(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 1 918 974 A2**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
07.05.2008 Bulletin 2008/19

(51) Int Cl.:
H01J 49/14 *(2006.01)* ***H01J 49/16*** *(2006.01)*

(21) Application number: **07119671.1**

(22) Date of filing: **30.10.2007**

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC MT NL PL PT RO SE SI SK TR
Designated Extension States:
AL BA HR MK RS

(30) Priority: **30.10.2006 CN 200610142595**
09.05.2007 US 746282

(71) Applicant: **National Sun Yat-Sen University**
Gushan District, Kaohsiung City 804 (TW)

(72) Inventors:
- **Shiea, Jentaie**
  Kaohsiung City (TW)
- **Yuan, Cheng-Hui**
  Taipei City (TW)

(74) Representative: **Collin, Jérôme et al**
Cabinet Régimbeau
20, rue de Chazelles
75847 Paris Cedex 17 (FR)

(54) **Mass spectrometer assembly and method for ambient liquid mass spectrometry**

(57) A mass spectrometer including an electrospray-assisted laser desorption ionization device, which includes: an electrospray unit including a nozzle; a voltage supplying member disposed to establish between the nozzle and a receiving unit a potential difference such that liquid drops of the electrospray medium formed at the nozzle are laden with charges, and such that the liquid drops are forced to leave the nozzle toward the receiving unit along a traveling path; a laser desorption unit adapted to irradiate a sample such that analytes contained in the sample are desorbed to fly along a flying path which intersects the traveling path so as to enable the analytes to be occluded in the liquid drops, and such that as a result of dwindling in size of the liquid drops when moving along the traveling path, charges of the liquid drops will pass on to the analytes occluded therein to form ionized analytes.

EP 1 918 974 A2

Description

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The invention relates to a method for mass spectrometry, more particularly to a method for ambient liquid mass spectrometry that is capable of conducting direct analysis of mass spectrometry on a liquid sample under atmospheric pressure. The present invention also relates to a mass spectrometer assembly for conducting the method of ambient liquid mass spectrometry.

2. Description of the Related Art

[0002] A method for mass spectrometry is called electrospray ionization mass spectrometry (ESI-MS), which involves ionizing proteins contained in a liquid sample, followed by a protein analysis. As illustrated in FIG.1, an electrospray ionization mass spectrometer (ESI-MS) 1 includes an electrospray ionization device 11.

[0003] The electrospray ionization device 11 performs an electrospray ionization procedure to ionize the proteins in the liquid sample. An electric field is established between an open end 111 of a capillary 112 and an entrance side 121 of a mass analyzer 12. Subsequently, the liquid sample is pushed through the capillary 112 toward the open end 111. As the electric field force overcomes the surface tension of the liquid sample at the open end 111 of the capillary 112, aerosol droplets containing multivalent electric charges and protein molecules are formed, and are pushed into the mass analyzer 12 through the entrance side 121. The multivalent electrons are attached to the protein molecules to form ionized protein molecules with relatively lower mass-to-charge ratio (m/z) values as the charged droplets dwindle in size when traveling through the air from the open end 111 of the capillary 112 toward the entrance side 121 of the mass analyzer 12. However, body fluids or other biochemical solutions normally contain a high concentration of various salts. A "desalination" pre-process, such as dialysis, is required to prevent the protein molecules from being ionized by acquiring charges from the salts that are present in the body fluids/biochemical solutions, to thereby result in a simpler ion peak configuration in the mass spectrum obtained from ESI-MS. However, professional personnel are required to execute the "desalination" pre-process, which is a tedious, time consuming and inconvenient process.

[0004] Other methods for mass spectrometry require converting an originally liquid-state sample into a solid-state sample prior to conducting the analysis. One of these methods is called the matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). In MALDI-MS, a water soluble organic acidic matrix of highly laser light absorbing small organic molecules is mixed with a liquid sample containing protein molecules before the mixture is dehydrated to form a crystal. A laser beam is irradiated on the surface of the crystal, causing ionization and desorption of the protein molecules. Under an electric field, the ionized protein molecules are introduced into a mass analyzer for mass spectrometric analysis. However, the desorption process of MALDI-MS needs to be conducted in vacuum. Furthermore, it is inconvenient and time consuming to transform liquid samples into solid samples in order to perform MALDI-MS. In addition, the matrix used in MALDI-MS is generally an organic acid, which affects the analyte (e.g., proteins) chemically, causing the structure of the analyte to change.

[0005] A special type of MALDI-MS called "surface-assisted laser desorption/ionization" (SALDI) mass spectrometry (SALDI-MS) is capable of conducting mass spectrometric analysis on a liquid sample, but a vacuum environment is still required. Moreover, a highly viscous solute, such as glycerin, is required for preparing the liquid sample, keeping the cost of instrumentation high and preparation of the sample tedious.

[0006] It can be seen from the above than conducting protein analysis directly on a liquid sample using mass spectrometry techniques presents a variety of difficulties and inconveniences. Since spatial analytic information on proteins of organs or tissues is extremely important in the medical and biotechnological fields, there exists a great need for a method of mass spectrometry that is capable of conducting rapid, convenient, and accurate protein analysis on a liquid sample under atmospheric pressure.

SUMMARY OF THE INVENTION

[0007] Therefore, the object of the present invention is to provide a laser desorption device, a mass spectrometer assembly, and a method for mass spectrometry that is capable of conducting mass analysis directly on a liquid sample under atmospheric pressure.

[0008] According to one aspect of the present invention, there is provided a method for mass spectrometry, which is named "ambient liquid mass spectrometry" (ALMS), and which includes the steps of:

placing, on a sample stage, a liquid sample including a solution that contains a plurality of analytes and a material

serving as a matrix for absorbing laser energy so as to assist in desorption of at least one of the analytes;
providing an electrospray unit that includes a nozzle configured to sequentially form liquid drops of an electrospray medium thereat;
providing a receiving unit that is disposed to admit therein ionized analytes that are derived from the liquid sample, and that are to be analyzed by a mass analyzer disposed downstream of the receiving unit, the receiving unit being spaced apart from the nozzle of the electrospray unit in a longitudinal direction so as to define a traveling path;
establishing a potential difference between the nozzle of the electrospray unit and the receiving unit, the potential difference being of an intensity such that the liquid drops are laden with a plurality of charges, and such that the liquid drops are forced to leave the nozzle as multiple-charged ones for heading toward the receiving unit along the traveling path; and
irradiating the liquid sample with a laser beam such that, upon irradiation, laser energy is passed on to at least one of the analytes contained in the solution of the liquid sample via the matrix so that said at least one of the analytes contained in the liquid sample is desorbed to fly along a flying path which intersects the traveling path so as to enable said at least one of the analytes to be occluded in the multiple-charged liquid drops, and such that as a result of dwindling in size of the multiple-charged liquid drops when approaching the receiving unit along the traveling path, charges of the liquid drops will pass on to said at least one of the analytes occluded therein to form a corresponding one of the ionized analytes.

[0009] According to another aspect of the present invention, there is provided a laser desorption device for use in a mass spectrometer assembly.

[0010] The mass spectrometer assembly includes a receiving unit, an electrospray unit, and a voltage supplying member. The laser desorption device includes a sample stage and a laser transmission mechanism. The sample stage and the laser transmission mechanism are arranged with the receiving unit, the electrospray unit, and the voltage supplying member in a manner such that all the steps of the abovementioned method can be duly carried out. The laser transmission mechanism can be one of an ultraviolet (UV) laser, an infrared (IR) laser, a nitrogen laser, an argon ion laser, a helium-neon laser, a carbon dioxide (CO_2) laser, and a garnet (Nd:YAG) laser.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Other features and advantages of the present invention will become apparent in the following detailed description of the preferred embodiments with reference to the accompanying drawings, of which:

FIG.1 is a schematic diagram of various components included in an electrospray ionization mass spectrometer (ESI-MS) of the prior art to illustrate relative positions of the components and operational method involved in the ESI-MS;
FIG. 2 is a schematic diagram of a laser desorption device and an electrospray unit for the first preferred embodiment of a mass spectrometer assembly implementing the method of ambient liquid mass spectrometry (ALMS) according to the present invention, illustrating desorption of analytes contained in a liquid sample so as to fly along a flying path that intersects a traveling path of multiple-charged liquid drops;
FIG.3 is a schematic diagram, illustrating occlusion of the analytes in the multiple-charged liquid drops, and formation of ionized analytes as a result of dwindling in size of the multiple-charged liquid drops having the analytes occluded therein;
FIG. 4 is a schematic side view of the first and fifth preferred embodiments of a mass spectrometer assembly implementing the method of ALMS according to the present invention;
FIG.5 is a fragmentary enlarged view of the second preferred embodiment of a mass spectrometer assembly implementing the method of ALMS according to the present invention, illustrating relative positions of an airstream supplying mechanism and a nozzle;
FIG.6 is a fragmentary sectional view of the third preferred embodiment of a mass spectrometer assembly implementing the method of ALMS according to the present invention, illustrating relative positions of a micro-tube, a nozzle, and a pump;
FIG. 7 is a schematic side view of the fourth and sixth preferred embodiments of a mass spectrometer assembly implementing the method of ALMS according to the present invention;
FIG.8(a) to 8(c) illustrate mass spectra obtained as experiment results of comparative example 1 and exemplary methods 1 and 2; and
FIG. 9(a) to 9(f) illustrate mass spectra obtained as experiment results of exemplary methods 3 to 8.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0012] Before the present invention is described in greater detail, it should be noted herein that like elements are

denoted by the same reference numerals throughout the disclosure. It is also noted herein that in the accompanying drawings, sizes of constituting elements and relative distances among the elements are not drawn to scale.

[0013] In this invention, a suitable matrix is added to a liquid sample prior to conducting a mass spectrometric analysis. Particularly, as shown in FIGS.2 to 4, while an electrospray ionization process is implemented to form sequentially multiple-charged liquid drops 511 of a liquid electrospray medium 51, a laser beam 821 is irradiated onto a liquid sample 4, which includes a solution 41 that contains analytes 412 and a material 413 serving as a matrix for absorbing laser energy, and which is disposed in the passage way of a receiving unit 6 adapted to admit therein ionized analytes 414 that are derived from the liquid sample 4 for mass spectrometric analysis. The obtained mass spectrometric analysis results established that this novel technique, referred to as "ambient liquid mass spectrometry" (ALMS), is practicable directly on liquid samples under atmospheric pressure.

[0014] As the liquid sample 4 is irradiated by the laser beam 821, laser energy which is absorbed by the matrix material 413 is presumably passed on to at least one of the analytes 412 via the matrix material 413 so that said at least one of the analytes 412 is desorbed, and is occluded in the multiple-charged liquid drops 511 formed during the electrospray ionization process. As a result of dwindling in size of the multiple-charged liquid drops 511 when approaching the receiving unit 6, charges of the liquid drops 511 will pass on to said at least one of the analytes 412 occluded therein to form a corresponding ionized analyte 414. The ionized analyte 414 is received by the receiving unit 6 for mass spectrometric analysis thereby.

[0015] Moreover, since water molecules are highly absorbent to infrared (IR) light, the water molecules contained in an aqueous solution serve as the matrix for absorbing laser energy and transferring the laser energy to the analytes. Therefore, with procedures similar to those disclosed hereinabove, an infrared laser beam is employed to irradiate directly on an aqueous solution to obtain accurate mass spectrometric analysis results.

[0016] The abovedescribed novel method of mass spectrometry, named "Ambient Liquid Mass Spectrometry" (ALMS), apparently opens up a new era for mass spectrometric analysis of liquid samples, especially on aqueous solutions containing proteins, under atmospheric pressure. Operation procedure of ALMS is relatively simple and rapid, and resolution thereof is higher than that of ESI-MS. ALMS is capable of accurately detecting molecular weights of analytes, even when the analytes are macromolecules, such as proteins, thereby showing an outstanding ability in protein identification. These advantages allow ALMS to quickly analyze biochemical and medical liquid samples so as to obtain reliable results, which is extremely favorable in relevant applications, such as immediate diagnosis of diseases.

[0017] As shown in FIGS. 2 to 4, the method of ALMS according to the present invention can be implemented by performing the following steps:

[0018] Place a liquid sample 4, on a sample stage 81, that includes a solution 41 containing a plurality of analytes 412 and a matrix material 413 for absorbing laser energy so as to assist in desorption of the analytes 412. In particular, the solution 41 includes a solvent 411 that contains the analytes 412 and the matrix material 413 therein.

[0019] Provide an electrospray unit 5 that includes a reservoir 52 for accommodating a liquid electrospray medium 51, and a nozzle 53 which is disposed downstream of the reservoir 52, and which is configured to sequentially form liquid drops 511 of the electrospray medium 51 thereat.

[0020] Provide a receiving unit 6 that is spaced apart from the nozzle 53 for receiving and analyzing ionized analytes 414 derived from the liquid sample 4.

[0021] Provide a detector 7 for detecting signals generated as a result of analyzing the ionized analytes 414 by the receiving unit 6, and for generating a mass spectrum of the liquid sample 4 from the signals.

[0022] Establish between the nozzle 53 and the mass analyzer 61 a potential difference which is of an intensity such that the liquid drops 511 are laden with a plurality of electric charges, and such that the liquid drops 511 are forced to leave the nozzle 53 as multiple-charged ones for heading toward the receiving unit 6 along a traveling path (X).

[0023] Irradiate the liquid sample 4 with a laser beam 821 such that at least one of the analytes 412 is desorbed to fly along a flying path (Y) which intersects the traveling path (X) so as to enable said at least one of the analytes 412 to be occluded in the multiple-charged liquid drops 511, and such that as a result of dwindling in size of the multiple-charged liquid drops 511 when approaching the mass analyzer 61 along the traveling path (X), charges of the liquid drops 511 will pass on to said at least one of the analytes 412 to form a corresponding one of the ionized analytes 414.

[0024] The electrospray medium forming the liquid drops is a solution normally used in electrospray methods. An example of the electrospray medium is a solution containing a volatile liquid such that the liquid portion in the liquid drops can vaporize prior to the receipt of the ionized analytes by the mass analyzer. Further, in order to help dissolve protein molecules and avoid interference due to an addition of salt in the volatile liquid, and to simplify the resultant mass spectrum, the volatile liquid is preferably one with a low polarity, such as isoacetonitrile, acetone, alcohol, etc. If the analyte in the liquid sample is a protein, and it is desired to investigate the un-denatured state of the protein, the electrospray medium is preferably a solution that contains a volatile liquid and that does not contain an acid, such as methanol aqueous solution.

[0025] Based on different requirements, "an aqueous solution containing methanol and acetic acid" and a "methanol aqueous solution" are used as the electrospray medium in the embodiments of the present invention, respectively. In

addition, it is assumed that the ion portion of the obtained analytes is multivalent with each charge being contributed by a proton (H^+) .

[0026] One of the main objects that the method of ALMS aims at is the detection of analytes from a liquid sample including a solution that contains the analytes and a matrix material. Therefore, no limitation is imposed on the types of solutions and the kinds of analytes detectable for the implementations of the present invention. Whether the solution is an aqueous solution, contains an organic solvent, or is a body fluid secreted by an organism and having a complicated composition, and whether the analytes are macroscopic molecules such as proteins, or are microscopic molecules such as ordinary compounds, mass spectrometric analysis results can be obtained through implementing the method of ALMS according to the present invention.

[0027] Therefore, the liquid sample under study can include various solutions, including organism's body fluids, chemical solutions, environment sampling solutions, or various eluates from liquid chromatography, etc. Preferably, the organism's body fluid can be selected from the group consisting of blood, tear, milk, perspiration, intestinal juice, brains fluid, spinal fluid, lymph, pus, blood serum, saliva, nasal mucus, urine, and excrement. When the liquid sample under study includes a chemical solution, the chemical solution can be an organic solution.

[0028] Preferably, the matrix material ismade fromamaterial that is non-transmissible by laser, such as gold, carbon, cobalt, iron, 2,5-dihydroxybenzoic acid (2,5-DHB), 3,5-dimethoxy-4-hydroxycinnamic: acid (sinapinic acid, (SA)), α-cyano-4-hydroxycinnamic acid (α-CHC), and a combination thereof. Better results are obtained when the material serving as the matrix has a particle diameter ranging from 50 nm to 50 μm.

[0029] When the solution included in the liquid sample is an aqueous solution, water molecules contained in the aqueous solution would be the material serving as the matrix, and an infrared laser beam is used for desorption. Incidentally, it is also practicable to add another material purposely into an aqueous solution to serve as the matrix. However, when it is desired to analyze an organic solution, a material serving as a matrix is added into the solution to form the liquid sample under study prior to implementing the method of ALMS. Descriptions related to detailed operational practices and mechanisms for the method of ALMS will be described in subsequent embodiments.

[Preferred embodiments]

[0030] The present invention is described in greater detail hereinbelow with respect to the preferred embodiments and exemplary applications presented. It should be noted herein that the embodiments and exemplary applications are for illustrative purposes only, and should not be considered as limitations imposed on the present invention.

Chemicals and Equipments Used

[0031] The preferred embodiments, exemplary methods, and comparison (experiment) example were conducted using the following chemicals and equipments:

1. Laser Transmission Mechanism:

   a. Ultraviolet (UV) Laser model no. VSL-3371, manufactured by Laser, Science Inc. of the United States. The laser beams transmitted by the ultraviolet laser have a wavelength of 337nm, a frequency of 10Hz, a pulse duration of 4ns, and a pulse energy of 100μJ.
   b. Infrared (IR) Laser model no. LS-2130SHP, manufactured by LOTIS TII of Russia. The laser beams transmitted by the infrared laser have a wavelength of 1064nm, a frequency of 2Hz, a pulse duration of 0.5ns, and a pulse energy of 50mJ.

2. Mass Analyzer (including the Detector): Quadrupole Time-of-Flight Mass Analyzer model no. BioTOF-Q, manufactured by Bruker Dalton company of Germany.
3. Electrospray Medium:

   a. Methanol: an HPLC solvent manufactured by Merck company of Germany.
   b. acetic acid: an HPLC solvent manufactured by Mallinckrodt company of Germany.

4. Analytes:

   a Protein Standard: insulin (molecular weight of 5733), myoglobin (molecular weight of 17566), lysozyme (molecular weight of 14305), and cytochrome c (molecular weight of 12232), all of which are high purity protein standards with concentrations of above 95% and manufactured by Sigma-Aldrich company of the United States.
   b. Hemin: molecular weight of 652.0, model no. H-2250 manufactured by Aldrich company of the United States.

c. 18-crown-6-ether: molecular weight of 264.32, model no. C0860 manufactured by Tokyo Chemical Industry Co., Ltd. of Japan.
d. 1-hexadecylamine: molecular weight of 241.46, model no. H740-8 manufactured by Aldrich company of the United States.
e. Methyl (triphenyl-phosphoranylidene) acetate: molecular weight of 334, model no. 64941 manufactured by Fluka company.
f. Cinnamic acid benzyl ester: molecular weight of 260, model no. C0358 manufactured by Tokyo Chemical Industry Co., Ltd. of Japan.
g. Cetylpyridinium chloride: molecular weight of 339.99 (note: Chemical equation of cetylpyridinium chloride is C21H38N-C1 with an average molecular weight of 339. 99 and a monoisotope molecular weight of 339.27, where C21H38N(304.30) is a cation and Cl(34.97) is an anion. Since a mass spectrometer detects cations, the molecular weight obtained is not 339.99), model no. 145-100G manufactured by AJAX Chemical.

5. Solvents:

a. Methanol (identical to the above)
b. Tetrahydrofuran (THF): model no. 9440-03 manufactured by J. T. Baker company of the United States.
c. Ethyl acetate: model no. 9282-03 manufactured by J. T. Baker company of the United States.
d. Methylene dichloride: a HPLC solvent manufactured by Merck company of Germany.
e. Tolene: an HPLC solvent manufactured by J. T. Baker company of the United States.
f. N-hexane: an HPLC solvent manufactured by J. T. Baker company of the United States.

6. Other chemicals:

a. H_2O_2: concentration of 30%, model no. 31642 manufactured by Riedel-de Haën company.
b. NaoH: model no. SK371842 manufactured by Nihon Shiyaku Industries Ltd.

7. Matrix Material:

a. Carbon powders: model no. 4206A manufactured by Merck company of Germany; particle diameter of below 50μm.
b. Gold nano-particles: provided privately; particle diameter of approximately 56nm.
c. α -cyano-4-hydroxycinnamic acid (α -CHC), an HPLC material manufactured by Sigma-Aldrich company of the United States.
d. 2,5-dihydroxybenzoic acid (2,5-DHB): model no. D0569 manufactured by Tokyo Chemical Industry Co., Ltd. of Japan.
e. 3,5-dimethoxy-4-hydroxycinnamic acid (Sinapinic acid (SA)) : model no. D1765 manufactured by Tokyo Chemical Industry Co., Ltd. of Japan.

8. Matrix-Assisted Laser Desorption Ionization Mass Spectrometer (MALDI-MS): model no. Autoflex MALDI/TOF, manufactured by Bruker Dalton company of Germany, and suitable for analyzing macromolecules in the linear mode.
9. Electrospray Ionization Mass Spectrometer (ESI-MS): including an electrospray unit, a mass analyzer, and a detector; the electrospray unit, the mass analyzer and the detector are identical to those used in the embodiments of the mass spectrometer assembly implementing the method of ALMS according to the present invention.
10. Relevant chemicals or equipments for bacterial extraction:

a. Glass beads: model no. 11079101 manufactured by Biospec Products, Inc.; diameter of 100 μm.
b. Sonicator: model no. XL2020 manufactured by Heat Systems, Inc.
c. Centrifuge: modelno. DSC-1524SDT TFA manufactured by Digisystem Laboratory Instruments, Inc.
d. Trifluroacetic acid: an analysis class acid with model no. 61030 manufactured by Riedel-de Haën company.
e. Acetonitrile (ACN) : an HPLC material with model no. UN1648 manufactured by Merck company of Germany.

First Preferred Embodiment - Mass Spectrometer Assembly

Implementing the Method of ALMS

[0032] Referring to FIG.4, the first preferred embodiment of a mass spectrometer assembly implementing the method of ALMS is adapted to conduct mass spectrometric analysis on a liquid sample 4. With reference back to FIG. 2 and

FIG. 3, the liquid sample 4 includes a solution 41 including a solvent that contains a plurality of analytes 412 and a material 413 serving as a matrix (also referred to as a matrix material 413) for assisting in desorption of at least one of the analytes 412. The mass spectrometer assembly includes an electrospray unit 5, a receiving unit 6, a voltage supplying member 3, and a laser desorption device 8.

[0033] The laser desorption device 8 includes a sample stage 81 on which the liquid sample 4 is placed, a laser transmission mechanism 82 that is capable of transmitting a laser beam 821 and that is disposed to irradiate the liquid sample 4, a lens 83 that is disposed to receive the laser beam 821 from the laser transmission mechanism 82 for focusing the energy carried by the laser beam 821, and a reflector 84 that is disposed to change the path of the laser beam 821. In this embodiment, the laser transmission mechanism 82 is an ultraviolet laser transmission mechanism 82a that is capable of transmitting the laser beam 821. In principle, the laser desorption device 8 is designed as long as the laser desorption device 8 is capable of irradiating the liquid sample 4 such that, upon irradiation, at least one of the analytes 412 contained in the solution 41 of the liquid sample 4 is desorbed. Therefore, in practice, the lens 83 and the reflector 84 can be varied in position as required, or can even be completely eliminated according to other embodiments of the present invention.

[0034] The sample stage 81 of the laser desorption device 8 includes a support member 811 that is made from a material non-transmissive by laser, and a hoister platform 812 that is provided for mounting of the support member 811 thereon, and that is movable. The support member 811 is provided for placement of the liquid sample 4, and has a support surface 813 for placement of the liquid sample 4 directly thereon. This way, an operator can begin performing the method of ALMS by dripping the liquid sample 4 on the support surface 813.

[0035] The receiving unit 6 is disposed to admit therein ionized analytes 414 that are derived from the liquid sample 4, and that are to be analyzed for mass spectrometric analysis. The receiving unit 6 includes a mass analyzer 61 disposed for analyzing the ionized analytes 414. The mass analyzer 61 is formed with a conduit 611 that is in air communication with the environment. The detector 7 is disposed to receive signals generated by the mass analyzer 61 as a result of analyzing the ionized analytes 414 so as to generate a mass spectrometric analysis result, i.e., a mass spectrum.

[0036] The electrospray unit 5 includes a reservoir 52 for accommodating a liquid electrospray medium 51, a nozzle 53 (in the embodiments of the present invention, the nozzle 53 is a capillary 53a) which is disposed downstream of the reservoir 52, and which is configured to sequentially form liquid drops 511 of the electrospraymedium 51 thereat, and a pump 54 disposed downstream of the reservoir 52 and upstream of the nozzle 53 for drawing the electrospray medium 51 into the nozzle 53. The nozzle 53 is spaced apart from the mass analyzer 61 of the receiving unit 6 in a longitudinal direction so as to define a traveling path (X).

[0037] The voltage supplying member 3 is disposed to establish between the nozzle 53 of the electrospray unit 5 and the mass analyzer 61 of the receiving unit 6 a potential difference which is of an intensity such that the liquid drops 511 are laden with a plurality of charges, and such that the liquid drops 511 are forced to leave the nozzle 53 as multiple-charged ones for heading toward the mass analyzer 61 along the traveling path (X).

[0038] In the first preferred embodiment, the nozzle 53 is made from a metal material, and a first central axis 532 of the nozzle 53 and a second central axis 612 of the conduit 611 in the mass analyzer 61 are substantially parallel to each other. The support member 811 of the sample stage 81 extends in the longitudinal direction such that the support surface 813 thereof defines a leveled plane in the longitudinal direction. The distance between projections of an outlet 531 of the nozzle 53 and an entrance 613 into the conduit 611 of the mass analyzer 61 on the leveled plane is approximately 8 mm. In addition, when the liquid sample 4 is placed on the support surface 813 of the support member 81, the shortest distance between the liquid sample 4 and the outlet 531 of the nozzle 53 is 1.5 mm.

[0039] When the laser transmission mechanism 82 of the laser desorption device 8 transmits the laser beam 821 to irradiate the liquid sample 4, upon irradiation, at least one of the analytes 412 contained in the solution 41 of the liquid sample 4 is desorbed to fly along a flying path (Y) which intersects the traveling path (X) so as to enable said at least one of the analytes 412 to be occluded in the multiple-charged liquid drops 511. As a result of dwindling in size of the multiple-charged liquid drops 511 when approaching the mass analyzer 61 of the receiving unit 6 along the traveling path (X), charges of the liquid drops 511 will pass on to said at least one of the analytes 412 to form a corresponding one of the ionized analytes 414. The ionized analytes 414 enter the mass analyzer 61 via the entrance 613 into the conduit 611 for subsequent mass spectrometric analysis.

Second Preferred Embodiment

[0040] With reference to FIG.5, the second preferred embodiment of a mass spectrometer assembly implementing the method of ALMS according to the present invention is similar to the first preferred embodiment. The only difference between the first and second preferred embodiments is that the electrospray unit 5' of the second preferred embodiment further includes an airstream supplying mechanism 55' for accelerating vaporization of the multiple-charged liquid drops 511 (refer to FIGS.2 to 4) to result in dwindling in size thereof when approaching the mass analyzer 61 (refer to FIG.5) along thetravelingpath (X). The airstream supplying mechanism 55' surrounds the nozzle 53, and supplies a nitrogen

airstream 5.51'. In particular, the temperature of the nitrogen airstream 551' can be controlled by the user between the room temperature and 325°C as is required.

Third Preferred Embodiment

[0041] As shown in FIG. 6, the third preferred embodiment of a mass spectrometer assembly implementing the method of ALMS according to the present invention is similar to the first preferred embodiment. The difference between the first and third preferred embodiments is that the nozzle 53" of the electrospray unit 5" of the third preferred embodiment is made from a non-metal material, and the electrospray unit 5" further includes a micro-tube 56". The micro-tube 56" includes a tubular body 561" connected between and disposed in fluid communication with the pump 54 and the nozzle 53", and a center portion 562" connected to the tubular body 561" and coupled to the voltage supplying member 3 (refer to FIG. 4) such that the potential difference is established between the micro-tube 56" and the mass analyzer 61 of the receiving unit 6.

Fourth Preferred Embodiment

[0042] Referring to FIG. 7, the fourth preferred embodiment of a mass spectrometer assembly implementing the method of ALMS according to the present invention is similar to the first preferred embodiment. The difference between the first and fourth preferred embodiments is that the sample stage 81'" of the laser desorption device 8"' includes a movable track 814"', and a support member set 815'" including a plurality of support members 816"' (only one is visible in FIG. 7) connected in sequence and mounted movably on the track 814"'.

[0043] To conduct mass spectrometric analysis using the mass spectrometer assembly of the fourth preferred embodiment, a plurality of liquid samples 4 (as shown in FIG.4) are first contained in containers 10 (e.g., test tubes or centrifuge tubes) (only one is visible in FIG. 7), respectively. Subsequently, each of the containers 10 is disposed on a corresponding one of the support members 816"'. Through control of a computer software, the support members 816"' move along the track 814"', carrying the liquid samples 4 thereon, such that the liquid samples 4 are sequentially disposed at a predefined location set by the operator. When each of the liquid samples 4 is disposed at the predefined location, the liquid sample 4 is irradiated by the laser beam 821 transmitted by the laser transmission mechanism 82 of the laser desorption device 8, and subsequent mass spectrometric analysis is conducted.

[0044] It should be noted herein that only one support member 816"' and one container 10 are visible in FIG.7 due to the direction of observation.

Fifth Preferred Embodiment

[0045] Referring to FIG.4, the fifth preferred embodiment of a mass spectrometer assembly implementing the method of ALMS according to the present invention is similar to the first preferred embodiment. The only difference between the first and the fifth preferred embodiments is that the laser transmission mechanism of the fifth preferred embodiment is an infrared (IR) laser 82c instead of the ultraviolet laser 82a as in the first preferred embodiment.

Sixth Preferred Embodiment

[0046] Referring to FIG. 6, the sixth preferred embodiment of a mass spectrometer assembly implementing the method of ALMS according to the present invention is similar to the fourth preferred embodiment. The only difference between the fourth and the sixth preferred embodiments is that the laser transmission mechanism of the sixth preferred embodiment is the infrared (IR) laser 82c (as shown in FIG.4) instead of the ultraviolet laser 82a.

[0047] It should be noted herein that each of the components of the mass spectrometer assembly according to the present invention can be designed to be movable so as to permit adjustments of the positions thereof by the user as are required, such that relative positions or distances among the various components of the mass spectrometer assembly can be determined. Similarly, parameters, such as the energy, frequency, incident angle of the laser beam irradiated by the laser transmission mechanism, and the composition and flow rate of the electrospray medium, etc., can be adjusted according to the objectives aimed, so as to obtain optimal detection results.

<Exemplary Methods and Comparative Example>

[0048] Presented hereinbelow are exemplary methods for the method of ALMS according to the present invention, along with a comparative example. In the exemplary methods and the comparative example, the liquid samples and electrospray medium are prepared following a certain proportion, or are obtained directly, under room temperature and atmospheric pressure. If it is not particularly pointed out, the liquid sample includes an aqueous solution, and the com-

position of the electrospray medium is [water : methanol : acetic acid = 50 : 50 : 0.1], and the flow rate of the electrospray medium is 150 μL per minute.

[0049] Further, if it is not particularly pointed out, the exemplary methods and the comparative example are conducted according to the third preferred embodiment of the present invention. In addition, the mass analyzer conducts the scans with a 2s/scan scanning rate. For each liquid sample presented, the molecular weight of the solvent is excluded from a scanning range of the mass analyzer.

Exemplary Methods 1 and 2 and Comparative Example 1 - Mass Spectrometric Analysis Conducted on Protein Standard Sample Solutions

[0050] In exemplary methods 1 and 2 and in comparative example 1, the electrospray medium used was a 20 vol% methanol aqueous solution, and the matrix material for the liquid sample was in the form of carbon powders with varying concentrations, respectively. The composition of the liquid sample used, and the figure number of corresponding mass spectrum for each of exemplary methods 1 and 2 and comparative example 1 are tabulated in Table 1 below.

Table 1

	Liquid Sample		Mass spectrum
	Analytes	Carbon powder Concentration	
Comparative example 1	myoglobin (10^{-5} M), cytochrome c (10^{-5} M), lysozyme (10^{-5} M)	0 mg/μL	FIG. 8(a)
Exemplary Method 1		0.4 mg/μL	FIG. 8(b)
Exemplary Method 2		0.8 mg/μL	FIG. 8(c)

[0051] Since the electrospray medium used does not contain any acid, the applicant predicted that the mass spectra obtained should present the formation of "un-denatured proteins". In other words, the molecular weight of myoglobin resulted from exemplary methods 1 and 2, where ALMS analysis was used, should be 17567 Da, instead of 16951 Da, which is the molecular weight of a denatured protein short of one heme molecular (molecular weight of 616 Da).

Results

[0052] It is clearly shown in FIGS. 8(b) and 8(c) that there are three ion peaks, which are respectively denoted by "■", "▲", "•", and whose molecular weights are calculated by a computer software to be 12232 Da, 14306 Da, and 17567 Da, respectively. The calculated molecular weights almost completely correspond to the molecular weights of myoglobin, cytochrome c, and lysozyme as provided by the manufacturer. In addition, it is obvious that the detected myoglobin is in an un-denatured state. The results confirm that the method of ALMS works effectively, and is capable of conducting direct detection on a liquid sample including a protein so as to obtain accurate and satisfactory quantitative results.

[0053] The reason for this success is that, upon irradiation, laser energy of the ultraviolet laser beam is passed on to at least one of the analytes (proteins) contained in the solution of the liquid sample via the matrix material (carbon powders) so that the analyte is successfully desorbed. On the other hand, the liquid sample used in comparative example 1 does not contain carbon powders or any other materials to serve as a matrix, the analytes could not be effectively desorbed from the liquid sample (or the volume of desorbed analytes was too small). Since no or a minimal number of analytes reached and was detected by the mass analyzer for mass spectrometric analysis, corresponding signals for the analytes could not be generated.

[0054] It should be noted herein that the peak shown in FIG. 8 (a) is an interference signal, and is relatively enlarged due to the absence of analyte signals.

Exemplary Methods 3 to 8 - ALMS Analysis Conducted on Liquid Samples Provided with Organic Solutions and Carbon Powders

[0055] The composition of the liquid sample used, and the figure number of corresponding mass spectrum for each of the exemplary methods 3 to 8 are tabulated in Table 2 below.

Table 2

	Liquid Sample			Mass Spectrum
	Material serving as Matrix	Solvent	Analytes and of Concentration thereof	
Exemplary Method 3	Carbon powder (0.8 mg/μL)	Methanol	Hemin ($2*10^{-3}$M)	FIG.9(a)
Exemplary Method 4		THF	18-crown-6-ether ($2*10^{-2}$M)	FIG.9 (b)
Exemplary Method 5		EA.	1-hexadecylamine ($1*10^{-3}$M)	FIG. 9 (c)
Exemplary Method 6		Methylene dichloride	Methyl (triphenyl-phosphoranylidene) acetate ($1*10^{-2}$M)	FIG. 9(d)
Exemplary Method 7		Tolene	Cinnamic acid benzyl ester ($2*10^{-2}$M)	FIG.9(e)
Exemplary Method 8		n-hexane	Cetylpyridinium chloride ($1*10^{-4}$M)	FIG. 9(f)

Results

[0056] It is clearly observed in FIGS.9(a) to 9(f) corresponding ion peaks formed by analytes of the liquid samples used in exemplary methods 3 to 8. In addition, the molecular weights obtained after calculation match with the known facts, confirming the operability of the method of ALMS on liquid samples provided with organic solutions and organic compounds.

[0057] In an experiment conducted by the applicant, a linear equation obtained through linear regression analysis demonstrates that glycosylated hemoglobin/ hemoglobin (HbA1/Hb) values of in blood of a diabetes patient obtained using ALMS analysis has a specific relationship with those obtained using ionic chromatography, which is a currently common method used in the medical field for obtaining the quantities of Hb and HbA1. Therefore, the (HbA1/Hb) values obtained using ALMS analysis should have a certain degree of credibility and reference value. In particular, it is reported that it takes approximately an hour, including preparation work on the samples, to conduct analysis using ionic chromatography. On the other hand, instantaneous detection and result can be obtained using ALMS analysis. Therefore, the method of ALMS should have the potential of replacing the method of ionic chromatography in providing the basis for diagnoses of diseases.

[0058] In addition, it is also verified by experiment that a highly credible mass spectrometric analysis result can be obtained when a liquid sample includes an aqueous solution, even if it is a body fluid with complicated composition and containing a large quantity of salts, after a simple diluting step, rapid and convenient analysis can be conducted using the method of ALMS, without adding an additional matrix material, by irradiating an infrared laser beam on the liquid sample. In this case, the "water molecules" contained in the aqueous solution serve as the "matrix" for transferring the laser energy to the analytes such that the analytes are desorbed and enter the mass analyzer for subsequent mass spectrometric analysis.

[0059] With reference to the results described hereinabove with respect to the exemplary methods and the comparative example, it is shown that the present invention is in deed capable of performing rapid and accurate mass spectrometric analysis directly on a liquid sample. In addition, no specific restriction is imposed on the sample to be analyzed, i.e., whether it is a body fluid with a complicated composition, or an organic solution, a protein solution, etc., qualitative information about the contents therein can be obtained through the method of ALMS according to the present invention. Moreover, other than qualitative information, relative quantitative information on various analytes in a liquid sample, such as compositional proportions of the analytes in the liquid sample, can also be reflected through the use of ALMS analysis. It is of special importance that when a liquid sample includes an aqueous solution, by irradiating the liquid sample with infrared laser, satisfactory detection results can be obtained through ALMS analysis.

[0060] In addition, a mass spectrometer assembly implementing the method of ALMS should be capable of being connected in series to other analytic instruments. A high performance liquid chromatograph (HPLC) is taken as an example hereinbelow for illustration. When a biochemical sample (normally including an aqueous solution) is eluted after passing through the HPLC, ALMS analysis can be conducted by irradiating laser on the eluted sample when it is disposed between the electrospray unit and the mass analyzer of the mass spectrometer assembly implementing the method of ALMS.

[0061] In sum, since.the method of ALMS according to the present invention is conducted directly under atmospheric pressure, instead of vacuum, and since operation time needed is extremely short, the cost of instrumentation for implementing the present invention, the technical requirements for manufacturing such instrumentation and for operation of such method have all greatly reduced as compared to matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) of the prior art. Further, it has been verified that the method of ALMS according to the present invention can be used to analyze various kinds of liquid samples, including protein aqueous solutions, body fluids, and organic solutions containing organic compounds, etc., can all be analyzed directly (with minimal sample preparation), as opposed to making the originally liquid samples into solid samples. In addition, satisfactory results can be obtained both for qualitative analysis (i.e., the determination of the identity of the analytes detected) and relative quantitative analysis (i.e., the quantity of various kinds of analytes contained in the liquid sample).

[0062] Due to the convenience and speed of the method of ALMS according to the present invention, and immediate results obtainable through use of such method, it is evident that the present invention is advantageous in related fields, where qualitative analysis of analytes in a large quantity of liquid samples or determination of relative concentrations of analytes in liquid samples is required, such as in medical fields, environmental examination, criminal judgment, academic research, etc.

[0063] The method of ALMS according to the present invention can also be applied to the analysis of a body fluid secreted by an organism. Through identities and relative concentrations of substances in an organism' s body fluid, the biological condition of the organism can be determined.

[0064] Moreover, the mass spectrometer assembly implementing the method of ALMS according to the present invention can be connected in series to other analytic instruments, such as a high performance liquid chromatograph (HPLC), so that an operator can conduct ALMS analysis so as to obtain information on the substances contained in the sample in sequence with conducting sample purification. This greatly enhances operational convenience and greatly reduces operational time when several analyses need to be conducted on identical samples.

[0065] While the present invention has been described in connection with what are considered the most practical and preferred embodiments, it is understood that this invention is not limited to the disclosed embodiments but is intended to cover various arrangements included within the spirit and scope of the broadest interpretation and equivalent arrangements.

Claims

1. A mass spectrometer assembly comprising:

a receiving unit disposed to admit therein ionized analytes that are derived from a liquid sample, and including a mass analyzer disposed for analyzing the ionized analytes; and

an electrospray unit including a reservoir for accommodating a liquid electrospray medium, and a nozzle which is disposed downstream of said reservoir, and which is configured to sequentially form a liquid drop of said electrospray medium thereat, said nozzle being spaced apart from said receiving unit in a longitudinal direction so as to define a traveling path;

a voltage supplying member disposed to establish between said nozzle and said receiving unit a potential difference which is of an intensity such that the liquid drop is laden with a plurality of charges, and such that the liquid drop is forced to leave said nozzle as a multiple-charged one for heading toward said receiving unit along the traveling path; and

a laser desorption device including

a sample stage on which the liquid sample is placed, the liquid sample including a solution that contains the analytes and a material serving as a matrix for absorbing laser energy; and

a laser transmission mechanism disposed to irradiate the liquid sample such that, upon irradiation, laser energy is passed on to at least one of the analytes contained in the solution of the liquid sample via the matrix so that said at least one of the analytes is desorbed to fly along a flying path which intersects the traveling path of the multiple-charged liquid drops of said electrospray medium so as to enable said at least one of the analytes to be occluded in said multiple-charged liquid drops, and such that as a result of dwindling in size of the multiple-charged liquid drops when approaching said receiving unit from said nozzle of said electrospray unit along the traveling path, charges of the liquid drops will pass on to said at least one of the analytes occluded therein to form a corresponding one of the ionized analytes.

2. The mass spectrometer assembly as claimed in Claim 1, wherein the solution of the liquid sample is an aqueous solution, the material serving as the matrix being water molecules contained in the aqueous solution, said laser

transmission mechanism being an infrared laser.

3. The mass spectrometer assembly as claimed in Claim 1, wherein said sample stage of said laser desorption device includes a movable track, and a support member having the liquid sample disposed thereon, and mounted movably on said track such that the liquid sample moves with said supporting member along said track.

4. The mass spectrometer assembly as claimed in Claim 1, wherein said sample stage of said laser desorption device includes a support member that is made from a material non-transmissible by laser, and that has a support surface for placement of the liquid sample directly thereon.

5. A method for mass spectrometry, comprising the steps of:

placing, on a sample stage, a liquid sample including a solution that contains a plurality of analytes and a material serving as a matrix for absorbing laser energy;
providing an electrospray unit that includes a nozzle configured to sequentially form liquid drops of an electrospray medium thereat;
providing a receiving unit that is disposed to admit therein ionized analytes that are derived from the liquid sample, and that are to be analyzed by a mass analyzer disposed downstream of the receiving unit, the receiving unit being spaced apart from the nozzle of the electrospray unit in a longitudinal direction so as to define a traveling path;
establishing a potential difference between the nozzle of the electrospray unit and the receiving unit, the potential difference being of an intensity such that the liquid drops are laden with a plurality of charges, and such that the liquid drops are forced to leave the nozzle as multiple-charged ones for heading toward the receiving unit along the traveling path; and
irradiating the liquid sample with a laser beam such that, upon irradiation, laser energy is passed on to at least one of the analytes contained in the solution of the liquid sample via the matrix so that said at least one of the analytes contained in the liquid sample is desorbed to fly along a flying path which intersects the traveling path so as to enable said at least one of the analytes to be occluded in the multiple-charged liquid drops, and such that as a result of dwindling in size of the multiple-charged liquid drops when approaching the receiving unit along the traveling path, charges of the liquid drops will pass on to said at least one of the analytes occluded therein to form a corresponding one of the ionized analytes.

6. The method as claimed in Claim 5, wherein the solution is an aqueous solution, the material serving as the matrix being water molecules contained in the aqueous solution, the laser beam being infrared laser beam.

7. The method as claimed in Claim 5, wherein the material serving as the matrix is made from a material that is non-transmissible by laser.

8. The method as claimed in Claim 5, wherein the solution included in the liquid sample includes a body fluid secreted by an organism.

9. The method as claimed in Claim 11, wherein the body fluid is selected from the group consisting of blood, tear, milk, perspiration, intestinal juice, brains fluid, spinal fluid, lymph, pus, blood serum, saliva, nasal mucus, urine, and excrement.

10. The method as claimed in Claim 5, wherein the solution included in the liquid sample is a protein solution.

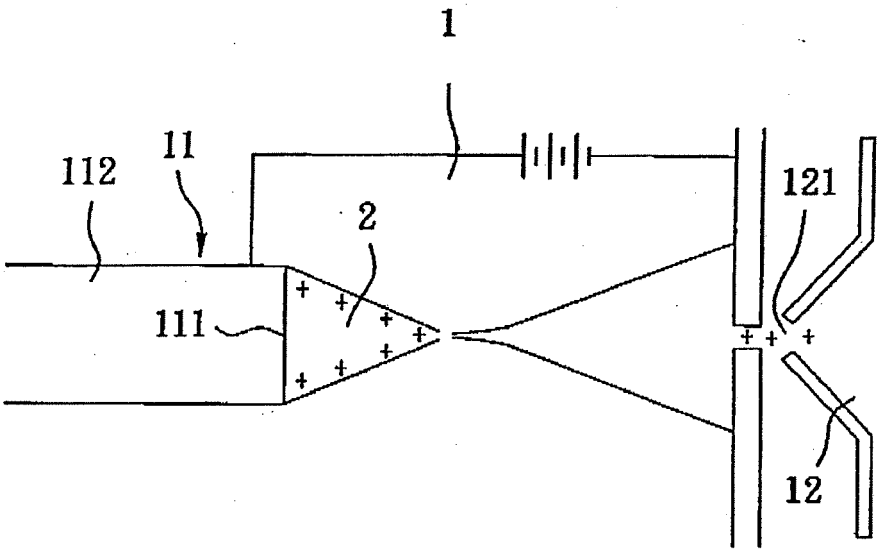

FIG. 1
PRIOR ART

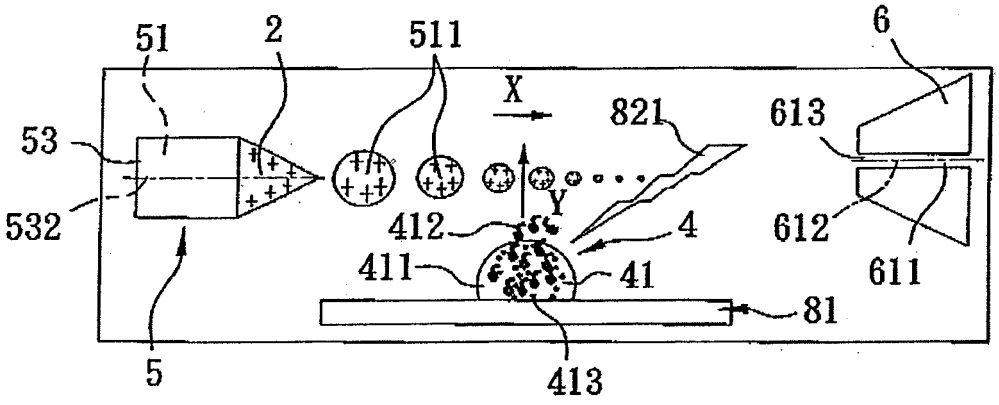
51
2
511
6
X
821
613
53
Y
532
412
4
612
411
41
611
81
5
413

FIG. 2

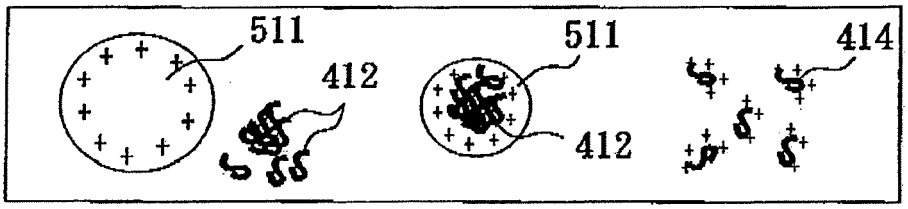
511
511
414
412
412

FIG. 3

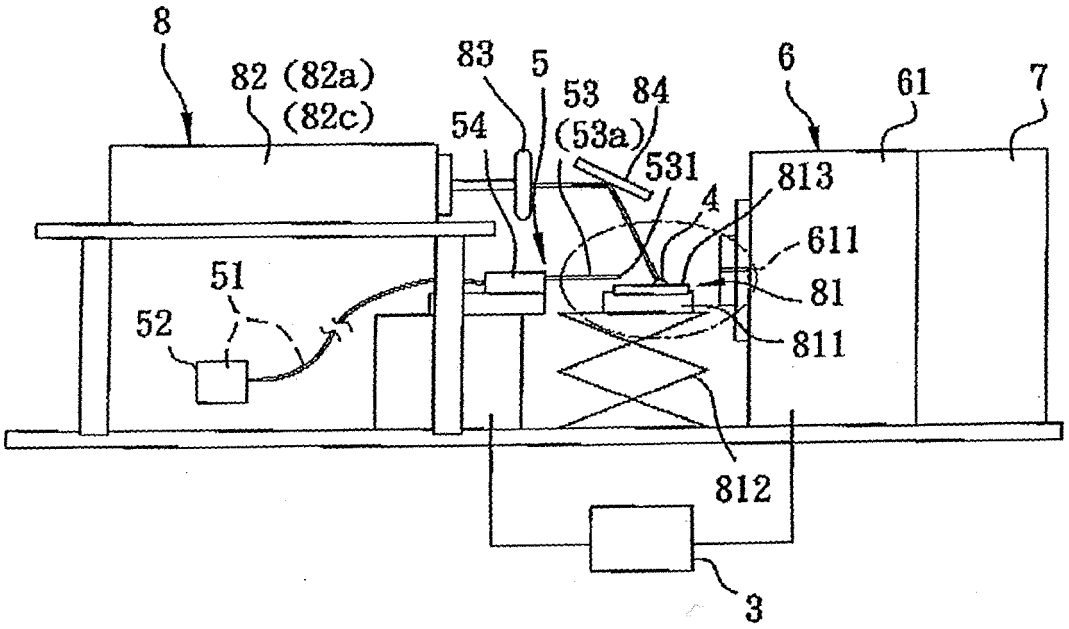
8
82 (82a)
(82c)
83
5
53
84
6
61
7
54
(53a)
531
813
4
611
51
81
52
811
812
3

FIG. 4

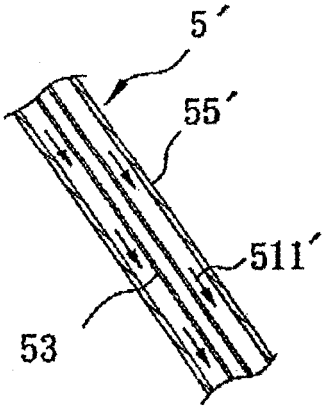
5'
55'
511'
53

FIG. 5

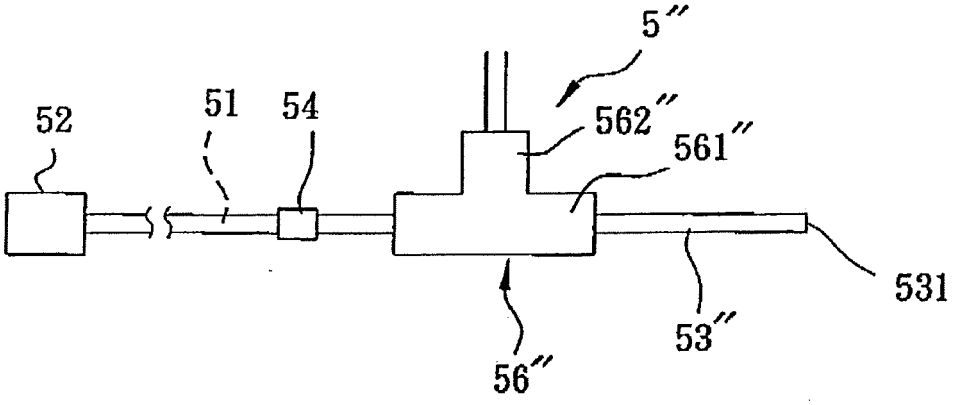
5''
52
51
54
562''
561''
531
56''
53''

FIG. 6

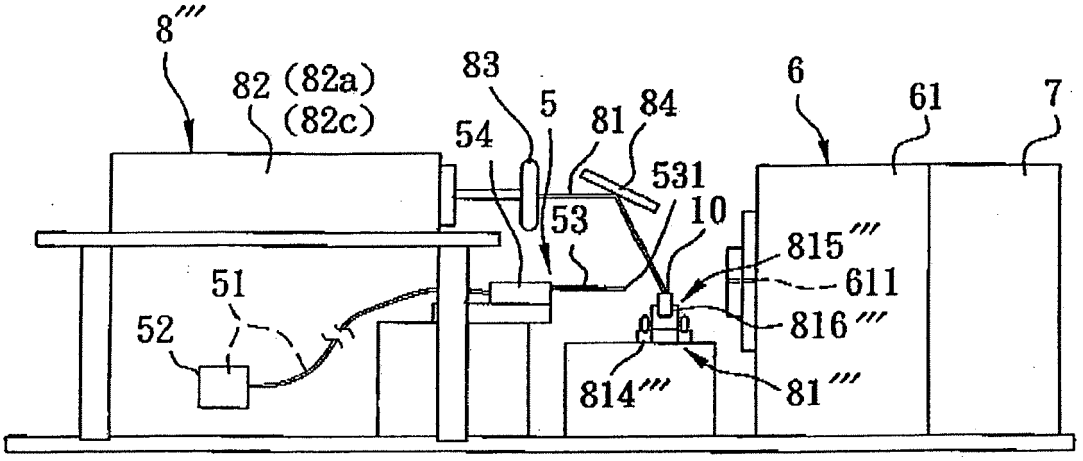
8'''
82 (82a)
(82c)
83
5
54
81
84
6
61
7
531
10
53
815'''
611
816'''
51
52
814'''
81'''

FIG. 7

FIG. 8(a)

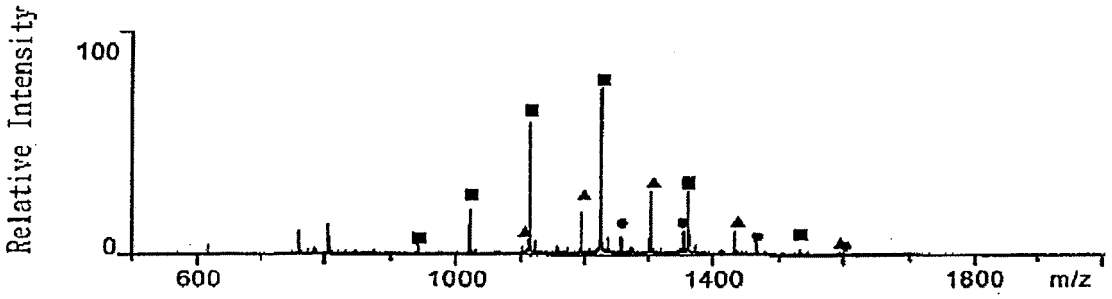

FIG. 8(b)

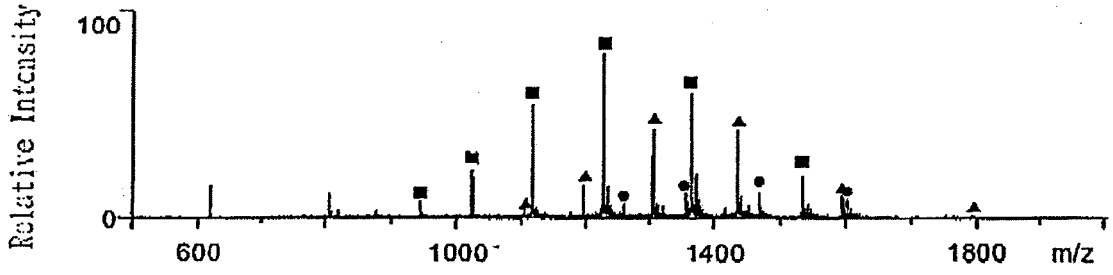

FIG. 8(c)

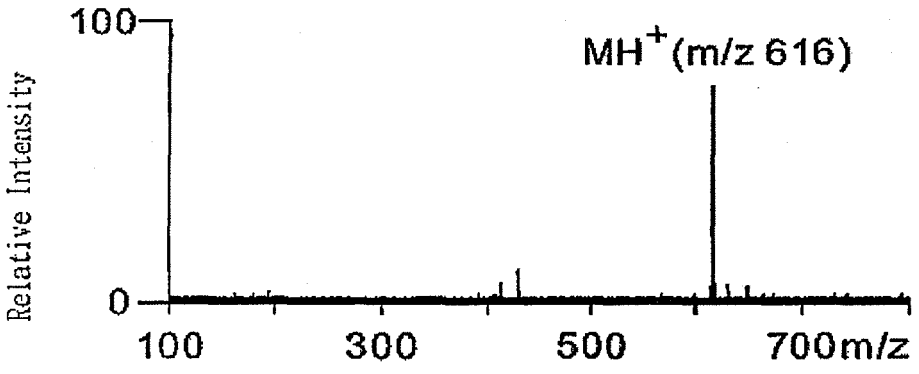

FIG. 9(a)

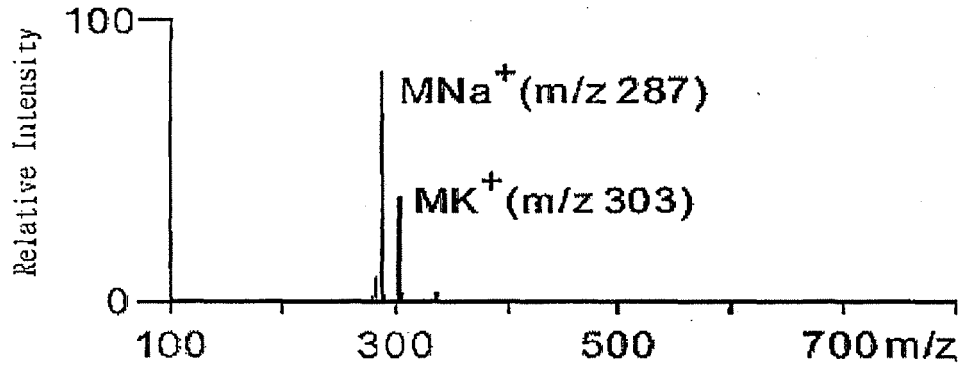

FIG. 9(b)

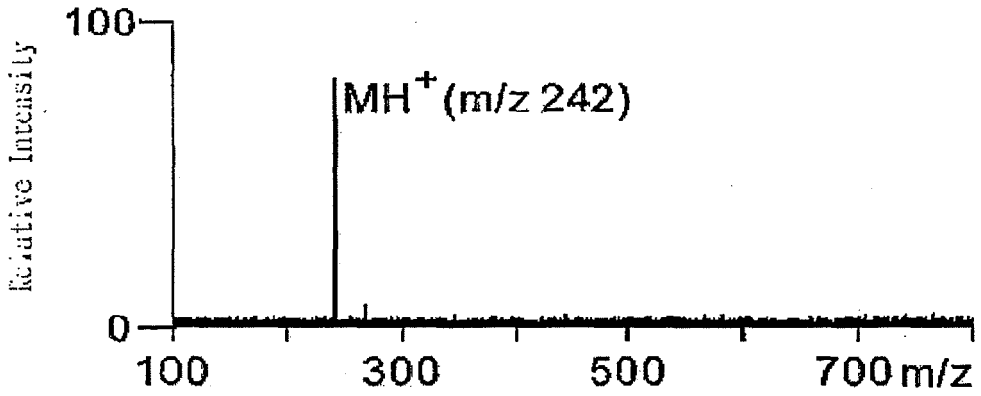

FIG. 9(c)

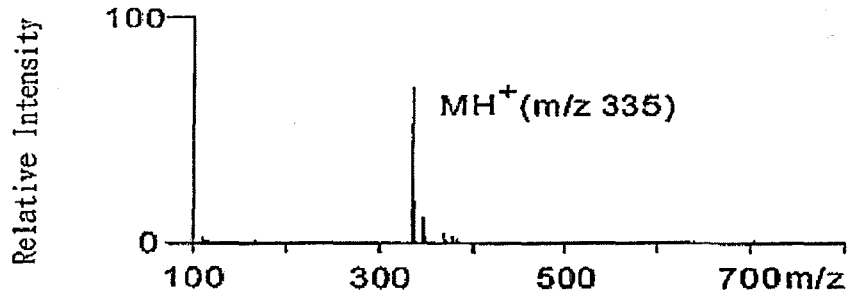

FIG. 9(d)

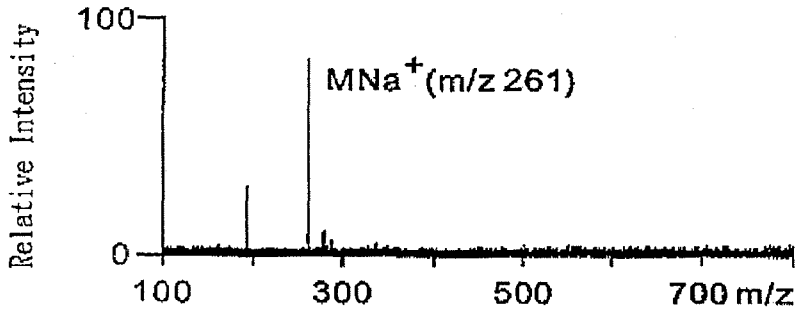

FIG. 9(e)

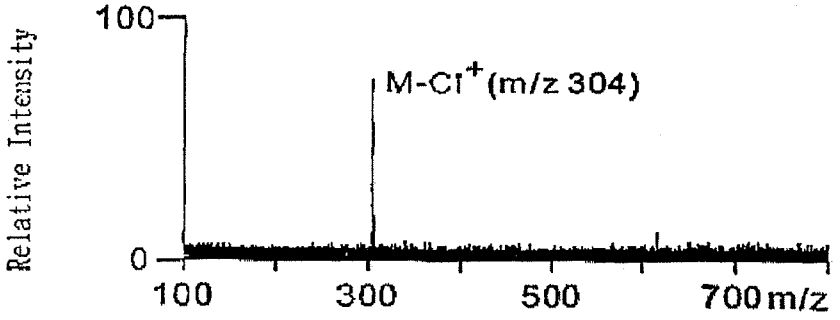

FIG. 9(f)