

plate and dried. These samples were extremely inhomogeneous, and one regularly had to search for spots on the sample ("hot spots") containing analyte molecules in order to perform an analysis of these substances. Quantitative work was impossible. Most investigations into the MALDI process have been performed with these samples, possibly explaining many of the inconsistencies in these investigations.

[0008] Methods are now available for some water-insoluble matrix substances, such as α -cyano-4-hydroxycinnamic acid (CHCA), which can produce thin layers consisting of only a single layer of closely spaced crystals, one micrometer or so in diameter, with very high reproducibility. A predominantly water-based solution of analyte molecules is applied to this thin layer of matrix crystals; the matrix crystals bind the analyte molecules on the surface without being dissolved themselves. The excess solvent can then be removed again by suction after thirty seconds to one minute, thus removing many impurities, such as salts. A large proportion of the analyte molecules are also removed, however, and this needs to be taken into consideration in quantitative investigations. The analyte molecules adsorbed on the surface can also be subsequently embedded into the small matrix crystals if an organic solvent which partially dissolves the matrix crystals is applied after the drying process. After vaporization of this solvent one obtains a very homogeneous sample, which delivers the same ion currents with the same analytical results from every spot. Sample support plates already prepared with thin layers of CHCA are now commercially available. Adequate investigations of the MALDI processes occurring on these thin-layer samples have yet to be published.

[0009] Laser desorption, which was previously only used in high vacuum, has for a few years also been used at atmospheric pressure, simplifying the sample introduction but not, as yet, increasing the detection sensitivity. This method is termed AP-MALDI (atmospheric pressure MALDI).

[0010] With the introduction of solid-state lasers into the MALDI technology instead of the previously used nitrogen lasers, it was found that the more homogeneous beam profile of these solid-state lasers decreases the ion yield. A method for inhomogeneous profiling has therefore been developed which increases the ion yield even beyond the ion yield of nitrogen lasers. This technique is described in the patent application publication DE 10 2004 044 196 A1 (A. Haase et al.), (patent application GB 2 421 352 A, U.S. Pat. No. 7,235, 781 C1).

[0011] For other types of mass spectrometer, such as time-of-flight mass spectrometers with orthogonal ion injection (OTOF), it is more favorable to use a continuous ion beam instead of pulsed ion generation. The patent publication WO 99/38 185 A2 (A. N. Krutchinski et al.) has already elucidated a method whereby the ion clouds from the usual MALDI processes were drawn out in RF ion guides and thus converted into ion currents which were at least temporarily constant in order to serve those types of mass spectrometers needing a constant ion current.

[0012] Whenever the term "mass of the ions" or simply "mass" is used here in connection with ions, it is always the "mass-to-charge ratio" m/z which is meant, i.e. the physical mass m of the ions divided by the dimensionless and absolute number z of the positive or negative elementary charges which this ion carries.

SUMMARY

[0013] The invention combines parameter values for the desorption process which, in the literature, have not been

regarded as favorable for the MALDI process, neither singly nor in combination, but which produce an unprecedentedly high degree of ionization.

[0014] By vaporizing sample material from very small sample spots of less than twenty micrometers diameter, preferably less than ten micrometers, and by also using laser light with very short pulse durations of less than one nanosecond, preferably less than 500 picoseconds, only relatively few analyte ions are produced in each laser spot; overall, however, the degree of ionization of the analyte molecules increases to values between one tenth of a percent and one percent when suitable matrix materials are used. This is more than ten times the degree of ionization obtained previously. This results in a ten- to twenty-fold increase in detection sensitivity for the analyte molecules, an unprecedented sensitivity for MALDI. It is advantageous to set such a low energy density that, with every pulse of laser light, only approximately one picogram or less of sample material is vaporized.

[0015] For use in normal MALDI time-of-flight mass spectrometers, it is favorable for several laser spots, for example 10 to 20, from each laser light pulse to be generated side by side on the sample so as to provide sufficient ions in each pulse for optimal utilization of the time-of-flight mass spectrometer and its measuring device for ions. Devices for generating several laser spots from a single laser light beam are described in the above-cited patent application publication DE 10 2004 044 196 A1 (A. Haase et al.).

[0016] For other types of mass spectrometer which operate more efficiently with a continuous ion beam, for example time-of-flight mass spectrometers with orthogonal ion injection, such a constant ion beam can be achieved by an extremely high repetition rate of the UV laser light pulses of above 20 kilohertz, preferably higher than 50 kilohertz. The desorption clouds generated in quick succession merge into each other in the surrounding vacuum and form the continuous ion current with an ion current strength that is optimal for many mass spectrometers even with only one laser spot per laser light pulse.

[0017] If only one laser spot per laser light pulse is generated, the energy supplied to the sample with every pulse of laser light only amounts to fractions of a microjoule; therefore the laser only requires a quite low overall power and can be correspondingly compact.

[0018] Such high repetition rates for the laser pulses produce a practically continuous ion beam current, even if individual plasma clouds are generated. In one embodiment, each plasma cloud can expand relatively undisturbed to a diameter of approx. one to two centimeters before the ions are captured by the suction effect of an ion funnel. The neutral gas molecules of the vaporization cloud can be pumped off efficiently. However, the desorption can also take place directly into an RF ion guide. It is favorable to slightly dampen the free expansion of the plasma clouds by introducing ambient gas.

[0019] The spots should be moved across the sample between laser shots to allow time for each vaporization crater produced to cool down. If several spots are generated in parallel, the cited patent application describes how such movement can be generated. If single spots are used, moving mirrors can be utilized, for example mirrors moved by piezo effects or galvanic effects, which can also be used in conjunction with movement of the sample support plate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. 1 gives a schematic representation of a time-of-flight mass spectrometer with orthogonal ion injection