic acid (AcOH) for 2 min using a pressure bomb at 500 psi. Columns were slurry packed with 5 μm Monitor- C_{18} particles suspended in 95% acetonitrile (ACN) 5% isopropanol (*i*PrOH) to a final length of 10 cm and then rinsed with 0.1% AcOH for 15 min at 500 psi. Pressure was increased to 1,000 psi for 2 min to test the stability of the column under high pressure. The end of the columns (end opposite the tip) were cut with a ceramic capillary cutter to within 1.5 cm of the packing material bed to minimize dead volume.

EXAMPLE II

Method of Making A Comparative Tube With Frit Not In the Emitter Tip

Traditional analytical columns with integrated emitter tips were constructed following a protocol described by Ficarro et al. *Anal. Chem.* 2009, 81, 3440-3447 hereby incorporated by reference in its entirety. In brief, a polymerized silica-based frit was fabricated by removing a 2.5 cm section of the polyimide coating about 5 cm from the end of the capillary tubing. The silicate solution was allowed to migrate via capillary action to about 1 cm past the exposed window. Next, polymerization was induced using a soldering iron at 375°C. The soldering iron was applied to the polyimide coating just below the exposed window for 10 sec per side. The excess silicate solution was then ejected using a pressure bomb at 500 psi. The frit was then reheated with the soldering iron for 2 sec per side and then rinsed with 0.1% AcOH for 10 reheat/rinse cycles in order to stabilize the frit. The frit was then heated with an ethanol burner for 2 sec (4X) in order to increase the flow rate of the column. The resulting char from the polyimide coating was removed with

methanol. Columns slurry packed with 5 μm Monitor- C_{18} particles suspended in 95% ACN 5% *i*PrOH to a final length of 10 cm and then rinsed with 0.1% AcOH for 15 min at 500 psi. Pressure was increased to 1,000 psi for 2 min to test the stability of the column under high pressure. Columns were then dried with nitrogen gas at 200 psi for 10 min to remove all moisture within the column which can damage the laser-based pipet puller. Finally, an integrated emitter tip of less than 2 μm diameter and having a void volume was formed 2-3 mm beyond the frit using a laser-based pipet puller. According to this aspect, the frit is within the tube and is not within the emitter tip. The end of the columns (end opposite the tip) were cut with a ceramic capillary cutter to within 1.5 cm of the packing material bed to minimize dead volume

EXAMPLE III

Performance Comparison

A bovine serum albumin standard tryptic peptide mixture was analyzed using an Agilent 1200 Series HPLC system coupled to an linear ion trap/Fourier transform (LTQ-FT) hybrid mass spectrometer (Thermo Finnigan). Peptides (0.2 pmol) were loaded onto a pre-column (360 μm o.d. x 75 μm i.d., packed with 3 cm of 5 μm Monitor- C_{18} resin) via a microautosampler and subsequently transferred to an analytical column at a flow rate of 200 nl/min. Peptides were eluted directly in to the LTQ-FT using the following gradient: 0-17 min, 0-70% solvent B (ACN with 0.1 M AcOH); 17-19 min, 70-95% B; 19-20 min, 95-0% B; 20-37 min 0% B. Solvent A was composed of aqueous 0.1 M AcOH. The mass spectrometer scan functions and HPLC solvent gradient were controlled by the Xcalibur data system. Spectra were acquired in the positive ion mode where each full MS survey scan (400-1800 m/z) was followed by 1 MS/MS scan of the most intense ion per survey spectrum, fragmenting selected ions via collision induced dissociation.

A column made according to Example I and a column made according to Example II and were used for the analysis of the same BSA standard tryptic peptide mixture as described above. Using Xcalibur software, extracted ion chromatograms (XIC) were generated for three signature peptides: 1) YIC*DNQDTISSK (2+) 722.6 m/z , 2) HLVDEPQNLIK (2+) 653.4 m/z , and 3) LVNELTEFAK (2+) 582.4 m/z (Fig. 2A-D). Peak area and peak width were calculated and recorded for each signature peptide along with its retention time to assess the performance of each analytical column. The data is provided in Table 1 below. The column of comparative Example II includes a tapered emitter tip having no frit therein, and accordingly having a void volume within the tapered emitter tip. The column of Example I includes the frit within the emitter tip and accordingly includes no or substantially no void volume. Without wishing to be bound by scientific theory, the lack of a void volume in the column of comparative Example I keeps the resolution initially afforded by the

chromatographic material, providing consistent and uniform peaks as indicated in the data of Table 1. In contrast, the variation in peak widths within and across the column of Example II may be due to slight differences in the void volumes created during tip fabrication. Importantly, the column of Example I demonstrated about a 2-fold increase in sensitivity. Without further wishing to be bound by scientific theory, having a porous frit within the emitter tip acts as an aerator which may increase ionization efficiency and also increases stability of the column.

Table 1

Peak (<i>m/z</i>)	Example I Column 1			Example I Column 2		
	RT (min)	Peak Area	Peak Width (min)	RT (min)	Peak Area	Peak Width (min)
722.6	15.87	2.38E+08	0.18	15.39	1.84E+08	0.16
653.4	17.07	1.15E+08	0.20	16.34	1.54E+08	0.18
582.4	18.24	1.43E+08	0.18	17.43	1.83E+08	0.18
Peak (<i>m/z</i>)	Example II Column 1			Example II Column 2		
	RT (min)	Peak Area	Peak Width (min)	RT (min)	Peak Area	Peak Width (min)
722.6	16.38	9.70E+07	0.28	16.46	8.48E+07	0.30
653.4	17.73	3.24E+07	0.56	17.91	3.62E+07	0.70
582.4	19.05	6.07E+07	0.58	19.34	6.98E+07	0.60

Given the benefit of the above disclosure and description of exemplary embodiments, it will be apparent to those skilled in the art that numerous alternative and different embodiments are possible in keeping with the general principles of the invention disclosed here. Those skilled in this art will recognize that all such various modifications and alternative embodiments are within the true scope and spirit of the invention. While the invention has been illustrated and described in detail in the drawings and foregoing description, such illustration and description is to be considered as exemplary and not restrictive in character, it being understood that, only the preferred embodiments have been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected. The appended claims are intended to cover all such modifications and alternative embodiments. It should be understood that the use of a singular indefinite or definite article (e.g., “a,” “an,” “the,” etc.) in this disclosure and in the following claims follows the traditional approach in patents of meaning “at least one” unless in a particular instance it is clear from context that the term is intended in that particular instance to mean specifically one and only one. Likewise, the term “comprising” is open ended, not excluding

additional items, features, components, etc. References identified herein are expressly incorporated herein by reference in their entireties unless otherwise indicated.

WHAT IS CLAIMED IS

1. A method of making a tube with a frit in an emitter tip having a channel and an orifice comprising

placing a polymerizable liquid into the channel of the emitter tip of the tube, wherein the
5 tube has an intermediate portion; and

polymerizing the polymerizable liquid into a polymerized porous frit within the channel of the emitter tip of the tube.

2. The method of claim 1 wherein the polymerizable liquid is introduced into the tube through the emitter tip.

10 3. The method of claim 1 wherein the emitter tip is a tapered emitter tip.

4. The method of claim 1 wherein the polymerized porous frit extends from the orifice of the emitter tip into the emitter tip.

5. The method of claim 1 wherein the polymerized porous frit extends a distance from the orifice of the emitter tip into the emitter tip.

15 6. The method of claim 1 wherein the polymerized porous frit extends from the orifice of the emitter tip into the emitter tip and into the intermediate portion of the tube.

7. The method of claim 1 wherein the polymerized porous frit occupies the channel within the emitter tip.

8. The method of claim 1 wherein the polymerized porous frit substantially occupies the
20 channel within the emitter tip.

9. The method of claim 1 wherein the polymerized porous frit completely occupies the channel within the emitter tip.

10. The method of claim 1 wherein the polymerizable liquid is placed into the channel of the emitter tip of the tube by capillary action.

25 11. The method of claim 1 wherein the polymerizable liquid is placed into the channel of the emitter tip of the tube by vacuum drawing the polymerizable liquid into the emitter tip.

12. The method of claim 1 wherein the polymerizable liquid is placed into the channel of the emitter tip of the tube by pressure forcing the polymerizable liquid into the emitter tip.

13. The method of claim 1 wherein the emitter tip of the tube is placed into the polymerizable liquid, the polymerizable liquid is placed into the emitter tip, the emitter tip is withdrawn from the polymerizable liquid, and the polymerizable liquid is polymerized into a polymerized porous frit within the emitter tip.

14. The method of claim 1 wherein the polymerizable liquid is polymerized into a polymerized porous frit by uniformly heating the emitter tip.

15. The method of claim 1 wherein the polymerizable liquid is polymerized into a polymerized porous frit by placing the emitter tip into a heating block and uniformly heating the emitter tip.

16. The method of claim 1 wherein the polymerizable liquid is polymerized into a polymerized porous frit by placing the emitter tip into a hollow cylindrical heating block and uniformly heating the emitter tip.

17. A tube comprising

a first end portion having a first orifice, an intermediate portion, and a second end portion having a second orifice, with the second end portion being an emitter tip, wherein a polymerized porous frit is within the emitter tip.

18. The tube of claim 17 wherein the emitter tip is a tapered emitter tip.

19. The tube of claim 17 wherein the polymerized porous frit extends from the second orifice into the emitter tip.

20. The tube of claim 17 wherein the polymerized porous frit extends a distance from the second orifice into the emitter tip.

21. The tube of claim 17 wherein the polymerized porous frit extends from the second orifice into the emitter tip and into the intermediate portion of the tube.

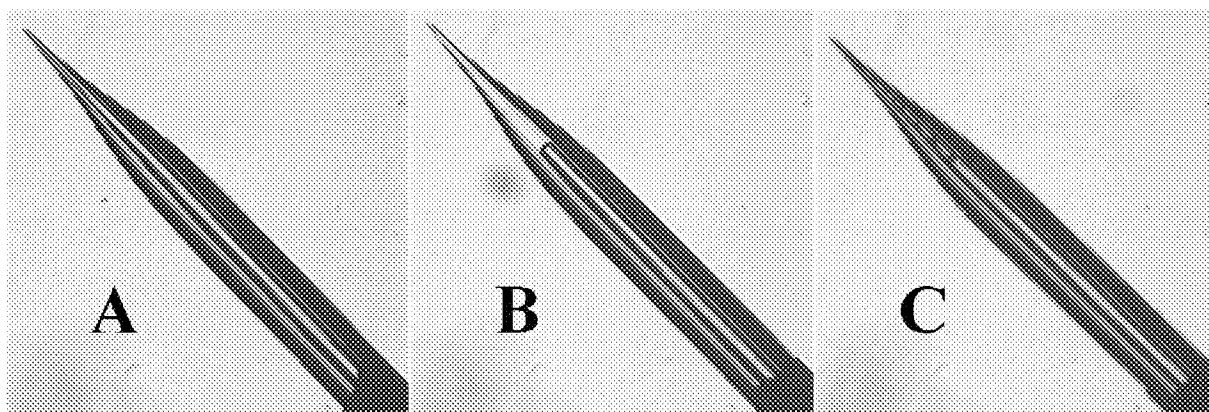
22. The tube of claim 17 wherein the polymerized porous frit occupies a channel within the emitter tip.

23. The tube of claim 17 wherein the polymerized porous frit substantially occupies a channel within the emitter tip.

24. The tube of claim 17 wherein the polymerized porous frit completely occupies a channel within the emitter tip.

1/1

Figure 1



A. CLASSIFICATION OF SUBJECT MATTER**B01D 15/08(2006.01)i, G01N 30/00(2006.01)i**

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)

B01D 15/08; B29C 44/32; B01D 59/44; G01N 30/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: capillary tube, capillary column, capillary, tube, frit, porous frit, polymerizable, polymerized, polymerizing, emitter tip, tapered

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010-0193683 A1 (MARTO, J. A.) 5 August 2010. See abstract; claims 1-2,7,9; paragraphs [0002], [0007], [0009] [0026], [0032], [0036], [0045]; and figures 1-2e.	1,3-9,13-24
Y		2,10-12
Y	US 5997746 A (VALASKOVIC, G. A.) 7 December 1999. See abstract; claims 1-2; column 1, lines 47- 67; column 2, lines 1-4, lines 34-37; column 5, lines 47-56, column 6, lines 34-45; and figures 1-4.	2,10-12
A		1,3-9,13-24
A	US 6395183 B1 (VALASKOVIC, G. et al.) 28 May 2002. See abstract; claims 1,2; column 1, lines 5-12; column 2, lines 21-39; column 5, lines 56-59; column 6, lines 45-48; and figures 6.	1-24
A	US 4793920 A (CORTES, H. et al.) 27 December 1988. See abstract; claim 1; column 1, lines 10-15; column 2, line 66-column 3, line 3; column 3, lines 55-68; column 7, lines 39-46, lines 59-65; and figures 2-4.	1-24
A	WO 2007-092227 A2 (BATTELLE MEMORIAL INSTITUTE.) 16 August 2007. See abstract; claims 1,6; paragraphs [0010], [0014], [0016]-[0017]; and figures 1-4.	1-24



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"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

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search

25 September 2013 (25.09.2013)

Date of mailing of the international search report

26 September 2013 (26.09.2013)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City,
302-701, Republic of Korea

Facsimile No. +82-42-472-7140

Authorized officer

LEE Dong Wook

Telephone No. +82-42-481-8163



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2013/045892

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010-0193683 A1	05/08/2010	EP 2164589 A1 WO 2008-150759 A1	24/03/2010 11/12/2008
US 05997746 A	07/12/1999	AU 1999-24480 A1 AU 1999-24480 B2 AU 1999-35696 A1 CA 2310091 A1 EP 1030717 A1 EP 1094874 A1 EP 1094874 B1 JP 2001-522668 A US 6190559 B1 US 6409651 B1 WO 99-24117 A1 WO 99-62610 A1	31/05/1999 27/06/2002 20/12/1999 20/05/1999 30/08/2000 02/05/2001 01/02/2006 20/11/2001 20/02/2001 25/06/2002 20/05/1999 09/12/1999
US 6395183 B1	28/05/2002	EP 1361912 A1 EP 1361912 B1 JP 2004-526134 A WO 02-058814 A1	19/11/2003 01/04/2009 26/08/2004 01/08/2002
US 04793920 A	27/12/1988	EP 0231684 A1 EP 0231684 B1 JP 62-282262 A	12/08/1987 19/06/1991 08/12/1987
WO 2007-092227 A2	16/08/2007	US 2007-0194224 A1 WO 2007-092227 A3	23/08/2007 26/06/2008