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(54) **MATRIX CARTRIDGE FOR MALDI**

(57) The present invention relates to a matrix cartridge for MALDI comprising a base layer coated with a film layer of matrix material preferably having a uniform thickness. The invention also relates to a process for

making the matrix cartridge. The matrix cartridge can be used in a sublimator to coat a substrate with a biological sample with a layer of matrix material.

EP 3 618 097 A1

Description

[0001] The present invention relates to a matrix cartridge for MALDI, a process for making such a matrix cartridge and the use of the cartridge to coat a sample for MALDI.

[0002] MALDI (matrix assisted laser desorption/ionization) mass spectrometry imaging (MSI) is a technique that is used for the identification, quantification and localisation of molecules in a sample such as a biological tissue sample. In this method the sample to be analysed is first pre-treated with a matrix of organic material that is applied as a film onto the (tissue) sample.

[0003] After preparing the matrix coated sample, the sample is targeted with a laser beam. By absorption of energy from the laser beam, analytes are released from the matrix (desorption) and arrive in ionised form in the gas phase (ionisation). In an analyser the mass to charge ratio (m/z) is further determined to identify, quantify and localize the molecules.

[0004] Conventionally, the samples are prepared by spray coating the matrix material onto the surface of a substrate containing the sample material. To this end the matrix material is dissolved in a water/organic solvent mixture, sprayed onto the sample material and dried. When the spray drops collide and crystallize, spatial information is lost. To prevent this, the matrix layer is created by spraying several times.

[0005] The challenge is to create a uniform matrix layer with minimal crystal size so that maximal spatial resolution can be achieved, while de-localisation is minimized.

[0006] Currently, these drawbacks are overcome by using a matrix sublimation technique. Here the matrix substance goes directly from the solid to the gaseous phase under the influence of heat and reduced pressure. Subsequently the gaseous matrix material is directly deposited onto the relatively cold surface of a condenser containing a substrate with the sample material. The deposition is at a molecular level as gaseous molecules recrystallize. This results in a relatively small crystal size of the matrix material (around 5 μm , compared to 20 μm with spraying).

[0007] Such a technique is for instance described by Joseph A. Hankin et al in J Am Soc Mass Spectrom, 2007 September; 18(9); 1646-1652.

[0008] In the known process a sublimator apparatus is put under vacuum. Then the matrix material is heated in a heating unit until sublimation takes place. The gaseous material is then deposited on a cooled sample plate containing the sample to be analysed.

[0009] In the known sublimation process, the matrix material is put as a powder at the base of the sublimator. In US2016/0035553, it is described that the matrix substance is contained in a vapour deposition source, which can be a boat type, a basket type, a crucible type and a wire type.

[0010] The drawback of adding the matrix material as a powder is that it is difficult to control the amount of matrix which influences the reproducibility of the measurements. Also, the use of a powder requires strict handling instructions for the operator, making the work more laborious and time consuming.

[0011] These drawbacks have now been overcome by a cartridge and process according to the invention.

[0012] Thus, the current invention provides a matrix cartridge for MALDI comprising a base layer coated with a film layer of matrix material. Preferably the film layer has a uniform thickness of from 0.1 μm to 1000 μm , more preferably from 0.5 μm to 500 μm , even more preferably from 1 μm to 300 μm .

[0013] The advantage of using the matrix cartridge according to the invention in preparing MALDI samples by sublimation is that it provides a predetermined amount of matrix so that it results in an accurate and reproducible deposition of the matrix material onto the sample to be analysed.

[0014] With uniform thickness of the film layer is meant that the thickness of the film layer does not deviate more than 20% from the average thickness of the film layer. For example, if the average thickness of the film layer is 100 μm , the thickness of the film layer anywhere on the surface of the base layer is between 80 and 120 μm . Preferably, the thickness of the film layer does not deviate more than 10%, more preferably not more than 5% or even not more than 1%, from the average thickness of the film layer over the entire surface of the base layer.

[0015] The film layer thickness can be determined by any method known in the art, for instance by using (electron) microscope technologies.

[0016] A uniform thickness of the film layer on the cartridge has the advantage that it will result in uniform heating of the matrix material during the sublimation process.

[0017] The amount of matrix material on the base layer is generally from 5 to 100 $\mu\text{g}/\text{mm}^2$, preferably from 10 to 50 $\mu\text{g}/\text{mm}^2$.

[0018] The film layer of matrix material is in direct contact with the base layer over at least 70 % of the surface of the base layer, preferably at least 90%, more preferably at least 99%, or even 100%. This means that there is no further material present between film layer and base layer.

[0019] The base layer is made of a material that it is heat conductive, so that it is particularly suitable for being heated in a uniform way in a sublimation process for preparing a sample for MALDI as will be discussed in more detail later. In particular it should be able to withstand temperatures of from 100 °C to 200 °C at reduced pressures (vacuum).

[0020] Suitable heat conductive materials are metals and glass. Metals are preferred in view of the heat transfer capacity. Examples of metal that can be used are stainless steel, aluminium and copper.

[0021] In a particular embodiment of the invention the base layer is made of metal and is further connected to heating means. The heating means can be heating element such as a heat conductive metal coil. The heating element has electrodes that can be connected to the sublimator. The heating means can be separate or the heating means can be integrated in the base layer.

[0022] According to another embodiment the matrix cartridge further comprises an adhesive layer. This makes it easier for the skilled person to adhere the matrix cartridge to a relevant position, such as a heating unit in a sublimator. Thus, according to this embodiment, the matrix cartridge further comprises an adhesive layer and a release layer. This means that the matrix cartridge is a layered system comprising, one adjacent to the other in the following order: film layer, base layer, adhesive layer and release layer.

[0023] In use, the release layer is removed from the adhesive layer and then the remaining cartridge is adhered to the heating unit in the sublimator. The adhesive layer is selected such that it is able to withstand the conditions in the sublimator in terms of high temperature and low pressure and that the cartridge can be easily removed after use. The selection of materials is readily available to the skilled person.

[0024] The dimensions of the cartridge will depend on the sublimator equipment to be used. In general the base plate has a size of about 10-150 mm in length and 10-150 mm in width. The thickness of the base plate will generally be from 0.1 to 10 mm.

[0025] Matrix materials for MALDI are known in the art. The matrix materials facilitate the production of intact gas-phase ions from the material in the sample to be analysed. A laser beam serves as the desorption and ionization source. The preferred matrix material is thus capable of absorbing radiation at a specific wavelength from the laser source (typically ultraviolet or infrared laser source). Further requirements are (amongst others) that it can be soluble in appropriate solvents and that it is stable in vacuum.

[0026] Examples of matrix materials are: α -cyano-4-hydroxycinnamic acid (CHCA), sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid), 2,5-dihydroxybenzoic acid (DHB), 2-(4-hydroxy phenyl azo) benzoic acid (HABA), succinic acid, 2,6-dihydroxy acetophenone, ferulic acid, caffeic acid (3,4-dihydroxy-cinnamic acid), 2,4,6-trihydroxy acetophenone, 3-hydroxypicolinic acid, 2-aminobenzoic acid, nicotinic acid, trans-3-indoleacrylic acid, isovanillin, dithranol and β -carboline (Norharmane). Due to ease of use and availability, preferred matrix materials are DHB, sinapic acid and Norharmane.

[0027] The total amount of matrix material in the film layer is generally from 10 to 500 mg, preferably 30 to 300 mg.

[0028] The invention further relates to a sealed container comprising the matrix cartridge for MALDI as described above and an inert gas. The sealed container consist of a reservoir suitable for containing the matrix cartridge and a seal layer, that closes of the reservoir. The seal layer is designed such that it hermetically closes of the reservoir and that it can easily be removed from the reservoir when it is needed to take out the matrix cartridge from the container.

[0029] According to a second aspect, the present invention provides a process for making the matrix cartridge for MALDI as described above.

[0030] According to a first embodiment of the process for making the matrix cartridge for MALDI, the process comprises the steps of:

- a) providing a base layer;
- b) dissolving a matrix material in a solvent;
- c) spraying the solution obtained in step b) onto the base layer to create a film layer;
- d) drying the film layer obtained in step c);
- e) optionally repeating step c) and d).

[0031] The base layer and matrix material are as defined above. For the solvent, any solvent suitable for creating a solution of the matrix material that can be sprayed is suitable. Examples of suitable solvents are: methanol, ethanol, water, chloroform, acetonitrile and mixtures thereof. The concentration of the matrix material in the solvent can be determined by the skilled person. In order to achieve a relatively fast process, the concentration should be as high as possible. For example, the concentration can be from 5 to 150 mg/ml.

[0032] The equipment for spraying the solution can be standard equipment available to the skilled person that allows to quickly spray a uniform layer of the matrix solution. An example is a HTX TM-sprayer.

[0033] Since the solution is of relative high concentration of matrix material and volatile solvents are generally used, air drying occurs at room temperature without further heating. However, slightly heating the sprayed film layer or applying a gas current to increase drying speed is not excluded.

[0034] As described above, to achieve the final film layer of matrix material, several spraying and drying steps can be applied, as needed, for example to create 10 to 20 layers. The total amount of matrix material and thickness of the film layer are as indicated above.

[0035] According to a second embodiment of the process for making the MALDI cartridge, the process comprises the steps of

- a) providing a base layer;
- b) placing a source of matrix material and the base layer in a vacuum chamber;
- c) heating the source of matrix material;
- d) cooling the base layer;
- e) creating reduced pressure in the vacuum chamber;
- f) bringing the vacuum chamber, source of matrix material and base layer back

to room temperature and normal pressure after a time sufficient to deposit a film layer of matrix material on the base layer.

[0036] Thus, according to this embodiment of the process, the matrix cartridge is manufactured using a sublimation process.

[0037] The base layer and matrix material are as defined above. The equipment for sublimation is known in the art. One example is a Matrix Vapor Deposition System iMLayer from Shimadzu.

[0038] The source of matrix material for this process can be the matrix material in solid (powder) form. It can be added to the sublimator in a suitable container, for instance a boat type container.

[0039] The matrix material will be heated to a suitable temperature to bring it in a gaseous state, in particular from 80 °C to 250 °C. The base layer is cooled to a low temperature so as to condense the matrix material on the base layer.

In general the temperature will be from -80 °C to 40 °C.

[0040] The reduced pressure in the vacuum chamber is from 0.1 to 1000 Pa, preferably from 1 to 10 Pa.

[0041] After deposition of the matrix material on the base layer in steps c), d) and e), the sublimator is brought back to normal temperature and pressure. For example, first the base layer is brought back to room temperature, then the pressure is brought back up to normal, followed by stopping the heating of the source of matrix material.

[0042] The matrix cartridge of the invention can be used to coat a sample to be analysed by MALDI with the matrix material using a sublimation technique. The sample to be analysed can be biological material, but can also be a polymer, organic or inorganic coating or a medicinal product (drug).

[0043] The present invention thus also relates to a process for coating a sample for MALDI with a matrix material, comprising the steps of:

- a) providing a matrix cartridge comprising a base layer coated with a film layer of matrix material according to any of claims 1 to 7;
- b) providing a substrate with a sample to be analysed;
- c) placing the matrix cartridge and substrate in a vacuum chamber;
- d) creating a reduced pressure in the vacuum chamber;
- e) heating the matrix cartridge;
- f) cooling the substrate;
- g) bringing the vacuum chamber, source of matrix material and base layer back to room temperature and normal pressure after a time sufficient to deposit a film of matrix material on the substrate covering the sample.

[0044] The matrix cartridge is as described above.

[0045] The cartridge will be heated to a suitable temperature to bring it in a gaseous state, in particular from 80 °C to 250 °C. The substrate is cooled to a low temperature so as to condense the matrix material on the substrate. In general the temperature will be from -80 °C to 40 °C.

[0046] The reduced pressure in the vacuum chamber is from 0.1 to 1000 Pa, preferably from 1 to 10 Pa.

[0047] The time for depositing the matrix material is generally in the order of minutes, e.g. from 0.5 to 30 minutes, preferably 0.5 to 5 minutes. The deposition time can be determined experimentally based on the signal obtained from the tissue. It can also be determined based on weight per area or density via light scattering.

[0048] After deposition of the matrix material on the substrate in steps d) to f), the sublimator is brought back to normal temperature and pressure. For example, first the substrate is brought back to room temperature, then the pressure is brought back up to normal, followed by stopping the heating of the matrix cartridge.

Examples

[0049] In the Examples a solution was prepared of the matrix in a suitable solvent. The matrix in the solvent was sprayed with a HTX sprayer with a certain velocity and flow rate. The matrix was sprayed in a pattern, either horizontal (HH) or criss-cross (CC) with a predetermined track spacing. Experimental conditions and results are shown in Table 1.

Table 1

Ex.	Matrix	Base layer	Solvent conc. (mg/ml)	Velocity (mm/min)	Flow rate (ml/min)	Track spacing (mm)	Temp. (°C)	Pattern	Layers	Final concentration (µg/mm ²)	Result
1	Norharmane	Stainless steel	MeOH:CHCl ₃ 30	1000	0.4	2	30	HH	8	36	Uniform
2	Norharmane	Glass	MeOH:CHCl ₃ 30	1000	0.4	2	30	HH	8	36	Uniform
3	DHB	Stainless steel	70% MeOH 40	1000	0.4	2	50	CC	6	36	Uniform
4	DHB	Glass	70% MeOH 40	1000	0.4	2	50	CC.	6	36	Uniform
5	DHB	Stainless steel	MeOH:CHCl ₃ 30	1000	0.4	2	40	CC	6	36	Uniform
6	Sinapic acid	Stainless steel	70% MeOH 30	1000	0.4	1	40	CC	3	36	Uniform

[0050] Ex. is example; Conc. is concentration; Temp. is temperature.

[0051] The matrix cartridge of example 2 was used to coat a sample of mini-pig intestine cryosections on a glass substrate by sublimation, resulting in a uniform coating of the matrix on the sample. The matrix cartridge of example 6 was used to coat a glass substrate by sublimation, resulting in a uniform coating of the matrix on the sample.

Claims

1. A matrix cartridge for MALDI comprising a base layer coated with a film layer of matrix material.
2. A matrix cartridge according to claim 1, wherein the film layer has a uniform thickness of from 0.1 μm to 1000 μm , preferably of from 0.5 μm to 500 μm .
3. The matrix cartridge according to claim 1 or 2, wherein the total amount of matrix material in the film layer is from 10 to 500 mg.
4. The matrix cartridge according to claim 1, wherein the base layer is made of metal or glass, preferably metal.
5. The matrix cartridge according to any of the preceding claims, wherein the matrix material is selected from the group consisting of 2,5-dihydroxybenzoic acid (DHB), 4-hydroxy-3,5-dimethoxycinnamic acid (sinapic acid) and β -carboiline (Norharmane).
6. The matrix cartridge according to any of the preceding claims wherein the base layer is a metal and the matrix cartridge is connected to heating means.
7. The matrix cartridge according to any of the preceding claims, wherein the matrix cartridge further comprises an adhesive layer adjacent to the base layer.
8. Sealed container containing the matrix cartridge for MALDI according to any of claims 1 to 7, and an inert gas.
9. A process for making a matrix cartridge for MALDI according to any of claims 1 to 7, comprising the steps of:
 - a) providing a base layer;
 - b) dissolving a matrix material in a solvent;
 - c) spraying the solution obtained in step b) onto the base layer to create a film layer;
 - d) drying the film layer obtained in step c);
 - e) optionally repeating step c) and d).
10. A process for making a matrix cartridge for MALDI according to any of the claims 1 to 7, comprising the steps of
 - a) providing a base layer;
 - b) placing a source of matrix material and the base layer in a vacuum chamber;
 - c) heating the source of matrix material;
 - d) cooling the base layer;
 - e) creating reduced pressure in the vacuum chamber;
 - f) bringing the vacuum chamber, source of matrix material and base layer back to room temperature and normal pressure after a time sufficient to deposit a film layer of matrix material on the base layer.
11. The process according to claim 9, wherein the source of matrix material is a container holding a powder of matrix material.
12. A process for coating a sample for MALDI with a matrix material, comprising the steps of
 - a) providing a matrix cartridge comprising a base layer coated with a film layer of matrix material according to any of claims 1 to 7;
 - b) providing a substrate with a sample to be analysed;
 - c) placing the matrix cartridge and substrate in a vacuum chamber;
 - d) creating a reduced pressure in the vacuum chamber;

EP 3 618 097 A1

- e) heating the matrix cartridge;
- f) cooling the substrate;
- g) bringing the vacuum chamber, source of matrix material and base layer back to room temperature and normal pressure after a time sufficient to deposit a film of matrix material on the substrate covering the sample.

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EUROPEAN SEARCH REPORT

Application Number
EP 18 19 2007

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	MICHAEL J. ROTH ET AL: "Thin-Layer Matrix Sublimation with Vapor-Sorption Induced Co-Crystallization for Sensitive and Reproducible SAMDI-TOF MS Analysis of Protein Biosensors", JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY., vol. 23, no. 10, 31 July 2012 (2012-07-31), pages 1661-1669, XP055228007, US ISSN: 1044-0305, DOI: 10.1007/s13361-012-0442-7 * section Thin Layer Transfer of MALDI Matrix; page 1663 *	1-12	INV. H01J49/16 H01J49/04
X	US 6 071 610 A (JARRELL JOSEPH A [US] ET AL) 6 June 2000 (2000-06-06) * column 5, line 34 - line 45 * * column 6, line 30 - line 52 *	1-12	
A,D	JOSEPH A. HANKIN ET AL: "Sublimation as a method of matrix application for mass spectrometric imaging", JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY., vol. 18, no. 9, 1 September 2007 (2007-09-01), pages 1646-1652, XP55548187, US ISSN: 1044-0305, DOI: 10.1016/j.jasms.2007.06.010 * page 1647 *	1-12	TECHNICAL FIELDS SEARCHED (IPC) H01J
The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 28 January 2019	Examiner Peters, Volker
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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**ANNEX TO THE EUROPEAN SEARCH REPORT
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EP 18 19 2007

5 This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
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REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 20160035553 A [0009]

Non-patent literature cited in the description

- **JOSEPH A. HANKIN et al.** *J Am Soc Mass Spectrom*, September 2007, vol. 18 (9), 1646-1652 [0007]