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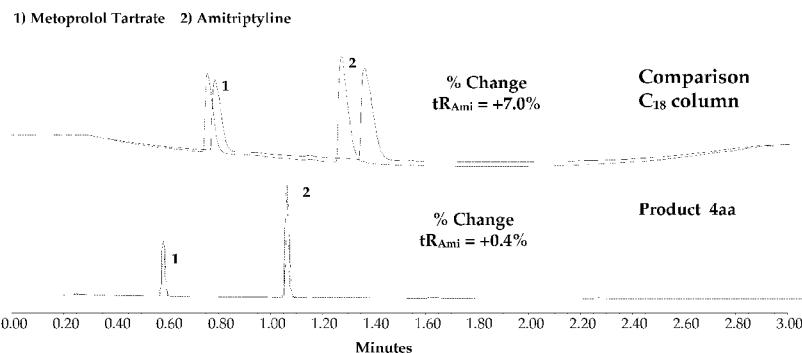
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(54) Title: HIGH PURITY CHROMATOGRAPHIC MATERIALS COMPRISING AN IONIZABLE MODIFIER

Figure 1



(57) Abstract: The present invention provides novel chromatographic materials, e.g., for chromatographic separations, processes for its preparation and separations devices containing the chromatographic material; separations devices, chromatographic columns and kits comprising the same; and methods for the preparation thereof. The chromatographic materials of the invention are high purity chromatographic materials comprising a chromatographic surface wherein the chromatographic surface comprises a hydrophobic surface group and one or more ionizable modifier

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**HIGH PURITY CHROMATOGRAPHIC MATERIALS COMPRISING
AN IONIZABLE MODIFIER**

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. provisional application Ser. No. 61/231,045, filed August 4, 2009, and U.S. provisional application Ser. No. 61/353,999, filed June 11, 2010, the disclosures of which are incorporated herein in their entireties by this reference.

BACKGROUND OF THE INVENTION

10 Packing materials for liquid chromatography (LC) are generally classified into two types: organic materials, *e.g.*, polydivinylbenzene, and inorganic materials typified by silica. Many organic materials are chemically stable against strongly alkaline and strongly acidic mobile phases, allowing flexibility in the choice of mobile phase pH. However, organic chromatographic materials generally result in columns with low efficiency, particularly with 15 low molecular-weight analytes. Many organic chromatographic materials not only lack the mechanical strength of typical chromatographic silica and also shrink and swell when the composition of the mobile phase is changed.

Silica is the material most widely used in High Performance Liquid Chromatography (HPLC), Ultra Performance Liquid Chromatography (UPLC), and Supercritical Fluid 20 Chromatography (SFC). The most common applications employ silica that has been surface-derivatized with an organic functional group such as octadecyl (C18), octyl (C8), phenyl, amino, cyano, *etc.* As stationary phases for HPLC, these packing materials result in columns that have high efficiency and do not show evidence of shrinking or swelling.

25 Current Hybrid Material Technologies (HMT) provide important solutions to traditional chromatographic problems experienced with silica based packing materials. HMT improvements include dramatically improved high and excellent low pH stability, great mechanical stability, good peak shape when used at pH 7, high efficiency, good retentivity, and desirable chromatographic selectivity.

However, two problems have been noted for some HMT and silica materials. The first 30 is poor peak shape for bases when used at low pH, which can negatively impact loadability and peak capacity when used at low pH.

A second problem observed for many HMT and silica materials is a change in acidic and basic analyte retention times (denoted 'drift') after a column is exposed to repeated changes in mobile phase pH (*e.g.*, switching repeatedly from pH 10 to 3).

Thus, there remains a need for alternative materials that provide superior peak shape and reduced drift.

SUMMARY OF THE INVENTION

5 The present invention provides novel chromatographic materials, *e.g.*, for chromatographic separations, processes for its preparation and separations devices containing the chromatographic material.

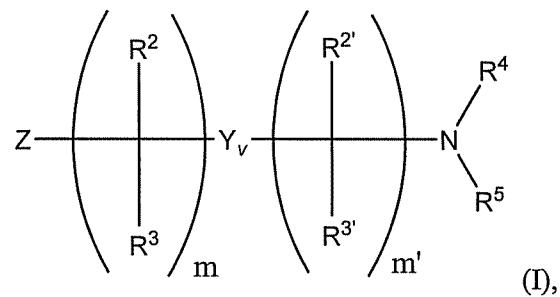
In one aspect the invention provides, a high purity chromatographic material (HPCM) comprising a chromatographic surface wherein the chromatographic surface comprises a 10 hydrophobic surface group and one or more ionizable modifiers with the proviso that when the ionizable modifier does not contain a Zwitterion, the ionizable modifier does not contain a quaternary ammonium ion moiety.

In certain aspects the HPCM may further comprise a chromatographic core material. In some aspects, the chromatographic core is a silica material; a hybrid inorganic/organic material; 15 a superficially porous material; or a superficially porous material.

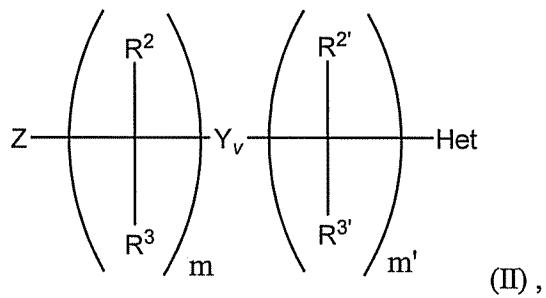
In another aspect the ionizable modifier contains a carboxylic acid group, a sulfonic acid group, a phosphoric acid group, a boronic acid group, an amino group, an imido group, an amido group, a pyridyl group, an imidazolyl group, an ureido group, a thionyl-ureido group or an aminosilane group.

20 In another aspect, the ionizable modifier is selected from the group of zirconium, aluminum, cerium, iron, titanium, salts thereof, oxides and combinations thereof.

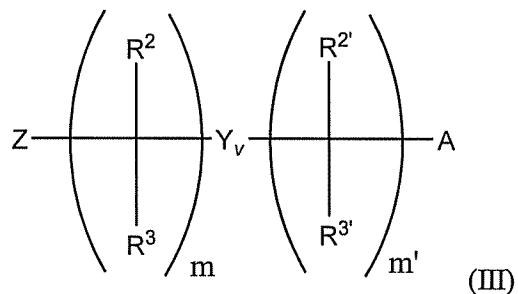
In another aspect, the ionizable modifier may be obtained from an ionizable modifying reagent selected from groups having formula (I)



25 the formula (II):



the formula (III):



or a combination thereof

5 wherein

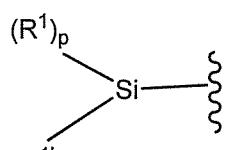
m is an integer from 1-8;

v is 0 or 1;

when v is 0, m' is 0;

when v is 1, m' is an integer from 1-8;

10 Z represents a chemically reactive group, including (but not limited to)



, -OH, -OR⁶, amine, alkylamine, dialkylamine, isocyanate, acyl chloride, triflate, isocyanate, thiocyanate, imidazole carbonate, NHS-ester, carboxylic acid, ester, epoxide, alkyne, alkene, azide, -Br, -Cl, or -I;

Y is an embedded polar functionality;

each occurrence of R¹ independently represents a chemically reactive group on silicon, including (but not limited to) -H, -OH, -OR⁶, dialkylamine, triflate, Br, Cl, I, vinyl, alkene, or -(CH₂)_mQ;

each occurrence of Q is -OH, -OR⁶, amine, alkylamine, dialkylamine, isocyanate, acyl chloride, triflate, isocyanate, thiocyanate, imidazole carbonate, NHS-ester, carboxylic acid, ester, epoxide, alkyne, alkene, azide, -Br, -Cl, or -I;

m" is an integer from 1-8

p is an integer from 1-3;

each occurrence of R¹ independently represents F, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl, fluoroalkyl, or fluoroaryl;

each occurrence of R², R²', R³ and R³' independently represents hydrogen, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl, -Z, or a group having the formula -Si(R')_bR"_a or -C(R')_bR"_a;

15 a and b each represents an integer from 0 to 3 provided that a + b = 3;

R' represents a C₁ - C₆ straight, cyclic or branched alkyl group;

R" is a functionalizing group selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, cyano, amino, diol, nitro, ester, a cation or anion exchange group, an alkyl or aryl group containing an embedded polar functionality and a chiral moiety.

20 R⁴ represents hydrogen, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl;

R⁵ represents hydrogen, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl;

each occurrence of R⁶ independently represents C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl;

Het represents a heterocyclic or heteroaryl ring system comprising at least one nitrogen atom; and

A represents an acidic ionizable modifier moiety or a dual charge ionizable modifier moiety.

In certain aspects, the where the ionizable modifying reagent is selected from formula (III), A represents a protected or unprotected alkyl, aryl, or arylalkyl groups containing 5 phosphoric, carboxylic, sulfonic, or boronic acid.

In certain other aspects, the where the ionizable modifying reagent is selected from formula (III), A represents a dual charge ionizable modifier. While not limited to theory; the dual charge ionizable modifier has two sub-groups that can display opposite charges. Under some conditions the dual charge ionizable modifier can act similarly to a zwitterions and

10 ampholytes to display both a positive and negative charge and maintain a zero net charge.

Under other conditions the dual charge ionizable may only have one group ionized and may display a net positive or negative charge.

Dual charge ionizable modifying reagents include, but are not limited to, alkyl, branched alkyl, aryl, cyclic, polycyclic, heterocyclic and polyheterocyclic groups 15 that can display a positive charge (commonly on a nitrogen or oxygen atom), and a negative charge through an acidic group that includes a carboxylic, sulfonic, phosphonic or boronic acid. Alternatively, some metal containing complexes can display both positive and negative charges.

Dual charge ionizable modifying reagents may also include, but are not limited to Zwitterion, ampholyte, amino acid, aminoalkyl sulfonic acid, aminoalkyl carboxylic acid, mono and di-methylaminoalkyl sulfonic acid, mono and di-methylaminoalkyl carboxylic acid, pyridinium alkyl sulfonic acid, and pyridinium alkyl carboxylic acid groups. Alternatively the dual charge ionizable modifier may include 2-(N-morpholino)ethanesulfonic acid, 3-(N-morpholino)propanesulfonic acid, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid, piperazine-N,N'-bis(2-ethanesulfonic acid), N-cyclohexyl-3-aminopropanesulfonic acid, N-25 cyclohexyl-2-hydroxyl-3-aminopropanesulfonic acid, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 6-Methyl-9,10-didehydro-ergoline-8-carboxylic acid, phenolsulfonphthalein, betaine, quinonoid, N,N-bis(2-hydroxyethyl)glycine, and N-[tris(hydroxymethyl)methyl]glycine groups.

In certain aspects, where the ionizable modifying reagent is selected from formulas (I), 30 (II) or (III),

m is 2 or 3.

In some aspects, where the ionizable modifying reagent is selected from formulas (I), (II) or (III), R¹ represents Cl, -OH, dialkylamino, methoxy or ethoxy.

In certain aspects, where the ionizable modifying reagent is selected from formulas (I), 35 (II) or (III), R¹ represents , methyl, ethyl, isobutyl, isopropyl or tert-butyl.

In other aspects where the ionizable modifying reagent is selected from formulas (I), (II) or (III), each occurrence of R² and R³ represents hydrogen.

In other aspects where the ionizable modifying reagent is selected from formulas (I), (II) or (III), each occurrence of R²' and R³' represents hydrogen.

5 In other aspects where the ionizable modifying reagent is selected from formula (I), each of R⁴ and R⁵ represents hydrogen.

In still other aspects where the ionizable modifying reagent is selected from formulas (II), Het is pyridyl, pyrimidinyl, pyridazinyl, pyrazinyl, piperidinyl, piperizinyl, hexahydropyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, pyrrolidinyl, pyrazolidinyl, 10 imidazolidinyl or triazinyl.

In other aspects where the ionizable modifying reagent is selected from formulas (I), (II) or (III), V is 1, m' is 3, and each occurrence of R², R²', R³ and R³' is hydrogen. In certain aspects, where the ionizable modifying reagent is selected from formulas (I), (II) or (III), V is 1, m' is 3, and each occurrence of R², R²', R³ and R³' is hydrogen, Y is carbamate, carbonate, 15 amide, urea, ether, thioether, sulfinyl, sulfoxide, sulfonyl, thiourea, thiocarbonate, thiocarbamate or triazole.

In yet other aspects, the ionizable modifying reagent is aminopropyltriethoxysilane, aminopropyltrimethoxysilane, 2-(2-(trichlorosilyl)ethyl)pyridine, 2-(2-(trimethoxyethyl)pyridine, 2-(2-(triethoxyethyl)pyridine, 2-(4-pyridylethyl)triethoxysilane, 2-20 (4-pyridylethyl)trimethoxysilane, 2-(4-pyridylethyl)trichlorosilane, chloropropyltrimethoxysilane, chloropropyltrichlorosilane, chloropropyltrichlorosilane, chloropropyltriethoxysilane, imidazolylpropyltrimethoxysilane, imidazolylpropyltrietoxysilane, imidazolylpropyltrichlorosilane, imidazolylpropyltrichlorosilane, imidazolylpropyltrichlorosilane, sulfopropyltrisilanol, carboxyethylsilanetriol, 2-(carbomethoxyethyl)methyldichlorosilane, 2-25 (carbomethoxyethyl)trichlorosilane, 2-(carbomethoxyethyl)trimethoxysilane, n-(trimethoxysilylpropyl)ethylenediamine triacetic acid, (2-diethylphosphatoethyl)triethoxysilane, 3-mercaptopropyltriethoxysilane, 3-mercaptopropyltrimethoxysilane, bis[3-(triethoxysilyl)propyl]disulfide, bis[3-(triethoxysilyl)propyl]tetrasulfide, 2,2-dimethoxy-1-thia-2-silacyclopentane, bis(trichlorosilylethyl)phenylsulfonyl chloride, 2-30 (chlorosulfonylphenyl)ethyltrichlorosilane, 2-(chlorosulfonylphenyl)ethyltrimethoxysilane, 2-(ethoxysulfonylphenyl)ethyltrimethoxysilane, 2-(ethoxysulfonylphenyl)ethyltrimethoxysilane, 2-(ethoxysulfonylphenyl)ethyltrichlorosilane, sulphonic acid phenethyltrisilanol, (triethoxysilyl 35 ethyl)phenyl phosphonic acid diethyl ester, (trimethoxysilyl ethyl)phenyl phosphonic acid diethyl ester, (trichlorosilyl ethyl)phenyl phosphonic acid diethyl ester, phosphonic acid phenethyltrisilanol, N-(3-trimethoxysilylpropyl)pyrrole, N-(3-triethoxysilylpropyl)-4,5-dihydroimidazole, bis(methyldimethoxysilylpropyl)-N-methylamine,

tris(trimethoxysilylpropyl)amine, bis(3-trimethoxysilylpropyl)-N-methylamine, (N,N-diethyl-3-aminopropyl)trimethoxysilane, N-(hydroxyethyl)-N-methylaminopropyltrimethoxysilane, 3-(N,N-dimethylaminopropyl)trimethoxysilane, bis(2-hydroxyethyl)-3-aminopropyltrimethoxysilane, N,N'-bis(hydroxyethyl)-N,N'-
5 bis(trimethoxysilylpropyl)ethylenediamine, or N,N-dimethyl-3-aminopropylmethyldimethoxysilane.

In some aspects, the ratio of the hydrophobic surface group : ionizable modifier in the HPCM of the invention is from about 2.5:1 to about 350:1; from about 3:1 to about 200:1; from about 4:1 to about 150:1; from about 4:1 to about 35:1; from about 5:1 to about 25:1; from
10 about 5:1 to about 22:1; from about 20:1 to about 100:1; or from about 25:1 to about 100:1.

In other aspects, the concentration of ionizable modifier in the HPCM of the invention is less than about 0.5 $\mu\text{mol}/\text{m}^2$; less than about 0.4 $\mu\text{mol}/\text{m}^2$; less than about 0.3 $\mu\text{mol}/\text{m}^2$; from about 0.01 $\mu\text{mol}/\text{m}^2$ to about 0.5 $\mu\text{mol}/\text{m}^2$; from about 0.01 $\mu\text{mol}/\text{m}^2$ to about 0.4 $\mu\text{mol}/\text{m}^2$; or from about 0.03 $\mu\text{mol}/\text{m}^2$ to about 0.3 $\mu\text{mol}/\text{m}^2$.

15 In another aspect, the hydrophobic surface group of the HPCM of the invention is a C_4 to C_{30} bonded phase. In certain aspects, the hydrophobic surface group is a C_{18} bonded phase. In other aspects, the hydrophobic surface group is an aromatic, phenylalkyl, fluoro-aromatic, phenylhexyl, pentafluorophenylalkyl or chiral bonded phase. In still other aspects, the hydrophobic surface group is an embedded polar bonded phase.

20 In certain aspects, the HPCM of the invention may be in the form of a particle, a granular material, a monolith, a superficially porous material, a superficially porous particle, a superficially porous monolith, or a superficially porous layer for open tubular chromatography.

In certain aspects, the HPCM of the invention may be in inorganic material (e.g., silica, alumina, titania, zirconia), a hybrid organic/inorganic material, an inorganic material (e.g.,
25 silica, alumina, titania, zirconia) with a hybrid surface layer, a hybrid material with an inorganic (e.g., silica, alumina, titania, zirconia) surface layer, or a hybrid material with a different hybrid surface layer. In other aspects, the HPCM of the invention may have ordered pore structure, non-periodic pore structuring, non-crystalline or amorphous pore structuring or substantially disordered pore structuring.

30 In one aspect, the HPCM of the invention does not have chromatographically enhancing pore geometry.

In another aspect, the HPCM of the invention has chromatographically enhancing pore geometry.

In certain aspects, the HPCM of the invention has a surface area of about 25 to 1100
35 m^2/g ; about 80 to 500 m^2/g ; or about 120 to 330 m^2/g .

In other aspects, the HPCM of the invention has a pore volume of about 0.15 to 1.5 cm³/g; or about 0.5 to 1.3 cm³/g.

In yet other aspects, the HPCM of the invention has a micropore surface area of less than about 110 m²/g; less than about 105 m²/g; less than about 80 m²/g; or less than about 50 m²/g.

In still yet other aspects, the HPCM of the invention has an average pore diameter of about 20 to 1500Å; about 50 to 1000Å; about 100 to 750Å; or about 110 to 500Å.

In still yet other aspects, when the HPCM of the invention is in the form of a particle, the HPCM of the invention has an average particle size of about 0.3-100 µm; about 0.5-20 µm; 10 0.8-10 µm; or about 1.0-3.5 µm.

In another aspect, the HPCM of the invention is hydrolytically stable at a pH of about 1 to about 14; at a pH of about 10 to about 14; or at a pH of about 1 to about 5.

In still another aspect, the HPCM of the invention has a quantified surface coverage ratio, B/A, from about 2.5 to about 300 wherein A represents the ionizable modifier and B 15 represents the hydrophobic group. In certain aspects, the quantified surface coverage ratio, B/A, is from about 3 to about 200, from about 4 to about 35 or from about 5 to about 22.

In another aspect, the HPCM of the invention may be surface modified. In certain aspects, the HPCM of the invention may be surface modified by coating with a polymer. In other aspects, the HPCM of the invention may be surface modified by coating with a polymer by 20 a combination of organic group and silanol group modification; by a combination of organic group modification and coating with a polymer; or by a combination of silanol group modification and coating with a polymer. In other aspects, the HPCM of the invention may be material has been surface modified by a combination of organic group modification, silanol group modification and coating with a polymer. In still other aspects, the HPCM of the 25 invention may be surface modified via formation of an organic covalent bond between the material's organic group and the modifying reagent.

In certain aspects, the HPCM of the invention may further comprising a nanoparticle dispersed within the material. In aspects further comprising a nanoparticle, the nanoparticle may be a mixture of more than one nanoparticle. In some aspects comprising a nanoparticle, 30 the nanoparticle is present in <20% by weight of the nanocomposite or in <5% by weight of the nanocomposite. In other aspects comprising a nanoparticle, the nanoparticle is crystalline or amorphous. In certain aspects, the nanoparticle is a substance which comprises one or more moieties selected from the group consisting of silicon carbide, aluminum, diamond, cerium, carbon black, carbon nanotubes, zirconium, barium, cerium, cobalt, copper, europium, 35 gadolinium, iron, nickel, samarium, silicon, silver, titanium, zinc, boron, oxides thereof, and nitrides thereof. In certain other aspects, the nanoparticle is a substance which comprises one or

more moieties selected from the group consisting of nano-diamonds, silicon carbide, titanium dioxide, cubic-boronitride. In another aspect, the nanoparticles are less than or equal to 200 nm in diameter; less than or equal to 100 nm in diameter; less than or equal to 50 nm in diameter; or less than or equal to 20 nm in diameter.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the drift with pH switching (from pH 3 to pH 10) using (a) a traditional, commercial C18 bonded material and (b) the material of the instant invention.

10 **Figure 2** depicts the peak shape of various analytes using (a) a traditional, commercial C18 bonded material and (b) the material of the instant invention.

15 **Figure 3** depicts a comparison of isocratic loading behavior for amitriptyline on 4.6 x 150 mm columns containing three different HPCM C18 materials: (a) Product 2e which has a high level of ionizable modifier shows fronting/Anti-Langmuirian peak shape suggesting a concave Langmuirian isotherm; (b) Product 2d which has a balanced level of ionizable modifier shows nearly symmetrical Gaussian/linear peak shape suggesting a linear Langmuirian isotherm; and (c) Product 2b which has a very low level of ionizable modifier shows tailing/Bi-Langmuirian peak shape suggesting a convex Langmuirian isotherm.

20 **Figure 4** depicts a comparison of isocratic loading behavior for amitriptyline on C18 columns (both 2.1 x 50 mm).

20

DETAILED DESCRIPTION OF THE INVENTION

25 The present invention provides novel chromatographic materials, *e.g.*, for chromatographic separations, processes for its preparation and separations devices containing the chromatographic material. The present invention will be more fully illustrated by reference to the definitions set forth below.

Definitions

30 “High Purity” or “high purity chromatographic material” includes a material which is prepared from high purity precursors. In certain aspects, high purity materials have reduced metal contamination and/or non-diminished chromatographic properties including, but not limited to, the acidity of surface silanols and the heterogeneity of the surface.

35 “Chromatographic surface” includes a surface which provides for chromatographic separation of a sample. In certain aspects, the chromatographic surface is porous. In some aspects, a chromatographic surface may be the surface of a particle, a superficially porous material or a monolith. In certain aspects, the chromatographic surface is composed of the surface of one or more particles, superficially porous materials or monoliths used in

combination during a chromatographic separation. In certain other aspects, the chromatographic surface is non-porous.

“Ionizable modifier” includes a functional group which bears an electron donating or electron withdrawing group. In certain aspects, the ionizable modifier contains one or more 5 carboxylic acid groups, amino groups, imido groups, amido groups, pyridyl groups, imidazolyl groups, ureido groups, thionyl-ureido groups or aminosilane groups, or a combination thereof. In other aspects, the ionizable modifier contains a group bearing a nitrogen or phosphorous atom having a free electron lone pair. In certain aspects, the ionizable modifier is covalently attached to the material surface and has an ionizable group. In some instances it is attached to 10 the chromatographic material by chemical modification of a surface hybrid group.

“Hydrophobic surface group” includes a surface group on the chromatographic surface which exhibits hydrophobicity. In certain aspects, a hydrophobic group can be a carbon bonded phase such as a C4 to C18 bonded phase. In other aspects, a hydrophobic surface group can contain an embedded polar group such that the external portion of the hydrophobic surface 15 maintains hydrophobicity. In some instances it is attached to the chromatographic material by chemical modification of a surface hybrid group. In other instances the hydrophobic group can be C4-C30, embedded polar, chiral, phenylalkyl, or pentafluorophenyl bonding and coatings.

“Chromatographic core” includes a chromatographic materials, including but not limited to an organic material such as silica or a hybrid material, as defined herein, in the form 20 of a particle, a monolith or another suitable structure which forms an internal portion of the materials of the invention. In certain aspects, the surface of the chromatographic core represents the chromatographic surface, as defined herein, or represents a material encased by a chromatographic surface, as defined herein. The chromatographic surface material may be disposed on or bonded to or annealed to the chromatographic core in such a way that a discrete 25 or distinct transition is discernable or may be bound to the chromatographic core in such a way as to blend with the surface of the chromatographic core resulting in a gradation of materials and no discrete internal core surface. In certain embodiments, the chromatographic surface material may be the same or different from the material of the chromatographic core and may exhibit different physical or physiochemical properties from the chromatographic core, 30 including, but not limited to, pore volume, surface area, average pore diameter, carbon content or hydrolytic pH stability

“Hybrid”, including “hybrid inorganic/organic material,” includes inorganic-based structures wherein an organic functionality is integral to both the internal or “skeletal” inorganic structure as well as the hybrid material surface. The inorganic portion of the hybrid material 35 may be, *e.g.*, alumina, silica, titanium, cerium, or zirconium or oxides thereof, or ceramic material. “Hybrid” includes inorganic-based structures wherein an organic functionality is

integral to both the internal or "skeletal" inorganic structure as well as the hybrid material surface. As noted above, exemplary hybrid materials are shown in U.S. Patent Nos. 4,017,528, 6,528,167, 6,686,035 and 7,175,913.

The term "alicyclic group" includes closed ring structures of three or more carbon atoms. Alicyclic groups include cycloparaffins or naphthenes which are saturated cyclic hydrocarbons, cycloolefins, which are unsaturated with two or more double bonds, and cycloacetylenes which have a triple bond. They do not include aromatic groups. Examples of cycloparaffins include cyclopropane, cyclohexane and cyclopentane. Examples of cycloolefins include cyclopentadiene and cyclooctatetraene. Alicyclic groups also include fused ring structures and substituted alicyclic groups such as alkyl substituted alicyclic groups. In the instance of the alicyclics such substituents can further comprise a lower alkyl, a lower alkenyl, a lower alkoxy, a lower alkylthio, a lower alkylamino, a lower alkylcarboxyl, a nitro, a hydroxyl, -CF₃, -CN, or the like.

The term "aliphatic group" includes organic compounds characterized by straight or branched chains, typically having between 1 and 22 carbon atoms. Aliphatic groups include alkyl groups, alkenyl groups and alkynyl groups. In complex structures, the chains can be branched or cross-linked. Alkyl groups include saturated hydrocarbons having one or more carbon atoms, including straight-chain alkyl groups and branched-chain alkyl groups. Such hydrocarbon moieties may be substituted on one or more carbons with, for example, a halogen, a hydroxyl, a thiol, an amino, an alkoxy, an alkylcarboxy, an alkylthio, or a nitro group. Unless the number of carbons is otherwise specified, "lower aliphatic" as used herein means an aliphatic group, as defined above (e.g., lower alkyl, lower alkenyl, lower alkynyl), but having from one to six carbon atoms. Representative of such lower aliphatic groups, e.g., lower alkyl groups, are methyl, ethyl, n-propyl, isopropyl, 2-chloropropyl, n-butyl, sec-butyl, 2-aminobutyl, isobutyl, tert-butyl, 3-thiopentyl and the like. As used herein, the term "nitro" means -NO₂; the term "halogen" designates -F, -Cl, -Br or -I; the term "thiol" means SH; and the term "hydroxyl" means -OH. Thus, the term "alkylamino" as used herein means an alkyl group, as defined above, having an amino group attached thereto. Suitable alkylamino groups include groups having 1 to about 12 carbon atoms, preferably from 1 to about 6 carbon atoms. The term "alkylthio" refers to an alkyl group, as defined above, having a sulphydryl group attached thereto. Suitable alkylthio groups include groups having 1 to about 12 carbon atoms, preferably from 1 to about 6 carbon atoms. The term "alkylcarboxyl" as used herein means an alkyl group, as defined above, having a carboxyl group attached thereto. The term "alkoxy" as used herein means an alkyl group, as defined above, having an oxygen atom attached thereto.

Representative alkoxy groups include groups having 1 to about 12 carbon atoms, preferably 1 to about 6 carbon atoms, e.g., methoxy, ethoxy, propoxy, tert-butoxy and the like. The terms

"alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous to alkyls, but which contain at least one double or triple bond respectively. Suitable alkenyl and alkynyl groups include groups having 2 to about 12 carbon atoms, preferably from 1 to about 6 carbon atoms.

The term "alkyl" includes saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups and cycloalkyl substituted alkyl groups. In certain embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone, *e.g.*, C1-C30 for straight chain or C3-C30 for branched chain. In certain embodiments, a straight chain or branched chain alkyl has 20 or fewer carbon atoms in its backbone, *e.g.*, C1-C20 for straight chain or C3-C20 for branched chain, and more preferably 18 or fewer. Likewise, preferred cycloalkyls have from 4-10 carbon atoms in their ring structure and more preferably have 4-7 carbon atoms in the ring structure. The term "lower alkyl" refers to alkyl groups having from 1 to 6 carbons in the chain and to cycloalkyls having from 3 to 6 carbons in the ring structure.

Moreover, the term "alkyl" (including "lower alkyl") as used throughout the specification and Claims includes both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxy, 20 phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulphydryl, alkylthio, arylthio, thiocarboxylate, sulfate, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, *e.g.*, with the substituents described above. An "aralkyl" moiety is an alkyl substituted with an aryl, *e.g.*, having 1 to 3 separate or fused rings and from 6 to about 18 carbon ring atoms, *e.g.*, phenylmethyl (benzyl).

The term "amino," as used herein, refers to an unsubstituted or substituted moiety of the formula -NRaRb, in which Ra and Rb are each independently hydrogen, alkyl, aryl, or heterocyclyl, or Ra and Rb, taken together with the nitrogen atom to which they are attached, form a cyclic moiety having from 3 to 8 atoms in the ring. Thus, the term "amino" includes cyclic amino moieties such as piperidinyl or pyrrolidinyl groups, unless otherwise stated. An "amino-substituted amino group" refers to an amino group in which at least one of Ra and Rb, is further substituted with an amino group.

The term "aromatic group" includes unsaturated cyclic hydrocarbons containing one or more rings. Aromatic groups include 5- and 6-membered single-ring groups which may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine and the like.

5 The aromatic ring may be substituted at one or more ring positions with, for example, a halogen, a lower alkyl, a lower alkenyl, a lower alkoxy, a lower alkylthio, a lower alkylamino, a lower alkylcarboxyl, a nitro, a hydroxyl, -CF₃, -CN, or the like.

The term "aryl" includes 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, unsubstituted or substituted benzene,

10 pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl and the like. The aromatic ring can be substituted at one or more ring positions with such substituents, *e.g.*, as described above for alkyl groups.

Suitable aryl groups include unsubstituted and substituted phenyl groups. The term "aryloxy" as

15 used herein means an aryl group, as defined above, having an oxygen atom attached thereto.

The term "aralkoxy" as used herein means an aralkyl group, as defined above, having an oxygen atom attached thereto. Suitable aralkoxy groups have 1 to 3 separate or fused rings and from 6 to about 18 carbon ring atoms, *e.g.*, O-benzyl.

The term "ceramic precursor" is intended to include any compound that results in the

20 formation of a ceramic material.

The term "chiral moiety" is intended to include any functionality that allows for chiral or stereoselective syntheses. Chiral moieties include, but are not limited to, substituent groups having at least one chiral center, natural and unnatural amino-acids, peptides and proteins, derivatized cellulose, macrocyclic antibiotics, cyclodextrins, crown ethers, and metal

25 complexes.

The term "embedded polar functionality" is a functionality that provides an integral polar moiety such that the interaction with basic samples due to shielding of the unreacted silanol groups on the silica surface is reduced. Embedded polar functionalities include, but are not limited to carbonate, amide, urea, ether, thioether, sulfinyl, sulfoxide, sulfonyl, thiourea,

30 thiocarbonate, thiocarbamate, ethylene glycol, heterocyclic, triazole functionalities or carbamate functionalities such as disclosed in U. S. Patent No. 5,374,755, and chiral moieties.

The language "chromatographically-enhancing pore geometry" includes the geometry of the pore configuration of the presently-disclosed materials, which has been found to enhance the chromatographic separation ability of the material, *e.g.*, as distinguished from other

35 chromatographic media in the art. For example, a geometry can be formed, selected or constructed, and various properties and/or factors can be used to determine whether the

chromatographic separations ability of the material has been “enhanced”, *e.g.*, as compared to a geometry known or conventionally used in the art. Examples of these factors include high separation efficiency, longer column life and high mass transfer properties (as evidenced by, *e.g.*, reduced band spreading and good peak shape.) These properties can be measured or

5 observed using art-recognized techniques. For example, the chromatographically-enhancing pore geometry of the present porous inorganic/organic hybrid materials is distinguished from the prior art materials by the absence of “ink bottle” or “shell shaped” pore geometry or morphology, both of which are undesirable because they, *e.g.*, reduce mass transfer rates, leading to lower efficiencies.

10 Chromatographically-enhancing pore geometry is found in hybrid materials containing only a small population of micropores. A small population of micropores is achieved in hybrid materials when all pores of a diameter of about < 34Å contribute less than about 110 m²/g to the specific surface area of the material. Hybrid materials with such a low micropore surface area (MSA) give chromatographic enhancements including high separation efficiency and good mass

15 transfer properties (as evidenced by, *e.g.*, reduced band spreading and good peak shape).

Micropore surface area (MSA) is defined as the surface area in pores with diameters less than or equal to 34Å, determined by multipoint nitrogen sorption analysis from the adsorption leg of the isotherm using the BJH method. As used herein, the acronyms “MSA” and “MPA” are used interchangeably to denote “micropore surface area”.

20 The term “functionalizing group” includes organic functional groups which impart a certain chromatographic functionality to a chromatographic stationary phase.

The term "heterocyclic group" includes closed ring structures in which one or more of the atoms in the ring is an element other than carbon, for example, nitrogen, sulfur, or oxygen. Heterocyclic groups can be saturated or unsaturated and heterocyclic groups such as pyrrole and 25 furan can have aromatic character. They include fused ring structures such as quinoline and isoquinoline. Other examples of heterocyclic groups include pyridine and purine. Heterocyclic groups can also be substituted at one or more constituent atoms with, for example, a halogen, a lower alkyl, a lower alkenyl, a lower alkoxy, a lower alkylthio, a lower alkylamino, a lower alkylcarboxyl, a nitro, a hydroxyl, -CF₃, -CN, or the like. Suitable heteroaromatic and 30 heteroalicyclic groups generally will have 1 to 3 separate or fused rings with 3 to about 8 members per ring and one or more N, O or S atoms, *e.g.* coumarinyl, quinolinyl, pyridyl, pyrazinyl, pyrimidyl, furyl, pyrrolyl, thienyl, thiazolyl, oxazolyl, imidazolyl, indolyl, benzofuranyl, benzothiazolyl, tetrahydrofuranyl, tetrahydropyranyl, piperidinyl, morpholino and pyrrolidinyl.

The term "metal oxide precursor" is intended to include any compound that contains a metal and results in the formation of a metal oxide, *e.g.*, alumina, silica, titanium oxide, zirconium oxide.

The term "monolith" is intended to include a collection of individual particles packed 5 into a bed formation, in which the shape and morphology of the individual particles are maintained. The particles are advantageously packed using a material that binds the particles together. Any number of binding materials that are well known in the art can be used such as, for example, linear or cross-linked polymers of divinylbenzene, methacrylate, urethanes, alkenes, alkynes, amines, amides, isocyanates, or epoxy groups, as well as condensation 10 reactions of organoalkoxysilanes, tetraalkoxysilanes, polyorganoalkoxysiloxanes, polyethoxysiloxanes, and ceramic precursors. In certain embodiments, the term "monolith" also includes hybrid monoliths made by other methods, such as hybrid monoliths detailed in US Patent No. 7,250,214; hybrid monoliths prepared from the condensation of one or more monomers that contain 0-99 mole percent silica (*e.g.*, SiO_2); hybrid monoliths prepared from 15 coalesced porous inorganic/organic particles; hybrid monoliths that have a chromatographically-enhancing pore geometry; hybrid monoliths that do not have a chromatographically-enhancing pore geometry; hybrid monoliths that have ordered pore structure; hybrid monoliths that have non-periodic pore structure; hybrid monoliths that have non-crystalline or amorphous molecular ordering; hybrid monoliths that have crystalline domains or regions; hybrid monoliths with a 20 variety of different macropore and mesopore properties; and hybrid monoliths in a variety of different aspect ratios. In certain embodiments, the term "monolith" also includes inorganic monoliths, such as those described in G. Guiochon / *J. Chromatogr. A* 1168 (2007) 101-168.

The term "nanoparticle" is a microscopic particle/grain or microscopic member of a powder/nanopowder with at least one dimension less than about 100 nm, *e.g.*, a diameter or 25 particle thickness of less than about 100 nm (0.1 mm), which may be crystalline or noncrystalline. Nanoparticles have properties different from, and often superior to those of conventional bulk materials including, for example, greater strength, hardness, ductility, sinterability, and greater reactivity among others. Considerable scientific study continues to be devoted to determining the properties of nanomaterials, small amounts of which have been 30 synthesized (mainly as nano-size powders) by a number of processes including colloidal precipitation, mechanical grinding, and gas-phase nucleation and growth. Extensive reviews have documented recent developments in nano-phase materials, and are incorporated herein by reference thereto: Gleiter, H. (1989) "Nano-crystalline materials," *Prog. Mater. Sci.* 33:223-315 and Siegel, R. W. (1993) "Synthesis and properties of nano-phase materials," *Mater. Sci. Eng.* 35 A168:189-197. In certain embodiments, the nanoparticles comprise oxides or nitrides of the following: silicon carbide, aluminum, diamond, cerium, carbon black, carbon nanotubes,

zirconium, barium, cerium, cobalt, copper, europium, gadolinium, iron, nickel, samarium, silicon, silver, titanium, zinc, boron, and mixtures thereof. In certain embodiments, the nanoparticles of the present invention are selected from diamonds, zirconium oxide (amorphous, monoclinic, tetragonal and cubic forms), titanium oxide (amorphous, anatase, brookite and 5 rutile forms), aluminum (amorphous, alpha, and gamma forms), and boronitride (cubic form). In particular embodiments, the nanoparticles of the present invention are selected from nano-diamonds, silicon carbide, titanium dioxide (anatase form), cubic-boronitride, and any combination thereof. Moreover, in particular embodiments, the nanoparticles may be crystalline or amorphous. In particular embodiments, the nanoparticles are less than or equal to 100 mm in 10 diameter, *e.g.*, less than or equal to 50 mm in diameter, *e.g.*, less than or equal to 20 mm in diameter.

Moreover, it should be understood that the nanoparticles that are characterized as dispersed within the composites of the invention are intended to describe exogenously added nanoparticles. This is in contrast to nanoparticles, or formations containing significant 15 similarity with putative nanoparticles, that are capable of formation *in situ*, wherein, for example, macromolecular structures, such as particles, may comprise an aggregation of these endogenously created.

The term “substantially disordered” refers to a lack of pore ordering based on x-ray powder diffraction analysis. Specifically, “substantially disordered” is defined by the lack of a 20 peak at a diffraction angle that corresponds to a d value (or d-spacing) of at least 1 nm in an x-ray diffraction pattern.

“Surface modifiers” include (typically) organic functional groups which impart a certain chromatographic functionality to a chromatographic stationary phase. The porous inorganic/organic hybrid materials possess both organic groups and silanol groups which may 25 additionally be substituted or derivatized with a surface modifier.

The language “surface modified” is used herein to describe the composite material of the present invention that possess both organic groups and silanol groups which may additionally be substituted or derivatized with a surface modifier. “Surface modifiers” include (typically) organic functional groups which impart a certain chromatographic functionality to a 30 chromatographic stationary phase. Surface modifiers such as disclosed herein are attached to the base material, *e.g.*, via derivatization or coating and later crosslinking, imparting the chemical character of the surface modifier to the base material. In one embodiment, the organic groups of a hybrid material, react to form an organic covalent bond with a surface modifier. The modifiers can form an organic covalent bond to the material’s organic group via a number 35 of mechanisms well known in organic and polymer chemistry including but not limited to nucleophilic, electrophilic, cycloaddition, free-radical, carbene, nitrene, and carbocation

reactions. Organic covalent bonds are defined to involve the formation of a covalent bond between the common elements of organic chemistry including but not limited to hydrogen, boron, carbon, nitrogen, oxygen, silicon, phosphorus, sulfur, and the halogens. In addition, carbon-silicon and carbon-oxygen-silicon bonds are defined as organic covalent bonds, whereas 5 silicon-oxygen-silicon bonds that are not defined as organic covalent bonds. A variety of synthetic transformations are well known in the literature, *see, e.g., March, J. Advanced Organic Chemistry, 3rd Edition, Wiley, New York, 1985.*

Chromatographic Surface materials

10 The invention provides, a high purity chromatographic material (HPCM) comprising a chromatographic surface wherein the chromatographic surface comprises a hydrophobic surface group and one or more ionizable modifiers with the proviso that when the ionizable modifier does not contain a Zwitterion, the ionizable modifier does not contain a quaternary ammonium ion moiety.

15 In certain aspects the HPCM may further comprise a chromatographic core material. In some aspects, the chromatographic core is a silica material; a hybrid inorganic/organic material; a superficially porous material; or a superficially porous particle. The chromatographic core material may be in the form of discreet particles or may be a monolith. The chromatographic core material may be any porous material and may be commercially available or may be 20 produced by known methods, such as those methods described in, for example, in U.S. Patent Nos. 4,017,528, 6,528,167, 6,686,035 and 7,175,913. In some embodiments, the chromatographic core material may be a non-porous core.

The composition of the chromatographic surface material and the chromatographic core material (if present) may be varied by one of ordinary skill in the art to provide enhanced 25 chromatographic selectivity, enhanced column chemical stability, enhanced column efficiency, and/or enhanced mechanical strength. Similarly, the composition of the surrounding material provides a change in hydrophilic/lipophilic balance (HLB), surface charge (*e.g.*, isoelectric point or silanol pKa), and/or surface functionality for enhanced chromatographic separation. Furthermore, in some embodiments, the composition of the chromatographic material may also 30 provide a surface functionality for available for further surface modification.

The ionizable modifiers and the hydrophobic surface groups of the HPCMs of the invention can be prepared using known methods. Some of the ionizable modifier reagents are commercially available. For example silanes having amino alkyl trialkoxysilanes, methyl amino alkyl trialkoxysilanes, and pyridyl alkyl trialkoxysilanes are commercially available. Other 35 silanes such as chloropropyl alkyl trichlorosilane and chloropropyl alkyl trialkoxysilane are also commercially available. These can be bonded and reacted with imidazole to create imidazolyl

alkyl silyl surface species, or bonded and reacted with pyridine to create pyridyl alkyl silyl surface species. Other acidic modifiers are also commercially available, including, but not limited to, sulfopropyltrisilanol, carboxyethylsilanetriol, 2-(carbomethoxy)ethylmethyldichlorosilane, 2-(carbomethoxy)ethyltrichlorosilane, 2-(carbomethoxy)ethyltrimethoxysilane, n-(trimethoxysilylpropyl)ethylenediamine, triacetic acid, (2-diethylphosphatoethyl)triethoxysilane, 2-(chlorosulfonylphenyl)ethyltrichlorosilane, and 2-(chlorosulfonylphenyl)ethyltrimethoxysilane.

5 (carbomethoxy)ethyltrimethoxysilane, n-(trimethoxysilylpropyl)ethylenediamine, triacetic acid, (2-diethylphosphatoethyl)triethoxysilane, 2-(chlorosulfonylphenyl)ethyltrichlorosilane, and 2-(chlorosulfonylphenyl)ethyltrimethoxysilane.

It is known to one skilled in the art to synthesize these types of silanes using common synthetic protocols, including grinard reactions and hydrosilylations. Products can be purified 10 by chromatography, recrystallization or distillation

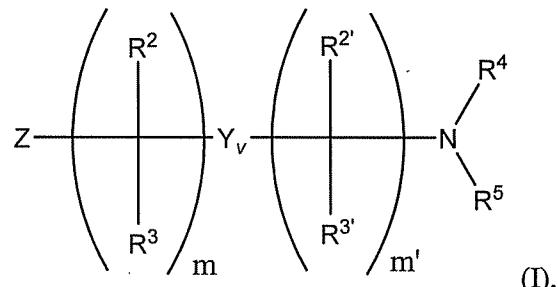
Other additives such as isocyanates are also commercially available or can be synthesized by one skilled in the art. A common isocyanate forming protocol is the reaction of a primary amine with phosgene or a reagent known as Triphosgene.

In some embodiments the ionizable modifier contains a carboxylic acid group, a sulfonic acid group, a phosphoric acid group, a boronic acid group, an amino group, an imido group, an amido group, a pyridyl group, an imidazolyl group, an ureido group, a thionyl-ureido group or an aminosilane group.

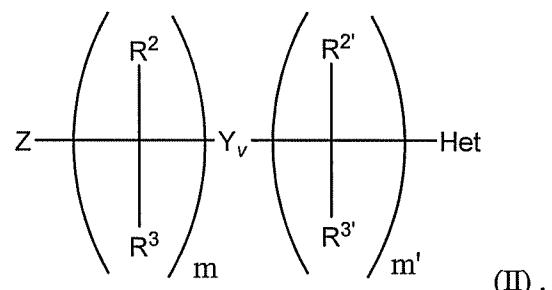
15

In other aspects the ionizable modifier reagent may be selected from groups formula

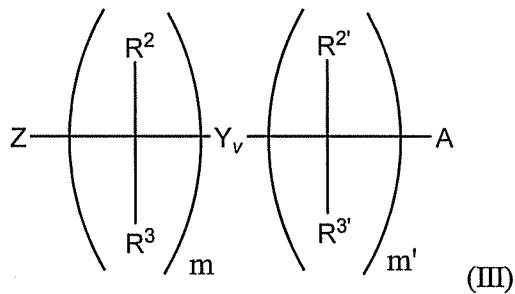
(I)



the formula (II):



the formula (III):



wherein

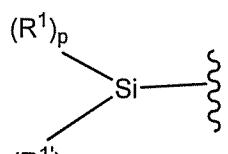
m is an integer from 1-8;

v is 0 or 1;

5 when v is 0, m' is 0;

when v is 1, m' is an integer from 1-8;

Z represents a chemically reactive group, including (but not limited to)



, -OH, -OR⁶, amine, alkylamine, dialkylamine, isocyanate, acyl chloride,

triflate, isocyanate, thiocyanate, imidazole carbonate, NHS-ester, carboxylic acid, ester,

10 epoxide, alkyne, alkene, azide, -Br, -Cl, or -I;

Y is an embedded polar functionality;

each occurrence of R^1 independently represents a chemically reactive group on silicon, including (but not limited to) -H, -OH, -OR⁶, dialkylamine, triflate, Br, Cl, I, vinyl, alkene, or $-(CH_2)_m^r Q$;

15 each occurrence of Q is -OH, -OR⁶, amine, alkylamine, dialkylamine, isocyanate, acyl chloride, triflate, isocyanate, thiocyanate, imidazole carbonate, NHS-ester, carboxylic acid, ester, epoxide, alkyne, alkene, azide, -Br, -Cl, or -I;

m'' is an integer from 1-8

p is an integer from 1-3;

each occurrence of R¹ independently represents F, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl, fluoroalkyl, or fluoroaryl;

each occurrence of R², R²', R³ and R³' independently represents hydrogen, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₂-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₄-C₁₈ heteroaryl, -Z, or a group having the formula -Si(R')_bR["]_a or -C(R')_bR["]_a;

a and b each represents an integer from 0 to 3 provided that a + b = 3;

R' represents a C₁ - C₆ straight, cyclic or branched alkyl group;

R" is a functionalizing group selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, cyano, amino, diol, nitro, ester, a cation or anion exchange group, an alkyl or aryl group containing an embedded polar functionality and a chiral moiety.

R⁴ represents hydrogen, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl;

R⁵ represents hydrogen, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl;

each occurrence of R⁶ independently represents C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl;

Het represents a heterocyclic or heteroaryl ring system comprising at least one nitrogen atom; and

A represents an acidic ionizable modifier moiety or a dual charge ionizable modifier moiety.

In yet other embodiments, the ionizable modifier is aminopropyltriethoxysilane, aminopropyltrimethoxysilane, 2-(2-(trichlorosilyl)ethyl)pyridine, 2-(2-(trimethoxy)ethyl)pyridine, 2-(2-(triethoxy)ethyl)pyridine, 2-(4-pyridylethyl)triethoxysilane, 2-(4-pyridylethyl)trimethoxysilane, 2-(4-pyridylethyl)trichlorosilane, chloropropyltrimethoxysilane, chloropropyltrichlorosilane, chloropropyltrichlorosilane, chloropropyltriethoxysilane, imidazolylpropyltrimethoxysilane, imidazolylpropyltriethoxysilane, imidazolylpropyl trichlorosilane, sulfopropyltrisilanol,

carboxyethylsilanetriol, 2-(carbomethoxy)ethylmethyldichlorosilane, 2-(carbomethoxy)ethyltrichlorosilane, 2-(carbomethoxy)ethyltrimethoxysilane, n-(trimethoxysilylpropyl)ethylenediamine triacetic acid, (2-diethylphosphatoethyl)triethoxysilane, 3-mercaptopropyltriethoxysilane, 3-mercaptopropyltrimethoxysilane, bis[3-(triethoxysilylpropyl]disulfide, bis[3-(triethoxysilylpropyl]tetrasulfide, 2,2-dimethoxy-1-thia-2-silacyclopentane, bis(trichlorosilylethyl)phenylsulfonyl chloride, 2-(chlorosulfonylphenyl)ethyltrichlorosilane, 2-(chlorosulfonylphenyl)ethyltrimethoxysilane, 2-(ethoxysulfonylphenyl)ethyltrimethoxysilane, 2-(ethoxysulfonylphenyl)ethyltrimethoxysilane, 2-(ethoxysulfonylphenyl)ethyltrichlorosilane, sulphonic acid phenethyltrisilanol, (triethoxysilylethyl)phenyl phosphonic acid diethyl ester, (trimethoxysilyl ethyl)phenyl phosphonic acid diethyl ester, (trichlorosilyl ethyl)phenyl phosphonic acid diethyl ester, phosphonic acid phenethyltrisilanol, N-(3-trimethoxysilylpropyl)pyrrole, N-(3-triethoxysilylpropyl)-4,5-dihydroimidazole, bis(methyldimethoxysilylpropyl)-N-methylamine, tris(triethoxysilylpropyl)amine, bis(3-trimethoxysilylpropyl)-N-methylamine, (N,N-diethyl-3-aminopropyl)trimethoxysilane, N-(hydroxyethyl)-N-methylaminopropyltrimethoxysilane, 3-(N,N-dimethylaminopropyl)trimethoxysilane, bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane, N,N'-bis(hydroxyethyl)-N,N'-bis(trimethoxysilylpropyl)ethylenediamine, or N,N-dimethyl-3-aminopropylmethyldimethoxysilane.

20 In certain embodiments, when the ionizable modifier is of the formula (III), the acidic ionizable modifiers is a protected or deprotected forms of trisilanol, trialkoxysilane or trichlorosilane; or a salt of sulfonic acid alkyl silanes, sulfonic acid phenylalkyl silanes, sulfonic acid benzylalkyl silanes, sulfonic acid phenyl silanes, sulfonic acid benzyl silanes, carboxylic acid alkyl silanes, carboxylic acid phenylalkyl silanes, carboxylic acid benzylalkyl silanes, 25 carboxylic acid phenyl silanes, carboxylic acid benzyl silanes, phosphoric acid alkyl silanes, phosphonic acid phenylalkyl silanes, phosphonic acid benzylalkyl silanes, phosphonic acid phenyl silanes, phosphonic acid benzyl silanes, boronic acid alkyl silanes, boronic acid phenylalkyl silanes, boronic acid benzylalkyl silanes, boronic acid phenyl silanes, boronic acid benzyl silanes.

30 In certain embodiments, when the ionizable modifier is of the formula (III), the acidic ionizable modifiers is a protected or deprotected version or a salt of sulfonic acid alkyl isocyanates, sulfonic acid phenylalkyl isocyanates, sulfonic acid benzylalkyl isocyanates, sulfonic acid phenyl isocyanates, sulfonic acid benzyl isocyanates carboxylic acid alkyl isocyanates, carboxylic acid phenylalkyl isocyanates, carboxylic acid benzylalkyl isocyanates, 35 carboxylic acid phenyl isocyanates, carboxylic acid benzyl isocyanates, phosphoric acid alkyl isocyanates, phosphonic acid phenylalkyl isocyanates, phosphonic acid benzylalkyl isocyanates,

phosphonic acid phenyl isocyanates, phosphonic acid benzyl isocyanates, boronic acid alkyl isocyanates, boronic acid phenylalkyl isocyanates, boronic acid benzylalkyl isocyanates, boronic acid phenyl isocyanates, or boronic acid benzyl isocyanates.

In certain embodiments, when the ionizable modifier reagent is selected from formula (III), A represents a dual charge ionizable modifier moiety. While not limited to theory; the dual charge ionizable modifier moiety has two sub-groups that can display opposite charges. Under some conditions the dual charge ionizable modifier moiety can act similarly to a zwitterions and ampholytes to display both a positive and negative charge and maintain a zero net charge. Under other conditions the dual charge ionizable modifier moiety may only have one group ionized and may display a net positive or negative charge. Dual charge ionizable modifier moieties include, but are not limited to, alkyl, branched alkyl, aryl, cyclic, polyaromatic, polycyclic, heterocyclic and polyheterocyclic groups that can display a positive charge (commonly on a nitrogen or oxygen atom), and a negative charge through an acidic group that includes a carboxylic, sulfonic, phosphonic or boronic acid. Alternatively, some metal containing complexes can display both positive and negative charges. Dual charge ionizable modifier moieties may also include, but are not limited to zwitterions, ampholyte, amino acid, aminoalkyl sulfonic acid, aminoalkyl carboxylic acid, mono and di-methylaminoalkyl sulfonic acid, mono and di-methylaminoalkyl carboxylic acid, pyridinium alkyl sulfonic acid, and pyridinium alkyl carboxylic acid groups. Alternatively the dual charge ionizable modifier moiety may be 2-(N-morpholino)ethanesulfonic acid, 3-(N-morpholino)propanesulfonic acid, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid, piperazine-N,N'-bis(2-ethanesulfonic acid), N-cyclohexyl-3-aminopropanesulfonic acid, N-cyclohexyl-2-hydroxyl-3-aminopropanesulfonic acid, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 6-Methyl-9,10-didehydro-ergoline-8-carboxylic acid, phenolsulfonphthalein, betaines, quinonoids, N,N-bis(2-hydroxyethyl)glycine, and N-[tris(hydroxymethyl)methyl]glycine groups.

In some embodiments, the ratio of the hydrophobic surface group : ionizable modifier in the HPCM of the invention is from about 4:1 to about 150:1; from about 20:1 to about 100:1; or from about 25:1 to about 100:1.

In other embodiments, the concentration of ionizable modifier in the HPCM of the invention is less than about 0.5 $\mu\text{mol}/\text{m}^2$; less than about 0.4 $\mu\text{mol}/\text{m}^2$; less than about 0.3 $\mu\text{mol}/\text{m}^2$; from about 0.01 $\mu\text{mol}/\text{m}^2$ to about 0.5 $\mu\text{mol}/\text{m}^2$; from about 0.1 $\mu\text{mol}/\text{m}^2$ to about 0.4 $\mu\text{mol}/\text{m}^2$; or from about 0.2 $\mu\text{mol}/\text{m}^2$ to about 0.3 $\mu\text{mol}/\text{m}^2$.

In still another aspect, the HPCM of the invention has a quantified surface coverage ratio, B/A, from about 2.5 to about 300 wherein A represents the ionizable modifier and B

represents the hydrophobic group. In certain aspects, the quantified surface coverage ratio, B/A, is from about 3 to about 200, from about 4 to about 35 or from about 5 to about 22.

In another aspect, the hydrophobic surface group of the HPCM of the invention is a C4 to C18 bonded phase. In certain aspects, the hydrophobic surface group is a C18 bonded phase.

5 In still other aspects, the hydrophobic surface group is an embedded polar bonded phase. In other aspects, the hydrophobic surface group is an aromatic, phenylalkyl, fluoro-aromatic, phenylhexyl, or pentafluorophenylalkyl bonded phase. In another aspect, the hydrophobic surface group is a C₄-C₃₀, embedded polar, chiral, phenylalkyl, or pentafluorophenyl bonding or coating.

10 In certain embodiments, the HPCM of the invention may be in the form of a particle, a monolith or a superficially porous material. In certain other aspects, the HPCM of the invention is a non-porous material.

In certain aspects, the HPCM of the invention may be an inorganic material (e.g., silica,), a hybrid organic/inorganic material, an inorganic material (e.g., silica) with a hybrid

15 surface layer, a hybrid particle with a inorganic (e.g., silica) surface layer, or a hybrid particle with a different hybrid surface layer.

In one embodiment, the HPCM of the invention does not have chromatographically enhancing pore geometry. In another embodiment, the HPCM of the invention has chromatographically enhancing pore geometry.

20 In certain embodiments, the HPCM of the invention has a surface area of about 25 to 1100 m²/g; about 80 to 500 m²/g; or about 120 to 330 m²/g.

In other embodiments, the HPCM of the invention a pore volume of about 0.15 to 1.7 cm³/g; or about 0.5 to 1.3 cm³/g.

In certain other embodiments, the HPCM of the invention is non-porous.

25 In yet other embodiments, the HPCM of the invention has a micropore surface area of less than about 110 m²/g; less than about 105 m²/g; less than about 80 m²/g; or less than about 50 m²/g.

In still yet other embodiments, the HPCM of the invention has an average pore diameter of about 20 to 1500 Å; about 50 to 1000 Å; about 100 to 750 Å; or about 150 to 500 Å.

30 In another embodiment, the HPCM of the invention is hydrolytically stable at a pH of about 1 to about 14; at a pH of about 10 to about 14; or at a pH of about 1 to about 5.

In another aspect, the invention provides materials as described herein wherein the HPCM material further comprises a nanoparticle or a mixture of more than one nanoparticles

35 dispersed within the chromatographic surface.

In certain embodiments, the nanoparticle is present in <20% by weight of the nanocomposite, <10% by weight of the nanocomposite, or <5% by weight of the nanocomposite.

In other embodiments, the nanoparticle is crystalline or amorphous and may be silicon carbide, aluminum, diamond, cerium, carbon black, carbon nanotubes, zirconium, barium, cerium, cobalt, copper, europium, gadolinium, iron, nickel, samarium, silicon, silver, titanium, zinc, boron, oxides thereof, or a nitride thereof. In particular embodiments, the nanoparticle is a substance which comprises one or more moieties selected from the group consisting of nanodiamonds, silicon carbide, titanium dioxide, and cubic-boronitride.

10 In other embodiments, the nanoparticles may be less than or equal to 200 nm in diameter, less than or equal to 100 nm in diameter, less than or equal to 50 nm in diameter, or less than or equal to 20 nm in diameter.

Surface Modification

15 The HPCM materials of the invention may further be surface modified.

Thus, in one embodiment, the material as described herein may be surface modified with a surface modifier having the formula $Z_a(R')_bSi-R''$, where $Z = Cl, Br, I, C_1 - C_5$ alkoxy, dialkylamino or trifluoromethanesulfonate; a and b are each an integer from 0 to 3 provided that $a + b = 3$; R' is a $C_1 - C_6$ straight, cyclic or branched alkyl group, and R'' is a functionalizing group.

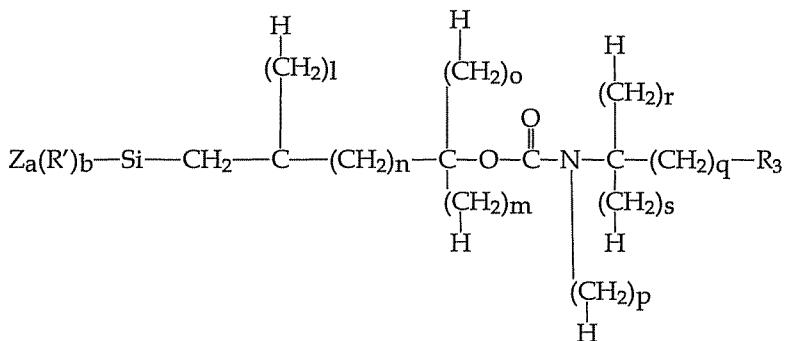
20 In another embodiment, the materials have been surface modified by coating with a polymer.

25 In certain embodiments, R' is selected from the group consisting of methyl, ethyl, propyl, isopropyl, butyl, t-butyl, sec-butyl, pentyl, isopentyl, hexyl and cyclohexyl. In other embodiments, R' is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, cyano, amino, diol, nitro, ester, a cation or anion exchange group, an alkyl or aryl group containing an embedded polar functionality and a chiral moiety. In certain embodiments, R' is selected from the group consisting of aromatic, phenylalkyl, fluoroaromatic, phenylhexyl, pentafluorophenylalkyl and chiral moieties.

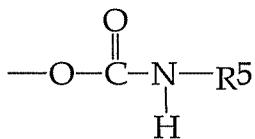
30 In one embodiment, R'' is a $C_1 - C_{30}$ alkyl group. In a further embodiment, R'' comprises a chiral moiety. In another further embodiment, R'' is a $C_1 - C_{20}$ alkyl group.

35 In certain embodiments, the surface modifier comprises an embedded polar functionality. In certain embodiments, such embedded polar functionality includes carbonate, amide, urea, ether, thioether, sulfinyl, sulfoxide, sulfonyl, thiourea, thiocarbonate, thiocarbamate, ethylene glycol, heterocyclic, or triazole functionalities. In other embodiments,

such embedded polar functionality includes carbamate functionalities such as disclosed in U. S. Patent No. 5,374,755, and chiral moieties. Such groups include those of the general formula



wherein l, m, o, r and s are 0 or 1, n is 0, 1, 2 or 3 p is 0, 1, 2, 3 or 4 and q is an integer from 0 to 5 19; R₃ is selected from the group consisting of hydrogen, alkyl, cyano and phenyl; and Z, R', a and b are defined as above. Preferably, the carbamate functionality has the general structure indicated below:



wherein R⁵ may be, e.g., cyanoalkyl, t-butyl, butyl, octyl, dodecyl, tetradecyl, octadecyl, or 10 benzyl. Advantageously, R⁵ is octyl, dodecyl, or octadecyl.

In certain embodiments, the surface modifier is selected from the group consisting of phenylhexyltrichlorosilane, pentafluorophenylpropyltrichlorosilane, octyltrichlorosilane, octadecyltrichlorosilane, octyldimethylchlorosilane and octadecyldimethylchlorosilane. In some embodiments, the surface modifier is selected from the group consisting of 15 octyltrichlorosilane and octadecyltrichlorosilane. In other embodiments, the surface modifier is selected from the group consisting of an isocyanate or 1,1'-carbonyldiimidazole (particularly when the hybrid group contains a (CH₂)₃OH group).

In another embodiment, the material has been surface modified by a combination of organic group and silanol group modification.

20 In still another embodiment, the material has been surface modified by a combination of organic group modification and coating with a polymer. In a further embodiment, the organic group comprises a chiral moiety.

In yet another embodiment, the material has been surface modified by a combination of silanol group modification and coating with a polymer.

25 In other embodiments, the material has been surface modified via formation of an organic covalent bond between the particle's organic group and the modifying reagent.

In still other embodiments, the material has been surface modified by a combination of organic group modification, silanol group modification and coating with a polymer.

In another embodiment, the material has been surface modified by silanol group modification.

5 In certain embodiments, the surface modified layer may be porous or non-porous.

Separation Devices and Kits

Another aspect provides a variety of separations devices having a stationary phase comprising the HPCM materials as described herein. The separations devices include, *e.g.*,

10 chromatographic columns, thin layer plates, filtration membranes, sample cleanup devices and microtiter plates.

The HPCM Materials impart to these devices improved lifetimes because of their improved stability. Thus, in a particular aspect, the invention provides a chromatographic column having improved lifetime, comprising

15 a) a column having a cylindrical interior for accepting a packing material, and
b) a packed chromatographic bed comprising the high purity chromatographic material as described herein.

In another particular aspect, the invention provides a chromatographic device, comprising

20 a) an interior channel for accepting a packing material and
b) a packed chromatographic bed comprising the high purity chromatographic material as described herein.

The invention also provides for a kit comprising the HPCM materials as described herein, as described herein, and instructions for use. In one embodiment, the instructions are for
25 use with a separations device, *e.g.*, chromatographic columns, thin layer plates, filtration membranes, sample cleanup devices and microtiter plates.

Synthesis of Materials of the Invention

The invention also provides methods for producing the high purity chromatographic
30 materials (HPCM) materials described herein.

In one embodiment, the invention provides a method for producing the HPCM described herein comprising the steps of:

a. reacting a chromatographic core with an ionizable modifying reagent to obtain a ionizable modified material; and

- b. reacting the resultant material with a hydrophobic surface modifying group.

In another embodiment, the invention provides a method for producing the High purity chromatographic materials described herein comprising the steps of:

- a. reacting a chromatographic core with hydrophobic surface modifying group to 5 obtain a surface modified material; and
- b. reacting the resultant material with an ionizable modifying reagent.

In another embodiment, the invention provides a method for producing the High purity chromatographic materials described herein comprising the steps of:

- a. reacting a chromatographic core with hydrophobic surface modifying group to 10 obtain a surface modified material; and
- b. reacting the resultant material with an endcapping surface group, and
- c. reacting the resultant material with an ionizable modifying reagent.

In another embodiment, the invention provides a method for producing the High purity chromatographic materials described here comprising the steps of:

- 15 a. reacting a chromatographic core with an ionizable modifying reagent to obtain an ionizable modified material; and
- b. reacting the resultant material to produce a hybrid surface layer; and
- c. reacting the resultant material with a hydrophobic surface modifying group.

In one aspect, the HPCM of the invention as described above is made with a charge 20 ratio, B'/A' , from about 3 to about 133 wherein A' represents the ionizable modifier reagent charged in the preparation and B' represents the hydrophobic group charged in the preparation. In certain aspects, the charge ratio, B'/A' , is from about 4 to about 80, from about 4 to about 15, or from about 6 to about 7.

25 In one embodiment, the methods described herein further comprise the step of endcapping remaining silanol groups.

In one embodiment, in the methods described the steps are performed simultaneously.

20 In another embodiment, the pore structure of the as-prepared high purity chromatographic materials us modified by hydrothermal treatment, which enlarges the openings of the pores as well as the pore diameters, as confirmed by nitrogen (N_2) sorption analysis. The hydrothermal treatment is performed by preparing a slurry containing the as-prepared hybrid 30 material and a solution of a base in water, heating the slurry in an autoclave at an elevated temperature, e.g., 100 to 200°C, for a period of 10 to 30 h. The use of an alkyl amine such as

trimethylamine (TEA) or Tris(hydroxymethyl) methyl amine or the use of sodium hydroxide is advantageous. The thus-treated material is cooled, filtered and washed with water and methanol, then dried at 80°C under reduced pressure for 16 h.

5 In certain embodiments, following hydrothermal treatment, the surfaces of the high purity chromatographic materials are modified with various agents. Such “surface modifiers” include (typically) organic functional groups which impart a certain chromatographic functionality to a chromatographic stationary phase. In certain aspects, when the HPCM is a hybrid material, it possesses both organic groups and silanol groups which may additionally be substituted or derivatized with a surface modifier.

10 The surface of the hydrothermally treated high purity chromatographic materials contains organic groups, which can be derivatized by reacting with a reagent that is reactive towards the materials’ organic group. For example, vinyl groups on the material can be reacted with a variety of olefin reactive reagents such as bromine (Br₂), hydrogen (H₂), free radicals, propagating polymer radical centers, dienes and the like. In another example, hydroxyl groups 15 on the material can be reacted with a variety of alcohol reactive reagents such as isocyanates, carboxylic acids, carboxylic acid chlorides and reactive organosilanes as described below. Reactions of this type are well known in the literature, *see, e.g.*, March, *J. Advanced Organic Chemistry*, 3rd Edition, Wiley, New York, 1985; Odian, G. *The Principles of Polymerization*, 2nd Edition, Wiley, New York, 1981.

20 In addition, the surface of the hydrothermally treated high purity chromatographic materials also contains silanol groups, which can be derivatized by reacting with a reactive organosilane. The surface derivatization of the high purity chromatographic materials is conducted according to standard methods, for example by reaction with octadecyltrichlorosilane or octadecyldimethylchlorosilane in an organic solvent under reflux conditions. An organic 25 solvent such as toluene is typically used for this reaction. An organic base such as pyridine or imidazole is added to the reaction mixture to catalyze the reaction. The product of this reaction is then washed with water, toluene and acetone. This material can be further treated by hydrolysis in a pH modified aqueous organic solution at ambient or elevated temperatures. An organic solvent such as acetone is typically used for this hydrolysis. Modification of pH can be 30 achieved using acid or base modifiers, including trifluoroacetic acid, formic acid, hydrochloric acid, acetic acid, sodium or ammonium formate, sodium, potassium or ammonium acetate, phosphate buffers, ammonium hydroxide, ammonium carbonate, or ammonium bicarbonate. The product of the hydrolysis is then washed with water, toluene and acetone and dried at 80°C to 100°C under reduced pressure for 16 h. The resultant materials can be further reacted with a 35 short-chain silane such as trimethylchlorosilane to endcap the remaining silanol groups, by using a similar procedure described above.

Surface modifiers such as disclosed herein are attached to the base material, *e.g., via* derivatization or coating and later crosslinking, imparting the chemical character of the surface modifier to the base material. In one embodiment, the organic groups of the high purity chromatographic materials react to form an organic covalent bond with a surface modifier. The 5 modifiers can form an organic covalent bond to the materials organic group via a number of mechanisms well known in organic and polymer chemistry including but not limited to nucleophilic, electrophilic, cycloaddition, free-radical, carbene, nitrene and carbocation reactions. Organic covalent bonds are defined to involve the formation of a covalent bond between the common elements of organic chemistry including but not limited to hydrogen, 10 boron, carbon, nitrogen, oxygen, silicon, phosphorus, sulfur and the halogens. In addition, carbon-silicon and carbon-oxygen-silicon bonds are defined as organic covalent bonds, whereas silicon-oxygen-silicon bonds that are not defined as organic covalent bonds.

The term “functionalizing group” includes organic functional groups which impart a certain chromatographic functionality to a chromatographic stationary phase, including, *e.g.,* 15 octadecyl (C₁₈) or phenyl. Such functionalizing groups are incorporated into base material directly, or present in, *e.g.,* surface modifiers such as disclosed herein which are attached to the base material, *e.g.,* via derivatization or coating and later crosslinking, imparting the chemical character of the surface modifier to the base material.

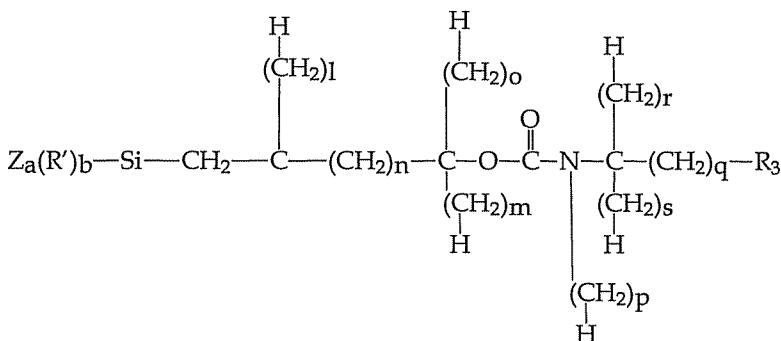
In certain embodiments, silanol groups are surface modified. In other embodiments, 20 organic groups are surface modified. In still other embodiments, the high purity chromatographic materials’ organic groups and silanol groups are both surface modified or derivatized. In another embodiment, the high purity chromatographic materials are surface modified by coating with a polymer. In certain embodiments, surface modification by coating with a polymer is used in conjunction with silanol group modification, organic group 25 modification, or both silanol and organic group modification. The ionizable modifier may be added to the material by silanol group modification, organic group modification, or by both silanol and organic group modification. The hydrophobic surface group may be added to the material by silanol group modification, organic group modification, or by both silanol and organic group modification.

30

More generally, the surface of high purity chromatographic materials may be modified by: treatment with surface modifiers including compounds of formula $Z_a(R')_bSi-R''$, where Z = Cl, Br, I, C₁ - C₅ alkoxy, dialkylamino, *e.g.,* dimethylamino, or trifluoromethanesulfonate; a and b are each an integer from 0 to 3 provided that a + b = 3; R' is a C₁ - C₆ straight, cyclic or 35 branched alkyl group, and R'' is a functionalizing group. In certain instances, such materials have been surface modified by coating with a polymer.

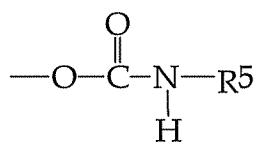
R' includes, *e.g.*, methyl, ethyl, propyl, isopropyl, butyl, t-butyl, sec-butyl, pentyl, isopentyl, hexyl or cyclohexyl; preferably, R' is methyl.

The functionalizing group R" may include alkyl, alkenyl, alkynyl, aryl, cyano, amino, diol, nitro, ester, cation or anion exchange groups, an alkyl or aryl group containing an 5 embedded polar functionalities or chiral moieties. Examples of suitable R" functionalizing groups include chiral moieties, C₁-C₃₀ alkyl, including C₁-C₂₀, such as octyl (C₈), octadecyl (C₁₈) and triacontyl (C₃₀); alkaryl, *e.g.*, C₁-C₄-phenyl; cyanoalkyl groups, *e.g.*, cyanopropyl; diol groups, *e.g.*, propyldiol; amino groups, *e.g.*, aminopropyl; and alkyl or aryl groups with embedded polar functionalities, *e.g.*, carbonate, amide, urea, ether, thioether, sulfinyl, 10 sulfoxide, sulfonyl, thiourea, thiocarbonate, thiocarbamate, ethylene glycol, heterocyclic, and triazole functionalities or carbamate functionalities such as disclosed in U. S. Patent No. 5,374,755, and chiral moieties. In certain embodiments, R" is selected from the group consisting of aromatic, phenylalkyl, fluoroaromatic, phenylhexyl, pentafluorophenylalkyl and chiral moieties. Such groups include those of the general formula



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wherein l, m, o, r and s are 0 or 1, n is 0, 1, 2 or 3 p is 0, 1, 2, 3 or 4 and q is an integer from 0 to 19; R₃ is selected from the group consisting of hydrogen, alkyl, cyano and phenyl; and Z, R', a and b are defined as above. Preferably, the carbamate functionality has the general structure indicated below:



20

wherein R⁵ may be, *e.g.*, cyanoalkyl, t-butyl, butyl, octyl, dodecyl, tetradecyl, octadecyl, or benzyl. Advantageously, R⁵ is octyl, dodecyl, or octadecyl.

In certain applications, such as chiral separations, the inclusion of a chiral moiety as a functionalizing group is particularly advantageous.

25

Polymer coatings are known in the literature and may be provided generally by polymerization or polycondensation of physisorbed monomers onto the surface without chemical bonding of the polymer layer to the support (type I), polymerization or

polycondensation of physisorbed monomers onto the surface with chemical bonding of the polymer layer to the support (type II), immobilization of physisorbed prepolymers to the support (type III) and chemisorption of presynthesized polymers onto the surface of the support (type IV). *see, e.g.,* Hanson, *et al.*, *J. Chromat. A*656 (1993) 369-380, the text of which is

5 incorporated herein by reference. As noted above, coating the hybrid material with a polymer may be used in conjunction with various surface modifications described in the invention.

Thus, in certain embodiments, the hydrophobic surface modifier is selected from the group consisting of phenylhexyltrichlorosilane, pentafluorophenylpropyltrichlorosilane, octyltrichlorosilane, octadecyltrichlorosilane, octyldimethylchlorosilane and

10 octadecyldimethylchlorosilane. In a further embodiment, the surface modifier is selected from the group consisting of octyltrichlorosilane and octadecyltrichlorosilane.

In another embodiment, the high purity chromatographic materials have been surface modified by a combination of organic group and silanol group modification.

15 In other embodiments, the high purity chromatographic materials have been surface modified by a combination of organic group modification and coating with a polymer.

In other embodiments, the high purity chromatographic materials have been surface modified by a combination of silanol group modification and coating with a polymer.

20 In another embodiment, the high purity chromatographic materials have been surface modified via formation of an organic covalent bond between the hybrid cores' and/or surrounding material materials' organic group and the modifying reagent.

In certain embodiments, the high purity chromatographic materials have been surface modified by a combination of organic group modification, silanol group modification and coating with a polymer.

25 In one embodiment, the high purity chromatographic materials have been surface modified by silanol group modification.

30 In another embodiment, the invention provides a method wherein the high purity chromatographic materials are modified by further including a porogen. In a further embodiment, the porogen is selected from the group consisting of cyclohexanol, toluene, mesitylene, 2-ethylhexanoic acid, dibutylphthalate, 1-methyl-2-pyrrolidinone, 1-dodecanol and Triton X-45. In certain embodiments, the porogen is toluene or mesitylene.

35 In one embodiment, the invention provides a method wherein the high purity chromatographic materials are further modified by including a surfactant or stabilizer. In certain embodiments, the surfactant is Triton X-45, Triton X100, Triton X305, TLS, Pluronic F-87, Pluronic P-105, Pluronic P-123, sodium dodecylsulfate (SDS), ammonia docecydsulfate, TRIS docecydsulfate, or Triton X-165. In certain embodiments, the surfactant is sodium dodecylsulfate (SDS), ammonia docecydsulfate, or TRIS docecydsulfate.

Certain embodiments of the synthesis of the HPCMs of the invention including hybrids, silica, particles, monoliths and superficially porous materials, are described above and further illustrated in the Examples below.

5 Examples

The present invention may be further illustrated by the following non-limiting examples describing the surface modification of porous chromatographic materials.

Materials

10 All reagents were used as received unless otherwise noted. Those skilled in the art will recognize that equivalents of the following supplies and suppliers exist and, as such, the suppliers listed below are not to be construed as limiting.

Characterization

15 Those skilled in the art will recognize that equivalents of the following instruments and suppliers exist and, as such, the instruments listed below are not to be construed as limiting.

The %C, %H, %N values were measured by combustion analysis (CE-440 Elemental Analyzer; Exeter Analytical Inc., North Chelmsford, MA) or %C by Coulometric Carbon Analyzer (modules CM5300, CM5014, UIC Inc., Joliet, IL). The specific surface areas (SSA), 20 specific pore volumes (SPV) and the average pore diameters (APD) of these materials were measured using the multi-point N₂ sorption method (Micromeritics ASAP 2400; Micromeritics Instruments Inc., Norcross, GA). The SSA was calculated using the BET method, the SPV was the single point value determined for P/P₀ > 0.98 and the APD was calculated from the desorption leg of the isotherm using the BJH method. Scanning electron microscopic (SEM) 25 image analyses were performed (JEOL JSM-5600 instrument, Tokyo, Japan) at 7 kV. Particle sizes were measured using a Beckman Coulter Multisizer 3 analyzer (30 µm aperture, 70,000 counts; Miami, FL). The particle diameter (dp) was measured as the 50% cumulative diameter of the volume based particle size distribution. The width of the distribution was measured as the 90% cumulative volume diameter divided by the 10% cumulative volume diameter (denoted 30 90/10 ratio). Multinuclear (¹³C, ²⁹Si) CP-MAS NMR spectra were obtained using a Bruker Instruments Avance-300 spectrometer (7 mm double broadband probe). The spinning speed was typically 5.0-6.5 kHz, recycle delay was 5 sec. and the cross-polarization contact time was 6 msec. Reported ¹³C and ²⁹Si CP-MAS NMR spectral shifts were recorded relative to tetramethylsilane using the external standards adamantane (¹³C CP-MAS NMR, δ 38.55) and 35 hexamethylcyclotrisiloxane (²⁹Si CP-MAS NMR, δ -9.62). Populations of different silicon environments were evaluated by spectral deconvolution using DMFit software. [Massiot, D.;

Fayon, F.; Capron, M.; King, I.; Le Calvé, S.; Alonso, B.; Durand, J.-O.; Bujoli, B.; Gan, Z.; Hoatson, G. *Magn. Reson. Chem.* 2002, 40, 70-76] Titrations were performed using a Metrohm 716 DMS Titrino autotitrator with 6.0232.100 pH electrode (Metrohm, Hersau, Switzerland, or equivalent).

5

EXAMPLE 1

BEH porous hybrid particles (15 g, Waters Corporation, Milford, MA; 6.5 %C; SSA = 186 m²/g; SPV = 0.79 cm³/g; APD = 151 Å) of the formula (O_{1.5}SiCH₂CH₂SiO_{1.5})(SiO₂)₄ (prepared following the method described in U.S. Patent 6,686,035) were refluxed in toluene 10 (100 mL, Fisher Scientific, Fairlawn, NJ) using a Dean-Stark trap for 1 hour. Reaction 1a used 7.2 g BEH material. Upon cooling the Component A silane additive was added, which included aminopropyltriethoxysilane (APTES, Gelest Inc., Morrisville, PA), 2-(2-(trichlorosilyl)ethyl)pyridine (2PE, Gelest Inc., Morrisville, PA), 2-(4-pyridylethyl)triethoxysilane (4PE, Gelest Inc., Morrisville, PA), N-trimethoxysilylpropyl-15 N,N,N-trimethylammonium chloride (QPTMS, 50% solution in methanol, Gelest Inc., Morrisville, PA) or chloropropyltrimethoxysilane (CPTMS, Gelest Inc., Morrisville, PA). The reaction was heated to reflux for 1 hour. Upon cooling, imidazole (Aldrich, Milwaukee, WI) and octadecyldimethylchlorosilane (Component B, ODMCS, Aldrich or Gelest) were added. The reaction was then heated to reflux for 3 hours. For reactions 1j and 1k, 200 mL of toluene 20 was used, and imidazole was added at the same time as the CPTMS. The reaction was then cooled and the product was filtered and washed successively with toluene, 1:1 v/v acetone/water and acetone (all solvents from Fisher Scientific). The product was then dried at 80 °C under reduced pressure for 16 hours. Reaction data is listed in Table 1. Product 1a was a control experiment that did not employ the use of a Component A silane additive. For products 1b-11 25 the Component A silane additive charges ranged between 0.03-10.6 μmol/m² and the charge molar ratio of Component B to A ranged from 0.19-66.6. Products 1k and 1l introduced a chloropropyl silane group to the particle which is known to react with imidazole to obtain an imidazole propyl group [A. M. Lazarin, Y. Gushikem and S. C. deCastro, *J. Mater. Chem.*, 2000, 10, 2526; B. Gadenne, P. Hesemann, J. J. E. Moreau *Chem. Commun.*, 2004, 1768]. The 30 reaction between the chloropropyl groups with imidazole was confirmed using ¹³C CP-MAS NMR spectroscopy.

The surface coverage of Component A silane additives was determined by the difference in particle %N after surface modification as measured by elemental analysis. As shown in Table 1, unbonded BEH particles as well as products 1a-1c did not have determinable 35 nitrogen content by this measurement. ND stands for none determined. The surface coverage of C₁₈-groups was determined by the difference in particle %C before and after the surface

modification as measured by elemental analysis. Surface coverage of C₁₈-groups could be corrected by factoring out carbon content due to Component A silane additive by assuming complete condensation of the silane additive (correction method I), or by using the value obtained from the Component A silane additive coverage calculation (correction method II).

5 For products 1b-1j the correction in C₁₈ coverage may be overestimated, but is still quite small (less than 0.11 $\mu\text{mol}/\text{m}^2$).

TABLE 1

| Product | dp (μm) | Component A | | | Component B | | | Silane Additive Coverage ($\mu\text{mol}/\text{m}^2$) | | | Corrected C ₁₈ Coverage ($\mu\text{mol}/\text{m}^2$) (□%C) | |
|---------|-------------------------|---|------------------------|---------------------|------------------|---------------------------|-------|--|----------|---|---|--|
| | | Silane Additive ($\mu\text{mol}/\text{m}^2$) | Silane Additive (g) | ODMCS Silane (g) | Imidazole (g) | Charge Molar Ratio B/A | %C | %N | (%N) | ($\mu\text{mol}/\text{m}^2$) ($\Delta\%$ C) | ($\mu\text{mol}/\text{m}^2$) ($\Delta\%$ C) | |
| 1a | 3.5 | --- | --- | 0.96 | 0.37 | --- | 13.02 | ND | --- | 1.71 | --- | |
| 1b | 3.4 | APTES | 0.03 | 0.019 | 1.99 | 0.78 | 66.6 | 13.34 | ND | 1.80 (I) | 1.80 (I) | |
| 1c | 3.4 | APTES | 0.06 | 0.036 | 1.92 | 0.75 | 33.3 | 13.54 | ND | 1.93 | 1.92 (I) | |
| 1d | 3.4 | APTES | 0.30 | 0.190 | 1.99 | 0.78 | 6.66 | 13.27 | 0.1 5 | 0.20 | 1.78 1.75 (I) | |
| 1e | 3.4 | APTES | 0.60 | 0.380 | 1.99 | 0.78 | 3.33 | 11.83 | 0.2 0 | 0.27 | 1.37 1.30 (I) | |
| 1f | 3.4 | 2PE | 0.30 | 0.199 | 1.92 | 0.75 | 6.66 | 13.87 | 0.1 3 | 0.26 | 2.03 1.93 (I) | |
| 1g | 4.8 | 4PE | 0.06 | 0.046 | 1.89 | 0.78 | 33.3 | 13.38 | 0.0 9 | 0.17 | 1.68 1.66 (I) | |
| 1h | 3.4 | 4PE | 0.30 | 0.223 | 1.92 | 0.75 | 6.66 | 13.48 | 0.1 4 | 0.28 | 1.91 1.81 (I) | |
| 1i | 4.8 | QPTMS | 0.06 | 0.089 | 1.89 | 0.78 | 33.3 | 12.97 | 0.1 0 | 0.23 | 1.56 1.54 (I) | |
| 1j | 3.4 | QPTMS | 0.30 | 0.427 | 1.92 | 0.75 | 6.66 | 13.17 | 0.1 3 | 0.31 | 1.81 1.74 (I) | |
| 1k | 3.4 | CPTMS | 1.20 | 0.658 | 1.92 | 3.76 | 1.66 | 12.66 | 0.5 8 | 0.74 | 1.66 1.49 (II) | |
| 1l | 3.4 | CPTMS | 10.6 | 5.840 | 1.92 | 3.76 | 0.19 | 10.15 | 1.4 5 | 1.99 | 0.95 0.45 (II) | |

EXAMPLE 2

Materials from Example 1 were modified with trimethylchlorosilane (TMCS, Gelest Inc., Morrisville, PA) using imidazole (Aldrich, Milwaukee, WI) in refluxing toluene (100 mL) for 4 hours. The reaction was then cooled and the product was filtered and washed successively 5 with water, toluene, 1:1 v/v acetone/water and acetone (all solvents from J.T. Baker) and then dried at 80 °C under reduced pressure for 16 hours. Reaction data are listed in Table 2.

TABLE 2

| Product | Precursor | Particles (g) | TMCS (g) | Imidazole (g) | %C |
|---------|-----------|---------------|----------|---------------|-------|
| 2a | 1a | 7.2 | 1.49 | 1.12 | 13.96 |
| 2b | 1b | 15.9 | 3.27 | 2.48 | 14.22 |
| 2c | 1c | 15.0 | 3.00 | 2.25 | 14.33 |
| 2d | 1d | 15.0 | 3.11 | 2.34 | 13.97 |
| 2e | 1e | 15.6 | 3.30 | 2.44 | 12.93 |
| 2f | 1f | 15.0 | 3.00 | 2.25 | 14.57 |
| 2g | 1g | 15.0 | 3.11 | 2.34 | 14.19 |
| 2h | 1h | 15.0 | 3.00 | 2.25 | 14.19 |
| 2i | 1i | 15.0 | 3.11 | 2.34 | 13.80 |
| 2j | 1j | 15.0 | 3.00 | 2.25 | 13.83 |
| 2k | 1k | 15.0 | 2.99 | 2.25 | 13.12 |
| 2l | 1l | 15.0 | 2.99 | 2.25 | 11.00 |

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EXAMPLE 3

BEH porous hybrid particles (Waters Corporation, Milford, MA; 6.5%C; SSA = 182-185 m²/g; SPV = 0.72-0.76 cm³/g; APD = 142-151 Å) of the formula (O_{1.5}SiCH₂CH₂SiO_{1.5})(SiO₂)₄ (prepared following the method described in U.S. Patent 6,686,035) were refluxed in toluene (5 mL/g, Fisher Scientific, Fairlawn, NJ) using a Dean-Stark trap for 1 hour. Upon cooling the Component A silane additive was added, which included aminopropyltriethoxysilane (APTES, Gelest Inc., Morrisville, PA) 2-(4-pyridylethyl)triethoxysilane (4PE, Gelest Inc., Morrisville, PA), or diethylphosphatoethyltriethoxysilane (DEPS, Gelest Inc. Morrisville, PA) or 2-(4-chlorosulfonylphenyl)ethyltrichlorosilane (SPETCS, 50% in toluene, Gelest Inc., Morrisville PA). The reaction was heated to reflux for 1 hour. Upon cooling, imidazole (Aldrich, Milwaukee, WI) and octadecyltrichlorosilane (Component B, ODTCS, Aldrich, Milwaukee, WI) were added. The reaction was then heated to reflux for 16 hours. Product 3c was reacted for 3 hours. Products 3af-3aj did not add a component B.

25 The reaction was then cooled and the product was filtered and was washed successively with toluene, 1:1 v/v acetone/water, and acetone (all solvents from J.T. Baker). The material was then refluxed in a acetone / aqueous 0.12 M ammonium acetate solution (Sigma Chemical

Co., St. Louis, MO) for 2 hours (hydrolysis-type A), acetone/aqueous 0.1 M ammonium bicarbonate (pH 8) solution for 20 hours at 50°C (hydrolysis-type B), or acetone/aqueous 0.1 M ammonium bicarbonate (pH 10) solution for 20 hours at 50°C (hydrolysis-type C). The reaction was then cooled and the product was filtered and washed successively with toluene, 1:1 v/v 5 acetone/water, and acetone (all solvents from J.T. Baker). The product was then dried at 80 °C under reduced pressure for 16 hours. Reaction data is listed in Table 3. The silane additive (Component A) charges ranged from 0.03-3.70 μ mol/m² and the molar ratio of charge molar ratio of Component B to A ranged from 4.3-133.4.

The surface coverage of C₁₈-groups was determined by the difference in particle %C 10 before and after the surface modification as measured by elemental analysis. Correction for coverage of C₁₈-groups, obtained by factoring out carbon content due to silane additive by assuming complete condensation of the silane additive, were small for this dataset (less than 0.15 μ mol/m²) and were not included in Table 3. Product 3aj had an ion-exchange capacity of 0.22 mequiv/g by titration.

TABLE 3

| Product | dp (μm) | Particles (g) | Component A | | | Component B | | | Charge Molar Ratio B/A | Hydrolysis Type | %C | C_{18} Coverage ($\mu\text{mol}/\text{m}^2$) |
|---------|-------------------------|------------------|--|--------------------|---------------------------|---|------------------------|------------------|---------------------------------|--------------------|-------|---|
| | | | Silane Additive ($\mu\text{mol}/\text{m}^2$) | Silane Additive | Silane Additive (g) | ODTCS Charge ($\mu\text{mol}/\text{m}^2$) | ODTCS Silane (g) | Imidazole (g) | | | | |
| 3a | 2.9 | 15 | APTES | 0.03 | 0.018 | 3.99 | 4.30 | 1.51 | 133.4 | A | 16.53 | 3.19 |
| 3b | 2.9 | 15 | APTES | 0.06 | 0.037 | 2.00 | 2.15 | 0.76 | 33.2 | A | 12.80 | 1.84 |
| 3c | 2.9 | 50 | APTES | 0.06 | 0.124 | 4.00 | 14.43 | 5.06 | 66.7 | A | 16.33 | 3.13 |
| 3d | 1.8 | 20 | APTES | 0.06 | 0.050 | 4.06 | 5.74 | 2.02 | 65.3 | A | 16.24 | 3.26 |
| 3e | 2.9 | 30 | APTES | 0.12 | 0.147 | 4.00 | 8.61 | 3.03 | 33.3 | A | 15.92 | 2.96 |
| 3f | 2.9 | 30 | APTES | 0.20 | 0.246 | 4.00 | 8.61 | 3.03 | 20.0 | A | 16.10 | 3.03 |
| 3g | 2.9 | 15 | 4PE | 0.06 | 0.045 | 1.99 | 2.14 | 0.76 | 33.0 | A | 13.13 | 2.05 |
| 3h | 2.9 | 15 | 4PE | 0.06 | 0.045 | 3.99 | 4.30 | 1.51 | 66.4 | A | 16.70 | 3.36 |
| 3i | 2.9 | 15 | 4PE | 0.30 | 0.224 | 1.99 | 2.14 | 0.76 | 6.6 | A | 13.33 | 2.12 |
| 3j | 3.9 | 20 | 4PE | 0.30 | 0.294 | 2.00 | 2.82 | 0.99 | 6.7 | A | 13.22 | 2.05 |
| 3k | 2.9 | 15 | 4PE | 0.30 | 0.224 | 3.99 | 4.30 | 1.51 | 13.3 | A | 16.64 | 3.33 |
| 3l | 3.9 | 15 | 4PE | 0.31 | 0.230 | 2.00 | 2.12 | 0.74 | 6.4 | A | 13.44 | 2.13 |
| 3m | 3.9 | 15 | 4PE | 0.31 | 0.230 | 2.00 | 2.12 | 0.74 | 6.4 | B | 13.12 | 2.02 |
| 3n | 3.9 | 20 | 4PE | 0.20 | 0.196 | 1.72 | 2.43 | 0.85 | 8.6 | B | 12.06 | 1.65 |
| 3o | 3.9 | 20 | 4PE | 0.40 | 0.392 | 2.28 | 3.22 | 1.13 | 5.7 | B | 13.84 | 2.27 |
| 3p | 3.9 | 20 | 4PE | 0.40 | 0.392 | 1.72 | 2.43 | 0.85 | 4.3 | C | 12.40 | 1.77 |
| 3q | 3.9 | 20 | 4PE | 0.20 | 0.196 | 2.28 | 3.22 | 1.13 | 11.4 | C | 13.68 | 2.21 |
| 3r | 3.5 | 20 | 4PE | 0.40 | 0.394 | 2.30 | 3.27 | 1.15 | 5.8 | B | 14.04 | 2.4 |
| 3s | 3.5 | 20 | 4PE | 0.40 | 0.394 | 2.30 | 3.27 | 1.15 | 5.8 | C | 14.16 | 2.44 |
| 3t | 1.8 | 20 | 4PE | 0.30 | 0.297 | 2.00 | 2.86 | 1.00 | 5.0 | B | 13.28 | 2.01 |
| 3u | 3.5 | 20 | 4PE | 0.35 | 0.346 | 2.53 | 3.59 | 1.26 | 7.2 | C | 14.41 | 2.46 |
| 3v | 3.5 | 20 | 4PE | 0.35 | 0.346 | 2.07 | 2.94 | 1.03 | 5.9 | C | 13.40 | 2.10 |
| 3w | 3.5 | 20 | 4PE | 0.25 | 0.246 | 2.53 | 3.59 | 1.26 | 10.1 | C | 14.59 | 2.52 |
| 3x | 3.5 | 20 | 4PE | 0.25 | 0.246 | 2.07 | 2.94 | 1.03 | 8.3 | C | 13.28 | 2.06 |
| 3y | 3.5 | 20 | 4PE | 0.20 | 0.197 | 2.70 | 3.83 | 1.35 | 13.5 | B | 14.88 | 2.71 |

| | | | | | | | | | | | | |
|-----|-----|----|--------|------|-------|------|------|------|------|---|-------|------|
| 3z | 3.5 | 20 | 4PE | 0.40 | 0.394 | 2.70 | 3.83 | 1.35 | 6.8 | B | 14.81 | 2.68 |
| 3aa | 3.5 | 20 | 4PE | 0.20 | 0.197 | 2.70 | 3.83 | 1.35 | 13.5 | C | 14.71 | 2.65 |
| 3ab | 3.5 | 20 | 4PE | 0.40 | 0.394 | 2.70 | 3.83 | 1.35 | 6.8 | C | 14.90 | 2.71 |
| 3ac | 1.8 | 40 | 4PE | 0.30 | 0.595 | 2.30 | 6.57 | 2.30 | 7.7 | C | 14.01 | 2.27 |
| 3ad | 3.5 | 40 | 4PE | 0.30 | 0.592 | 2.30 | 6.53 | 2.29 | 7.7 | C | 13.97 | 2.38 |
| 3ae | 4.9 | 22 | 4PE | 0.30 | 0.316 | 2.30 | 3.49 | 1.23 | 7.7 | C | 13.90 | 2.28 |
| 3af | 3.9 | 15 | 4PE | 0.03 | 0.022 | --- | --- | --- | --- | C | 6.46 | --- |
| 3ag | 3.9 | 15 | 4PE | 0.06 | 0.044 | --- | --- | --- | --- | C | 6.38 | --- |
| 3ah | 3.9 | 15 | 4PE | 3.70 | 2.700 | --- | --- | --- | --- | C | 7.80 | --- |
| 3ai | 4.0 | 30 | DEPS | 3.00 | 5.400 | --- | --- | --- | --- | C | 7.62 | --- |
| 3aj | 4.0 | 40 | SPETCS | 1.00 | 4.90 | --- | --- | 5.00 | --- | C | 7.94 | --- |

EXAMPLE 4

Materials from Example 3 were modified with triethylchlorosilane (TECS, Gelest Inc., Morrisville, PA) or tert-butyldimethylchlorosilane (TBDMCS, Gelest Inc., Morrisville, PA) using imidazole (Aldrich, Milwaukee, WI) in refluxing toluene (5 mL/g) for 4-20 hours. The reaction was cooled and the product was filtered and washed successively with water, toluene, 1:1 v/v acetone/water and acetone (all solvents from J.T. Baker) and then dried at 80 °C under reduced pressure for 16 hours. Reactions 4a-4g and 4m-4ab were reacted for 4 hours, reactions 4h-4l were reacted for 20 hours. Additional trimethylchlorosilane (TMCS, Gelest Inc., Morrisville, PA) and imidazole was added to reactions 4m-4ab and the reaction was heated for an additional 16 hours. Selected products were further reacted with TMCS (reaction 4k) or hexamethyldisilazane (reaction 4c, Gelest Inc., Morrisville, PA) in a similar process. Reaction data are listed in Table 4.

TABLE 4

| Product | Precursor | Particle s (g) | Silane | Silane (g) | Imidazole (g) | %C |
|---------|-----------|----------------------|--------|---------------|------------------|-------|
| 4a | 3a | 15 | TECS | 4.18 | 2.27 | 17.32 |
| 4b | 3b | 15 | TECS | 4.18 | 2.27 | 14.53 |
| 4c | 3c | 50 | TECS | 13.95 | 7.75 | 17.58 |
| 4d | 3d | 10 | TECS | 2.79 | 1.51 | 17.27 |
| 4e | 3d | 10 | TBDMCS | 2.79 | 1.51 | 16.99 |
| 4f | 3e | 32 | TECS | 9.00 | 4.85 | 16.96 |
| 4g | 3f | 32 | TECS | 8.4.0 | 4.55 | 17.10 |
| 4h | 3g | 15 | TBDMCS | 4.18 | 2.27 | 14.54 |
| 4i | 3h | 15 | TBDMCS | 4.18 | 2.27 | 17.44 |
| 4j | 3i | 15 | TBDMCS | 4.18 | 2.27 | 14.62 |
| 4k | 3j | 20 | TBDMCS | 5.48 | 2.97 | 14.61 |
| 4l | 3k | 15 | TBDMCS | 4.18 | 2.27 | 17.30 |
| 4m | 3l | 15 | TECS | 2.06 | 1.12 | 15.14 |
| 4n | 3m | 15 | TECS | 2.06 | 1.12 | 14.82 |
| 4o | 3n | 20 | TECS | 2.74 | 1.49 | 14.28 |
| 4p | 3o | 20 | TECS | 2.74 | 1.49 | 15.43 |
| 4q | 3p | 20 | TECS | 2.74 | 1.49 | 15.26 |
| 4r | 3q | 20 | TECS | 2.74 | 1.49 | 15.36 |
| 4s | 3r | 20 | TECS | 2.74 | 1.49 | 15.61 |
| 4t | 3s | 20 | TECS | 2.74 | 1.49 | 15.67 |
| 4u | 3t | 20 | TECS | 2.74 | 1.49 | 14.96 |
| 4v | 3u | 20 | TECS | 2.38 | 1.29 | 15.92 |
| 4w | 3v | 20 | TECS | 2.24 | 1.22 | 14.99 |
| 4x | 3w | 20 | TECS | 2.21 | 1.20 | 15.97 |
| 4y | 3x | 20 | TECS | 2.25 | 1.22 | 14.97 |
| 4z | 3ac | 20 | TECS | 2.78 | 1.50 | 15.71 |
| 4aa | 3ad | 10 | TECS | 2.76 | 1.50 | 15.52 |
| 4ab | 3ae | 22 | TECS | 2.70 | 1.47 | 15.51 |
| 4ac | 3ad | 10 | TBDMCS | 3.40 | 3.00 | 15.36 |

EXAMPLE 5

BEH porous hybrid particles (Waters Corporation, Milford, MA; 3.9 μm , 6.68 %C; SSA = 182 m^2/g ; SPV = 0.75 cm^3/g ; APD = 148 \AA) of the formula $(\text{O}_{1.5}\text{SiCH}_2\text{CH}_2\text{SiO}_{1.5})(\text{SiO}_2)_4$ (prepared following the method described in U.S. Patent 6,686,035) were refluxed in toluene (5 mL/g, Fisher Scientific, Fairlawn, NJ) using a Dean-Stark trap for 1 hour. Upon cooling the Component A silane additive was added, which included aminopropyltriethoxysilane (APTES, Gelest Inc., Morrisville, PA), 2-(4-pyridylethyl)triethoxysilane (4PE, Gelest Inc., Morrisville, PA), or 2-(carbomethoxy)ethyltrichlorosilane (CMETCS, Gelest Inc., Morrisville, PA). The reaction was heated to reflux for 1 hour. For reactions 5f and 5g a mixture of APTES and 10 CMETCS were used. Upon cooling, imidazole (Aldrich, Milwaukee, WI) or diisopropyl ethylamine (DIPEA, Aldrich, Milwaukee, WI) and the Component B silane was added, which included phenylhexyltrichlorosilane (PTCS), octyltrichlorosilane (OTCS, Aldrich, Milwaukee, WI), pentafluorophenylpropyltrichlorosilane (PFPPTCS), or octadecyldimethylchlorosilane (ODMCS, Aldrich, Milwaukee, WI). Products 5a-5h used imidazole. Products 5i-5t used 15 DIPEA. The reaction was then heated to reflux for 16 hours.

The reaction was cooled and the product was filtered and was washed successively with toluene, 1:1 v/v acetone/water, and acetone (all solvents from J.T. Baker). The material was then hydrolyzed as detailed in Example 3. Products 5a-5h used hydrolysis type A. Products 5i-5u used hydrolysis type C. The reaction was then cooled and the product was filtered and 20 washed successively with toluene, 1:1 v/v acetone/water, and acetone (all solvents from J.T. Baker). The product was then dried at 70 $^{\circ}\text{C}$ under reduced pressure for 16 hours. Reaction data is listed in Table 5. The Component A silane additive charges ranged from 0.03-0.35 $\mu\text{mol}/\text{m}^2$ and the charge molar ratio of Component B to A ranged from 6.5-133.3. The surface coverage was determined by the difference in particle %C before and after the surface 25 modification as measured by elemental analysis.

TABLE 5

| Product | dp (μm) | Particles (g) | Component A | | | Component B | | | Charge Molar Ratio B/A | %C | Surface Coverage ($\mu\text{mol}/\text{m}^2$) | |
|---------|-------------------------|------------------|--|--------------------|--|---|-------------------|--------------------------|---------------------------------|-------|---|------|
| | | | Silane Additive ($\mu\text{mol}/\text{m}^2$) | Silane Additive | Silane Additive ($\mu\text{mol}/\text{m}^2$) | Primary Silane Charge ($\mu\text{mol}/\text{m}^2$) | Primary Silane | Primary Silane (g) | | | | |
| 5a | 3.9 | 15 | APTES | 0.03 | 0.018 | PTCS | 2 | 1.16 | 0.74 | 66.7 | 9.75 | 1.22 |
| 5b | 3.9 | 15 | APTES | 0.03 | 0.018 | PTCS | 4 | 2.33 | 1.49 | 133.3 | 12.27 | 2.32 |
| 5c | 3.9 | 15 | APTES | 0.06 | 0.036 | PTCS | 2 | 1.16 | 0.74 | 33.3 | 10.09 | 1.37 |
| 5d | 3.9 | 15 | APTES | 0.06 | 0.036 | PTCS | 4 | 2.33 | 1.49 | 66.7 | 12.19 | 2.28 |
| 5e | 3.9 | 15 | 4PE | 0.31 | 0.22 | OTCS | 2 | 1.35 | 0.74 | 6.5 | 9.435 | 1.88 |
| 5f | 3.9 | 15 | APTES; CMETCS | 0.06, 0.06 | 0.036, 0.036 | ODTCS | 4 | 4.24 | 1.49 | 66.7 | 15.94 | 2.72 |
| 5g | 3.9 | 15 | APTES; CMETCS | 0.03, 0.03 | 0.018, 0.018 | ODTCS | 4 | 4.24 | 1.49 | 133.3 | 16.02 | 2.74 |
| 5h | 3.9 | 15 | CMETCS | 0.06 | 0.036 | ODTCS | 4 | 4.24 | 1.49 | 66.7 | 15.86 | 2.69 |
| 5i | 1.8 | 41 | 4PE | 0.30 | 0.60 | PFPPTCS | 2.30 | 5.90 | 4.40 | 7.7 | 10.30 | 2.34 |
| 5j | 1.8 | 40 | 4PE | 0.35 | 0.68 | PFPPTCS | 2.29 | 5.60 | 4.20 | 6.5 | 9.93 | 2.39 |
| 5k | 3.5 | 40 | 4PE | 0.30 | 0.59 | PFPPTCS | 3.00 | 7.55 | 5.68 | 10.0 | 10.61 | 2.65 |
| 5l | 3.5 | 40 | 4PE | 0.30 | 0.59 | PFPPTCS | 2.30 | 5.78 | 4.35 | 7.7 | 10.06 | 2.24 |
| 5m | 4.9 | 70 | 4PE | 0.30 | 1.04 | PFPPTCS | 2.29 | 10.10 | 7.60 | 7.7 | 10.46 | 2.61 |
| 5n | 3.5 | 40 | 4PE | 0.30 | 0.59 | PTCS | 2.30 | 4.98 | 4.35 | 7.7 | 11.43 | 2.15 |
| 5o | 1.8 | 40 | 4PE | 0.30 | 0.57 | PTCS | 2.31 | 4.80 | 4.20 | 7.7 | 11.18 | 2.16 |
| 5p | 3.0 | 16 | 4PE | 0.30 | 0.24 | PTCS | 2.01 | 1.79 | 1.79 | 6.7 | 12.26 | 2.66 |
| 5q | 4.5 | 70 | 4PE | 0.30 | 1.04 | PTCS | 2.30 | 8.70 | 7.60 | 7.7 | 11.11 | 2.05 |
| 5r | 4.5 | 70 | 4PE | 0.30 | 1.04 | PTCS | 2.30 | 8.70 | 7.60 | 7.7 | 11.41 | 2.19 |
| 5s | 3.5 | 350 | 4PE | 0.30 | 5.40 | PTCS | 2.30 | 45.50 | 39.70 | 7.7 | 12.16 | 2.43 |
| 5t | 3.5 | 80 | 4PE | 0.30 | 1.25 | PTCS | 2.31 | 10.60 | 9.20 | 7.7 | 11.81 | 2.30 |
| 5u | 3.5 | 40 | 4PE | 0.30 | 0.592 | PTCS | 2.31 | 5.00 | 2.3 | 7.7 | 11.89 | 2.39 |

EXAMPLE 6

Material from Example 5 was modified with triethylchlorosilane (TECS, Gelest Inc., Morrisville, PA) or tert-butyldimethylchlorosilane (TBDMCS, Gelest Inc., Morrisville, PA) using imidazole (Aldrich, Milwaukee, WI) in refluxing toluene (5 mL/g) for 17-20 hours.

5 Additional TMCS and imidazole was added to reactions 6e, 6i-6o, and 6q after 4 hours and the reaction was heated for an additional 16 hours. For reactions 6i and 6j diisopropyl ethylamine (DIPEA, Aldrich, Milwaukee, WI) was used in place of imidazole. The reaction was then cooled and the product was filtered and washed successively with water, toluene, 1:1 v/v acetone/water and acetone (all solvents from J.T. Baker) and then dried at 70 °C under reduced

10 pressure for 16 hours. Samples of product 6p were further hydrolyzed in an aqueous acetonitrile solution or hydrolysis C in Example 3. No noticeable change in carbon content was observed. Reaction data are listed in Table 6.

TABLE 6

| Product | Precursor | Particles (g) | Silane | Silane (g) | Base (g) | %C |
|---------|-----------|---------------|--------|------------|----------|-------|
| 6a | 5a | 15 | TBDMCS | 4.11 | 2.23 | 11.36 |
| 6b | 5b | 15 | TBDMCS | 4.11 | 2.23 | 13.44 |
| 6c | 5c | 15 | TBDMCS | 4.11 | 2.23 | 11.71 |
| 6d | 5d | 15 | TBDMCS | 4.11 | 2.23 | 13.33 |
| 6e | 5e | 15 | TECS | 2.06 | 1.12 | 11.56 |
| 6f | 5f | 15 | TBDMCS | 4.11 | 2.23 | 16.66 |
| 6g | 5g | 15 | TBDMCS | 4.11 | 2.23 | 16.76 |
| 6h | 5h | 15 | TBDMCS | 4.11 | 2.23 | 16.89 |
| 6i | 5l | 20 | TECS | 2.57 | 2.64 | 11.55 |
| 6j | 5n | 19 | TECS | 2.59 | 2.66 | 12.90 |
| 6k | 5o | 40 | TECS | 5.31 | 2.88 | 13.60 |
| 6l | 5p | 10 | TECS | 1.45 | 0.79 | 13.91 |
| 6m | 5q | 67 | TECS | 9.20 | 5.00 | 13.75 |
| 6n | 5r | 70 | TECS | 9.20 | 5.00 | 13.06 |
| 6o | 5s | 20 | TECS | 2.92 | 1.58 | 13.80 |
| 6p | 5s | 40 | TBDMCS | 9.21 | 4.99 | 13.32 |
| 6q | 5u | 36 | TECS | 5.00 | 2.70 | 13.43 |

15

EXAMPLE 7

BEH porous hybrid particles (15 g, 1.7 µm, Waters Corporation, Milford, MA; 6.5%; SSA = 92 m²/g; SPV = 0.73 cm³/g; APD = 311 Å) of the formula (O_{1.5}SiCH₂CH₂SiO_{1.5})(SiO₂)₄ prepared following the method described in U.S. Patent 6,686,035) were refluxed in toluene (100 mL, Fisher Scientific, Fairlawn, NJ) using a Dean-Stark trap for 2 hours. Upon cooling the Component A silane additive aminopropyltriethoxysilane (0.018 g, 0.06 µmol/m² charge, Gelest Inc., Morrisville, PA) was added and the reaction was heated to reflux for 1 hour. Upon

cooling, imidazole (5.06 g, Aldrich, Milwaukee, WI) and the Component B silane tert-butyldimethylchlorosilane (2.08 g, Gelest Inc., Morrisville, PA) were added. The reaction was then heated to reflux for 20 hours. The reaction was then cooled and the product was filtered and washed successively with water, toluene, 1:1 v/v acetone/water and acetone (all solvents from J.T. Baker) and then dried at 80 °C under reduced pressure for 16 hours. The surface coverage of product 7a, determined by the difference in particle %C before and after the surface modification (7.88 %C) as measured by elemental analysis, was determined to be 2.50 μmol/m².

EXAMPLE 8

10 Porous silica particles (Waters Corporation, Milford, MA; 3.5 μm; SSA = 251 m²/g; SPV = 0.80 cm³/g; APD = 119 Å) were refluxed in toluene (5 mL per gram of silica, Fisher Scientific, Fairlawn, NJ) using a Dean-Stark trap for 1 hour. Upon cooling the Component A silane additive was added, which included aminopropyltriethoxysilane (APTES, Gelest Inc., Morrisville, PA) or 2-(4-pyridylethyl)triethoxysilane (4PE, Gelest Inc., Morrisville, PA).

15 Product 8a used APTES. Products 8b-d used 4PE. The reaction was heated to reflux for 1 hour. Upon cooling, imidazole (Aldrich, Milwaukee, WI) or diisopropyl ethylamine (DIPEA, Aldrich, Milwaukee, WI) and the Component B silane was added, which included phenylhexyltrichlorosilane (PTCS), pentafluorophenylpropyltrichlorosilane (PFPPTCS), or octadecyldimethylchlorosilane (ODMCS, Aldrich, Milwaukee, WI). Products 8a and 8b used

20 Imidazole. Products 8c and 8d used DIPEA. The reaction was then heated to reflux for 20 hours. The reaction was then cooled and the product was filtered and was washed successively with toluene, 1:1 v/v acetone/water, and acetone (all solvents from J.T. Baker). The material was then refluxed in an acetone / aqueous 0.1 M ammonium acetate solution (Sigma Chemical Co., St. Louis, MO) for 3.5 hours. Products 8b, 8c and 8d were heated at 50 °C for 20 hours.

25 The reaction was then cooled and the product was filtered and washed successively with toluene, 1:1 v/v acetone/water, and acetone (all solvents from J.T. Baker). The product was then dried at 80 °C under reduced pressure for 16 hours. The surface coverage of the product, determined by the difference in particle %C before and after the surface modification as measured by elemental analysis. Products 8a, was further reacted in a similar manner as

30 described for product 4c, to yield products 8e. Products 8b and 8d were further reacted in a similar manner as described for product 4m, to yield products 8f and 8g. Reaction data are listed in Table 7.

EXAMPLE 9

35 Samples of porous particles from Example 2, 4, 6, and 8 were used for the separation of a mixture of neutral, polar and basic compounds listed in Table 8. The 2.1x100 mm

chromatographic columns were packed using a slurry packing technique. The chromatographic system consisted of an ACQUITY UPLC® System and an ACQUITY UPLC® Tunable UV detector. Empower 2 Chromatography Data Software (Build 2154) was used for data collection and analysis. Mobile phase conditions were: 20 mM K₂HPO₄/KH₂PO₄, pH 5 7.00±0.02 / methanol (40/60 v/v); flow rate: 0.25 mL/min; temperature: 30 °C; detection: 254 nm; analytes: uracil, propranolol, butylparaben, naphthalene, dipropylphthalate, acenaphthene, and amitriptyline. Columns 4g and 8a were tested at 23°C.

It can be seen that columns packed with particles from Examples 2, 4, 6, 7 and 8 provide sufficient retention and resolution in the separation of neutral, polar, and basic 10 compounds under these conditions. Relative retention is the retention time of the analyte divided by the retention time of acenaphthene. Therefore values less than one, indicate less retention than acenaphthene, and values greater than one, indicate more retention than acenaphthene. (Relative retention is a well known parameter in the field of HPLC.)

TABLE 7

| Product | Particles (g) | Component A | | | Component B | | | Endcap | | | | |
|---------|---------------|---|---------------------|--|--------------------|--------------------|----------|------------------------|-------|---|----------------|----------|
| | | Silane Additive Charge (μmol/m ²) | Silane Additive (g) | Primary Silane Charge (μmol/m ²) | Primary Silane (g) | Primary Silane (g) | Base (g) | Charge Molar Ratio B/A | %C | Surface Coverage (μmol/m ²) | Endcap Product | Final %C |
| 8a | 40 | 0.060 | 0.13 | ODTCS | 4.0 | 15.58 | 5.47 | 66.7 | 14.77 | 3.45 | 8e | 16.00 |
| 8b | 25 | 0.30 | 0.507 | ODTCS | 2.3 | 5.60 | 2.00 | 7.6 | 11.48 | 2.53 | 8f | 13.39 |
| 8c | 25 | 0.30 | 0.507 | PPPTCS | 2.3 | 5.00 | 3.7 | 7.6 | 6.25 | 2.71 | -- | -- |
| 8d | 25 | 0.30 | 0.507 | PTCS | 2.3 | 4.30 | 3.7 | 7.6 | 7.94 | 2.49 | 8g | 10.31 |

TABLE 8

| Product | Retention Factor: Acenaphthene | Relative Retention: | | | |
|---------|--------------------------------|----------------------------|-----------------------------|----------------------------|-----------------------------------|
| | | Propranolol / Acenaphthene | Butylparaben / Acenaphthene | Naphthalene / Acenaphthene | Dipropyl phthalate / Acenaphthene |
| 2a | 8.45 | 0.218 | 0.300 | 0.458 | 0.540 |
| 2b | 8.79 | 0.218 | 0.291 | 0.455 | 0.520 |
| 4f | 13.49 | 0.145 | 0.210 | 0.422 | 0.382 |
| 4g | 13.80 | 0.140 | 0.219 | 0.431 | 0.382 |
| 4j | 8.87 | 0.232 | 0.287 | 0.458 | 0.505 |
| 4k | 8.14 | 0.215 | 0.292 | 0.459 | 0.482 |
| 4m | 8.09 | 0.210 | 0.285 | 0.452 | 0.457 |
| 4n | 8.24 | 0.215 | 0.284 | 0.450 | 0.458 |
| 4o | 7.58 | 0.232 | 0.296 | 0.456 | 0.502 |
| 4p | 8.86 | 0.204 | 0.265 | 0.444 | 0.440 |
| 4q | 7.28 | 0.229 | 0.300 | 0.459 | 0.460 |
| 4r | 9.39 | 0.192 | 0.248 | 0.439 | 0.435 |
| 4s | 9.38 | 0.189 | 0.270 | 0.270 | 0.441 |
| 4t | 9.59 | 0.183 | 0.264 | 0.321 | 0.426 |
| 4u | 8.91 | 0.208 | 0.275 | 0.444 | 0.455 |

| | | | | | |
|---|-------|-------|-------|-------|--------|
| 4z | 10.52 | 0.169 | 0.247 | 0.432 | 1.425 |
| 4aa | 9.92 | 0.183 | 0.255 | 0.429 | 1.488 |
| 4ac | 9.56 | 0.182 | 0.248 | 0.439 | 1.488 |
| 6e | 3.23 | 0.298 | 0.412 | 0.444 | 1.651 |
| 6f | 10.51 | 0.195 | 0.214 | 0.543 | 1.648 |
| 6g | 10.61 | 0.191 | 0.212 | 0.443 | 0.650 |
| 6h | 10.52 | 0.193 | 0.213 | 0.443 | 0.418 |
| 8a | 18.32 | 0.130 | 0.196 | 0.419 | 0.418 |
| Commercial <2 μ m Hybrid C ₁₈ Column | 10.49 | 0.168 | 0.228 | 0.436 | 0.421 |
| Commercial <2 μ m Silica C ₁₈ Column | 13.39 | 0.213 | 0.252 | 0.426 | 0.429 |
| Commercial <2 μ m Silica C ₁₈ Column | 17.85 | 0.153 | 0.194 | 0.417 | 0.432 |
| Commercial <2 μ m Silica C ₁₈ Column | 6.70 | 2.663 | 0.284 | 0.480 | 0.495 |
| | | | | | 1.404 |
| | | | | | 17.912 |

EXAMPLE 10

Samples of porous particles from Example 2, 4, 6, and 8 were evaluated for USP peak tailing factors using the mobile phase and test conditions of Example 9. The results are shown in Table 9. Peak tailing factors is a well known parameter in the field of HPLC (a lower value corresponds to reduced tailing). It is evident that columns packed with particles from Examples 2, 4, 6, 7 and 8 have comparable tailing factors to commercially available C₁₈-columns.

TABLE 9

| Product | Tailing Factor for: | | | | | |
|---|----------------------------|--------------|-------------|--------------------|--------------|---------------|
| | Propranolol | Butylparaben | Naphthalene | Dipropyl-phthalate | Acenaphthene | Amitriptyline |
| 2a | 1.00 | 1.42 | 1.54 | 1.26 | 1.25 | 1.37 |
| 2b | 1.86 | 1.30 | 1.24 | 1.26 | 1.15 | 1.98 |
| 4f | 1.03 | 1.37 | 1.28 | 1.33 | 1.26 | 1.88 |
| 4g | 0.95 | 1.31 | 1.25 | 1.28 | 1.22 | 1.91 |
| 4j | 1.51 | 1.19 | 1.17 | 1.16 | 1.17 | 3.44 |
| 4k | 1.32 | 1.16 | 1.16 | 1.14 | 1.20 | 1.45 |
| 4m | 1.25 | 1.28 | 1.29 | 1.28 | 1.26 | 1.55 |
| 4n | 1.67 | 1.12 | 1.11 | 1.09 | 1.06 | 1.41 |
| 4o | 1.18 | 1.16 | 1.16 | 1.13 | 1.11 | 1.31 |
| 4p | 1.79 | 1.18 | 1.18 | 1.15 | 1.13 | 1.91 |
| 4q | 1.57 | 1.15 | 1.17 | 1.14 | 1.16 | 1.60 |
| 4r | 1.52 | 1.17 | 1.17 | 1.15 | 1.15 | 2.39 |
| 4s | 1.09 | 1.41 | 1.27 | 1.29 | 1.14 | 1.14 |
| 4t | 1.27 | 1.41 | 1.23 | 1.32 | 1.13 | 1.31 |
| 4u | 1.37 | 1.16 | 1.18 | 1.16 | 1.17 | 2.19 |
| 4z | 1.22 | 1.39 | 1.45 | 1.42 | 1.31 | 2.44 |
| 4aa | 1.61 | 1.25 | 1.30 | 1.24 | 1.22 | 2.42 |
| 4ac | 1.50 | 1.31 | 1.51 | 1.40 | 1.50 | 2.58 |
| 6e | 1.34 | 1.24 | 1.25 | 1.23 | 1.29 | 1.47 |
| 6f | 1.96 | 1.23 | 1.29 | 1.26 | 1.31 | 2.66 |
| 6g | 1.92 | 1.24 | 1.28 | 1.25 | 1.29 | 2.69 |
| 6h | 1.92 | 1.22 | 1.27 | 1.25 | 1.29 | 2.81 |
| 8a | 1.06 | 1.11 | 1.08 | 1.11 | 1.08 | 2.76 |
| Commercial <2 µm Hybrid C ₁₈ Column | 0.88 | 1.34 | 1.24 | 1.29 | 1.14 | 1.15 |
| Commercial <2 µm Silica C ₁₈ Column | 0.96 | 1.17 | 1.10 | 1.33 | 1.10 | 6.95 |
| Commercial <2 µm Silica C ₁₈ Column | 0.95 | 1.35 | 1.22 | 1.32 | 1.10 | 1.77 |
| Commercial <2 µm Silica C ₁₈ Column | 4.19 | 1.34 | 1.29 | 1.28 | 1.12 | 1.34 |

EXAMPLE 11

Samples of porous particles from Example 2-8 were used for the separation of a mixture of neutral, polar and basic compounds listed in Table 10. The 2.1x100 mm chromatographic columns were packed using a slurry packing technique. Columns packed with 5 products 5i-5m, 6i-6o, and 8c-8g used 2.1x50 mm chromatographic columns. The chromatographic system consisted of an ACQUITY UPLC® System and an ACQUITY UPLC® Tunable UV detector. Empower 2 Chromatography Data Software (Build 2154) was used for data collection and analysis. Mobile phase conditions were: 15.4 mM ammonium formate, pH 3.00±0.02 / acetonitrile (65/35 v/v); flow rate: 0.25 mL/min; temperature: 30 °C; detection: 254 10 nm; analytes: uracil, pyrenesulfonic acid, desipramine, amitriptyline, butylparaben, and toluene. Columns 4g and 8a were tested at 23 °C.

It can be seen that columns packed with particles from Examples 2-8 provide sufficient retention and resolution in the separation of neutral, polar, and basic compounds under these conditions. Relative retention is the retention time of the analyte divided by the retention time 15 of toluene. Therefore values less than one, indicate less retention than toluene, and values greater than one, indicate more retention than toluene (relative retention is a well known parameter in the field of HPLC).

TABLE 10

| Product | Retention Factor: Toluene | Relative Retention: | | | |
|---------|------------------------------|---------------------------------|-------------------------|---------------------------|--------------------------|
| | | Pyrenesulfonic acid/ Toluene | Desipramine/ Toluene | Amitriptyline/ Toluene | Butylparaben/ Toluene |
| 2a | 10.95 | 0.148 | 0.275 | 0.373 | 0.965 |
| 2b | 11.73 | 0.669 | 0.180 | 0.244 | 0.975 |
| 3ac | 10.31 | 1.913 | 0.151 | 0.215 | 1.073 |
| 3ad | 9.70 | 2.137 | 0.138 | 0.197 | 1.076 |
| 4j | 11.37 | 0.491 | 0.184 | 0.250 | 1.081 |
| 4k | 10.82 | 0.216 | 0.231 | 0.311 | 1.101 |
| 4m | 10.75 | 0.227 | 0.219 | 0.297 | 1.115 |
| 4n | 10.76 | 0.245 | 0.216 | 0.291 | 1.125 |
| 4o | 10.31 | 0.219 | 0.234 | 0.316 | 1.098 |
| 4p | 11.07 | 0.177 | 0.242 | 0.328 | 1.074 |
| 4q | 9.69 | 0.354 | 0.188 | 0.250 | 1.211 |
| 4r | 11.31 | 0.154 | 0.243 | 0.329 | 1.001 |
| 4s | 11.73 | 0.180 | 0.251 | 0.339 | 1.087 |
| 4t | 11.76 | 0.199 | 0.237 | 0.319 | 1.099 |
| 4u | 11.34 | 0.229 | 0.240 | 0.326 | 1.140 |
| 4z | 12.78 | 0.208 | 0.233 | 0.316 | 1.044 |
| 4aa | 12.14 | 0.213 | 0.229 | 0.312 | 1.051 |
| 4ac | 11.46 | 0.405 | 0.177 | 0.241 | 1.026 |
| 5i | 3.64 | 5.053 | 0.244 | 0.326 | 1.151 |
| 5j | 3.28 | 5.353 | 0.223 | 0.302 | 1.179 |
| 5k | 3.86 | 3.910 | 0.331 | 0.437 | 1.114 |

| | | | | | |
|---|-------|-------|-------|-------|--------|
| 5l | 3.18 | 5.334 | 0.228 | 0.306 | 1.171 |
| 5m | 3.34 | 5.170 | 0.228 | 0.307 | 1.163 |
| 6e | 6.26 | 0.651 | 0.190 | 0.243 | 1.218 |
| 6f | 11.91 | 1.614 | 0.194 | 0.267 | 0.865 |
| 6g | 12.09 | 0.771 | 0.244 | 0.334 | 0.854 |
| 6h | 12.18 | 0.109 | 0.305 | 0.420 | 0.849 |
| 6i | 5.75 | 0.467 | 0.339 | 0.433 | 1.119 |
| 6j | 6.78 | 0.643 | 0.246 | 0.329 | 1.156 |
| 6k | 6.48 | 0.484 | 0.258 | 0.340 | 1.111 |
| 6l | 6.48 | 0.403 | 0.293 | 0.387 | 1.081 |
| 6m | 6.51 | 0.503 | 0.248 | 0.326 | 1.147 |
| 6n | 6.44 | 0.467 | 0.260 | 0.342 | 1.138 |
| 6o | 6.38 | 0.435 | 0.280 | 0.368 | 1.105 |
| 7a | 1.41 | 1.261 | 0.290 | 0.364 | 1.258 |
| 8a | 20.48 | 0.444 | 0.169 | 0.231 | 0.808 |
| 8c | 5.09 | 4.280 | 0.386 | 0.526 | 1.129 |
| 8f | 18.35 | 0.181 | 0.152 | 0.206 | 0.997 |
| 8g | 9.73 | 0.457 | 0.210 | 0.277 | 1.112 |
| Commercial <2 µm Hybrid C ₁₈ Column | 12.38 | 0.103 | 0.299 | 0.408 | 0.894 |
| Commercial <2 µm Silica C ₁₈ Column | 17.24 | 0.099 | 0.289 | 0.393 | 0.924 |
| Commercial <2 µm Silica C ₁₈ Column | 1.57 | 1.030 | 2.882 | 3.934 | 10.172 |
| Commercial <2 µm Silica C ₁₈ Column | 9.61 | 0.170 | 0.595 | 0.888 | 1.076 |

EXAMPLE 12

Samples of porous particles from Example 2-8 were evaluated for USP peak tailing factors using the mobile phase and test conditions of Example 11. The results are shown in 5 Table 11. Peak tailing factor is a well known parameter in the field of HPLC (a lower value corresponds to reduced tailing). It is evident that columns packed with particles from Examples 2-8 provide have comparable tailing factors to commercially available C₁₈-columns.

TABLE 11

| Product | Tailing Factor for: | | | | |
|---|---------------------|-------------|---------------|--------------|---------|
| | Pyrenesulfonic acid | Desipramine | Amitriptyline | Butylparaben | Toluene |
| 2a | 24.51 | 1.81 | 2.21 | 1.06 | 1.03 |
| 2b | 4.60 | 1.63 | 1.81 | 1.00 | 1.01 |
| 3ac | 1.89 | 2.16 | 2.32 | 1.06 | 1.02 |
| 3ad | 1.17 | 1.69 | 1.66 | 1.03 | 1.01 |
| 4j | 1.86 | 1.65 | 2.06 | 1.04 | 1.03 |
| 4k | 1.68 | 1.63 | 1.95 | 1.06 | 1.01 |
| 4m | 1.60 | 1.45 | 1.54 | 1.14 | 1.05 |
| 4n | 1.58 | 1.36 | 1.51 | 1.00 | 0.98 |
| 4o | 1.43 | 1.46 | 1.70 | 1.04 | 1.01 |
| 4p | 1.76 | 1.54 | 1.81 | 1.05 | 1.01 |
| 4q | 1.25 | 1.40 | 1.61 | 1.03 | 0.99 |
| 4r | 1.72 | 1.69 | 2.04 | 1.05 | 1.04 |
| 4s | 1.67 | 1.90 | 2.51 | 1.03 | 1.02 |
| 4t | 1.75 | 1.82 | 2.38 | 1.02 | 1.00 |
| 4u | 1.78 | 2.05 | 2.56 | 1.05 | 1.00 |
| 4z | 2.18 | 2.84 | 3.26 | 1.08 | 1.05 |
| 4aa | 1.90 | 1.80 | 2.03 | 1.05 | 1.05 |
| 4ac | 2.24 | 1.90 | 2.04 | 1.09 | 1.07 |
| 5i | 1.46 | 1.54 | 1.49 | 1.08 | 0.97 |
| 5j | 1.27 | 1.68 | 1.61 | 1.09 | 1.12 |
| 5k | 1.50 | 1.38 | 1.36 | 1.12 | 1.05 |
| 5l | 1.65 | 1.48 | 1.47 | 1.36 | 1.23 |
| 5m | 1.19 | 1.21 | 1.17 | 1.01 | 1.01 |
| 6e | 1.37 | 1.30 | 1.35 | 1.09 | 1.05 |
| 6f | 2.30 | 1.50 | 1.66 | 1.12 | 1.07 |
| 6g | 2.83 | 1.61 | 1.79 | 1.11 | 1.06 |
| 6h | 2.35 | 1.77 | 2.05 | 1.10 | 1.07 |
| 6i | 2.60 | 1.36 | 1.38 | 1.08 | 1.12 |
| 6j | 1.51 | 1.40 | 1.39 | 1.08 | 1.10 |
| 6k | 1.88 | 1.64 | 1.64 | 1.08 | 0.85 |
| 6l | 1.49 | 1.24 | 1.29 | 0.99 | 0.83 |
| 6m | 1.39 | 1.26 | 1.29 | 1.08 | 1.09 |
| 6n | 1.46 | 1.30 | 1.32 | 1.08 | 1.04 |
| 6o | 1.53 | 1.44 | 1.43 | 1.08 | 1.07 |
| 7a | 1.67 | 1.54 | 1.57 | 1.18 | 1.15 |
| 8a | 3.40 | 1.49 | 1.62 | 1.04 | 1.05 |
| 8c | 1.30 | 1.17 | 1.16 | 1.00 | 0.92 |
| 8f | 1.55 | 1.44 | 1.57 | 1.06 | 1.05 |
| 8g | 1.61 | 1.42 | 1.45 | 1.10 | 1.08 |
| Commercial <2 µm Hybrid C ₁₈ Column | 1.71 | 2.76 | 3.32 | 1.03 | 1.03 |
| Commercial <2 µm Silica C ₁₈ Column | 1.40 | 2.81 | 3.58 | 1.01 | 1.02 |

| | | | | | |
|--|------|------|------|------|------|
| Commercial <2 μ m Silica C ₁₈ Column Commercial <2 μ m Silica C ₁₈ Column | 1.75 | 3.20 | 3.82 | 1.04 | --- |
| | 1.65 | 2.20 | 2.65 | 1.06 | 1.01 |

EXAMPLE 13

Samples of porous particles from Example 2, 4-8 were used for the separation of a mixture of neutral and basic compounds listed in Table 12. The 2.1x 50 mm chromatographic columns were packed using a slurry packing technique. The chromatographic system consisted of an ACQUITY UPLC® System and an ACQUITY UPLC® Tunable UV detector. Empower Chromatography Data Software (Build 1154) was used for data collection and analysis.

Gradient conditions: 15-65% acetonitrile (solvent B) over 4.6 minutes in 0.1% formic acid (Solvent A) followed by a 1.4 minute hold; flow rate: 0.4 mL/min; temperature: 30 °C; detection: 260 nm; basic test mix prepared in 16.7% methanol: uracil, metoprolol tartate, papaverine, amitriptyline; neutral test mix prepared in 16.7% methanol: uracil, prednisone, caffeine. Columns packed with products 5l, 5n, 6i, 6j, 8c and 8f used 15-95% acetonitrile. Comparison Column A and B were commercially available and contained 2.7 μ m C₁₈-bonded superficially porous silica packing material. Comparison Column C was commercially available and contained 1.7 μ m porous hybrid particles of the formula (O_{1.5}SiCH₂CH₂SiO_{1.5})(SiO₂)₄, that was surface modified with ODTCS followed by endcapping.

Peak capacities were calculated using the average of the peak widths (4 □) over three injections. The determination of peak capacity and the problems caused by poor peak shape and resulting poor peak capacities for basic analytes in low pH gradient separations is well known in the field of HPLC and UPLC. By comparing the ratio of peak capacities for a basic analyte (amitriptyline) to a neutral analyte (prednisone) under these test conditions, a better comparison of basic analyte chromatographic performance can be made. A peak capacity ratio near one indicates similar performance of the basic and neutral analytes. A peak capacity ratio less than 0.8 indicates a substantial decrease in chromatographic performance. A peak capacity greater than one indicates an improvement in chromatographic performance for the basic analytes over the neutral analyte.

Differences due to changes in particle size can be observed by comparing the peak capacity ratios for columns packed with products 4u, 4j, and 4n. While these products are of similar Component A and B type and charges, they range in particle size from 1.8 μ m (product 4u), 2.9 μ m (product 4j) and 3.9 μ m (product 4n). The peak capacity ratios were determined to

be 0.86, 1.09 and 1.02, respectively. We can conclude that the particle size impacts performance under these conditions, especially for < 2 μm packing materials. Product 4u still has significant improvements in peak capacity ratios over Comparison Columns A-D.

The impact of Component A silane additive can be observed by comparing the peak 5 capacity ratios for columns packed with product 2a and 2d. These products are of similar size, Component B silane type and Component B silane charge. Product 2a does not contain a Component A silane additive. Product 2d was prepared with APTES charged at 0.3 $\mu\text{mol}/\text{m}^2$. The peak capacity ratios were determined to be 0.72 and 1.18, respectively. We can conclude that Component A silane additive type improves performance under these conditions.

10 Differences in Component A silane additive type can be observed by comparing the peak capacity ratios for columns packed with products 4c and 4i. These products are of similar size and Component B silane charge. While they were prepared using the same Component A silane additive charge, the Component A silane additive type was APTES for product 4c and 4PE for product 4i. The peak capacity ratios were determined to be 0.74 and 0.38, respectively.
15 We conclude that Component A silane additive type impacts performance under these conditions.

Differences in Component A silane additive charge can be observed by comparing the peak capacity ratios for columns packed with products 4h and 4j. These products are of similar size, Component B silane type and Component B silane charge. While these products were 20 prepared with the same Component A silane additive type, the Component A charge varied from 0.06 $\mu\text{mol}/\text{m}^2$ (product 4h) to 0.3 $\mu\text{mol}/\text{m}^2$ (product 4j). The peak capacity ratios were determined to be 0.67 and 1.09, respectively. We conclude that Component A silane additive charge impacts performance under these conditions.

Differences in Component B silane type can be observed by comparing the peak 25 capacity ratios for columns packed with product 4k and 6e. These products are of similar size, Component A silane additive type and Component A silane additive charge. While these products were prepared with the same Component B silane charge, the Component B silane type was ODTCS for product 4k and OTCS for product 6e. The peak capacity ratios were determined to be 1.02 and 0.34, respectively. We conclude the Component B silane type 30 impacts performance under these conditions.

Differences in Component B silane charge can be observed by comparing the peak capacity ratios for columns packed with products 4l and 4j. These products are of similar size, Component A silane additive type and charge. While these products were prepared with the same Component B silane, the Component B silane charge was 4 $\mu\text{mol}/\text{m}^2$ (product 4l) and 35 2 $\mu\text{mol}/\text{m}^2$ (product 4j). The peak capacity ratios were determined to be 0.45 and 1.09,

respectively. We conclude the Component B silane charge impacts performance under these conditions.

TABLE 12

| Product | <u>A</u> Amitriptyline Pc | <u>B</u> Prednisone Pc | Ratio A/B |
|---------|---------------------------------|------------------------------|--------------|
| 2a | 95 | 132 | 0.72 |
| 2d | 99 | 84 | 1.18 |
| 4c | 88 | 119 | 0.74 |
| 4h | 66 | 98 | 0.67 |
| 4i | 33 | 88 | 0.38 |
| 4j | 109 | 100 | 1.09 |
| 4k | 88 | 86 | 1.02 |
| 4l | 49 | 109 | 0.45 |
| 4m | 97 | 96 | 1.01 |
| 4n | 86 | 84 | 1.02 |
| 4o | 169 | 155 | 1.09 |
| 4p | 150 | 148 | 1.01 |
| 4q | 144 | 149 | 0.96 |
| 4r | 147 | 157 | 0.94 |
| 4s | 176 | 181 | 0.97 |
| 4t | 181 | 177 | 1.02 |
| 4u | 202 | 235 | 0.86 |
| 4z | 169 | 209 | 0.81 |
| 4aa | 162 | 163 | 0.99 |
| 4ac | 179 | 154 | 1.16 |
| 5i | 237 | 219 | 1.08 |
| 5j | 231 | 218 | 1.06 |
| 5k | 160 | 147 | 1.09 |
| 5l | 165 | 144 | 1.15 |
| 5m | 126 | 116 | 1.09 |
| 5n | 171 | 137 | 1.25 |
| 6a | 113 | 86 | 1.32 |
| 6b | 105 | 92 | 1.14 |
| 6c | 123 | 89 | 1.39 |
| 6d | 39 | 92 | 0.43 |
| 6e | 33 | 98 | 0.34 |
| 6f | 86 | 104 | 0.82 |
| 6g | 79 | 105 | 0.75 |
| 6h | 26 | 100 | 0.26 |
| 6i | 169 | 160 | 1.06 |
| 6j | 184 | 156 | 1.18 |
| 6o | 177 | 154 | 1.15 |
| 6p | 185 | 152 | 1.22 |
| 7a | 174 | 217 | 0.80 |
| 8c | 157 | 143 | 1.10 |
| 8f | 174 | 153 | 1.14 |
| 8g | 188 | 150 | 1.25 |

| | | | |
|---------------------|----|-----|------|
| Comparison Column A | 65 | 245 | 0.27 |
| Comparison Column B | 41 | 248 | 0.16 |
| Comparison Column C | 76 | 267 | 0.28 |

EXAMPLE 14

5 Samples of porous particles from Example 2 and 4 were evaluated for efficiency difference upon increased loading of basic analytes. The 4.6 x 150 mm chromatographic columns were packed using a slurry packing technique. The chromatographic system consisted of an Alliance HPLC® System and a Waters 996 PDA detector. Empower 2 Chromatography Data Software (Build 2154) was used for data collection and analysis; injection volume 20 µL;

10 flow rate: 1.0 mL/min; temperature: 30 °C; detection: 230 nm; analytes: amitriptyline or propranolol (prepared 60 µg/mL in mobile phase). Loading range on Table 13: 0.1 µg-2.5 µg analyte on column. In order to have comparable retention factors (0.9-2.0), mobile phase conditions were modified for separations using amitriptyline [0.05% TFA in acetonitrile/water (60/40 v/v)] and propranolol [0.05% TFA in acetonitrile/water (70/30 v/v)]. Comparison

15 column A was commercially and contained 5 µm C₁₈-bonded porous silica packing material. Comparison column B was commercially available and contained 5 µm porous hybrid packing of the formula (O_{1.5}SiCH₂CH₂SiO_{1.5})(SiO₂)₄, that was surface modified with ODTCS followed by endcapping. Comparison columns C and D were commercially available and contained 5 µm porous silica packing that was surface modified with an organofunctional silane followed

20 by C₁₈ surface modification.

The observation of decreased efficiency and worsening of peak shape for basic analytes at increased loadings when used under low pH isocratic conditions is well known in the field of HPLC and UPLC. Not limited to theory, this worsening of separation performance for basic analytes has been attributed with analyte overloading. As tabulated in Table 12, the decreased

25 performance at increased loadings is determined as the percent loss in column efficiency between 0.1-1.2 µg or 0.1-2.5 µg loading of amitriptyline or propranolol.

Similar results were obtained for amitriptyline and propranolol at the 1.2 µg and 2.5 µg loadings. Columns that performed well on this test, including columns containing products 2c, 2g, and 4g, had a low loss in efficiency (<20%) at the 1.2 µg analyte loading. These columns

30 had comparable performance to Comparison Columns A and C and improved performance over Comparison Columns B and D. These well-performing columns had a further decrease in efficiency between 1.2 µg and 2.5 µg loadings of approximately 100%. Other columns tested

had a greater loss in efficiency (>20%) at 1.2 μg analyte loading, as well as a further decrease in efficiency between 1.2 μg and 2.5 μg loadings of approximately 25-50%.

The impact of Component A silane additive type can be observed by comparing the loss in amitriptyline efficiency (1.2 μg on column) for columns packed with product 2c and 2g.

5 These products have the same Component B silane type and Component B silane charge. While they were prepared using the same Component A silane additive charge, the Component A silane additive was APTES for product 2c and 4PE for product 2g. The losses in amitriptyline efficiency were determined to be 4% and 13%, respectively.

The impact of Component A silane charge can be observed by comparing the loss in 10 amitriptyline efficiency (1.2 μg on column) for columns packed with product 4c, 4f, and 4g.

These products have the same Component B silane type and Component B silane charge. While they were prepared using the same Component A silane additive type, the Component A silane charge was 0.06 $\mu\text{mol}/\text{m}^2$ for product 4c, 0.12 $\mu\text{mol}/\text{m}^2$ for product 4f and 0.20 $\mu\text{mol}/\text{m}^2$ for product 4g. The losses in amitriptyline efficiency were determined to be 40%, 34% and 10%, 15 respectively.

The impact of Component B silane type can be observed by comparing the loss in amitriptyline efficiency (1.2 μg on column) for columns packed with product 2c and 4b. These products have the same Component A silane additive type and Component A silane additive charge. While they were prepared using the same Component B silane charge, the Component 20 B silane type was ODMCS for product 2c and ODTCS for product 4b. The losses in amitriptyline efficiency were determined to be 4% and 43%, respectively.

TABLE 13

| Product | %Loss in efficiency for | | | |
|------------------------|--|--|--------------------------------------|--------------------------------------|
| | Amitriptyline (1.2 µg on Column) | Amitriptyline (2.5 µg on Column) | Propranolol (1.2 µg on Column) | Propranolol (2.5 µg on Column) |
| 2c | 4% | 8% | 5% | 10% |
| 2g | 13% | 30% | 15% | 30% |
| 4a | 61% | 77% | 57% | 75% |
| 4b | 43% | 63% | 37% | 60% |
| 4c | 40% | 59% | 41% | 59% |
| 4f | 34% | 52% | 34% | 52% |
| 4g | 10% | 18% | 8% | 11% |
| Comparison Column A | -4% | -3% | 2% | 4% |
| Comparison Column B | 51% | 72% | 47% | 71% |
| Comparison Column C | 6% | 14% | 8% | 18% |
| Comparison Column D | 20% | 44% | 26% | 49% |

EXAMPLE 15

The general procedure for modifying surface silanol groups to result in the display of hydrophobic surface group and ionizable modifier that is detailed in Examples 1, 3, 5, 7 and 8 is applied to modify the surface silanol groups of different porous materials. Included in this are monolithic, spherical, granular, superficially porous and irregular materials that are silica, hybrid inorganic/organic materials, hybrid inorganic/organic surface layers on hybrid inorganic/organic, silica, titania, alumina, zirconia, polymeric or carbon materials, and silica surface layers on hybrid inorganic/organic, silica, titania, alumina, zirconia or polymeric or carbon materials. The particles size for spherical, granular or irregular materials vary from 5-500 µm; more preferably 15-100 µm; more preferably 20-80 µm; more preferably 40-60 µm. The APD for these materials vary from 30 to 2,000 Å; more preferably 40 to 200 Å; more preferably 50 to 150 Å. The SSA for these materials vary from 20 to 1000 m²/g; more preferably 90 to 800 m²/g; more preferably 150 to 600 m²/g; more preferably 300 to 550 m²/g. The TPV for these materials vary from 0.3 to 1.5 cm³/g; more preferably 0.5 to 1.2 cm³/g; more preferably 0.7 to 1.1 cm³/g. The macropore diameter for monolithic materials vary from 0.1 to 30 µm, more preferably 0.5 to 25 µm, more preferably 1 to 20 µm.

The ionizable modifier, component A, is selected from groups used in Examples 1, 3, 5, 7 and 8 or is selected from a group having formula (I), formula (II) or formula (III) including an acidic ionizable modifier including, but not limited to, protected and unprotected versions of alkyl, aryl, and arylalkyl groups containing phosphoric, carboxylic, sulfonic, and boronic acids

Preferred silane ionizable modifying reagents of formula I and II include 4-pyridyl alkyl trialkoxysilane, 3-pyridyl alkyl trialkoxysilane, 2-pyridyl alkyl trialkoxysilane, imidazole alkyl trialkoxysilane, aminoalkyl trialkoxysilane, and mono- and di-alkylaminoalkyl trialkoxysilane.

Preferred silane ionizable modifying reagents of formula III include the trisilanol, trialkoxysilane or trichlorosilane, the protected and deprotected acid forms, chloro forms, as well as salts of sulfonic acid alkyl silanes, sulfonic acid phenylalkyl silanes, sulfonic acid benzylalkyl silanes, sulfonic acid phenyl silanes, sulfonic acid benzyl silanes, carboxylic acid alkyl silanes, carboxylic acid phenylalkyl silanes, carboxylic acid benzylalkyl silanes, carboxylic acid phenyl silanes, carboxylic acid benzyl silanes, phosphoric acid alkyl silanes, phosphonic acid phenylalkyl silanes, phosphonic acid benzylalkyl silanes, phosphonic acid phenyl silanes, phosphonic acid benzyl silanes, boronic acid alkyl silanes, boronic acid phenylalkyl silanes, boronic acid benzylalkyl silanes, boronic acid phenyl silanes, boronic acid benzyl silanes.

15

EXAMPLE 16

Residual silanol groups from select materials prepared in Example 15 are further reacted following protocols detailed in Examples 2, 4, and 6.

EXAMPLE 17

20

In a general procedure propanol hybrid surrounded hybrid particles (product 17a) were prepared in a multistep procedure as follows;

25

Acetoxypropyltrimethoxysilane (700 g, Gelest Inc., Morrisville, PA) was mixed with ethanol (374 g, anhydrous, J.T. Baker, Phillipsburgh, NJ) and an aqueous solution of 0.01 M Acetic Acid (22 g, J.T. Baker, Phillipsburgh, NJ) in a flask. The resulting solution was agitated and refluxed for 16 hours in an atmosphere of argon or nitrogen. Alcohol was removed from the flask by distillation at atmospheric pressure. Residual alcohol and volatile species were removed by heating at 110° C for 17 hours in a sweeping stream of argon or nitrogen. The resulting polyorganoalkoxy siloxanes was a clear viscous liquid had a viscosity of 95 cP.

30

This polyorganoalkoxy siloxanes was added to a suspension of BEH porous hybrid

particles (20 g, Waters Corporation, Milford, MA; 6.5% C; SSA = 190 m²/g; SPV = 0.80 cm³/g; APD = 155 Å) of the formula (O_{1.5}SiCH₂CH₂SiO_{1.5})(SiO₂)₄ (prepared following the method described in U.S. Patent 6,686,035) in dry toluene (Fisher Scientific, Fairlawn, NJ; 5 mL/g).

This reaction was heated at 80 °C for one hour and 110 °C for 20 hours using a Dean-Stark trap to remove residual water. The reaction was cooled to room temperature and particles were

35

isolated on 0.5 µm filtration paper and washed repeatedly using ethanol (anhydrous, J.T. Baker,

Phillipsburgh, NJ). The material was then heated to 50 °C in a suspension with ethanol (3 mL/g, anhydrous, J.T.Baker, Phillipsburgh, NJ), deionized water (7 mL/g) and 30% ammonium hydroxide (20 g; J.T.Baker, Phillipsburgh, NJ) for 4 hours. The reaction was then cooled and the product was filtered and washed successively with water and methanol (Fisher Scientific, 5 Fairlawn, NJ). The product was then dried at 80 °C under reduced pressure for 16 hours.

The particles were then mixed with an aqueous solution of 0.3 M tris(hydroxymethyl)aminomethane (TRIS, Aldrich Chemical, Milwaukee, WI) at a slurry concentration of 5 mL/g. The pH of the resultant slurry was adjusted to 9.8 using acetic acid (J.T. Baker, Phillipsburgh, NJ). The slurry was then enclosed in a stainless steel autoclave and 10 heated to 155 °C for 20 hours. After cooling the autoclave to room temperature, the product was isolated on 0.5 µm filtration paper and washed with water and methanol (Fisher Scientific, Suwanee, GA). The particles were then dried at 80 °C under vacuum for 16 hours.

The particles were then dispersed in a 1 molar hydrochloric acid solution (Aldrich, Milwaukee, WI) for 20 h at 98 °C. The particles were isolated on 0.5 µm filtration paper and 15 washed with water to a neutral pH, followed by acetone (HPLC grade, Fisher Scientific, Fairlawn, N.J.). The particles were dried at 80 °C under vacuum for 16 h. Products obtained by this approach have 8.1-8.6 %C; SSA = 150-166 m²/g; SPV = 0.6-0.7 cm³/g; APD = 134-145 Å). Structural analysis was performed using NMR spectroscopy. Surface coverage of propanol groups, determined by the difference in particle %C using elemental analysis, was 3.2-20 3.8 µmol/m².

EXAMPLE 18

Propanol hybrid surrounded hybrid particles from Example 17 were modified with octadecyl isocyanate (ODIC, Aldrich Chemical), pentafluorophenyl isocyanate (PFPIC, Aldrich 25 Chemical), 2,2-Diphenylethyl isocyanate (DPEIC, Aldrich Chemical), 4-cyanophenyl isocyanate (4CPIC, Aldrich Chemical), or 3-cyanophenyl isocyanate (3CPIC, Aldrich Chemical) in dry toluene (5 mL/g, J.T.Baker) under an argon blanket. The suspension was heated to reflux (110 °C) for 16 h and then cooled to <30°C. The particles were transferred to a filter apparatus and washed exhaustively with toluene and acetone. The material was then treated as detailed in the 30 hydrolysis section of Example 3, or the material was heated for an hour at 50 °C in a 1:1 v/v mixture of acetone and 1% trifluoroacetic acid (Aldrich, Milwaukee, WI) solution (10 mL/g particles) (Hydrolysis D). The reaction was then cooled and the product was filtered and washed successively with acetone and toluene (heated at 70 °C). The product was then dried at 70 °C under reduced pressure for 16 hours. Reaction data is listed in Table 14. The surface

coverage of carbamate groups was determined by the difference in particle %C before and after the surface modification as measured by elemental analysis.

TABLE 14

| Product | dp (μm) | Particles (g) | Component B | | | Hydrolysi s Type | %C | Carbamate Surface Coverage ($\mu\text{mol}/\text{m}^2$) |
|---------|-------------------------|------------------|-------------|---------------------------|--|------------------------|-------|--|
| | | | Isocyanate | Isocyanate mass (g) | Isocyanate Charge ($\mu\text{mol}/\text{m}^2$) | | | |
| 18a | 3.0 | 25 | ODIC | 11.9 | 10.0 | B | 15.93 | 2.55 |
| 18b | 3.0 | 60 | ODIC | 28.9 | 10.0 | B | 15.86 | 2.47 |
| 18c | 3.0 | 40 | ODIC | 19.3 | 10.0 | B | 15.28 | 2.26 |
| 18d | 3.0 | 40 | ODIC | 19.3 | 10.0 | B | 15.28 | 2.26 |
| 18e | 4.0 | 50 | ODIC | 27.3 | 11.8 | B | 15.49 | 2.47 |
| 18f | 3.5 | 25 | ODIC | 12.0 | 10.0 | B | 14.58 | 2.07 |
| 18g | 3.5 | 25 | ODIC | 6.0 | 5.0 | B | 13.04 | 1.52 |
| 18h | 3.5 | 15 | ODIC | 7.2 | 10.0 | B | 14.3 | 2.00 |
| 18i | 3.5 | 15 | ODIC | 7.2 | 10.0 | B | 14.55 | 2.09 |
| 18j | 3.5 | 15 | ODIC | 7.2 | 10.0 | B | 14.55 | 2.09 |
| 18k | 3.5 | 10 | ODIC | 4.8 | 10.0 | B | 13.64 | 1.76 |
| 18l | 3.5 | 10 | ODIC | 4.8 | 10.0 | B | 13.78 | 1.81 |
| 18m | 3.5 | 10 | ODIC | 4.8 | 10.0 | B | 13.87 | 1.85 |
| 18n | 4.9 | 10 | ODIC | 3.6 | 8.0 | B | 12.97 | 1.58 |
| 18o | 4.9 | 33 | ODIC | 12.4 | 8.0 | B | 14.45 | 2.12 |
| 18p | 3.5 | 50 | ODIC | 18.9 | 8.0 | B | 14.82 | 2.19 |
| 18q | 3.5 | 50 | ODIC | 18.9 | 8.0 | B | 14.78 | 2.18 |
| 18r | 3.5 | 50 | ODIC | 18.9 | 8.0 | B | 14.95 | 2.24 |
| 18s | 3.5 | 60 | ODIC | 23.6 | 8.0 | B | 15.10 | 2.20 |
| 18t | 4.9 | 60 | ODIC | 22.6 | 8.0 | B | 15.24 | 2.35 |
| 18u | 3.0 | 20 | PFPIC | 6.7 | 10.0 | D | 12.24 | 3.98 |
| 18v | 3.5 | 12 | DPEIC | 4.3 | 10.0 | C | 14.05 | 2.36 |
| 18w | 3.0 | 45 | 4CPIC | 10.4 | 10.0 | D | 13.01 | 3.63 |
| 18x | 4.9 | 40 | 4CPIC | 3.1 | 3.45 | C | 11.88 | 2.90 |
| 18y | 4.9 | 40 | 3CPIC | 3.1 | 3.45 | C | 11.96 | 2.97 |

5

EXAMPLE 19

The materials of Example 18 were further modified aminopropyltriethoxysilane (APTES, Gelest Inc., Morrisville, PA), 2-(4-pyridylethyl)triethoxysilane (4PE, Gelest Inc., Morrisville, PA) or 2-(2-pyridylethyl)trimethoxysilane (2PE, Gelest Inc., Morrisville, PA). in 10 refluxing toluene (5 mL/g) for 20 hours. Products 19a and 19b were reacted for 4 hours. The reaction was then cooled and the product was filtered and washed successively with water, toluene, 1:1 v/v acetone/water and acetone (all solvents from J.T. Baker). The material was then treated as detailed in the hydrolysis section of Example 3, or the material was heated for an hour at 50 °C in a 1:1 v/v mixture of acetone and 1% trifluoroacetic acid (Aldrich,

Milwaukee, WI) solution (10 mL/g particles) (Hydrolysis D). The reaction was then cooled and the product was filtered and washed successively water, toluene, 1:1 v/v acetone/water and acetone and then dried at 70 °C under reduced pressure for 16 hours. Reaction data is listed in Table 15.

5

TABLE 15

| Product | Precursor | Particles (g) | Component A | | | Charge Molar Ratio B/A | Hydrolysis Type | %C |
|---------|-----------|---------------|-----------------|-------------------|---|------------------------|-----------------|-------|
| | | | Silane Additive | Silane mass (g) | Silane Additive Charge (μmol/m ²) | | | |
| 19a | 18a | 10 | APTES | 0.01 | 0.03 | 333 | none | 16.82 |
| 19b | 18a | 10 | APTES | 0.02 | 0.05 | 200 | none | 16.82 |
| 19c | 18b | 30 | 4PE | 0.04 | 0.03 | 333 | none | 15.79 |
| 19d | 18b | 15 | 4PE | 0.04 | 0.06 | 167 | A | 15.81 |
| 19e | 18b | 20 | 4PE | 0.04 | 0.05 | 200 | B | 15.82 |
| 19f | 18b | 30 | 4PE | 0.08 | 0.06 | 167 | none | 15.79 |
| 19g | 18b | 17 | 4PE | 0.08 | 0.11 | 91 | A | 15.78 |
| 19h | 18b | 20 | 4PE | 0.08 | 0.09 | 111 | B | 15.79 |
| 19i | 18b | 30 | 4PE | 0.04 | 0.03 | 333 | B | 15.79 |
| 19j | 18b | 30 | 4PE | 0.08 | 0.06 | 167 | A | 15.79 |
| 19k | 18c | 30 | 4PE | 0.08 | 0.06 | 167 | B | 15.18 |
| 19l | 18d | 30 | 4PE | 0.08 | 0.06 | 167 | A | 15.22 |
| 19m | 18d | 30 | 4PE | 0.08 | 0.06 | 167 | D | 15.18 |
| 19n | 18d | 10 | 4PE | 0.01 | 0.03 | 439 | B | 15.17 |
| 19o | 18e | 10 | 4PE | 0.25 | 0.59 | 20 | B | 15.00 |
| 19p | 18e | 10 | APTES | 0.01 | 0.04 | 295 | B | 15.34 |
| 19q | 18e | 10 | APTES | 0.00 ₄ | 0.01 | 1180 | B | 15.32 |
| 19r | 18f | 25 | 2PE | 0.04 | 0.04 | 250 | B | 14.73 |
| 19s | 18g | 24 | 2PE | 0.04 | 0.04 | 125 | B | 13.32 |
| 19t | 18h | 10 | 4PE | 0.09 | 0.20 | 50 | C | 14.20 |
| 19u | 18i | 10 | 4PE | 0.13 | 0.30 | 33 | C | 14.36 |
| 19v | 18k | 9 | 4PE | 0.11 | 0.30 | 33 | C | 13.78 |
| 19w | 18l | 8 | 4PE | 0.04 | 0.10 | 100 | C | 13.63 |
| 19x | 18m | 9 | 4PE | 0.19 | 0.50 | 20 | C | 13.87 |
| 19y | 18n | 9 | 2PE | 0.10 | 0.30 | 27 | C | 13.27 |
| 19z | 18o | 30 | 4PE | 0.39 | 0.30 | 27 | C | 14.41 |
| 19aa | 18p | 30 | 4PE | 0.39 | 0.30 | 27 | C | 14.82 |
| 19ab | 18q | 30 | 4PE | 0.39 | 0.30 | 27 | C | 14.79 |
| 19ac | 18r | 30 | 4PE | 0.39 | 0.30 | 27 | C | 14.81 |
| 19ad | 18s | 55 | 4PE | 0.74 | 0.30 | 27 | C | 14.87 |
| 19ae | 18t | 30 | 4PE | 0.39 | 0.30 | 27 | C | 14.62 |
| 19af | 18u | 6 | 4PE | 0.01 | 0.03 | 333 | none | 11.69 |
| 19ag | 18v | 9 | 4PE | 0.12 | 0.31 | 32 | C | 14.07 |
| 19ah | 18w | 8 | 4PE | 0.01 | 0.03 | 347 | none | 12.77 |
| 19ai | 18x | 10 | 4PE | 0.21 | 0.51 | 7 | C | 11.83 |
| 19aj | 18x | 10 | 4PE | 0.12 | 0.29 | 12 | C | 11.80 |
| 19ak | 18y | 10 | 4PE | 0.21 | 0.51 | 7 | C | 11.93 |
| 19al | 18y | 10 | 4PE | 0.12 | 0.29 | 12 | C | 11.87 |

EXAMPLE 20

Selected materials of Example 19 were further modified by endcapping as detailed in Example 4. Data is listed in Table 16.

5

TABLE 16

| Product | Precurs or | %C |
|---------|---------------|-------|
| 20a | 19i | 16.73 |
| 20b | 19i | 16.72 |
| 20c | 19j | 16.40 |
| 20d | 19j | 16.67 |
| 20e | 19k | 16.11 |
| 20f | 19l | 15.84 |
| 20g | 19m | 16.06 |
| 20h | 19n | 15.46 |
| 20i | 19o | 15.67 |
| 20j | 19p | 16.00 |
| 20k | 19q | 16.18 |
| 20l | 19r | 15.50 |
| 20m | 19s | 13.91 |

EXAMPLE 21

Propanol hybrid surrounded hybrid particles from Example 17 were modified with 2-(4-pyridylethyl)triethoxysilane (4PE, Gelest Inc., Morrisville, PA) in refluxing toluene (5 mL/g) 10 for 20 hours. The reaction was then cooled and the product was filtered and washed successively with water, toluene, 1:1 v/v acetone/water and acetone (all solvents from J.T. Baker). The material was then treated as hydrolysis C of Example 3. The reaction was then cooled and the product was filtered and washed successively water, toluene, 1:1 v/v acetone/water and acetone. Selected products were then dried at 70 °C under reduced pressure 15 for 16 hours. Reaction data is listed in Table 17.

TABLE 17

| Product | dp (μm) | Particles (g) | Component A | | Hydrolysis Time (hr) | Vacuum Dried (Y/N) |
|---------|-------------------------|------------------|-----------------------|--|----------------------------|--------------------------|
| | | | Silane mass (g) | Silane Additive Charge ($\mu\text{mol}/\text{m}^2$) | | |
| 21a | 3.6 | 35 | 0.15 | 0.10 | 2 | Y |
| 21b | 3.6 | 35 | 0.30 | 0.20 | 2 | Y |
| 21c | 4.8 | 31 | 0.13 | 0.10 | 2 | Y |
| 21d | 4.8 | 31 | 0.26 | 0.20 | 2 | Y |
| 21e | 3.4 | 35 | 0.31 | 0.20 | 2 | Y |
| 21f | 4.8 | 35 | 0.30 | 0.20 | 2 | Y |
| 21g | 4.8 | 35 | 0.30 | 0.20 | 2 | Y |
| 21h | 3.4 | 35 | 0.31 | 0.20 | 2 | Y |
| 21i | 3.6 | 35 | 0.30 | 0.20 | 2 | Y |
| 21j | 4.8 | 25 | 0.31 | 0.30 | 20 | N |
| 21k | 4.8 | 35 | 0.16 | 0.15 | 20 | N |
| 21l | 4.8 | 30 | 0.19 | 0.15 | 20 | N |
| 21m | 3.6 | 35 | 0.23 | 0.15 | 20 | N |

EXAMPLE 22

5 Products from Example 21 were modified with isocyanate as detailed in Example 18 using hydrolysis C. Reaction data is listed in Table 18.

TABLE 18

| | | | Component B | | | | | |
|---------|-----------|------------------|-------------|---------------------------|---|---------------------------------|-------|--|
| Product | Precursor | Particles (g) | Isocyanate | Isocyanate mass (g) | Isocyanate Charge ($\mu\text{mol}/\text{m}^2$) | Charge Molar Ratio B/A | %C | Carbamate Surface Coverage ($\mu\text{mol}/\text{m}^2$) |
| 22a | 21a | 20 | ODIC | 7.60 | 8.00 | 80 | 14.86 | 2.21 |
| 22b | 21b | 20 | ODIC | 7.60 | 8.00 | 40 | 14.92 | 2.23 |
| 22c | 21c | 20 | ODIC | 7.30 | 8.00 | 80 | 14.62 | 2.20 |
| 22d | 21d | 20 | ODIC | 7.30 | 8.00 | 40 | 14.73 | 2.24 |
| 22e | 21e | 20 | ODIC | 7.85 | 8.00 | 40 | 14.83 | 2.10 |
| 22f | 21f | 20 | ODIC | 7.52 | 8.00 | 40 | 14.55 | 2.09 |
| 22g | 21h | 20 | ODIC | 7.85 | 8.00 | 40 | 14.69 | 2.05 |
| 22h | 21i | 20 | ODIC | 7.56 | 8.00 | 40 | 14.33 | 2.01 |
| 22i | 21j | 20 | ODIC | 7.28 | 8.00 | 27 | 14.00 | 1.96 |
| 22j | 21k | 20 | ODIC | 7.28 | 8.00 | 53 | 14.04 | 1.98 |
| 22k | 21l | 30 | ODIC | 10.92 | 8.00 | 53 | 13.99 | 1.96 |
| 22l | 21m | 35 | ODIC | 13.24 | 8.00 | 53 | 14.18 | 1.96 |
| 22m | 21a | 10 | 3CPIC | 0.80 | 3.47 | 35 | 10.08 | 1.32 |
| 22n | 21b | 10 | 3CPIC | 0.80 | 3.47 | 17 | 10.22 | 1.43 |

EXAMPLE 23

The concentration of surface pyridyl groups (ionizable modifier) were quantified for select materials prepared in Examples 3, 4, 21 and 22 using the following procedure. 2-(4-pyridylethyl)triethoxysilane (1.12 μ mol, Gelest Inc., Morrisville, PA) in methanol (0.4 mL, 5 HPLC grade) was added to a sample (0.2000 g) from Example 3, 21 or 22. The sample was then digested using sodium hydroxide solution (4.0 mL, 2.5 M) at 64 °C for 60 minutes. The sample was filtered through a Millex-LCR filter (0.45 μ m, 25 mm, Millipore) and was extracted with hexane (HPLC grade). The aqueous layer was then analyzed using a UV/Visible spectrophotometer (300-240 nM, 0.1 nM interval, scan speed = 120 nM/min, slit width = 2 nM).
10 The concentration of pyridyl groups were calculated using the absorbance at two wavelengths with corrections made for base particle contribution to absorbance. The results are listed in Table 19. These results indicate a reduced concentration of pyridylethyl groups (component A) on the surface than was charged. Using the determined coverage of component B we can determine the determined surface coverage ratio of B/A. The result of this is a larger range of
15 surface coverage ratio of B/A (8-190) than molar charge ratio (6-80).

TABLE 19

| | Component A | Component B | | Component A | Component B | |
|---------|--|---|------------------------|--|---|----------------------------|
| Product | Ionizable Modifier Charge ($\mu\text{mol}/\text{m}^2$) | Hydrophobic Group Charge ($\mu\text{mol}/\text{m}^2$) | Charge Molar Ratio B/A | Ionizable Modifier Coverage ($\mu\text{mol}/\text{m}^2$) | Hydrophobic Group Coverage ($\mu\text{mol}/\text{m}^2$) | Surface Coverage Ratio B/A |
| 3ah | 3.70 | --- | --- | 0.890 | --- | --- |
| 4v | 0.35 | 2.53 | 7 | 0.160 | 2.45 | 15 |
| 4w | 0.35 | 2.07 | 6 | 0.250 | 2.09 | 8 |
| 4x | 0.25 | 2.53 | 10 | 0.140 | 2.51 | 18 |
| 4y | 0.25 | 2.07 | 8 | 0.210 | 2.05 | 8 |
| 8b | 0.30 | 2.3 | 8 | 0.28 | 2.53 | 9 |
| 8f | 0.30 | 2.3 | 8 | 0.27 | 2.53 | 9 |
| 21a | 0.10 | --- | --- | 0.020 | --- | --- |
| 21b | 0.20 | --- | --- | 0.029 | --- | --- |
| 21c | 0.10 | --- | --- | 0.020 | --- | --- |
| 21d | 0.20 | --- | --- | 0.025 | --- | --- |
| 21e | 0.20 | --- | --- | 0.017 | --- | --- |
| 21f | 0.20 | --- | --- | 0.013 | --- | --- |
| 21g | 0.20 | --- | --- | 0.011 | --- | --- |
| 21h | 0.20 | --- | --- | 0.019 | --- | --- |
| 21i | 0.20 | --- | --- | 0.031 | --- | --- |
| 22a | 0.10 | 8.00 | 80 | 0.019 | 2.21 | 116 |
| 22b | 0.20 | 8.00 | 40 | 0.028 | 2.23 | 80 |
| 22c | 0.10 | 8.00 | 80 | 0.018 | 2.20 | 122 |
| 22d | 0.20 | 8.00 | 40 | 0.022 | 2.24 | 102 |
| 22e | 0.20 | 8.00 | 40 | 0.015 | 2.10 | 140 |
| 22f | 0.20 | 8.00 | 40 | 0.011 | 2.09 | 190 |
| 22g | 0.20 | 8.00 | 40 | 0.017 | 2.05 | 121 |
| 22h | 0.20 | 8.00 | 40 | 0.029 | 2.01 | 69 |
| 22i | 0.30 | 8.00 | 27 | 0.038 | 1.96 | 52 |
| 22j | 0.15 | 8.00 | 53 | 0.028 | 1.98 | 71 |
| 22k | 0.15 | 8.00 | 53 | 0.030 | 1.96 | 65 |
| 22l | 0.15 | 8.00 | 53 | 0.025 | 1.96 | 78 |

EXAMPLE 24

5 To a suspension of 5 μm BEH porous hybrid particles (25 g, Waters Corporation, Milford, MA; 6.5%C; SSA = 190 m^2/g ; SPV = 0.80 cm^3/g ; APD = 155 \AA) of the formula $(\text{O}_{1.5}\text{SiCH}_2\text{CH}_2\text{SiO}_{1.5})(\text{SiO}_2)_4$ (prepared following the method described in U.S. Patent 6,686,035) in dry toluene (250 mL, Fisher Scientific) was added Component A, 2-(4-pyridylethyl)triethoxysilane (0.2182 g, 0.2 $\mu\text{mol}/\text{m}^2$, Gelest Inc., Morrisville, PA), before
10 following the general procedure for preparing propanol hybrid surrounded hybrid particles detailed in Example 17. Product 24a of this reaction incorporated a low level of ionizable

modifier during the formation of the propanol hybrid surrounded hybrid particles having 8.3 %C and 3.70 $\mu\text{mol}/\text{m}^2$ propanol groups.

EXAMPLE 25

5 A portion product 24a (16.2 g) from Example 24 was reacted with Component B, octadecyl isocyanate (7.51 g, 10 $\mu\text{mol}/\text{m}^2$), in a similar process detailed in Example 18, using hydrolysis C. The product of this reaction had 14.27 %C and 2.00 $\mu\text{mol}/\text{m}^2$ carbamate groups. This resulting product 25a had a molar charge ratio of B/A of 50.

10

EXAMPLE 26

The general procedure to prepare a propanol hybrid surrounded core material, detailed in Examples 17 are applied to different porous materials. Included in this are core materials detailed in Example 15.

15

EXAMPLE 27

Modification of the surface of these propanol hybrid surrounded core materials prepared in Example 26 with a component B hydrophobic group is accomplished using silane approaches detailed in Examples 1, 3 or 5 or with isocyanate approaches detailed in Examples 18.

Further modification of the surface of these materials with a component A ionizable 20 modifier is accomplished using silane approached detailed in Examples 19. Alternatively the surface propanol groups is reacted with ionizable modifying reagents of formula type I, or II where Z is isocyanate or 1-carbamoyl imidazole, following the approach detailed in Example 18. Preferred ionizable modifiers include 4-pyridyl alkylisocyanates, 3-pyridyl alkylisocyanates, 2-pyridyl alkylisocyanates, imidazole alkylisocyanates, 1-(N-(4-pyridyl 25 alkyl)carbamoyl)imidazole, 1-(N-(3-pyridyl alkyl)carbamoyl)imidazole, 1-(N-(2-pyridyl alkyl)carbamoyl)imidazole, and 1-(N-(imidazol-1-yl-alkyl)carbamoyl)imidazole.

Alternatively the surface propanol groups is reacted with ionizable modifying reagents of formula III where Z is isocyanate or 1-carbamoyl imidazole, following the approach detailed in Example 18. Preferred ionizable modifiers include acid-protected and acid-non-protected 30 versions of isocyanato-alkyl sulfonic acid, isocyanato-alkyl carboxylic acid, isocyanato-alkyl phosphoric acid, isocyanato-alkyl boronic acid, [(imidazole-1-carbonyl)-amino]-alkyl sulfonic acid, [(imidazole-1-carbonyl)-amino]-alkyl carboxylic acid, [(imidazole-1-carbonyl)-amino]-alkyl phosphoric acid, [(imidazole-1-carbonyl)-amino]-alkyl boronic acid, isocyanato-aryl sulfonic acid, isocyanato-aryl carboxylic acid, isocyanato-aryl phosphoric acid, isocyanato-aryl 35 boronic acid, [(imidazole-1-carbonyl)-amino]-aryl sulfonic acid, [(imidazole-1-carbonyl)-

amino]-aryl carboxylic acid, [(imidazole-1-carbonyl)-amino]-aryl phosphoric acid, [(imidazole-1-carbonyl)-amino]-aryl boronic acid, isocyanato-aryl alkyl sulfonic acid, isocyanato-aryl alkyl carboxylic acid, isocyanato-aryl alkyl phosphoric acid, isocyanato-aryl alkyl boronic acid, [(imidazole-1-carbonyl)-amino]-aryl alkyl sulfonic acid, [(imidazole-1-carbonyl)-amino]-aryl 5 alkyl carboxylic acid, [(imidazole-1-carbonyl)-amino]-aryl alkyl phosphoric acid, [(imidazole-1-carbonyl)-amino]-aryl alkyl boronic acid, isocyanato-alkyl aryl alkyl sulfonic acid, isocyanato-alkyl aryl alkyl carboxylic acid, isocyanato-alkyl aryl alkyl phosphoric acid, isocyanato-alkyl aryl alkyl boronic acid, [(imidazole-1-carbonyl)-amino]-alkyl aryl alkyl sulfonic acid, [(imidazole-1-carbonyl)-amino]-alkyl aryl alkyl carboxylic acid, [(imidazole-1-carbonyl)-amino]-alkyl aryl alkyl phosphoric acid, and [(imidazole-1-carbonyl)-amino]-alkyl aryl alkyl 10 boronic acid.

EXAMPLE 28

Modification of the surface of these propanol hybrid surrounded core materials as detailed 15 in Example 27, but the ionizable group is reacted before the hydrophobic group.

EXAMPLE 29

The general procedure to prepare a propanol hybrid surrounded core material having an ionizable group, detailed in Examples 24 is applied to different core materials. Included in this 20 are core materials detailed in Example 15. Modification of the surface of these propanol hybrid surrounded core materials with a hydrophobic group is accomplished using silane approaches detailed in Examples 1, 3 or 5 or is accomplished using isocyanate approaches detailed in Examples 18.

25

EXAMPLE 30

Further modification of the surface of materials prepared in Examples 27-29 is accomplished using approaches detailed in Examples 2, 4, 6, and 20 or surface propanol groups are future reacted with alkyl isocyanate or aryl isocyanates as detailed in Example 18.

30

EXAMPLE 31

The general approach to prepare a hybrid surrounded hybrid particle is used to prepare new hybrid surrounded materials that have reactive surface groups other than silanols and propanol groups, following a general approach detailed in Example 17 and 24, using core materials detailed in Example 15. When hybrid surfaces are prepared that have vinyl, haloalkyl, 35 aminoalkyl, epoxy or phenyl groups, different reactions are performed to attach the hydrophobic or ionizable modifier. Vinyl groups are modified using radical addition, metathesis, epoxidation

and hydrosilylation. Haloalkyl groups are modified by nucleophilic displacement and Grinard reactions. Aminoalkyl groups are reacted with acids, isocyanates or nucleophilic displacement. Epoxy groups are hydrolyzed to present surface alcohol groups, or reactions with amines. Phenyl groups are substituted with chloromethyl, sulfonic or nitro groups. Ionizable modifying 5 reagents of formula type I, II or III result where Z represents a chemically reactive group, including (but not limited to) a silane, silanol, ether, amine, alkylamine, dialkylamine, isocyanate, acyl chloride, triflate, isocyanate, thiocyanate, imidazole carbonate, 1-carbamoyl imidazole, NHS-ester, carboxylic acid, ester, epoxide, alkyne, alkene, azide, -Br, -Cl, or -I.

Further modifications of these materials is accomplished as detailed in Examples 27-30.

10

EXAMPLE 32

In a general procedure propanol surrounded particles containing an ionizable modifier are prepared in a multistep procedure. Products 3af-3ah from Example 3 are reacted with acetoxypropyltrichlorosilane in dry toluene using imidazole. The reaction is heated to reflux for 15 20 hours before cooling, filtering, and washing with toluene, 1:1 v/v acetone/water, and acetone. The material is refluxed acetone/aqueous 0.1 M ammonium bicarbonate (pH 10) solution for 20 hours at 50°C. The reaction is cooled and the product is filtered and is washed successively with toluene, 1:1 v/v acetone/water, and acetone. The product is then hydrolyzed in 1 N HCl for 20 hours at an elevated temperature. The reaction is cooled and the product is filter and is 20 washed with water and acetone. The product is dried at 80 °C under reduced pressure for 16 hours. Products prepared by this approach have surface pyridylethyl and propanol groups.

EXAMPLE 33

The general procedure to prepare a propanol hybrid surrounded core material using 25 acetoxypropyltrichlorosilane or a polyorganoalkoxy siloxane, having an initial modification with ionizable modifier is applied to different core materials. Included in this are core materials detailed in Example 15. The modification of these core materials with an ionizable modifier is accomplished using silane approaches detailed in Examples 1, 3 or 5, or is accomplished using ionizable modifying reagents of formula I, II or III detailed in Example 15. The general 30 approach to modify core materials with acetoxypropyltrichlorosilane is detailed in Example 33. The general approach to modify core materials with acetoxypropyltrichlorosilane is detailed in Example 17.

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EXAMPLE 34

Acetoxypropyltrimethoxysilane (323 g, Gelest Inc., Morrisville, PA) was mixed with 2-(4-pyridylethyl)triethoxysilane (13.04 g, Gelest Inc., Morrisville, PA), ethanol (218 g, anhydrous, J.T. Baker, Phillipsburgh, NJ) and an aqueous solution of 2.2 M Acetic Acid (26 g, 5 J.T. Baker, Phillipsburgh, NJ) in a flask. The resulting solution was agitated and refluxed for 16 hours in an atmosphere of argon or nitrogen. Alcohol was removed from the flask by distillation at atmospheric pressure. Residual alcohol and volatile species were removed by heating at 110 °C for 5 hours in a sweeping stream of argon or nitrogen. The resulting polyorganoalkoxy siloxane, Product 34a, was a clear viscous liquid had a viscosity of 27 cP.

10

EXAMPLE 35

In a general procedure, propanol hybrid surrounded core materials containing an ionizable modifier are prepared by a multistep procedure where Product 34a from Example 34 is used in place of the polyorganoalkoxy siloxane in Example 17.

15

Alternatively this general procedure to prepare add the ionizable modifier before the preparation of the propanol hybrid surrounded core material is applied to different core materials. Included in this are core materials detailed in Example 15.

EXAMPLE 36

20

Modification of the surface of materials prepared in Examples 31-33 and 35 with a hydrophobic group is accomplished using silane approaches detailed in Examples 1, 3 or 5 or with isocyanate approaches detailed in Examples 18.

EXAMPLE 37

25

Secondary surface modification of materials prepared in Examples 36 is accomplished using approaches detailed in Examples 2, 4, 6, and 20 or with isocyanate approaches detailed in Examples 18

EXAMPLE 38

30

Products prepared in Examples 15, 16, 19-22, 24-25, 27-33, and 35-37 are chromatographically evaluated as detailed in Examples 9-14. Concentration of ionizable modifier are determined as detailed in Example 23.

EXAMPLE 39

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Samples of porous particles from Product 4aa and a 3 µm commercially available C₁₈ column were evaluated for changes in retention of ionized analytes when exposed to mobile

phases of different pH. The 2.1 x 50 mm chromatographic columns were packed using a slurry packing technique. The chromatographic system consisted of an ACQUITY UPLC® System and an ACQUITY UPLC® Tunable UV detector. Empower Chromatography Data Software (Build 1154) was used for data collection and analysis; injection volume 2 μ L; flow rate: 0.8 mL/min; temperature: 30 °C; detection: 260 nm; analytes: metoprolol and amitriptyline. Data were compared before (initial) and after (final) 7 cycles; each cycle included alternately 7 injections in a 0.1 % formic acid/acetonitrile gradient followed by 17 injections in a 10 mM ammonium bicarbonate (pH 10)/acetonitrile gradient. Both acidic and pH 10 gradients ran from 5 to 95 % acetonitrile in 2.5 minutes.

As shown in Figure 1, changes in retention of ionized analytes when exposed to mobile phases of different pH is a problem that is known in the art. The commercially available C₁₈ column experienced a 7% change in retention for amitriptyline, while Product 4aa experienced a 0.4% change in retention for amitriptyline under these conditions. While not limited to theory, it has been proposed that slow surface equilibration is to blame. Because conventional high-purity reversed-phase columns have much reduced surface charge at low pH, very small changes in surface charge may cause a large change in retention for ionized analytes. This effect is exacerbated by the use of low-ionic-strength mobile phases. The change in selectivity is not due to loss of bonded phase because the change is reversible, and no loss of retention is observed for neutral analytes. Storage and/or equilibration of columns in the low-pH mobile phase (allowing time for diffusion) will eventually return them to their original selectivity. This slow equilibration does not occur at elevated pH because of the relatively high concentration of deprotonated silanols.

These data indicate that, unlike the commercially available C₁₈ column, Product 4aa can be used in method development screens of high and low pH gradient conditions with the assurance that the method will work on an unused column.

EXAMPLE 40

Similar to Example 13, samples of porous particles from Example 4j and a commercially available C₁₈ column were used for the separation of a mixture of neutral and basic compounds. The basic test mix prepared included uracil, metoprolol tartate, labetalol, amitriptyline and the neutral test mix included uracil, prednisone, caffeine. The comparison C₁₈ column was commercially available and contained 3.5 μ m porous hybrid particles of the formula (O_{1.5}SiCH₂CH₂SiO_{1.5})(SiO₂)₄ that was surface modified with ODTCS followed by endcapping.

As shown in Figure 2, the results for Product 4j has drastic improvements in peak shape for basic analytes under these conditions, compared to the comparison C₁₈ column that did not

have any ionizable modifier added. This great improvement can also be demonstrated in improved peak capacities, as detailed in Example 13.

EXAMPLE 41

5 Samples of porous particles from Example 2 were evaluated for isocratic loading behavior for amitriptyline. The 4.6 x 150 mm chromatographic columns were packed using a slurry packing technique. The chromatographic system consisted of an Alliance HPLC® System and a Waters 996 PDA detector. Empower 2 Chromatography Data Software (Build 2154) was used for data collection and analysis; injection volume 20 µL; flow rate: 1.0 mL/min;
10 temperature: 30 °C; detection: 230 nm; analyte: amitriptyline (prepared 60 µg/mL in mobile phase) loading range: 0.3 µg - 1.2 µg analyte on column; mobile phase: 0.05% TFA in 40 % acetonitrile.

Deterioration of peak shape of basic analytes with increasing loading concentration is a well known problem for separations performed on HPCM at low pH. The effect of surface 15 charge on peak profiles can be observed, as shown in Figure 3, by comparing the change in peak profiles with increasing analyte concentration for Products 2b, 2d, and 2e. Product 2e has a high level of ionizable modifier shows fronting/Anti-Langmuirian peak shape suggesting a concave Langmuirian isotherm; (b) Product 2d has an optimal level of ionizable modifier shows nearly symmetrical Gaussian/linear peak shape suggesting a linear Langmuirian isotherm; (c) 20 Product 2b has a very low level of ionizable modifier shows tailing/Bi-Langmuirian peak shape suggesting a convex Langmuirian isotherm. The importance of maintaining good peak shape with increased analyte loading is well known in the art. Product 2d has an optimized surface charge to give high efficiencies for loads that far exceed those attainable on ordinary reversed-phase columns.

25

EXAMPLE 42

Samples of porous particles from Product 4aa and a 3 µm commercially available C₁₈ column were evaluated for isocratic loading behavior for amitriptyline. The 2.1 x 50 mm chromatographic columns were packed using a slurry packing technique. The chromatographic 30 system consisted of an ACQUITY UPLC® System and an ACQUITY UPLC® Tunable UV detector. Empower Chromatography Data Software (Build 1154) was used for data collection and analysis; injection volume 1.5 µL; flow rate: 0.2 mL/min; temperature: 30 °C; detection: 260 nm; analyte: amitriptyline loading range: 0.05 µg – 6.0 µg analyte on column; mobile phase: 0.05% TFA in 39% (for Commercially Available 3 µm C₁₈ Column) or 37% (Product 35 4aa) acetonitrile. It is clear, as shown in Figure 4, that Product 4aa maintains nearly linear-

isotherm behavior for amitriptyline at mass loads that approach those used in purification applications.

EXAMPLE 43

5 BEH porous hybrid particles (20 g, Waters Corporation, Milford, MA; 4.0 μm , 6.78 %C; SSA = 183 m^2/g ; SPV = 0.70 cm^3/g ; APD = 139 \AA) of the formula $(\text{O}_{1.5}\text{SiCH}_2\text{CH}_2\text{SiO}_{1.5})(\text{SiO}_2)_4$ (prepared following the method described in U.S. Patent 6,686,035) was slurried in water (60 mL) for addition of 3-(trihydroxysilyl)propyl sulfuric acid (6 g, 50% solution). The solution was heated at 90 $^{\circ}\text{C}$ for 20 hours. The reaction was cooled 10 and the product was filtered and washed with water and acetone. The product was then dried at 70 $^{\circ}\text{C}$ under a reduced pressure for 16 hours. The product had 7.29% C and an ion-exchange capacity of 0.160 mequiv/g by titration after subtracting the silanol contribution of an unbonded BEH particle. The surface coverage was determined by the difference in particle %C before and after the surface modification as measured by elemental analysis to be 1.01 $\mu\text{mol}/\text{m}^2$.

15

EXAMPLE 44

Superficially porous silica particles (20 g, 1.3 μm , SSA = 90-205 m^2/g ; SPV = 0.1-0.3 cm^3/g ; APD = 80-130 \AA) are reacted in a similar manner as detailed in Example 3 to yield a C₁₈ bonded material that has an optimal concentration of an ionizable modifier, such as 4PE or 20 APTES. This material (product 43a) is endcapped as detailed in Example 4, and evaluated as detailed in Examples 9-14, 41 and 42. The materials are evaluated as detailed in Examples 9-14, 41 and 42 and are compared to similar materials that do have the addition of the Component A ionizable modifier.

25

EXAMPLE 45

The process of Example 44 is performed using Superficially porous silica particles having a particle size of 0.3-2.0 μm . The materials are evaluated as detailed in Examples 9-14, 41 and 42.

30

EXAMPLE 46

The process of Example 44 is performed using Superficially porous silica particles having a particle size of 2-3 μm . The materials are evaluated as detailed in Examples 9-14, 41 and 42.

35

EXAMPLE 47

The process of Example 44 is performed using Superficially porous silica particles having a particle size greater than 3 μm . The materials are evaluated as detailed in Examples 9-14, 41 and 42.

5

EXAMPLE 48

The process of Examples 44-47 are performed using a C₄-C₁₂, C₃₀, embedded polar, chiral, phenylalkyl, or pentafluorophenyl bonding and coatings in place of C₁₈ bonding. The materials are evaluated as detailed in Examples 9-14, 41 and 42.

10

EXAMPLE 49

The process of Examples 44-48 are performed without the endcapping step prior to characterization. The materials are evaluated as detailed in Examples 9-14, 41 and 42.

15

EXAMPLE 50

BEH porous hybrid particles (2.9 μm , Waters Corporation, Milford, MA; 6.38%C; SSA = 86 m^2/g ; SPV = 0.68 cm^3/g ; APD = 297 \AA) of the formula (O_{1.5}SiCH₂CH₂SiO_{1.5})(SiO₂)₄ (prepared following the method described in U.S. Patent 6,686,035) were refluxed in toluene (9 20 mL/g, Fisher Scientific, Fairlawn, NJ) using a Dean-Stark trap for 2 hours. Upon cooling the Component A silane additive 2-(4-pyridylethyl)triethoxysilane was added and the reaction was heated to reflux for 1 hour. Upon cooling, imidazole (Aldrich, Milwaukee, WI) and the Component B silane tert-butyldimethylchlorosilane (TBDMCS, Gelest Inc., Morrisville, PA) or octadecyltrichlorosilane (ODTCS, Gelest Inc., Morrisville, PA) was added. The reaction was 25 then heated to reflux for 20 hours. The reaction was then cooled and the product was filtered and washed successively with water, toluene, 1:1 v/v acetone/water and acetone (all solvents from J.T. Baker) and then was hydrolyzed as detailed in Example 3, hydrolysis type C. The product was filtered and washed successively with toluene, 1:1 v/v acetone/water, and acetone. The product was dried at 70°C under reduced pressure for 16 hours. Reaction data are listed in 30 Table 20. The surface coverage of these products was determined by the difference in particle %C before and after the surface modification as measured by elemental analysis. Product 50b was further endcapped as detailed in Example 4 to yield a final carbon content of 10.52 %C.

35

Table 20

| | | Component A | Component B | | | | |
|---------|---------------|---------------------|----------------|--------------------|----------|------|---|
| Product | Particles (g) | Silane Additive (g) | Primary Silane | Primary Silane (g) | Base (g) | %C | Surface Coverage ($\mu\text{mol}/\text{m}^2$) |
| 50a | 20 | 0.139 | TBDMC S | 2.6 | 1.4 | 7.74 | 2.50 |
| 50b | 15 | 0.104 | ODTCS | 0.9 | 0.3 | 9.50 | 1.95 |

EXAMPLE 51

5 Superficially porous silica particles (1.35 μm , SSA = 55 m^2/g ; SPV = 0.15 cm^3/g ; APD = 107 \AA , 1.2 μm non-porous core, 0.1 μm thick porous shell) were refluxed in toluene (9 mL/g, Fisher Scientific, Fairlawn, NJ) using a Dean-Stark trap for 2 hours. Upon cooling a Component A ionizable modifier 2-(4-pyridylethyl)triethoxysilane (4PE, Gelest Inc., Morrisville, PA) was added for product 51a and reaction was heated to reflux for 1 hour before 10 cooling. No Component A ionizable modifier was added for product 51b. Imidazole (Aldrich, Milwaukee, WI) and octadecyltrichlorosilane (ODTCS, Gelest Inc., Morrisville, PA) were added. The reaction was then heated to reflux for 20 hours. The reaction was then cooled and the product was filtered and washed successively with water, toluene, 1:1 v/v acetone/water and acetone (all solvents from J.T. Baker) and then was hydrolyzed as detailed in Example 3, 15 hydrolysis type C. The product was filtered and washed successively with toluene, 1:1 v/v/ acetone/water, and acetone. The product was dried at 70°C under reduced pressure for 16 hours. Reaction data are listed in Table 21. The surface coverage of these products was determined by the difference in particle %C before and after the surface modification as measured by elemental analysis. These products were further endcapped as detailed in Example 20 4.

Table 21

| Product | Particles (g) | 4PE (g) | ODTCS (g) | Base (g) | %C | Surface Coverage ($\mu\text{mol}/\text{m}^2$) | Final %C |
|---------|---------------|---------|-----------|----------|------|---|----------|
| 51a | 15 | 0.067 | 0.74 | 0.26 | 2.84 | 2.42 | 3.37 |
| 51b | 15 | --- | 0.74 | 0.26 | 3.31 | 2.86 | 3.73 |

25

EXAMPLE 52

Following the protocol detailed in Example 13, peak capacity comparisons were made for Products 51a and 51b, as detailed in Table 22. The determination of peak capacity and the problems caused by poor peak shape and resulting poor peak capacities for basic analytes in low 30 pH gradient separations is well known in the field of HPLC and UPLC. Increased peak capacity

ratios correlate with improved performance for basic analytes under these test conditions. Products 51a and 51b have the same feed material and were both similarly bonded, the only difference between these materials is the inclusion of the Component A ionizable modifier for product 51a. Improvements in peak capacity ratios were obtained for Product 51a over 51b, 5 which is due to the introduction of the Component A ionizable modifier.

TABLE 22

| Product | A Amitriptyline Pc | B Prednisone Pc | Ratio A/B |
|---------|--------------------------|-----------------------|--------------|
| 51a | 126 | 204 | 0.62 |
| 51b | 45 | 184 | 0.24 |

10

EXAMPLE 53

Porous silica particles are hybrid coated, C₁₈-bonded and are endcapped in a process similar to the one detailed in US Patent 7,563,367B to yield product 53a. Alternatively, an ionizable modifier reagent, Component A (as detailed in Example 15) is added at different 15 points in this process. Product 53b introduced the Component A additive before hybrid coating. Product 53c introduces the Component A additive before C₁₈-bonding. Product 53d introduces the Component A additive before endcapping. Product 53e introduces the Component A additive after endcapping. The materials are evaluated as detailed in Examples 9-14, 41 and 42.

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EXAMPLE 54

Superficially porous silica particles are hybrid coated, C₁₈-bonded and are endcapped in a process similar to the one detailed in US Patent 7,563,367B to yield product 54a. Alternatively, an ionizable modifier, Component A (as detailed in Example 15) is added at 25 different points in this process. Product 54b introduced the Component A additive before hybrid coating. Product 54c introduces the Component A additive before C₁₈-bonding. Product 54d introduces the Component A additive before endcapping. Product 54e introduces the Component A additive after endcapping. The materials are evaluated as detailed in Examples 9-14, 41 and 42.

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EXAMPLE 55

BEH porous hybrid particles (4.0 μ m, 25 g, Waters Corporation, Milford, MA; 6.78%C; SSA = 183 m²/g; SPV = 0.70 cm³/g; APD = 139 \AA) of the formula (O_{1.5}SiCH₂CH₂SiO_{1.5})(SiO₂)₄ 35 (prepared following the method described in U.S. Patent 6,686,035) were refluxed in toluene

(375 mL, Fisher Scientific, Fairlawn, NJ) using a Dean-Stark trap. Upon cooling the zirconium n-propoxide (70% in n-propanol, 4.28 g, Gelest Inc., Morrisville, PA) was added and the reaction was stirred at ambient temperature for an hour and then heated to reflux overnight. The reaction was then cooled and the product was filtered and washed successively with toluene and 5 1% formic acid, and then was hydrolyzed in 1% formic acid for 1.5 hours at ambient temperature. Product 55a was filtered and washed with copious amounts of water and acetone. The product was dried at 80 °C under reduced pressure for 16 hours.

EXAMPLE 56

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Product 55a is further modified as detailed in Examples 1-8 and 15. The materials are evaluated as detailed in Examples 9-14, 41 and 42.

EXAMPLE 57

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The process of Examples 1, 3, 5, 7, 8, 15, 19, 21, 24, 27-29, 31-33, 35, 43-51, 53-55 are performed by using one or more ionizable modifiers selected from the group (not limited to) alkoxides, halides, salts and complexes of zirconium, aluminum, cerium, iron, titanium, and other ionizable or amphoteric groups. These products are endcapped as detailed in Example 4.

20

The materials are evaluated as detailed in Examples 9-14, 41 and 42.

EXAMPLE 58

25

A chromatographic column containing a packed bed of 1-5 µm chromatographic material that is C₁₈-bonded is evaluated as detailed in Examples 9-14, 41 and 42. This column is then flushed through with a dilute solution of a Component A, ionizable modifier in a suitable solvent for an extended time period to allow for incorporation of the ionizable modifier on the chromatographic bed. Examples of ionizable modifiers are included in Example 15 and 57. The column is further washed with a suitable solvent and is evaluated as detailed in Examples 9-14, 41 and 42.

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EXAMPLE 59

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C₁₈-bonded and endcapped 1-5 µm chromatographic materials are modified with a Component A, ionizable modifier. Examples of chromatographic materials are included in Example 15. Examples of ionizable modifiers are included in Example 15 and 57. The materials are evaluated as detailed in Examples 9-14, 41 and 42 and are compared to the C₁₈-bonded and endcapped material that does not contain an ionizable modifier.

EXAMPLE 60

The process of Example 58 and 59 are performed on superficially porous materials. Evaluations are performed as detailed in Examples 9-14, 41 and 42.

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EXAMPLE 61

The process of Example 58-60 are preformed on chromatographic materials that are C₄-C₁₂, C₃₀, embedded polar, chiral, phenylalkyl, or pentafluorophenyl bonding and coatings in place of C₁₈ bonding. Evaluations are performed as detailed in Examples 9-14, 41 and 42.

10 **Incorporation By Reference**

The entire contents of all patents published patent applications and other references cited herein are hereby expressly incorporated herein in their entireties by reference.

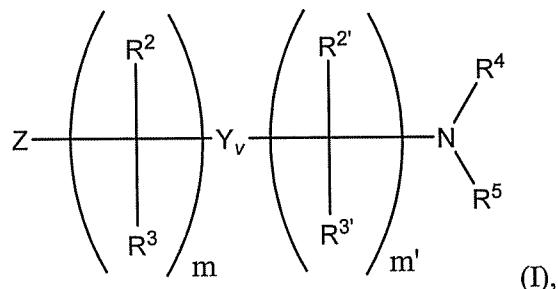
Equivalents

15

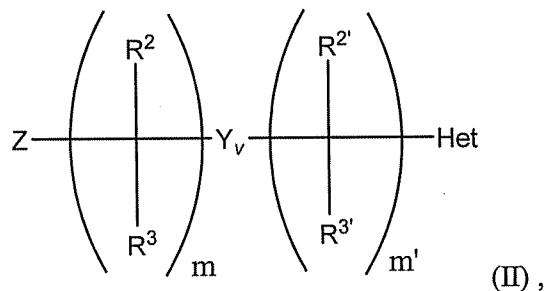
Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

What is Claimed is:

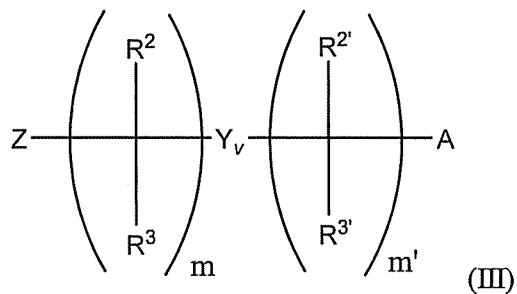
1. A high purity chromatographic material comprising a chromatographic surface wherein the chromatographic surface comprises a hydrophobic surface group and one or more ionizable modifiers with the proviso that when the ionizable modifier does not contain a Zwitterion, the ionizable modifier does not contain a quaternary ammonium ion moiety.
2. The high purity chromatographic material of Claim 1 further comprising a chromatographic core material.
3. The high purity chromatographic material of Claim 1, wherein the ionizable modifier contains a carboxylic acid group, a sulfonic acid group, an arylsulfonich group, a phosphoric acid group, a boronic acid group, an amino group, an imido group, an amido group, a pyridyl group, an imidazolyl group, an ureido group, a thionyl-ureido group or an aminosilane group.
4. The high purity chromatographic material of Claim 1, wherein the ionizable modifier is obtained from an ionizable modifying reagent selected from groups having the formula (I)



the formula (II):



20 the formula (III):



or a combination thereof

wherein

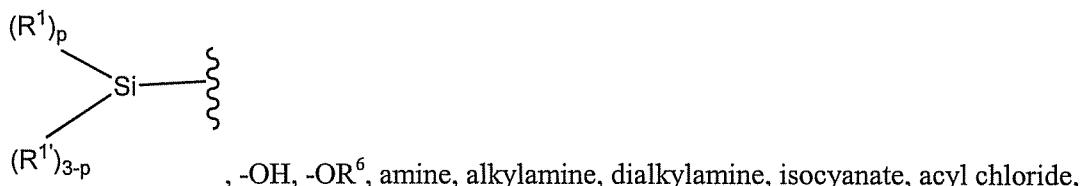
m is an integer from 1-8;

5 v is 0 or 1;

when v is 0, m' is 0;

when v is 1, m' is an integer from 1-8;

Z represents a chemically reactive group, including (but not limited to)



Y is an embedded polar functionality;

each occurrence of R¹ independently represents a chemically reactive group on silicon, including (but not limited to) -H, -OH, -OR⁶, dialkylamine, triflate, Br, Cl, I, vinyl, alkene, or -

15 $(CH_2)_{m''}Q$;

each occurrence of Q is -OH, -OR⁶, amine, alkylamine, dialkylamine, isocyanate, acyl chloride, triflate, isocyanate, thiocyanate, imidazole carbonate, NHS-ester, carboxylic acid, ester, epoxide, alkyne, alkene, azide, -Br, -Cl, or -I;

m'' is an integer from 1-8

20 p is an integer from 1-3;

each occurrence of R^{1'} independently represents F, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl, fluoroalkyl, or fluoroaryl;

each occurrence of R², R^{2'}, R³ and R^{3'} independently represents hydrogen, C₁-C₁₈ alkyl, 5 C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₂-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₄-C₁₈ heteroaryl, -Z, or a group having the formula -Si(R')_bR["]_a or -C(R')_bR["]_a;

a and b each represents an integer from 0 to 3 provided that a + b = 3;

R' represents a C₁ - C₆ straight, cyclic or branched alkyl group;

R" is a functionalizing group selected from the group consisting of alkyl, alkenyl, 10 alkynyl, aryl, cyano, amino, diol, nitro, ester, a cation or anion exchange group, an alkyl or aryl group containing an embedded polar functionality and a chiral moiety.

R⁴ represents hydrogen, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl;

R⁵ represents hydrogen, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, 15 C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl;

each occurrence of R⁶ independently represents C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, C₂-C₁₈ alkynyl, C₃-C₁₈ cycloalkyl, C₁-C₁₈ heterocycloalkyl, C₅-C₁₈ aryl, C₅-C₁₈ aryloxy, or C₁-C₁₈ heteroaryl;

Het represents a heterocyclic or heteroaryl ring system comprising at least one nitrogen 20 atom; and

A represents an acidic ionizable modifier moiety or a dual charge ionizable modifier moiety.

5. The high purity chromatographic material of Claim 4, wherein the ionizable modifying reagent has the formula I.
- 25 6. The high purity chromatographic material of Claim 4, wherein the ionizable modifying reagent has the formula II.
7. The high purity chromatographic material of Claim 4, wherein the ionizable modifying reagent has the formula III.

8. The high purity chromatographic material of Claim 4, wherein m is 2 or 3.
9. The high purity chromatographic material of Claim 4, wherein R¹ represents Cl.
10. The high purity chromatographic material of Claim 4, wherein R¹ represents -OH.
11. The high purity chromatographic material of Claim 4, wherein R¹ represents
5 dialkylamino, methoxy or ethoxy.
12. The high purity chromatographic material of Claim 4, wherein R^{1'} represents methyl.
13. The high purity chromatographic material of Claim 4, wherein R^{1'} represents ethyl.
14. The high purity chromatographic material of Claim 4, wherein R^{1'} represents isobutyl.
15. The high purity chromatographic material of Claim 4, wherein R^{1'} represents isopropyl.
- 10 16. The high purity chromatographic material of Claim 4, wherein R^{1'} represents tert-butyl.
17. The high purity chromatographic material of Claim 4, wherein each occurrence of R²
and R³ represents hydrogen.
18. The high purity chromatographic material of Claim 4, wherein each of R⁴ and R⁵
represents hydrogen.
- 15 19. The high purity chromatographic material of Claim 4, wherein Het is pyridyl,
pyrimidinyl, pyridazinyl, pyrazinyl, piperidinyl, piperazinyl, hexahdropyrimidinyl,
pyrrolyl, pyrazolyl, imidazolyl, pyrrolidinyl, pyrazolidinyl, imidazolidinyl or triazinyl.
- 20 20. The high purity chromatographic material of Claim 4, wherein the ionizable modifying
reagent is aminopropyltriethoxysilane, aminopropyltrimethoxysilane, 2-(2-
(trichlorosilyl)ethyl)pyridine, 2-(2-(trimethoxy)ethyl)pyridine, 2-(2-
(triethoxy)ethyl)pyridine, 2-(4-pyridylethyl)triethoxysilane, 2-(4-
pyridylethyl)trimethoxysilane, 2-(4-pyridylethyl)trichlorosilane,
chloropropyltrimethoxysilane, chloropropyltrichlorosilane, chloropropyltrichlorosilane,
chloropropyltriethoxysilane, imidazolylpropyltrimethoxysilane,
imidazolylpropyltriethoxysilane, imidazolylpropyl trichlorosilane, sulfopropyltrisilanol,
carboxyethylsilanetriol, 2-(carbomethoxy)ethylmethyldichlorosilane, 2-
(carbomethoxy)ethyltrichlorosilane, 2-(carbomethoxy)ethyltrimethoxysilane, n-
25 (trimethoxysilylpropyl)ethylenediamine triacetic acid, (2-

diethylphosphatoethyl)triethoxysilane, 3-mercaptopropyltriethoxysilane, 3-mercaptopropyltrimethoxysilane, bis[3-(triethoxysilyl)propyl]disulfide, bis[3-(triethoxysilyl)propyl]tetrasulfide, 2,2-dimethoxy-1-thia-2-silacyclopentane, bis(trichlorosilylethyl)phenylsulfonyl chloride, 2-

5 (chlorosulfonylphenyl)ethyltrichlorosilane, 2-(chlorosulfonylphenyl)ethyltrimethoxysilane, 2-(ethoxysulfonylphenyl)ethyltrimethoxysilane, 2-(ethoxysulfonylphenyl)ethyltrimethoxysilane, 2-(ethoxysulfonylphenyl)ethyltrichlorosilane, sulphonic acid phenethyltrisilanol, 10 (triethoxysilyl ethyl)phenyl phosphonic acid diethyl ester, (trimethoxysilyl ethyl)phenyl phosphonic acid diethyl ester, (trichlorosilyl ethyl)phenyl phosphonic acid diethyl ester, phosphonic acid phenethyltrisilanol, N-(3-trimethoxysilylpropyl)pyrrole, N-(3-triethoxysilylpropyl)-4,5-dihydroimidazole, bis(methyldimethoxysilylpropyl)-N-methylamine, tris(triethoxysilylpropyl)amine, bis(3-trimethoxysilylpropyl)-N-methylamine, (N,N-diethyl-3-aminopropyl)trimethoxysilane, N-(hydroxyethyl)-N-methylaminopropyltrimethoxysilane, 3-(N,N-dimethylaminopropyl)trimethoxysilane, bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane, N,N'-bis(hydroxyethyl)-N,N'-bis(trimethoxysilylpropyl)ethylenediamine, or N,N-dimethyl-3-aminopropylmethyldimethoxysilane.

15

20 21. The high purity chromatographic material of Claim 1 wherein the ratio of the hydrophobic surface group : ionizable modifier is from about 2.5:1 to about 350:1.

22. The high purity chromatographic material of Claim 1 wherein the ratio of the hydrophobic surface group : ionizable modifier is from about 3:1 to about 200:1.

23. The high purity chromatographic material of Claim 1 wherein the ratio of the hydrophobic surface group : ionizable modifier is from about 4:1 to about 35:1.

25

26. The high purity chromatographic material of Claim 1 wherein the concentration of ionizable modifier is less than about 0.5 $\mu\text{mol}/\text{m}^2$.

27. The high purity chromatographic material of Claim 1 wherein the concentration of ionizable modifier is less than about 0.4 $\mu\text{mol}/\text{m}^2$.

30 28. The high purity chromatographic material of Claim 1 wherein the concentration of ionizable modifier is less than about 0.3 $\mu\text{mol}/\text{m}^2$.

27. The high purity chromatographic material of Claim 1 wherein the concentration of ionizable modifier is from about 0.01 $\mu\text{mol}/\text{m}^2$ to about 0.5 $\mu\text{mol}/\text{m}^2$.
28. The high purity chromatographic material of Claim 1 wherein the concentration of ionizable modifier is from about 0.01 $\mu\text{mol}/\text{m}^2$ to about 0.4 $\mu\text{mol}/\text{m}^2$.
- 5 29. The high purity chromatographic material of Claim 1 wherein the concentration of ionizable modifier is from about 0.03 $\mu\text{mol}/\text{m}^2$ to about 0.3 $\mu\text{mol}/\text{m}^2$.
30. The high purity chromatographic material of Claim 1 wherein the hydrophobic surface group is a C4 to C30 bonded phase, an aromatic, a phenylalkyl, a fluoro-aromatic, a phenylhexyl, a pentafluorophenylalkyl, or a chiral bonded phase.
- 10 31. The high purity chromatographic material of Claim 1 wherein the hydrophobic surface group is a C18 bonded phase.
32. The high purity chromatographic material of Claim 1 wherein the hydrophobic surface group is an embedded polar bonded phase.
- 15 33. The high purity chromatographic material of Claim 1 wherein the chromatographic core is a silica material
34. The high purity chromatographic material of Claim 1 wherein the chromatographic core is a hybrid inorganic/organic material.
35. The high purity chromatographic material of Claim 1 wherein the chromatographic core is a superficially porous material.
- 20 36. The high purity chromatographic material of Claim 1 wherein the chromatographic core is a an inorganic material with a hybrid surface layer, a hybrid material with an inorganic surface layer, or a hybrid material with a different hybrid surface layer.
37. The high purity chromatographic material of Claim 1 wherein the material is in the form of a particle.
- 25 38. The high purity chromatographic material of Claim 1 wherein the material is in the form of a monolith.
39. The high purity chromatographic material of Claim 1 wherein the material is in the form of a superficially porous material.

40. The high purity chromatographic material of Claim 1 wherein the material does not have chromatographically enhancing pore geometry.
41. The high purity chromatographic material of Claim 1 wherein the material has chromatographically enhancing pore geometry.
- 5 42. The high purity chromatographic material of Claim 40 or 41, wherein said chromatographic material has a surface area of about 25 to 1100 m²/g.
43. The high purity chromatographic material of Claim 42, wherein said chromatographic material has a surface area of about 80 to 500 m²/g.
- 10 44. The high purity chromatographic material of Claim 43, wherein said chromatographic material has a surface area of about 120 to 330 m²/g.
45. The high purity chromatographic material of Claim 40 or 41, wherein said chromatographic material has a pore volume of about 0.15 to 1.7 cm³/g.
46. The high purity chromatographic material of Claim 45, wherein said chromatographic material has a pore volume of about 0.5 to 1.3 cm³/g.
- 15 47. The high purity chromatographic material of Claim 40 or 41, wherein said chromatographic material has a micropore surface area of less than about 110 m²/g.
48. The high purity chromatographic material of Claim 47, wherein said chromatographic material has a micropore surface area of less than about 105 m²/g.
49. The high purity chromatographic material of Claim 47, wherein said chromatographic material has a micropore surface area of less than about 80 m²/g.
- 20 50. The high purity chromatographic material of Claim 47, wherein said chromatographic material has a micropore surface area of less than about 50 m²/g.
51. The high purity chromatographic material of Claim 40 or 41, wherein said chromatographic material has an average pore diameter of about 20 to 1500 Å.
- 25 52. The high purity chromatographic material of Claim 51, wherein said chromatographic material has an average pore diameter of about 50 to 1000 Å.
53. The high purity chromatographic material of Claim 52, wherein said chromatographic material has an average pore diameter of about 100 to 750 Å.

54. The high purity chromatographic material of Claim 53, wherein said chromatographic material has an average pore diameter of about 110 to 500Å.
55. The high purity chromatographic material of Claim 1, wherein said chromatographic material is hydrolytically stable at a pH of about 1 to about 14.
- 5 56. The high purity chromatographic material of Claim 55, wherein said chromatographic material is hydrolytically stable at a pH of about 10 to about 14.
57. The high purity chromatographic material of Claim 55, wherein said chromatographic material is hydrolytically stable at a pH of about 1 to about 5.
- 10 58. The high purity chromatographic material of Claim 1, wherein the material has been surface modified.
59. The high purity chromatographic material of Claim 58, wherein the material has been surface modified by coating with a polymer.
- 15 60. The high purity chromatographic material of Claim 58, wherein the material has been surface modified by coating with a polymer by a combination of organic group and silanol group modification
61. The high purity chromatographic material of Claim 58, wherein the material has been surface modified by a combination of organic group modification and coating with a polymer.
- 20 62. The high purity chromatographic material of Claim 58, wherein the material has been surface modified by a combination of silanol group modification and coating with a polymer.
63. The high purity chromatographic material of Claim 58, wherein the material has been surface modified via formation of an organic covalent bond between the material's organic group and the modifying reagent.
- 25 64. The high purity chromatographic material of Claim 58, wherein the material has been surface modified by a combination of organic group modification, silanol group modification and coating with a polymer.
65. The high purity chromatographic material of Claim 1, further comprising a nanoparticle dispersed within the material.

66. The high purity chromatographic material of Claim 65, wherein nanoparticle is a mixture of more than one nanoparticle.
67. The high purity chromatographic material of Claim 65, wherein the nanoparticle is present in <20% by weight of the nanocomposite.
- 5 68. The high purity chromatographic material of Claim 65, wherein the nanoparticle is present in <5% by weight of the nanocomposite.
69. The high purity chromatographic material of Claim 65, wherein the nanoparticle is crystalline or amorphous.
70. The high purity chromatographic material of Claim 65, wherein the nanoparticle is a substance which comprises one or more moieties selected from the group consisting of silicon carbide, aluminum, diamond, cerium, carbon black, carbon nanotubes, zirconium, barium, cerium, cobalt, copper, europium, gadolinium, iron, nickel, samarium, silicon, silver, titanium, zinc, boron, oxides thereof, and nitrides thereof.
- 10 71. The high purity chromatographic material of Claim 65, wherein the nanoparticle is a substance which comprises one or more moieties selected from the group consisting of nano-diamonds, silicon carbide, titanium dioxide, cubic-boronitride.
- 15 72. The high purity chromatographic material of Claim 65, wherein the nanoparticles are less than or equal to 200 nm in diameter.
73. The high purity chromatographic material of Claim 65, wherein the nanoparticles are less than or equal to 100 nm in diameter.
- 20 74. The high purity chromatographic material of Claim 65, wherein the nanoparticles are less than or equal to 50 nm in diameter.
75. The high purity chromatographic material of Claim 65, wherein the nanoparticles are less than or equal to 20 nm in diameter.
- 25 76. A method for preparing a high purity chromatographic material according to Claim 2 comprising the steps of:
 - a. reacting a chromatographic core with ionizable modifying reagent to obtain a ionizable modified material; and

b. reacting the resultant ionizable material with a hydrophobic surface modifying group.

77. A method for preparing a chromatographic material according to Claim 2 comprising the steps of:

5 a. reacting a chromatographic core with hydrophobic surface modifying group to obtain a bonded material; and

b. reacting the resultant bonded material with an ionizable modifying reagent.

78. The method of Claim 76 or 77 further comprising the step of endcapping any remaining silanol groups.

10 79. The method of Claim 76 or 77 wherein the steps are performed simultaneously.

80. The method of Claim 76 or 77 further comprising the step of surface modifying the resulting material.

81. The method of Claim 76 or 77 further comprising subjecting the resulting chromatographic material to hydrothermal treatment.

15 82. A separations device having a stationary phase comprising the high purity chromatographic material of any one of Claims 1-75 or 90 or 91.

83. The separations device of Claim 82, wherein said device is selected from the group consisting of chromatographic columns, thin layer plates, filtration membranes, microfluidic separation devices, sample cleanup devices, solid supports, microchip separation devices, and microtiter plates.

20 84. The separations device of Claim 82, wherein the separations device is useful for applications selected from the group consisting of solid phase extraction, high pressure liquid chromatography, combinatorial chemistry, synthesis, biological assays, ultra performance liquid chromatography, ultra fast liquid chromatography, ultra high pressure liquid chromatography, supercritical fluid chromatography, and mass spectrometry.

25 85. A chromatographic column, comprising

a) a column having a cylindrical interior for accepting a packing material and

b) a packed chromatographic bed comprising The high purity chromatographic material of any one of Claims 1-75.

86. A kit comprising The high purity chromatographic material of any one of Claims 1-75,
5 and instructions for use.

87. The kit of Claim 86, wherein the instructions are for use with a separations device.

88. The kit of Claim 87, wherein the separations device is selected from the group consisting of chromatographic columns, thin layer plates, microfluidic separation devices, filtration membranes, sample cleanup devices and microtiter plates.

10 89. A chromatographic device, comprising

a) an interior channel for accepting a packing material and
b) a packed chromatographic bed comprising The high purity chromatographic material of any one of Claims 1-75.

15 90. The high purity chromatographic material of Claim 1, wherein the material is non-porous.

91. The high purity chromatographic material of Claim 1, wherein the ionizable modifier is selected from the group of zirconium, aluminum, cerium, iron, titanium, salts thereof, oxides and combinations thereof.

20 92. The high purity chromatographic material of Claim 4, wherein

V is 1, m' is 3, and each occurrence of R², R^{2'}, R³ and R^{3'} is hydrogen.

93. The high purity chromatographic material of Claim 92, wherein Y is carbamate, carbonate, amide, urea, ether, thioether, sulfinyl, sulfoxide, sulfonyl, thiourea, thiocarbonate, thiocarbamate or triazole.

Figure 1

1) Metoprolol Tartrate 2) Amitriptyline

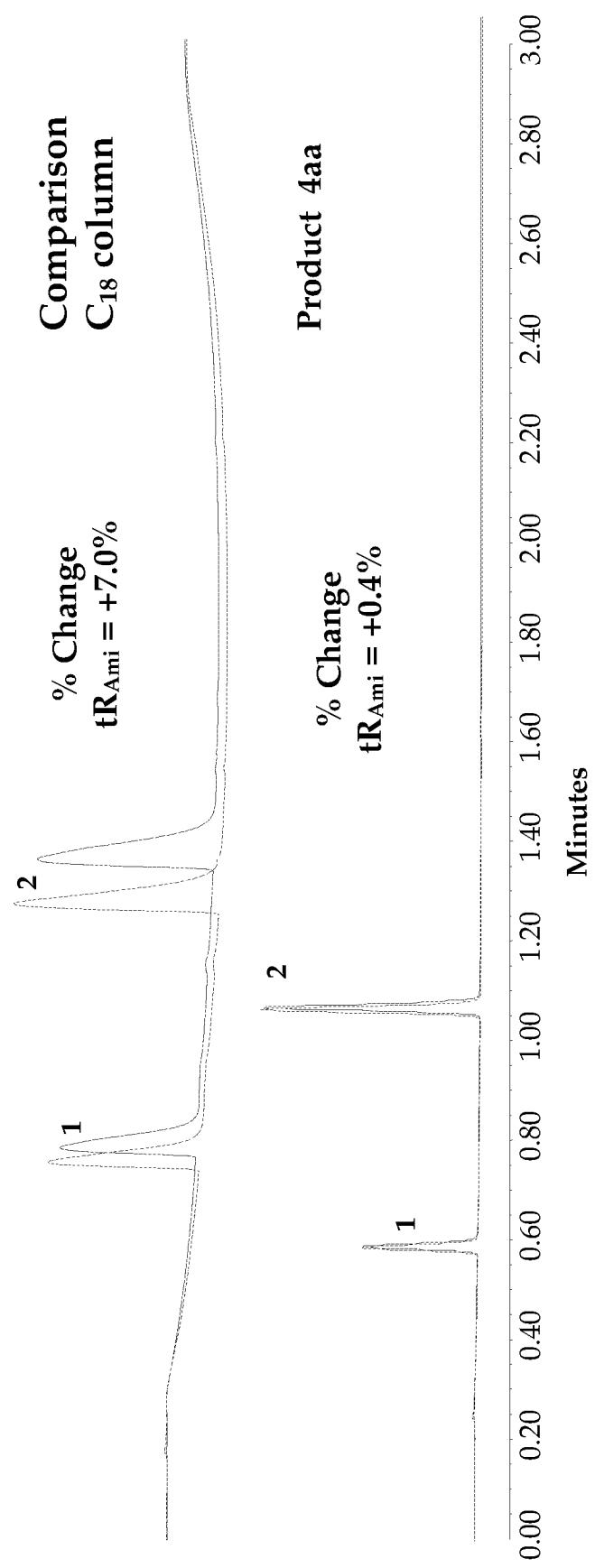


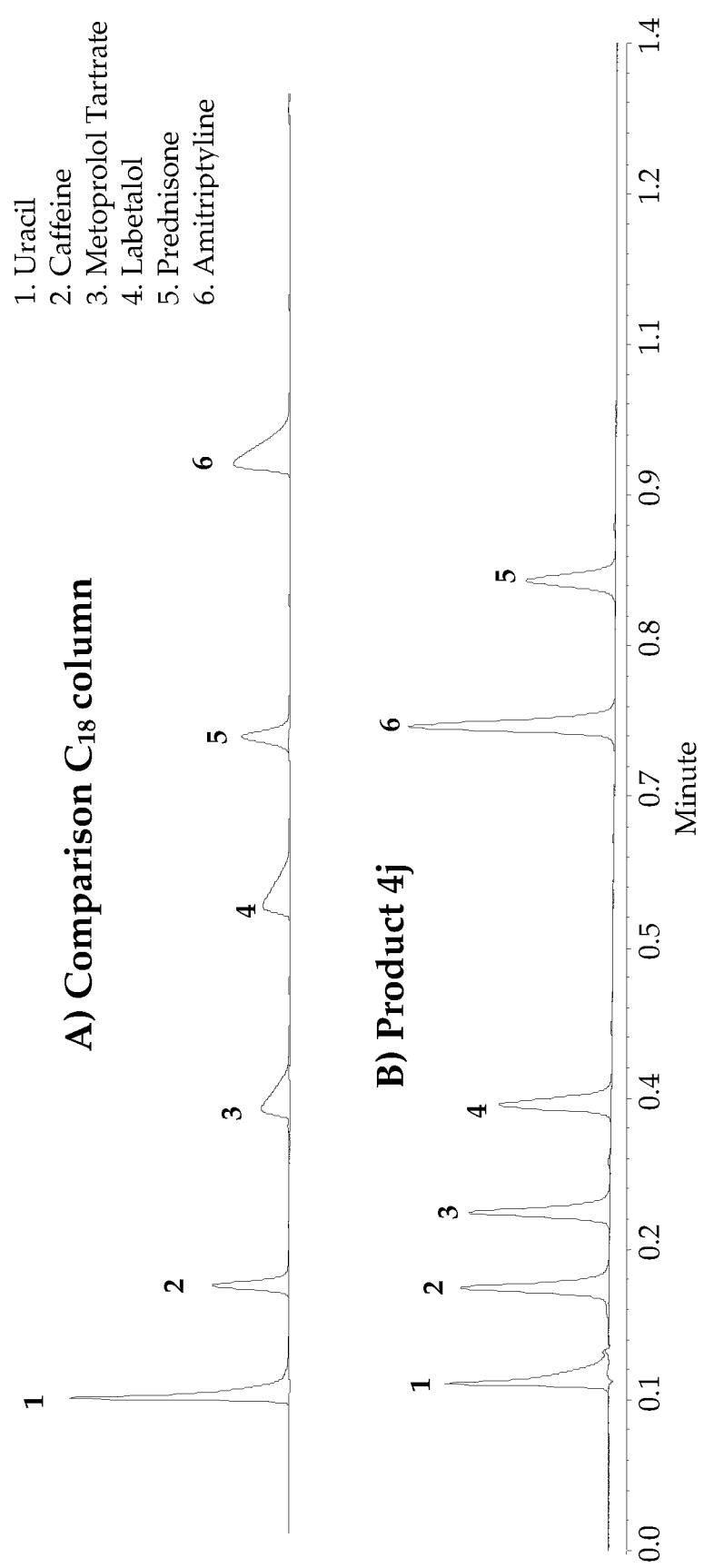
Figure 2

Figure 3

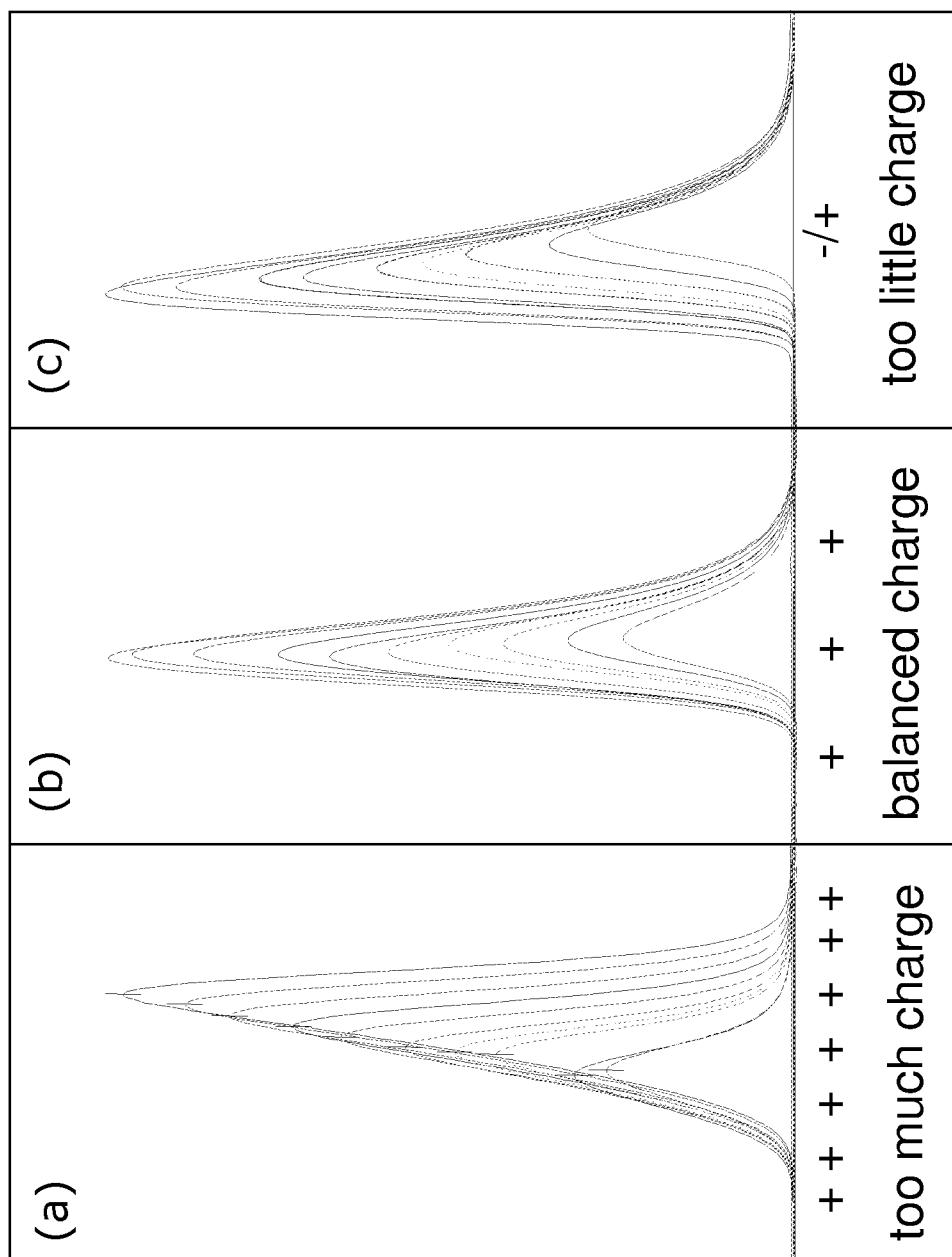
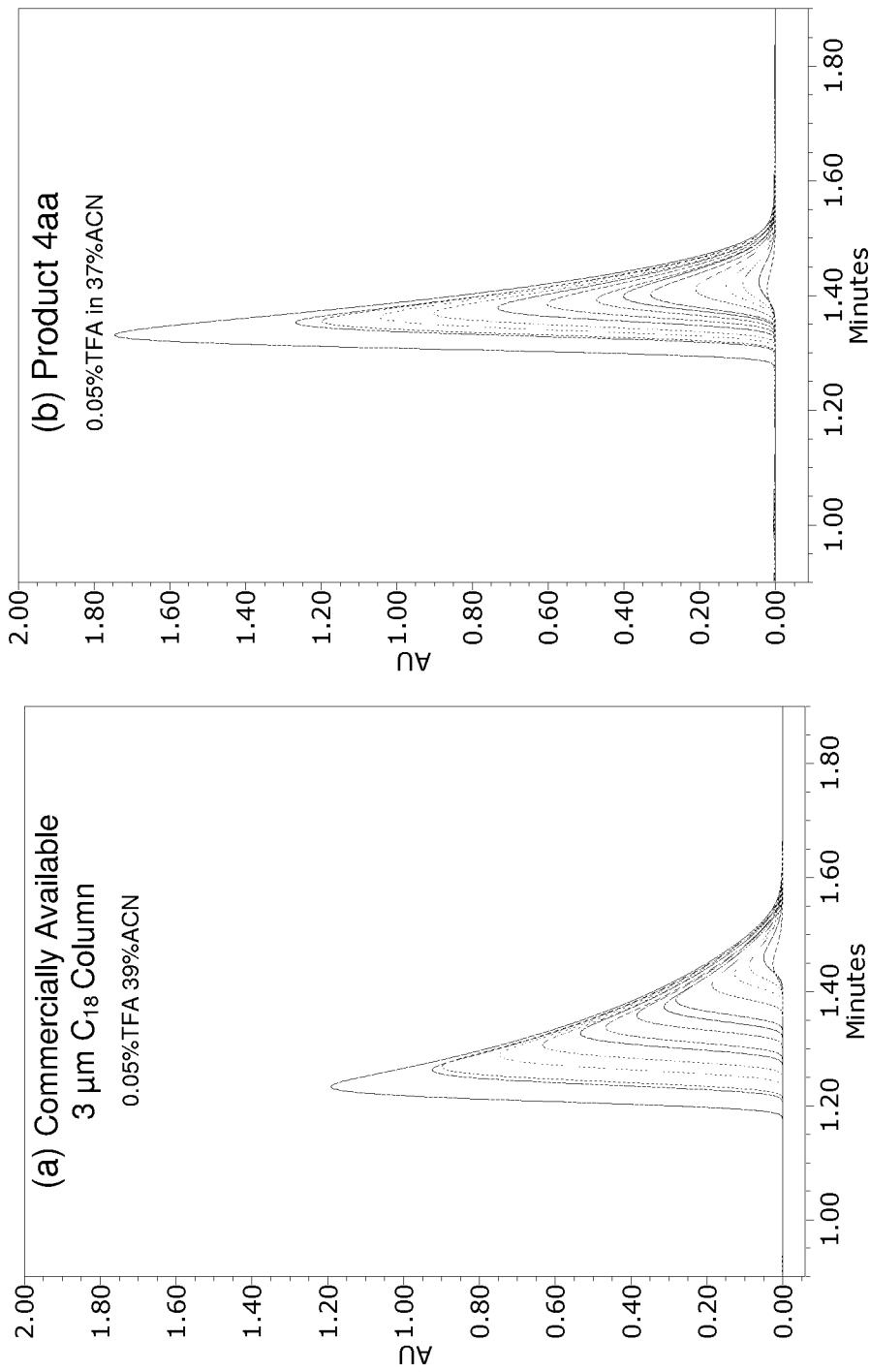


Figure 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/44390

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C08J 9/00 (2010.01)

USPC - 521/134

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: 521/134

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 521/139, 64; 210/656, 198.2, 635 (text search) Find search terms below

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

PubWEST (PGPB, USPT, EPAB, JPAB), Google Scholar
chromatograph\$, material, silica, hybrid, adsorbent, stationary phase, silane, ionizable, ionisable, modifier, bonded phase, porous, nonporous, hydrolysis, stability, \$chlorosilane, \$triethoxysilane, \$trimethoxysilane

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------------|--|--|
| X ---- Y | US 7,125,488 B2 (LI) 24 October 2006 (24.10.2006) col 3, ln 50 to col 4, ln 26; col 5, ln 8-14; col 7, ln 30-52; col 12, 32-49; col, 18, ln 41-44; col 21, ln 48; col 23, ln 11-14; col 23, ln 49 to col 24, ln 24 | 1-44, 47-58, 76-80, 90-93 ----- 45-46, 59-75, 81 |
| Y | US 2005/0230298 A1 (JIANG et al.) 20 October 2005 (20.10.2005) para [0007], [0051], [0067], [0071], [0092] | 45-46, 59-64, 81 |
| Y | US 2007/0189944 A1 (KIRKLAND et al.) 16 August 2007 (16.08.2007) para [0026], [0029], [0046], [0049] | 65-75 |

Further documents are listed in the continuation of Box C.

* 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 another 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

28 September 2010 (28.09.2010)

Date of mailing of the international search report

01 OCT 2010

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/44390

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 82-89 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.