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(54) Atmospheric-pressure ionization device and method for analysis of a sample

(57) The atmosperic-pressure ionization device for connection to an analysis device especially a mass analysis device, comprising:

a) an atmospheric-pressure ionization chamber;
b) a passageway configured for delivery of ions to the analysis device;
c) a sample support positioned within said ionization chamber;
d) a matrix placed on said support and containing an analyte;
e) a laser for directing laser energy to said sample and to induce ionization of said analyte to form analyte ions;
f) means for directing at least a portion of said ionized analyte into said passageway.

The sample support comprises a tube configured for delivery of the sample to an exit of the capillary. The laser is directed to said exit to induce ionization of said analyte at said exit. The sample is flowing through the tube to said exit. The tube is preferably a capillary tube.

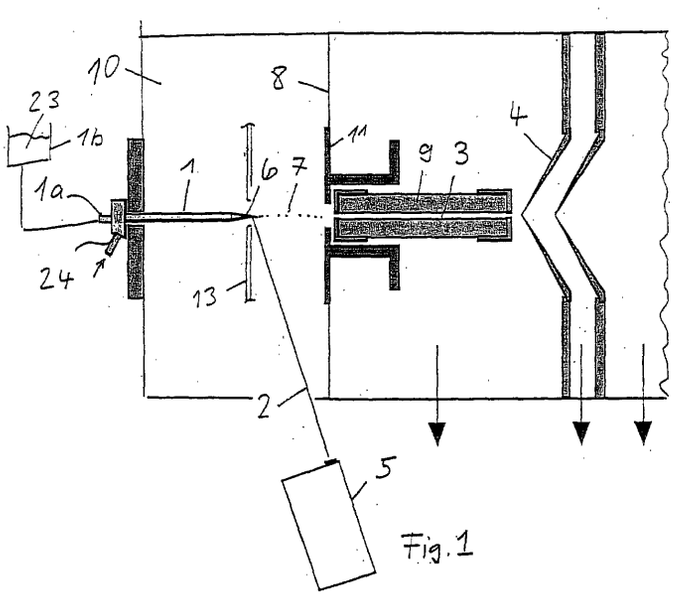

Fig. 1

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Description

[0001] The invention relates to an atmospheric-pressure ionization device according to claim 1.

[0002] A number of arrangements are known for dosing small samples of liquid into a mass spectrometer. Most of these are operated in off-line mode. These will shortly be described later. First an explanation of the prior art on-line arrangements in association with mass spectrometry and in particular matrix assisted laser desorption ionization (MALDI) would be given.

[0003] Continuous mass spectrometric monitoring of a reaction mixture or the effluent from a separation device requires continuous introduction of a minute stream of sample into the vacuum of the mass spectrometer. Established techniques use either pervaporation through a solid polymer membrane or mass flow through a capillary or porous membrane for this purpose. In addition a number of mechanical devices, including the moving belt interface, Rotating Ball Inlet (ROBIN), and the vacuum deposition interface, have been designed to introduce continuously the sample into the mass spectrometer. Each technique has its own advantages and disadvantages and no single technique has universal applicability.

[0004] Continuous fluid introduction through a capillary includes thermospray, electrospray ionization (ESI), particle beam and continuous-flow fast atom bombardment (CF-FAB). Thermospray and ESI operate by the generation (at ambient pressure) of a fine mist of droplets from the sample solution and the evaporation of the solvent from the droplets to yield ions of the analyte. The analyte molecules, being charged, can be electrically guided to enter the mass spectrometer. In the particle beam technique the remnant after vaporization of the droplets forms particles which are guided into the analyzer by translational momentum. In the CF-FAB interface the sample liquid is not nebulized but mixed with glycerol and made to flow onto the target area of an Argon atom source in the mass spectrometer.

[0005] The introduction of the ionization techniques matrix assisted laser desorption ionization and electrospray ionization has made great impact on the analysis of biochemically important compounds. MALDI is a method that allows the production of intact gas-phase ions from large nonvolatile and thermally labile molecules such as proteins, oligonucleotides, and synthetic polymers. In the thirteen years since its introduction, MALDI has become a standard method for the mass spectrometric analysis of large biomolecules. Molecules with molecular weights in excess of several hundred thousand can be desorbed and ionized intact. The MALDI process may be divided into the following two steps.

[0006] The first step involves preparing a sample by mixing the analyte with a molar excess of matrix. The typical matrix is an aromatic acid that strongly absorbs ultraviolet wavelength laser light. The matrix is believed to serve three purposes: absorption of energy from the laser light to desorb the analytes, isolation of the analytes from each other, and promotion of ionization.

[0007] The second step of the MALDI process involves desorption of bulk portions of the solid sample by intense, short duration pulses of laser light. The laser light causes a portion of the matrix and analyte sample to be volatilized and ionized. Usually the molecular masses of the resulting gas-phase ions are determined by time-of-flight TOF mass spectrometry. Ion extraction and detection can be pulsed synchronously with the pulsed production of ions by the laser.

[0008] In the electrospray interface, liquid sample is sprayed at atmospheric pressure from a capillary exit kept at a high potential (1-4 kV). The electrically induced spray of charged microdroplets desolvate into multiple charged ions, which are captured through a capillary restrictor where they are conducted into the low vacuum area of the mass spectrometer.

[0009] MALDI has several advantages over ESI, including spectral simplicity and tolerance to non-volatile buffers. An ESI spectrum depicts multiply charged ions. On one hand, their presence allows the detection of large ions at relatively low m/z, but on the other hand, it complicates the interpretation of the spectra recorded from complex mixtures of analytes. Second, the sensitivity is severely compromised by the presence of salts, impurities and organic buffers often present in biological samples. One of the main advantages that ESI has over MALDI is the capability to spray continuously an ion cloud'into the mass spectrometer. It is more difficult to couple MALDI directly to liquid samples, because samples are generally first dried on a solid surface before insertion into the mass spectrometer. The solution containing the dissolved analyte and matrix is generally applied to a metal probe tip or sample stage. As the solvent evaporates, the analyte and matrix co-crystallize to form a solid crystalline layer of analyte and matrix on the surface of the the sample holder. Conventional MALDI sources are operated under high vacuum, and hence, changing the sample holder for renewed sample deposition requires breaking the vacuum which severely limits its sample through-put and generally requires user intervention. For these reasons, there have been a number of studies and efforts at on-line coupling of MALDI to liquid samples.

[0010] A very important application for on-line mass spectrometry is the analysis of effluents from liquid based separation devices, including High Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE). In both of these techniques a complex mixture containing several analytes may be separated into its single components. The utilization of one of these techniques is often necessitated when analyzing biological samples, as no analytical device is capable in disclosing all the compounds contained in such a sample during one measurement. Hence a two-dimensional analysis has to be performed. In molecular biology mass spectrometry is generally the method of choice for the

determination of protein identity. Hence, the direct coupling between a separation device and the mass spectrometer is worth working on. Commercial combinations of HPLC and Electrospray Ionization mass spectrometry are available and widely used. Concerning MALDI analysis this combination has not yet been offered commercially, but several research groups have demonstrated the ability of MALDI MS to analyse flowing liquid streams and HPLC effluents. The different attempts done in developing a versatile on-line MALDI interface have shed light on a number of problem areas associated with this challenging approach. The main problem inherent with the on-line coupling of liquid samples and MALDI mass spectrometry have been ascribed to the necessity of crystallization of analyte and matrix, giving rise to clogging of the interface.

[0011] To cope with clogging problems the introduction of the sample has been approached by either pneumatic nebulization creating an aerosol or by use of liquid matrices for continuous flow probes.

[0012] The challenge of interfacing MALDI with liquid samples containing a crystalline matrix has additionally been approached by mechanical introduction of the sample. Most recently a MALDI source operating at ambient pressure has been introduced, which offers a great potential for on-line measurements. These prior arrangements are thoroughly described in the following.

[0013] A continuous flow (CF) probe, similar to a CF fast atom bombardment (FAB) interface, has been used for the analysis of a flowing sample with MALDI MS. Applying a liquid matrix has until recently been the only way to cope with clogging problems in CF probes. In CF-MALDI, the choice of liquid matrix is very limited at present. There are only two known liquid matrices, 3-nitrobenzyl alcohol and 2-nitrophenyl octyl ether, that are suitable for MALDI analysis carried out with ultraviolet (UV) wavelengths. A mixture of analyte and liquid MALDI matrix is delivered through the CF probe at low flow rates (ca 4.5 μl min^{-1}). The mixture of analyte and matrix is desorbed directly from the probe tip, located in the vacuum of the mass spectrometer, by a 266 nm laser. Due to the limited number of suitable liquid matrices for UV MALDI applications and the rather poor detection limits obtained with the interface, this approach has only met limited success. **Reference:** R.M. Whittal, et al. *Journal of Chromatography A,* **794,** (1998) 367-375.

[0014] Recently, a CF-probe employing MALDI at infrared wavelengths (2.8 μm) and 0.1% glycerol in ethanol as a matrix has been developed for continuous liquid introduction into the vacuum of the mass spectrometer. One potential advantage to IR MALDI is the ability to employ protic solvents as matrices, using the OH stretch absorption near 3 μm. A large number of potential liquid IR MALDI matrices are available compared to UV MALDI. Water is an obvious choice for an IR matrix because it absorbs strongly at the IR wavelength employed and it is the natural solvent for biomolecules. However, a great deal of work has to be done in order to cope with water freezing at the CF capillary end leading into vacuum. Strong material ablation associated with IR MALDI may reduce this problem, but experimental evidence is needed to disclose the applicability of water as matrix for CF IR-MALDI. **Reference:** S. Lawson and K.K. Murray. *Rapid Communications* in *Mass Spectrometry,* **14,** (2000) 129-134.

[0015] More recently a semi-continuous interface making use of a capillary for sample introduction has been demonstrated for MALDI MS. We have used term semi-continuous in order to distinguish this approach from other continuous flow techniques, because in the present version matrix and analyte is allowed to co-crystallize on a frit. The interface utilizes a porous frit connected to the high vacuum end of a CF-capillary. The liquid solution containing analyte and matrix flow through the frit into the vacuum of the mass spectrometer. The volatile solvent of the sample evaporate rapidly leaving a crystalline layer of matrix and analyte on the vacuum side of the frit. The regeneration of the interface is achieved by a combination of flushing the frit with pure solvent and laser ablation. The authors claim that the interface should allow the direct on-line coupling of liquid capillary chromatography with MALDI-MS, however the study offers only cursory examination of such a potential. **Reference:** Q. Zhan, et al. *Rapid Communications in Mass Spectrometry,* **13,** (1999) 2278-2283.

[0016] In the aerosol MALDI method, the solution containing matrix and analyte is sprayed into the mass sepctrometer where the solvent evaporates. The dried aerosol particles are ionized with a pulsed laser and analyzed by time-of-flight MS. The mass resolution for aerosol MALDI in TOF MS is often hampered by the large ion spatial distribution in the acceleration region of the ion source, but incorporating a reflectron in the flight tube partly compensate for the spread in ion energies. Typically high flow-rates, e.g. 0.5 mL/min, are applied in the aerosol MALDI technique. This inefficient sample utilization has partly been solved by introducing and ionizing single aerosol particles. Utilizing a pneumatic nebulizer with a flow rate of only 5-10 μL min^{-1} efficient aerosol generation could be obtained. By irradiating single aerosol particles with a 337 nm pulsed nitrogen laser, the produced ions were accelerated perpendicular to the particle beam into a reflectron time-of-flight mass spectrometer. **Reference:** L. He and K.K. Murray *Journal of Mass Spectrometry,* **34,** (1999) 909-914.

[0017] WO9853308 (Preisler) discloses a device for continuous vacuum deposition of matrix and analyte from a solution onto a moving surface inside the mass spectrometer could be used to obtain MALDI analysis of a flowing liquid stream. The device makes use of a rotating quartz wheel onto which the liquid is deposited through a narrow fused silica capillary that is kept in contact with the wheel. When the wheel is rotating, deposited sample is transported into the ion source region where MALDI takes place. Promising results have been obtained, and the system is compatible wih crystalline

matrices because clogging at the capillary exit is prevented due to the physical contact with the rotating wheel. The sample consisting of analytes and matrix was deposited in the form of a uniform narrow trace. The design resulted in excellent spot-to-spot reproducibility and attomole sensitivity. Capillary electrophoresis could be directly coupled with the interface. A major disadvantage of the system is the limited operation time because cleaning of the wheel is needed after it has made a 360 degrees cycle lasting about 3 minutes. However, the authors have approached this problem in a second generation interface, where the solution from the infusion capillary is deposited on a disposable Mylar tape. With a total tape length of 80 meters, uninterrupted deposition for about 24 hours has been demonstrated. Additionally a multiplex system utilizing a 12-capillary array has been adapted to the interface. The beam of the MALDI laser was scanned across the tape with 12 deposited traces providing multiplex MS for high thoughput analysis without compromising data quality.

[0018] The rotating ball inlet (ROBIN) as disclosed in WO-A-9920329 is an alternative means of mechanical introduction of liquid samples. The ROBIN MALDI interface represents a development of ROBIN which was originally designed for on-line analysis of volatile compounds. The principle of the inlet is that sample adhering to the surface of a ball is continuously carried past a polymer gasket into the vacuum chamber of the mass spectrometer. Volatile components evaporate from the surface of the ball when exposed to the vacuum. Nonvolatiles, including crystalline matrix and biopolymers, may be desorbed and ionized by laser irradiation of the ball surface in the vacuum of the mass spectrometer. This new interface was recently adapted to on-line MALDI. Here, the matrix and analyte solution is delivered through a capillary to a polymer gasket held tightly against the rotating ball. When the ball rotates it drags sample solution into the MS, where the solvent evaporates leaving a thin crystalline deposit of analyte and matrix on the surface of the ball. Using 2,5-dihydroxybenzoic acid (DHB) as the matrix and using 355 nm laser radiation the ROBIN MALDI interface showed ist ability to perform flow injection analysis of injected protein samples. Since the liquid sample was introduced as a very thin layer there was not enough material on one spot to form macrocrystals of matrix or solutes. Thus there is no risk of clogging the interface because of crystal formation.

[0019] Conventional MALDI sources are operated under high vacuum in order to achieve un-restriccted ion motion in the mass spectrometer. Hence, sample introduction into the MS requires an arrangement that ensures that the high vacuum will not be breached. Generally the sample probe is evacuated in a seperately pumped chamber before inserted in the high vacuum chamber of the MS.

[0020] US 5,965,884 and EP 0 964 427 A disclose a MALDI source which operates at atmospheric pressure (AP-MALDI). Many problems associated with conventional MALDI sources may be solved with the AP MALDI source. As the novel approach basically introduces the ions continuously into the MS, a much higher sample through-put rate is obtained and automation can be implemented more easily. The AP MALDI source operates at ambient pressure and may be useful for the analysis of organic molecules and permits easier construction of a sample switching device. The device includes a ionization enclosure including a passageway for delivery of ions to a mass analysis device. A holder maintains a matrix containing the sample and laser energy is directed onto said sample maintained by the holder to desorb and ionize the analyte. At least a portion of the ionized analyte is directed into the passageway.

[0021] The laser intensity is often attenuated in conventional MALDI time-of-flight instruments in order to reduce the spatial and energetic spead of the abblated MALDI plume. Lower laser intensity is obviously less inefficient in producing ions. Due to rapid thermalization of the ions by collision with ambient gas before fragmentation may occur, higher laser energies may be utilized for AP MALDI to produce more ions per time unit. Additionally the positional accuracy and geometry of the MALDI probe and associated ion optics is not critical to the mass assignment and resolution as it is for conventional MALDI. AP MALDI is not affected by this geometry as long as the produced ions are channelled into the MS. Furthermore AP-MALDI is capable in analysing samples not compatible with high vacuum conditions, including electrophoresis gels and polymer membranes which are prone to shrink when exposed to low pressures.

[0022] It is an object of the present invention to provide an atmospheric-pressure ionization device which facilitates the fully automated analysis system for many laboratories.

[0023] A tube is provided with a sample solution and said solution is irradiated by an ionizing laser at the exit of said capillary, the said laser either indirectly (through charge transfer reactions) or directly ionizes compounds in said sample solution whereby the resulting ions are transported by gas assisted and/or electrical means into the vacuum of the mass spectrometer. The tube permits easier connection to an apparatus for example a chromatograph or another apparatus for liquid separation. The tube itself may be configured for liquid separation.

[0024] We further know that some liquid solvents work well as matrices for MALDI mass spectrometry, especially compounds which absorb at or near 2800 nm have shown to be very applicable.

[0025] According to the invention a continuous flow probe adapted for atmospheric pressure MALDI MS is disclosed which is suited for continuous measurement of a sample stream. Apart from measurements on injected discrete samples this device may also be used for the monitoring of sample streams taken from reac-

tors or effluents from liquid chromatographs and another separation apparatus.

[0026] The atmospheric-pressure ionization device comprises a tube configured for delivery of the sample to an exit of the tube and that the laser that is directed to said exit to induce ionization of said analyte at said exit.

[0027] The invention is described in greater detail hereinafter relative to non-limitative embodiments and the attached drawings, wherein:

Fig. 1 is a schematic representation of an atmospheric-pressure ionization device of the invention,
Fig. 2 is a partial section and
Fig. 3 is a measured spectrum of cyano-4-hydroxycinnamic acid.

[0028] Fig. 1 illustrates a cross sectional view of the atmospheric pressure MALDI source. The liquid sample 23 to be analyzed flows through a tube 1. The tube 1 is provided with an inlet 1a which is connected to a device 1b, in which the sample 23 is stored. The device 1b may be a liquid chromatograph or another separation apparatus or just a container. A laser beam 2 generated in a laser 5 is focused onto an exit 6 of the tube 1 in order to irradiate the liquid sample. Analytes dissolved in the sample 23 are ionized by the laser ablation and generated ions 7 captured into a mass spectrometer 8 or another suitable analysis device through a channel 3 leading into the ion optics 4 of the of mass spectrometer 8 for subsequent mass analysis. The sample 23 is ionized in a chamber 10. The atmosphere within the chamber 10 may be air or a suitable gas to suppress oxidation of the analyte.

[0029] Fig. 2 illustrates a cross sectional view of the device according to Fig. 1 comprising a flange 11 having an inlet opening 19 leading into an analysis device 20, e.g. mass spectrometer, an ion focusing plate 13, a tube 16 and optionally a frit 15. The sample flow in the direction of arrow 18 is supposed to form a droplet 14 at the tube exit which serves as a taget for an ionizing laser beam 2. The sample may be sprayed at said exit of the tube and the laser is directed to the microdroplets of the sprayed sample. The production of microdroplets and ion transport may be assisted by a gas flow in direction of the connection 24 into the sheath 17 surrounding the tube 16. The gas leaves the sheath 17 at a circular opening 25. Produced ions 21 are directed towards the inlet opening 19 of the anlaysis device 20 by virtue of an electric potential difference between flange 11 and focusing plate 13.

[0030] The tube 1 may be a capillary tube, a liquid separation column or a channel in a chip. The diameter of the passage 16 is preferably in the range of 1 μm to 10 mm and the length is preferably in the range of a few mm e.g. 10 mm to several meters. For larger diameters the formation of the droplet may be assisted by the frit 15. A frit 15 is not needed for smaller diameters and in the case when the tube 1 is a capillary tube. The flow rate of the sample within the tube 1 is in the range of 1 to 5 $\mu l\ min^{-1}$.

[0031] Fig. 3 illustrates the measured spectra of cyano-4-hydroxycinnamic acid (M=198.3Da), a typical MALDI matrix, introduced as a solution in methanol. A nitrogen laser (337nm) was used to ablate the matrix. In the absence of the laser light, no ions were observed at all. The two peaks labeled show the protonated matrix with one water molecule attached to it ($M + H + H_2O^+$, m/z=207) and a protonated matrix cluster with one water molecule attached to it ($(2M + H + H_2O^+$,m/z=397). Peaks below 200 Da are supressed by the octupol ion guide in the MS instrument.

Claims

1. Atmospheric-pressure ionization device for connection to an analysis device especially a mass analysis device, comprising:

   a) an atmospheric-pressure ionization chamber;
   b) a passageway configured for delivery of ions to the analysis device
   c) a sample support positioned within said ionization chamber;
   d) a matrix placed on said support and containing an analyte;
   e) a laser for directing laser energy to said sample and to induce ionization of said analyte to form analyte ions;
   f) means for directing at least a portion of said ionized analyte into said passageway;

   characterized in that the sample support comprises a tube configured for delivery of the sample to an exit of the capillary and the laser that is directed to said exit to induce ionization of said analyte at said exit.

2. Device according to claim 1, **characterized in that** the sample is flowing through the tube to said exit.

3. Device according to claim 1 or 2, **characterized in that** said sample forms a droplet at said exit.

4. Device according to claim 3, **characterized in that** the laser is directed to said droplet to induce ionization of analyte in said droplet.

5. Device according to claims 1 to 3, **characterized in that** said sample is sprayed at said exit of the tube and that the laser is directed to said droplets.

6. Device according to claims 1 to 5, **characterized in**

that the analysis device is an ion mobility spectrometer.

7. Device according to claim 1 to 6, **characterized in that** said tube exit is fitted with a porous material.

8. Device according to claim 1 to 7, **characterized in that** porous material is a frit.

9. Device accoring to claims 1 to 8, **characterized in that** the porous material is a membrane.

10. Device according to claims 1 to 9, **characterized in that** a target area is positioned in the proximity of an inlet opening of said analysis device.

11. Device according to claims 1 to 10, **characterized in that** said laser is operated at a wavelength by which at least a part of the sample is ionized through absorption of laser light of a type selected from 266nm, 2800nm, 10600nm or combination therof.

12. Device according to claims 1 to 11, **characterized in that** at least a portion of said ionized analyte is directed to said passageway by electric- or gas-assisted means.

13. Device according to claim 1, **characterized in that** the tube is a capillary tube.

14. Method for analysis of a sample that may contain at least one analyte, comprising:

a) providing a matrix containing that sample;
b) delivering said sample to a tube in which the matrix flows to an exit at a front end of the tube;
c) said matrix forming at least one droplet at said exit;
d) maintaining said matrix containing said sample in a condition of ambiant pressure while directing laser energy onto said droplet at said exit end to desorb and ionize at least a portion of the at least one analyte, and
e) directing at least a portion of the ionized analyte into a analysis device.

15. Method according to claim 14, **characterized in that** the matrix is sprayed at said exit and that the droplets of said spray are irritated by a laser whereby analytes from said matrix are ionized for subsequent analysis.

16. Method according to claim 14 or 15, **characterized in that** the ions produced by the laser irradiation at said exit are transferred to a passageway by electrical or gas-assisted means.

17. Method according to claim 14, **characterized in that** the sample is delivered through a capillary tube.

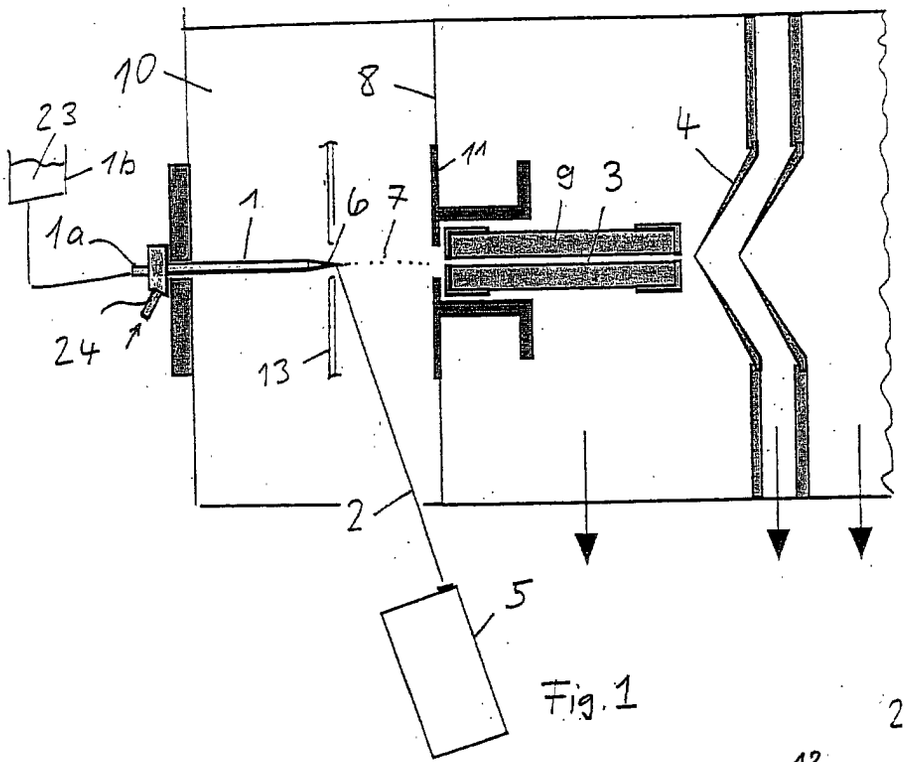

Fig. 1

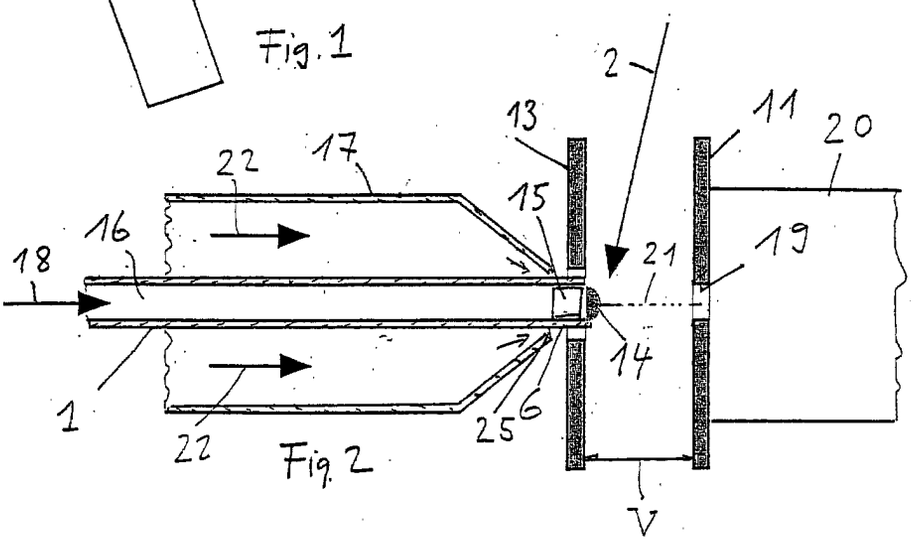

Fig. 2

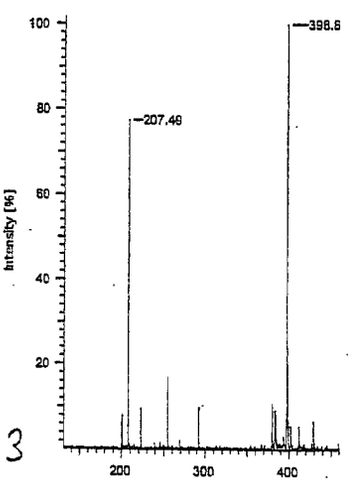

Fig. 3

European Patent Office

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Application Number
EP 00 81 0890

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	6 September 2001	Hulne, S

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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T : theory or principle underlying the invention
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EUROPEAN SEARCH REPORT

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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

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 81 0890

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