Title (en)

**SELECTIVE DETECTION AND ANALYSIS OF SMALL MOLECULES**

Title (de)

SELEKTIVE DETEKTION UND ANALYSE VON KLEINEN MOLEKÜLEN

Title (fr)

DÉTECTION ET ANALYSE SÉLECTIVES DE PETITES MOLÉCULES

Publication

EP 2676287 A2 20131225 (EN)

Application

EP 12714045 A 20120217

Priority

• IN 407DE2011 A 20110217

• IN 2012000113 W 20120217

Abstract (en)

[origin: WO2012111028A2]

The invention relates to a material, process and method for the selective analysis of small molecules. Particularly the invention provides a material and a technique for the analysis of small molecules excluding other large molecular weight (MW) analytes. The process involves selective detection of low molecular weight molecules from a sample comprising the steps of placing said sample with SBA-15particles; and subjecting the same to desorption ionization mass spectrometry, wherein low molecular weight molecules are selectively detectedover the higher molecular weight molecules. A kit for the selective analysis of small molecules is also provided.

Inventor

• **GHOSH DIPANKAR (IN)**

• PANCHAGNULA VENKATESWARLU (IN)

• DHAWARE DEEPIKA (IN)

Applicant

• JAWAHAR LAL NEHRU UNIVERSITY (IN)

• COUNCIL SCIENT IND RES (IN)

IPC 8 full level (invention and additional information)

**H01J 49/16** (2006.01); **B01J 20/10** (2006.01); **B01J 20/28** (2006.01); **G01N 33/68** (2006.01); **H01J 49/04** (2006.01)

CPC (invention and additional information)

**G01N 33/6851** (2013.01); **B01J 20/103** (2013.01); **B01J 20/28045** (2013.01); **B01J 20/28083** (2013.01); **B01J 20/283** (2013.01); **B01J 20/3204** (2013.01); **B01J 20/3236** (2013.01); **H01J 49/0418** (2013.01); G01N 1/405 (2013.01); G01N 2560/00 (2013.01)

Citation (search report)

See references of WO 2012111028A2

Designated contracting state (EPC)AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

EPO simple patent family WO 2012111028 A2 20120823; WO 2012111028 A3 20121011; EP 2676287 A2 20131225; EP 2676287 B1 20160120; SG 194207 A1 20131129;US 2013323849 A1 20131205

FIELD OF THE INVENTION

This invention relates to a material, process and method for the selective analysis of small molecules. Particularly the invention provides a material and a technique for the analysis of small molecules excluding other large molecular weight (MW) analytes.

BACKGROUND OF THE INVENTION

Analysis of biomolecules is a challenge due to the complex nature of biological samples. Most biological samples contain a diverse range of analytes with different biochemical functionalities, molecular sizes and masses as well as presence in varying abundances. Furthermore, these can also be present in various physical manifestations such as liquids, vapors/gaseous phases containing volatiles, solids and various forms of soft matter such as tissues, emulsions, networks, composites etc. Thus, maneuvering a given biological sample to analyze and understand the biochemical components present therein is an enormous task. Such information is of vital importance in healthcare/medical applications and numerous other commercial applications that are non medical/healthcare in nature.

One of the approaches followed in such situations is to study the sample profile and simplify it, but this may result in compromising the scope of the analysis. Also currently, separation, isolation and detection are performed separately moving from one platform to another severely compromising analytical performance.

'Matrix assisted laser desorption/ionization (MALDI) mass spectrometry' is a useful and popular analytical tool for large molecules such as peptides, polymers and proteins. However, due to inherent peaks from the 'matrix' that interfere in the low molecular region, MALDI is quite unpopular for small molecule analysis. In MALDI MS, all the analytes including the matrix itself are detected. Suspected ion suppression from the matrices has also been considered to be a major bottleneck for small molecule analysis using MALDI MS.

Laser desorption ionization mass spectrometry (LDI MS) is commonly referred to the techniques, which do not use organic matrices (unlike MALDI MS). The consequence of this is that smaller molecules which cannot be analyzed mass spectrometrically using

MALDI MS can now be analyzed along with larger molecules such as peptides. It must be emphasized that all the prior art using LDI or MALDI describe analysis of both small and large molecular weight analytes and none of these refer or imply "preferential" or "selective" analysis over the other classes of analytes. Thus prior art on LDI MS even if illustrating small molecule analysis do not make any reference to "selectivity" or "preference" over the rest of the molecules and quite often demonstrate the larger utility of LDI for both smaller and larger molecular weight analytes. A few specific examples of the prior art are detailed below in this regard.

To overcome the limitations and disadvantages of MALDI MS, a wide range of materials have been demonstrated for use as alternatives to the organic matrices in LDI MS. These include polymers, surfactants, activated carbon, carbon nanotubes and inorganic materials (like germanium nanodots(Seino, 2007 #33), platinum nanoflowers (Kawasaki, Yonezawa et al. 2007), metal oxide nanoparticles (Kinumi, Saisu et al. 2000), silicon nanowires (Go, Apon et al. 2005), metal oxide porous films (Chen and Chen 2004) etc.). The use of metal oxide particles and films being the most preferred as one could perform a 'matrix-free' analysis in real sense by removing/reducing the contribution from the matrix in the small molecule region (m/z <700).

One example of 'matrix-free' analysis is porous silicon (not silica), popularly known as DIOS (desorption ionization on porous silicon), which was investigated for its use in mass spectrometry as an alternative to MALDI MS. Wafers of silicon were produced and commercialized as 'DIOS target plates' for the analysis of proteins, peptides and many other analytes using laser desorption/ionization mass spectrometry (Siuzdak, Buriak et al. 2000) (Wei, Buriak et al. 1999) (Shen, Thomas et al. 2001) (Thomas, Shen et al. 2001). It is believed that the silicon wafer "softly" transmits the UV laser energy to the analytes leading to their desorption and ionization followed by mass spectral identification, usually in a 'time-of-flight' tube where analytes traveling in a tube with applied electrical potential get separated by virtue of their mass by charge

ratios (m/z). This material and the method is universal and does not discriminate or offer selectivity between various types of analytes. The primary objective of this prior art is to provide an alternative to MALDI MS. Thus in this example described DIOS enables the detection of small as well as large MW analyte detection minus the 'matrix' interference. US 6958480 claims a method of performing 'matrix-free' laser desorption/ionization mass spectrometry using a nanocomposite comprising mesoporous silica thin film on porous silicon, glass etc. This composite film is prepared by dispersing a silica precursor (tetraethyl orthosilicate TEOS) in a surfactant solution that is spread on yet another material, a silica wafer or a glass slide in this embodiment, to make it into a composite material in the form of a thin film. The final nanocomposite thin film is obtained by removing the surfactant employed as a template by means of exposing the films to deep UV light or by calcination at elevated temperatures. An analyte is then placed on this composite thin film, which is subsequently subjected to laser desorption/ionization mass spectrometry as described above. The thickness of the film is in the range of 70-300 nm with pore sizes of 1-50 nm. This nanocomposite film presents better performance characteristics over the above mentioned DIOS plates, but is used essentially for the same purpose of universally analyzing samples. Its advantage is as a superior method relative to the traditional MALDI MS. Thus, this nanocomposite based mesoporous technology also enables the detection of small as well as large MW analytes in a given sample minus any interference from the matrix. The invention does not disclose any selective analysis or ionization resulting from the material or the method. The inventors in the above mentioned patent also disclosed that peptides such as Angiotensin II, Bradykinin 1-7, P14R and ACTH 18-39 fragments were detected using this method wherein LDI MS was performed by placing a sample on the nanocomposite (Dattelbaum, Hicks et al. 2008). An article titled "Mesoporous silica for Desorption-Ionization Mass Spectrometry" by the same authors A. Dattelbaum et al published in Nanotech, Vol. 1, Ch. 5, pages 225 - 228, 2005. (Srinivas Iyer and Andrew M. Dattelbaum 2005) discloses use of mesoporous silica thin film nanocomposites for mass spectrometric analysis of both tryptophan (lower m z) and angiotensin (peptide).

It is also noteworthy that "porous silicon" wafer alone is already covered as a patent by another group for its utility in LDI MS (no selectivity has ever been covered) that is termed as DIOS as detailed in line numbers 51 to 60 (Siuzdak et. al.). Despite this, the combination of porous silicon and mesoporous silica has been granted a patent for the same utility, which is LDI MS (US 6958480). In summary, large W analytes can only be analyzed in the LDI MS mode using either the "organic matrix" such as 2,5-dihydroxybenzoic acid (DHB) and a-cyano hydroxy cinnamic acid a-CHCA) or the "porous silicon wafer" or "a nanocomposite thin film comprising mesoporous silica" or a few other materials.

An article titled "Selective binding and enrichment for low-molecular weight biomarker molecules in human plasma after exposure to nanoporous silica particles" by Rosa Terracciano, e.t al. published in Proteomics, Volume 6 Issue 1 1, pg 3243 - 3250, 2006, having DOI 10.1002/pmic.200500614. US 2008/0277578 Al discloses biomarker capturing strategy based on nanoporous silica particles. The strategy as described herein comprises of exposing a plasma sample to a silica particle thereby enriching low molecular weight biomolecules (m/z 800-10,000) that are peptides and proteins followed by a separate MALDI-MS analysis of the biomolecules extracted from the silica using conventional matrices like a-cyano hydroxy cinnamic acid (a-CHCA). In this document the definition of low molecular weight molecules includes analytes molecular weight range 800 to 10,000. In this document silica porous particles (not to be mistaken with mesoporous SBA-15 prepared using template assisted synthesis) or beads are used for ONLY enrichment. Analytes are then removed for conventional MALDI MS using various matrices such as a-CHCA. It is noteworthy that various methods exist for enrichment of various classes of molecules that operate without a concurrent mass spectral dimension. A case in point is the ĺĪ02 enrichment of phosphopeptides that is a commercial preset with extensive prior art. Also, the porous silica used has non uniform pore size and not as well defined as SB A- 15. The document enriches peptides and proteins with m/z 800- 10,000.

The present invention/method involves selective exclusion of these peptides and proteins and detecting only the smaller analytes (MW typically less than 1000 Da): This is achieved in the present invention by a material SBA-15, which enables desorption and mass spectral analysis of small molecules from mixtures containing large analytes. Signals for the small molecule analytes are obtained directly from the material itself without the addition of an external matrix.

In summary, there is no prior art on a material, process or method using mesoporous SBA-15 that can be used for the selective mass spectrometric determination of small molecular weight (typically less than lOOOm/z) analytes while excluding large molecular weight analytes in complex mixtures.

Therefore, a quick-yet selective material and method involving minimal or no sample preparation, labeled or label-free detection that enables simultaneous mass spectral analysis is an extremely valuable tool. Such tool is significantly benefit research in biological, chemical and related sciences. Such a material and method finds wide applicability in diverse areas such as drug discovery research, proteomics, metabolomics, and in general any other application where an efficient analytical solution is needed.

With the miniaturization of mass spectrometers (Shimma S., et. al Analytical Chemistry 2010, 82, 8456; Ouyang, Z. et. al, Analytical Chemistry 2009, 81, 2421 ;

Kissinger, P.T. et. al, http://www.ivdtechnologv.com/article/developing-point-care-mass- spectrometer), and potential use of these instruments at point of care and point of action

(such as hospitals, airports, defense applications), it is also imperative that a material enabling a method and process to selectively detect, distinguish and identify analytes of interest would be of enormous utility and commercial as well as societal importance.

The invention described herein involves (a) a process / product using pure porous material that is distinct from the prior art using various other materials or various physical forms and (b) leading to a unique utility not covered by the prior art (selective or preferential mass spectral detection of low MW molecules that are not peptides or proteins; not just LDI MS or enrichment of peptides as in the prior art).

OBJECT OF THE INVENTION

Therefore, an object of the present invention is to provide a process for the selective analysis of small molecules.

Another object of the invention is to provide a material for the selective detection of small molecules.

Yet another object of the invention is to develop a kit for the selective detection of small molecules excluding large molecular weight analytes in complex mixtures.

SUMMARY OF THE INVENTION

This invention provides a material, process and method for the selective analysis of small molecules. Particularly the invention provides a material and a technique for the analysis of small molecules excluding other large molecular weight (MW) analytes.

In one embodiment, the invention provides a process for the selective detection of low molecular weight molecules from a sample comprising the steps of placing said sample with SBA-15 particles; and subjecting the same to desorption ionization mass spectrometry, wherein low molecular weight molecules are selectively detected over the higher molecular weight molecules.

In another embodiment, wherein SBA-15 or sample are present in a solvent system. The solvent system is selected from organic solvents such as. methanol, acetonitrile and ethyl acetate. In preferred embodiment SBA-15 and methanol are present in ratio of 5: 1 (w/v).

In another- embodiment, either SBA-15 and / or the sample are chemically or physically modified. In another embodiment, the said modifier is a chemical or physical agent that introduces positive or negative charges to low molecular weight analytes to improve their selective mass spectrometric analysis.

In another embodiment, sample to be detected is used with the modifiers that modify the charge present on the surface of molecules to be detected. To introduce positive charge, the said modifiers are selected from alkali metal salts. In the preferred embodiment said modifier is lithium chloride. The modifiers are present in a ratio ranging from 1 : 1- 7: 1 (w/v) with SBA-15.

In yet-another embodiment, the modifier enables sensitive detection and MS/MS analysis.

In another embodiment the molecules detected have molecular weight less than 1000 (m/z).

In another embodiment the sample detected is a fluid of biological or synthetic origin comprising a mixture of small molecular weight analytes and large molecular weight analytes. The said sample could also be a solid, semi solid, gel, amorphous mixture, or a surface of biological or synthetic origin comprising a mixture of small molecular weight analytes and large molecular weight analytes. In preferred embodiment the molecules detected are selected from bacterial quorum sensing molecules, amino acids, synthetic or modified small molecules, lipids, fatty acids and their derivatives, therapeutic, pharmaceutical and drug molecules, metabolites, food, pesticide and environmental samples.

In another embodiment, wherein the said material and process will be used for clinical diagnostics, forensics, dope and narcotic analysis, environmental analysis, microbial community analysis and quorum sensing, pesticide analysis, food analysis, industrial fermentation, active pharmaceutical ingredient (API) or drug discovery or for high throughput mass spectrometry research use and practice wherein the samples comprise a mixture of small molecular weight analytes and large molecular weight analytes.

In another embodiment, the detection of small molecules involves minimal, optimal or no sample preparation.

In another embodiment, SBA-15 particles are employed as particles as powder or suspension in reagents or on a surface or as a free standing film or as a part of a device such as a chromatographic or mass spectrometric surface or device.

In another embodiment the invention provides a process of performing selective laser desorption ionization mass spectrometry comprising preparing mesoporous SB A- 15 particles having a desired pore size by preparing a suspension of SBA-15 particles in a solvent system selected from organic solvents preferably methanol optionally subjecting solution of step a to sonication dispersing SBA-15 in a solvent system comprising triflouoroacetic acid (TFA), mixing said suspension of SBA-15 with a sample of analytes/molecules, subjecting the solution of previous step to a laser in an ion generating section of a mass spectrometer to desorb and ionize the sample; and detecting the desorbed and ionized sample. In further embodiment sample to be detected is modified with the charge modifiers. In another embodiment the ratio of SBA and TFA is 1 : 1 [v/w].

In another embodiment is provided a chemically modified SBA-15.

In another embodiment is provided use of SBA-15 modified or unmodified for the detection of low molecular weight molecules (Less than 1000 m/z) while excluding molecules of molecular weight 1000 (m/z) or above. In yet another embodiment is provided a kit for the detection of small molecules from a complex mixture of analytes comprising first part consisting of SBA-15 and second part containing chemical reagents, wherein said SBA-15 is in the form of a powder or suspension in reagents or on a surface or as a free standing film or as a part of a device such as a chromatographic or mass spectrometric surface or device . In another embodiment the first part consists of SBA-15 powder in methanol in a ratio of 5: 1 (w/v). In further embodiment, in said kit is provided a third tube consisting of charge modifiers. In another embodiment said kit comprises SBA-15 layered with a charge modifier.

In further embodiment kit comprises a tube consisting of standard compounds. In another embodiment kit also comprises a surface for spotting the sample. The surface is a steel multi well plate. In another said kit SBA-15 is coated on a surface.

In another embodiment the invention provides a process of selective detection of small molecules from a sample comprising complex sample of molecules using a kit comprising the steps of preparing a solution of sample to be detected by mixing said sample with the reagents of second tube in a ratio of 1 : 1 , mixing the two solutions, spotting said solution on the surface provided in the kit and subjecting the same to laser desorption ionization mass spectrometry.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a material, process and method for the selective detection and analysis or estimation of small molecules over larger molecules. In particular the invention provides selective laser desorption/ionization mass spectrometric determination of small molecular weight molecules while excluding high molecular weight molecules from a mixture comprising high molecular weight and low molecular weight analytes/molecules.

The analytes to be analysed are present originate from a biological source or are from a synthetic source containing small and larger molecules. The complex sample mixture is analyzed using the process and method described before. The process excludes detection of high MW molecules such as proteins and peptides and such like that are detected by conventional mass spectrometric techniques. The process selectively detects and analyse low molecular weight molecules using appropriately prepared mesoporous SBA-15 embodiments. The invention also provides preparation of rnesoporous SBA-15 particles. A variety of methods are employed for the synthesis of rnesoporous materials such as SBA-15. The choice of template/polymer/surfactant depends on the characteristics desired like the pore size and overall morphology of the particles formed. Varying the process of synthesis can thus provide one with materials with different structural morphology, which can yield a similar and/or desired selectivity or exclusion.

The rnesoporous SBA-15 particles are prepared in a particular solvent system such as methanol or acetonitrile (ACN) or ethyl acetate and sonicated well to disperse the same. The solvent systems used to disperse the SBA-15 particles can contain a 0.1% trifluoroacetic acid (TFA) (50:50 v/v).

The SBA-15 particles are employed as particles as powder or suspension in reagents or on a surface or as a free standing film or as a part of a device such as a chromatographic or mass spectrometric surface or device. The SBA-15 may be present as a coating on a surface for detection of molecules. Time for drying of the SBA-15 coating 1 to 5 min at 25 degrees C and air drying. The activation of SBA-15 by physical and chemical treatments also improves performance features such as sensitivity. The activation of SBA-15 may be done by either of the steps: Laser induced activation under high vacuum conditions, Microwave induced activation, Thermal activation using heating under controlled conditions, and other chemical or physical induced treatments for activation and enhanced analytical performance.

In one aspect rnesoporous SBA-15 particles are chemically modified or a modifier is used in accordance with the sample to be detected to ensure selectivity towards a particular class or group of analytes.

The following are examples of process arid chemical modifications performed: (1) Addition of lithium chloride in methanol (7.5mg/mL) to the suspension of SBA-15 in methanol enabled selective mass spectral detection of amino acids in the presence of peptides from a test mixture containing both. The following amino acids present in the test mixture with their lithiated adducts that were selectively detected: leucine (1 144.1 1 17), isoleucine (144.1 117), valine (130.0986), tyrosine (194.0905), tryptophan (217.1066), phenylalanine (178.0944), methionine (162.0683), lysine (159.1246), while the peptides bradykinin 1-7, angiotensin II, P14R and ACTH 18-39 [m/z 757.4075, 1056.5511, 1533.866, 2465.2067 respectively] were selectively excluded. Without the addition of lithium chloride, amino acids were not detected at all.

(2) Addition of lithium chloride (7.5mg/mL) to the suspension of SBA- 15 in methanol for N-acyl homoserine lactones followed by the above described process of detection selectively yielded only lithiated adducts in the mass spectra. Without lithiation, numerous adducts such as protonated, sodiated, and potassiated were formed complicating the spectra leading to poor resolution, sensitivity and detection of the analytes.

(3) SBA-15 was also chemically modified to introduce a light absorbing moiety, such as (a) alpha-CHCA into the mesopores with an aim to eliminate selectivity and to show that selectivity can be tuned with chemical modification. The chemical modification was performed by attaching covalently a silylated amine such as 3-amino propyl triethoxy silane (200uL, lmmol) to the free silanol groups on SBA-15 and then this so formed ionic precursor was reacted with the anion of the standard MALDI matrices like alpha-cyano-4-hydroxy cinnamic acid (alpha (a)-CHCA; 1 19mg, lmmol) followed by thorough workup and cleaning to remove any adsorbed alpha-CHCA. This formed an ionic macro complex which was used further for the LDI-MS studies. The insertion of alpha-CHCA into the mesoporous structure of SBA-15 was characterized using IR spectroscopy, SEM and TEM. IR spectroscopy indicated the amine functionalization and TEM

indicated the porosity expected of the SBA-15 materials. Absence of alpha-CHCA crystals in the SEM images indicated that the alpha-CHCA was inserted into the pore. It is also noteworthy that the SEM images of modified and unmodified SBA-15 were similar (Figure 9 indicating that their function was determined only by either the presence or absence of alpha CHCA.

With this modification, a mixture of amino acids and peptides mentioned earlier in this document were tested. Peptides hitherto excluded were detected in high mass spectral intensities clearly showing that the selectivity towards small molecules was lost, while the amino acid signal was being interfered by some of the alpha-CHCA peaks. This example proves two concepts. Firstly, it shows evidence that the SBA-15 can be modified to tune the selectivity. Secondly, this result demonstrates the basis of selectivity. A light absorbing moiety such as alpha-CHCA absorbs the laser energy and utilizes it towards universal analyte desorption and ionization. In the absence of any such light absorbing moiety, SB A-15 excludes the larger molecules from getting desorbed and ionized for mass analysis, while the available thermal energy is sufficient for smaller molecules to undergo desorption, ionization and mass spectral detection.

Figure 9 reveals that the introduction of a light absorbing moiety such as alpha(a)- CHCA that is routinely used as MALDI matrix, into the porous structure of the SBA-15, selective detection of small molecules was lost Peptides, hitherto undetected using unmodified SBA-15 suspension (bottom frame), were now detected in very high mass spectral signal intensities as can be seen from the above spectra clearly demonstrating a loss of selectivity towards smaller molecules (top frame). In addition to demonstrating tuning of selectivity through chemical modification, this experiment also confirms that the absence of a light absorbing chemical structure as the basis for selective small molecule detection with the SB A- 15. A test mixture of amino acids and four peptide standards were used in this experiment. Using SBA-15 alone, the amino acids selectively detected as shown in the spectra above were: leucine (144.1 1 17), isoleucine (144.1117), valine (130.0986), tyrosine (194.0905), tryptophan (217.1066), phenylalanine (178.0944), methionine (162.0683), lysine (159.1246). With CHCA modified SBA-15, all the four peptides that were previously selectively excluded were now detected due to the loss of selectivity (bradykinin 1 -7, angiotensin II, P14R and ACTH 18-39, m/z 757.4075, 1056.5511, 1533.866, 2465.2067 respectively]. Furthermore due to the interference with CHCA peaks, only three amino acids (leucine (144.1 1 17), isoleucine (144.1117) and valine (130.0986)) were only detected from the mix with CHCA modified SBA-15. The remaining amino acid peaks were either getting suppressed or not detected in the modified SBA-15 due to matrix interference.

The lower molecular weight analytes include but is not limited to (a) Bacterial quorum sensing molecules (b) Amino acids (c) Synthetic or modified small molecules (d) Lipids, fatty acids and their derivatives (e) Therapeutic, pharmaceutical and drug molecules (f) metabolites (g) food, pesticide and environmental and such similar lower molecular weight analytes.

The following are the examples of molecules that have been selectively detected using the material, process and method described in this invention: Amino acids such as leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine, arginine, histidine, methionine, lysine, serine, proline ([M-H+2Li], m/z 144.1117, 144.1117, 130.0986, 194.0905, 217.1066, 178.0949,187.1325, 168.1013, 159.1246, 162.0683, 1 18.0567, 128.0834 respectively) and modified amino acids such as ornithine ([M-H+2Li] m/z 139.1042), hydroxyproline ([M-H+2Li], m/z 144.0812), phenylglycine ([M+Li], m/z 164.0823), symmetric dimethyl arginine ([M+H] m/z 203.1508), asymmetric dimethyl arginine ([M+H] m/z 203.1508), lipids and fatty acids and / or their esterified derivatives such as nervonic acid methyl ester, lignoceric acid methyl ester, tricosanoic acid methyl ester, cis-13, 16-docosadienoic acid methyl ester, erucic acid methyl ester, behenic acid methyl ester, heneicosanoic acid methyl ester, cis-5, 8, I I, 14, 17-eicosapentaenoic acid methyl ester, gamma linoleic acid methyl ester, linoleic acid methyl ester, myristoleic acid methyl ester, lauric acid methyl ester, undecanoic acid methyl ester, capric acid methyl ester, caproic acid methyl ester, butyric acid methyl ester ([M+Li3 m/z 387.3810, 389.3923, 375.3856, 357.3324, 359.3495, 361.3628, 347.3495, 329.2624 (M-H+2Li), 299.2562, 305.2614 (M-H+2П), 247.2229, 249.2400, 221.2075, 207.1923, 193^ 178, 137.1154, 115.0983 (M-H+2Li) respectively); N-acyl homoserine lactones class of bacterial sensing molecules, triglycerides (or their esters) such as: tripalmitin ([M+Li], m z 813.7601), glyceryl tridodecanoate ([M+Li], m/z 645.5756), glyceryl tridecanoate ([M+Li], m/z 561.4822), glyceryl trioctanoate ( [M+Li], m/z 477.381 1), glyceryl trimyristate ([M+Li], m/z 729.6661); carbohydrates such as glucose ([M+Li], m/z 187.0825), fructose ([M+Li], 187.0825), lactose ([M+Li], m/z 349.1424), galactose ([M+Li], m/z 349.1424); sucrose ([M+Li], 349.1424) heterocyclic compounds such as . mixtures of triazines 2,4-diamino-l,3,5-triazine, 2,4-diamino-6-methyl-l,3,5triazine, 2,4- diamino-6-(2-flu0ro*phenyl)- 1 ,3 ,5triazine, 2,4-diamino-6-(4-

methoxyphenyl)- 1 ,3 ,5 triazine ([M+H] m/z 1 12.0623, . 126.0753, 206.0830, 218.1013 respectively); pharmaceutical and drug molecules such as verapamil ([M+Li], m/z 461.2912), ibuprofen ([M+Li], m/z 213.1440), griseofulvin ([M+Li], m/z 359.0718), acetaminophen (paracetamol)( 158.0789); urea cycle metabolites and renal biomarkers such as creatinine, ornithinine, arginine, uracil, citrulline, ([M-H+2Li], m/z 126.0783, 139.1042, 187.1348, 125.0525, 188.111 1 respectively); cardiovascular metabolites such as symmetric ([M+H] m/z 203.1508) and asymmetric dimethyl arginine ([M+H] m/z 203.1508); metabolites implicated in early detection of diabetes: Leucine/isoleucine, valine, phenyl alanine and tryptophan, other bacterial and microbial metabolites; metabolites routinely used in new born, neonatal, pre and post natal screening investigations such as phenylalanine, tyrosine, arginine, clinical samples and such like.

The present process of detection or analysis requires optimal, minimal or no sample preparation. Example of minimal sample preparation include extraction of the small molecules from complex samples into suitable solvent followed by selective detection using the process described herein. Example of no sample preparation include direct usage of complex samples from their biological matrices such as plasma, serum, urine, fluids, cultures followed by the selective detection as described above. Example of optimal sample preparation are wherein the above biological samples are made to interact over a predetermined time duration with the materials in the above described process and method.

A standard sample test mixture is prepared by mixing low molecular weight analytes for e.g. amino acids or lipids or N-acyl homoserine lactones and high molecule analytes for e.g. peptides or proteins in a solvent ideally compatible for all the analytes like ACN:TFA (0.1%) or methanol: (0.1%)TFA. In one aspect the standard sample test mixture containing low molecular weight analytes is treated with an alkali metal salt such as lithium chloride to generate cationized adducts.

The sample mixture to be analysed can also be a complex biological mixture or an extract thereof in an organic solvent such as ethyl acetate etc. or can be a synthetic impure mixture containing both low and high molecular weight analytes.

The present invention provides a process for the selective detection and analysis or estimation of small molecules over larger molecules. In particular the invention provides selective laser desorption/ionization mass spectrometric determination of small molecular weight biomolecules while excluding high molecular weight analytes from a mixture comprising high MW and low MW analytes.

In accordance with the objectives of the invention, a process of selective detection and analysis or estimation of small molecules using mesoporous SBA-15 particles is disclosed herein. Other modes of mass analysis including various other ionization sources as well as mass analyzers/detectors or a combination thereof along with other embodiments of sample preparation can also be used in conjunction with the SBA-15 particles for selective detection of low molecular weight analytes and/or selective exclusion of large molecular weight analytes such as peptides and proteins or for SLDI. The invention also provides a kit comprising SBA-15 for the selective mass analysis. In one embodiment with different embodiments: In one embodiment, the kit would comprise of a tube with the unique suspension containing a predetermined concentration of (5: 1, w/y) SBA-15 powder in methanol. A sample mixture to be analyzed is prepared having reagents such as ethyl acetate, acetonitrile, trifluoroacetic acid, methanol and standard compounds. Contents from the unique suspension as described above were mixed in a 1 : 1 ratio (or a similar ratio). The analytes and the unique suspension are made to interact for a predetermined duration with the contents of the suspension. In one embodiment such as analysis of the examples mentioned, the duration was less than a minute. However this duration of interaction can also be tailored differently to enable specific tasks namely: adsorption, concentration, storage and transport of time and environment sensitive as well as labile analytes in liquid solution. The latter is applicable for sensitive and point-of-care applications such as microbial samples, clinical samples, diagnostics samples, bio terrorism agents, volatile matter and explosives.

In another embodiment kit comprises powder of SBA-15 to which samples of interest are added followed by selective mass analysis. In another embodiment SBA- 15 is in the form of a free standing thin film. In another embodiment kit comprises a coating of the SBA-15 suspension on a predetermined surface such as a glass, quartz, fused silica, ceramic or a stainless steel surface. To this surface coating, a sample of interest is added followed by selective mass analysis. Such a coating can also be present on other

materials for example gold, platinum, copper surface or an alloy made of these or other metals or a surface made of non metals.

Another, embodiment of the kit comprises of SBA-15 with physical or chemical modifiers such as lithium chloride in suspension. To this mixture of SBA-15 and modifier, analytes or samples of interest are added followed by selective mass analysis.

Other manifestations include the modifications such as lithium chloride mixed in the SBA-15 powder or in a free standing SBA-15 film or lithium chloride and SBA- 15 in methanol coated on a surface described above. A separate set of necessary reagents or standards will also be part of the kit. In yet another embodiment, SBA-15 is mixed with a modifier of predefined ratio such as 1 : 2 (w/w) to introduce charges (positive or negative depending on the modifier) to low molecular weight non polar analytes for enabling selective mass analysis.

In yet another embodiment, SBA-15 is mixed with lithium chloride in methanol (7:1 w/v) results in selective lithiation over sodiated and potassiated adducts of quorum sensing molecules like homoserine lactones, autoinducer peptides and others from bacterial biofilm cultures in synthetic media or biological fluids. The lithiated quorum sensing molecules exhibit higher signal to noise ratio enabling MS and MS/MS analysis.

In yet another embodiment the kit comprises of SBA-15 with or without sequentially layered modifier such a lithium chloride in a predetermined ratio such as 1 :2 (w/w) that is exposed to sample of interest. A sample of interest can be aspirated through physical suction or passed through a cartridge containing the sequentially layered materials followed by selective mass analysis from SBA-15.

In still another significant embodiment, SBA-15 in its manifestations described above when placed on a sample surface ablates the surface layers enabling their transition into vapor or gaseous phase followed by selective mass analysis. This can also be achieved by placing the sample on top of the SBA-15 for mass analysis.

**References:**


1. Chen, C.-T. and Y.-C. Chen (2004). "Desorption/ionization mass spectrometry on nanocrystalline titania sol-gel-deposited films." Rapid CommuiTr-Mass Spectrom. 18: 1956-1964.
2. Dattelbaum, A. M., R. K. Hicks, et al. (2008). "Surface assisted laser desorption-ionization mass spectrometry on patterned nanoporous silica thin films." Microporous and Mesoporous Materials 114: 193-200.
3. Go, E. P., J. V. Apon, et al. (2005). "Desorption/ionization on Silicon Nanowires." Analytical Chemistry 77(6): 1641-1646.
4. Kawasaki, H., T. Yonezawa, et al. (2007). "Platinum Nanoflowers for Surface-Assisted Laser Desorption/ionization Mass Spectrometry of Biomolecules." J. Phys. Chem. C 111: 16278-16283. Kinumi, T., T. Saisu, et al. (2000). "Matrix-assisted laser desorption/ionization time-of- flight mass spectrometry using an inorganic particle matrix for small molecule analysis." Journal of Mass Spectrometry 35: 417-422.
5. Shen, Z., J. J. Thomas, et al. (2001). "Porous Silicon as a Versatile Platform for Laser
6. Desorption/ionization Mass Spectrometry." Analytical Chemistry 73(3): 612-619. Siuzdak, G., E.; (US), J. U. Buriak, et al. (2000). Improved desorption/ionization of analytes from porous light-absorbing semiconductor.
7. Srinivas Iyer, L. A. N. U. and L. A. N. U. Andrew M. Dattelbaum (2005). Sample Desorption/ionization from Mesoporous Silica, The Regents of the University of California, Los Alamos, NM(US).
8. Thomas, J. J., Z. Shen, et al. (2001). "Desorption/ionization on silicon (DIOS): A diverse mass spectrometry platform for protein characterization." PNAS 98(9): 4932-4937. Wei, J., J. M. Buriak, et al. (1999). "Desorption-ionization mass spectrometry on porous silicon." Nature 399: 243-246.
9. Zhao, D., P. Yang, et al. (1998). "Continuous Mesoporous Silica Films with Highly
10. Ordered Large Pore Structures." Advanced Materials 10(16): 1380-1385



For further information, contact:
Dr. Dipankar Ghosh
Special Center for Molecular Medicine
Jawaharlal Nehru University
New Delhi - 110067
Tel/Fax : 91-11-26738781
Email ghoshd@jnu.ac.in