

**[0050]** The mobilities of the ion species in a mixture of ions can be read from the mobility spectrum. The mobility spectrum does not, however, provide any indication of the masses of the ions, which provide information as to identity of the ion species.

**[0051]** In order to obtain the masses of the ions, the input region of the mobility spectrometer in FIG. 4 can be coupled to a time-of-flight mass analyzer (e.g., a time-of-flight mass spectrometer with orthogonal ion injection), as shown in FIG. 5. The Laval nozzle plate and the four apertured diaphragms shown in FIG. 5 form a mobility filter 41. The ions that pass the mobility filter 41 are collected by an ion funnel located in the axis of the supersonic gas jet and are guided via ion guides 43, 44 and 45 to a pulser 46. The pulser 46 pulses out a segment of the ion beam perpendicular to the previous direction of flight, and forms an ion beam 47. The ion beam 47 is reflected in an energy-focusing reflector 48, and the mass spectrum is measured by an ion detector 15. The differential pumping system includes the vacuum chambers 26 to 29, which are evacuated by the pumps 16 to 19. The flight tube 30 is evacuated by a pump 40.

**[0052]** Relatively quick sequences of mass spectra may be acquired since the time-of-flight mass spectrometer operates with an acquisition rate of approximately 5,000 to 10,000 mass spectra per second. The mass spectra, however, merely include a few thousand ions and therefore have relatively high noise. A plurality of successive individual mass spectra therefore may be added together to form sum mass spectra. Approximately 25 to 100 mass spectra of good quality can be obtained in one second since adding together 100 to 200 individual mass spectra results in mass spectra with high signal-to-noise which can be evaluated relatively efficiently. The times of flight of the ion signals of the mass spectra are converted to masses, making it possible to obtain relatively accurate mass values with standard deviations of, for example, merely a few millionths of the mass (ppm) or better. From the series of mass spectra, the integral intensity curves  $I_n=f(V)$  can be extracted for ions of individual masses  $m_n$  (or individual mass ranges) as shown in FIG. 7. The derivative  $dI_n/dV$ , with respect to the height  $V$  of the field barrier, provides the mobility spectra for the ions. It is apparent therefore that several isomeric ion species with different structures or conformations can be present.

**[0053]** The chain of ion guides in the time-of-flight mass spectrometer in FIG. 5 includes a mass filter 43 and a collision cell 44. Such a time-of-flight mass spectrometer can also be used to acquire "daughter" ion mass spectra of selected ion species. In order to acquire daughter ion mass spectra, the "parent" ions are selected in the mass filter 43 before being fragmented in the collision cell 44. Since the mobility filter 41 can transmit both species of two isomeric ions or the species with the lower mobility, daughter ion spectra can be acquired either from the mixture of both species or from the single species allowed through. The daughter ion spectra may provide information on different structures or different conformations.

**[0054]** The mobilities measured using the aforescribed methods provide extremely precise values for the mobility factor  $K_0$ , as long as the influence of the mobility factor  $K_1$ , which describes the influence of the field strength  $E$  on the form of the ions, can be neglected. It shall be emphasized here that precision measurements in drift regions and precision measurements at field barriers can deviate slightly from one another due to the influence of  $K_1$ . In drift regions, the drift velocity  $v$  of the ions is measured at a constant field strength  $E$ . At field barriers, in contrast, the field strength  $E$ , which produces a specific drift velocity  $v$  of around 780 meters per

second, is measured. These field strengths, however, are comparable. In the drift regions, for example, the field strengths are constant at between 2 and 4 volts per millimeter, depending on setting. When the apertured diaphragms are each 4 mm apart, the voltages at field barriers are varied between 5 and 20 volts, and in some embodiments up to 32 volts, which are spread over the 8 millimeters separating the first and the third apertured diaphragms and thus result in a maximum of around 4 volts per millimeter (usually less) at the highest point of the field barrier.

**[0055]** The mobility spectra acquisition methods may be calibrated using known ion mobilities. The calibration function  $K_0=f(V)$  is substantially linear over wide ranges. After calibrating an acquisition method, the mobility spectra can be converted from the field barrier coordinates  $V$  to the mobility values  $K_0$ . From these calibrated spectra, the values  $K_0$  for the mobilities of the individual ion species and the mobility resolution  $R_{mob}=K_0/\Delta K_0$  of the method can be determined.

**[0056]** It is advantageous to use monoatomic helium as the drift gas for comparisons of measured mobility values  $K_0$  with computed mobilities for different conformations of one ion species in order to simplify the calculations. Helium can be used as the curtain gas 22 in an electrospray ion source, passing together with the ions through the inlet capillary 23 into the vacuum system, where the Laval nozzle forms it into a supersonic gas jet.

**[0057]** Where no such comparison of measured and computed mobility values is planned, nitrogen, clean air or other gases can be introduced as the curtain gas 22 in the electrospray ion source to be used to form the supersonic gas jet. It should be noted, however, that nitrogen and other gases produce mobility values which are different than those of helium. A further advantageous gas for mobility measurements is argon.

**[0058]** The gas, used to form the supersonic gas jet, is added as curtain gas 22 in the electrospray ion source. The curtain gas takes up the ions and guides them through the inlet capillary 23 into the first stage of the vacuum system. The curtain gas 22 can be heated to around 200 to 300 degrees Celsius in order to contribute to the desolvation of the ions in the capillary. A relatively large drop in temperature may occur in the inlet capillary, and in the transitions of the differential pumping stages. A method has also been described, however, in which the curtain gas is cooled, for example, down to the temperature of liquid nitrogen, before being introduced into the inlet capillary. Advantageously, the cooled curtain gas can increase the mobility resolution. The temperature of the curtain gas can also be used to investigate temperature-dependent conformational changes of the ions as a result of changes to the folding.

**[0059]** The increase in mobility resolution is due at least in part to an almost complete absence of diffusion broadening of the ion signals. Any diffusion broadening of the ion signal that does occur before the field barrier is reached, however, has relatively no effect. Similarly, even after the ions have passed through the field barrier, diffusion broadening of the ion signals is typically only detrimental when the height  $V$  of the field barrier is rapidly changed. In these circumstances, therefore, the ions should be quickly guided to the ion detector or mass analyzer in order to reduce the diffusion broadening. On the way to the ion detector or mass spectrometer, however, the temperature of the adiabatically cooled gas jet is very low which strongly reduces the diffusion broadening.

**[0060]** The ion mobility spectra represent the distribution of the ions over different conformational or structural isomers. The structural isomers are usually relatively stable, and therefore are almost always measured in accordance with the