

# Velocity Distributions of Analyte Molecules in Matrix-assisted Laser Desorption from Computer Simulations

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**The mass dependence of the velocity distributions of analyte molecules in matrix-assisted laser desorption is analyzed based on the results of molecular dynamics simulations. The spread of the velocities along the direction of the flow is found to be independent of the mass of the analyte molecules and to reflect the entrainment of the analyte molecules in the expanding matrix. The radial velocity distributions for both matrix molecules and analyte molecules of different masses, on the other hand, fit well to a Maxwell–Boltzmann distribution with the same temperature, suggesting the association of the spread in the radial velocities with the thermal motion in the plume. A consistent analytical description of the complete velocity distribution for matrix molecules and analyte molecules of different masses is proposed based on the approximation of a range of stream velocities and a single temperature in the ejected plume. © 1998 John Wiley & Sons, Ltd.**

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Since the invention of matrix-assisted laser desorption/ionization (MALDI) by Hillenkamp *et al.*<sup>1</sup> this technique has become a major tool for analysis of nonvolatile compounds.<sup>2</sup> Present experimental and theoretical efforts are aimed at improving the mass resolution of the technique, especially in the high mass region. One of the major factors that defines the resolution of mass spectra is the distribution of the initial velocities of ejected molecules. An adequate and consistent analytical description of the velocity distributions of matrix and analyte molecules in MALDI is thus essential to understanding and overcoming of the limitations of the technique.

The experimentally observed distributions are commonly described in terms of modified Maxwell–Boltzmann distribution on a stream velocity.<sup>3–5</sup> Using two fitting parameters of the distribution, namely, the temperature and the stream velocity of the plume, it is usually possible to satisfactorily describe experimental data. The problem, however, arises when one wants to correlate the fitting parameters with real physical parameters of the ejected plume. The association of the spread of velocities along the direction of flow solely with the thermal motion can be misleading and yields an overestimated value for temperature.<sup>3,6,7</sup> Moreover, angular resolved measurements reveal that the fitting parameters have an apparent dependence on angle of the measurement.<sup>5–8</sup> The fitted temperature and the stream velocity thus have to be considered only as fit parameters and their correlation with the real physical characteristics of the ablated plume is not straightforward.

The radial (parallel to the surface of the irradiated sample) velocity distributions of both matrix and analyte ions are found to be considerably narrower than the axial (normal to the surface) distributions,<sup>6,7</sup> leading to a forward-

peaked ejection. The difference between the axial and radial velocities is larger for analytes. The axial velocity distributions of the neutral matrix and analyte molecules measured by laser post-ionization are found to be nearly identical.<sup>3</sup> The same is true for the analyte ions of different molecular masses.<sup>7,9,10</sup> On the other hand, considerably smaller radial velocity spread was recently reported for higher mass analyte ions as compared to the matrix ions.<sup>7</sup> As a consequence, the analyte ions exhibit a higher degree of forward peaking than ions arising from the matrix. Both the radial and axial velocity distributions have an important influence on design and performance of MALDI mass spectrometers and a complete and consistent analytical description of the velocities of the ejected molecules in laser ablation is needed.

A consistent description of the velocity distributions of matrix and analyte molecules can result only from the understanding of the processes in the irradiated material leading to the ejection as well as the processes in the dense ejected plume at the early stages of plume expansion. This understanding requires a combination of theoretical, computational and experimental efforts aimed at the detailed investigation of the mechanisms of laser ablation. An approach that appeared to be a useful part of such investigation is a computer simulation using the molecular dynamics technique.<sup>11–14</sup> A breathing sphere model developed recently for molecular dynamics simulations of laser ablation of organic solids<sup>13</sup> has significantly expanded the time- and length-scales accessible for the simulations. This allows one not only to study the microscopic and mesoscopic mechanisms of laser ablation of organic films<sup>13</sup> but also to obtain sufficient data to analyze velocity distributions of ejected molecules.<sup>14</sup> The results of the simulations have shown that the total velocity distribution of matrix molecules ejected in laser ablation can be described by a single temperature,  $T$ , and a range of flow velocities. The Maxwell–Boltzmann distribution on a stream velocity has

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accordingly been modified to account for the range of stream velocities from zero to  $u_{\max}$  in the ejected plume. The modification has resulted in the following total velocity distribution of ejected molecules.<sup>14</sup>

$$dN(v, T, u_{\max}) = \frac{m}{4\pi kT u_{\max}} \exp\left\{-\frac{m(v_x^2 + v_y^2)}{2kT}\right\} \\ \times \left\{ \operatorname{erf}\left[\sqrt{\frac{m}{2kT}}v_z\right] - \operatorname{erf}\left[\sqrt{\frac{m}{2kT}}(v_z - u_{\max})\right] \right\} dv_x dv_y dv_z \quad (1)$$

where *erf* is the standard error function, *m* is the mass of the particle,  $v_x$ ,  $v_y$ ,  $v_z$  are velocity components and *k* is Boltzmann's constant.

We find that both axial and radial total velocity distributions of matrix molecules are well represented by the modified distribution providing a consistent way to describe the complete velocity distribution with a single set of parameters. The two parameters of the proposed equation are angle independent and have clear physical meaning, namely, the temperature that describes the thermal motion in the plume and the maximum stream velocity.

The simulations presented in Ref. 14 were performed for a two-dimensional model of a one-component organic solid of small organic molecules. In the present work, we extend our earlier simulation by incorporating larger/heavier molecules of different masses into the matrix of small molecules. These simulations are performed in order to analyze the mass dependence of the velocity distributions of analyte molecules and provide a consistent analytical description of the velocity distributions of matrix and analyte molecules in MALDI.

## COMPUTATIONAL METHOD

The simulation of laser ablation of a molecular solid is performed using the breathing sphere model that has been described in detail elsewhere.<sup>13</sup> Briefly, the model assumes that each molecule can be represented by a single particle that has the true translational degrees of freedom but an approximate internal degree of freedom. This internal (breathing) mode allows one to reproduce a realistic rate of the conversion of internal energy of the molecules excited by the laser to the translational motion of the other molecules. Since the molecules rather than the atoms are the particles of interest in the model, the size of the simulated system can be significantly extended. Moreover, since we are not following high frequency atomic vibrations, we can use a much longer timestep in the numerical integration and keep track of the processes in the simulated system for a longer time.

The results presented in the present paper are obtained for the two-dimensional version of the breathing sphere model. The parameters of the intermolecular potential are chosen to represent the van der Waals interaction in a molecular solid.<sup>13,15</sup> A mass of 100 Da has been attributed to each particle. A computational cell of dimensions  $81 \times 210$  nm (58 800 matrix molecules) is used. Periodic boundary conditions in the direction parallel to the surface are imposed. These conditions simulate the situation in which the radius of the laser beam is large compared to the penetration depth

so that the effect of the edges of a laser spot can be neglected. In other words, processes occurring in the center of the laser beam are being examined.

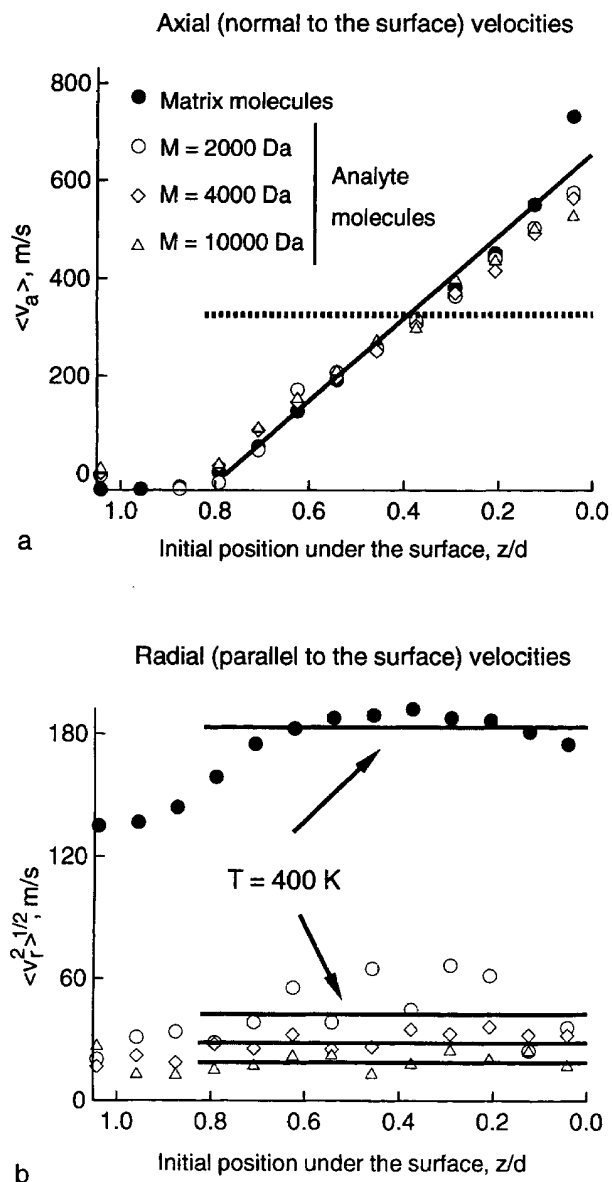
Analyte molecules of three masses, 2000 Da, 4000 Da and 10 000 Da, are introduced into the model. The sizes of the analyte molecules are chosen so that they are  $\sim 10\%$  heavier than the matrix molecules that would occupy the same space. The molar concentration of analyte molecules is less than 0.05% in these simulations. The positions for analyte molecules to be introduced are chosen at random and the results for ten runs with different initial configurations are averaged for each type of analyte in order to obtain statistically significant data on the velocity distributions. Small adjustments of the random choice of positions were made to assure a uniform distribution of analyte positions with depth over the ten runs.

The laser irradiation is simulated by vibrational excitation of matrix molecules that are randomly chosen during the laser pulse duration. In this case an implicit assumption is that the laser radiation is absorbed by the matrix molecules and internally converted to vibrational energy. The vibrational excitations are performed by depositing a quantum of energy equal to the photon energy into the kinetic energy of internal vibration (breathing mode) of the molecules to be excited. Laser pulses of 15 ps in duration at a wavelength of 337 nm are used in the simulations. The photon energy is scaled down by a factor of two in order to account for the lower cohesive energies in the two-dimensional system as compared to the three-dimensional case. The total number of photons entering the model solid during the laser pulse is determined from the laser fluence that is set to be  $\sim 50\%$  higher than the threshold fluence for the ablation.<sup>13</sup>

## RESULTS AND DISCUSSION

The simulations performed for a neat matrix sample have shown that the average axial velocities of molecules ejected in laser ablation depend nearly linearly on their initial position under the surface.<sup>14</sup> In the present study the analyte molecules of three different masses are embedded into the matrix at low concentration and the axial and radial velocity components of both matrix and analyte molecules are analyzed. The average axial velocities of analyte molecules, plotted in Fig. 1(a), follow the same linear dependence on the initial position as do the matrix molecules and do not exhibit any perceptible dependence on the molecular mass. The only apparent disparity between the axial velocities of matrix and analyte molecules is for the molecules ejected from the top of the surface region. The high velocities of the matrix molecules from the top layers are found to be due to the collisional redistribution of the axial momentum within the expanding plume. Collisions between the lighter matrix molecules and the heavier analyte molecules do not appear to be as effective in accelerating the analyte molecules to the highest maximum velocities as for the matrix molecules. Considering the data points in Fig. 1 one should take into account a limited statistic for analytes. Less than 100 analyte molecules of each mass are ejected in the 30 molecular dynamics simulations and each point in Fig. 1 results from the averaging over ten analyte molecules, versus a few thousand matrix molecules.

The radial velocities of the ejected matrix molecules have no significant correlation with the initial position under the surface, Fig. 1(b). At the same time they exhibit a strong mass dependence. We find that the average radial velocity



**Figure 1.** Average velocities of matrix molecules and analyte molecules of different masses as a function of their initial position under the surface,  $z$ , relative to the laser penetration depth,  $d$ . Points represent the velocities at 500 ps after the laser pulse in the molecular dynamics simulations. (a) Mean axial velocity  $\langle v_a \rangle$ . The solid line represents the approximation of a range of stream velocities from zero to  $u_{\max} = 650$  m/s. The dashed line shows the approximation of a single stream velocity. (b) Root mean square radial velocity  $\langle v_r^2 \rangle^{1/2}$ . All four lines represent a temperature of 400 K for matrix molecules and analyte molecules of different masses.

of the analyte molecules decreases as the square root of their masses. The value of a root mean square radial velocity that corresponds to a temperature of 400 K\* are denoted by the solid lines in Fig. 1 and appear to be good approximations of the sets of data points for matrix molecules and analyte molecules of all masses. The radial velocity component does not contain a contribution from the forwarded flow of

\*The two-dimensional model has only one radial degree of freedom, thus the root mean square radial velocity of the particles is related to temperature by  $\langle v_r^2 \rangle^{1/2} = (kT/m)^{1/2}$ .

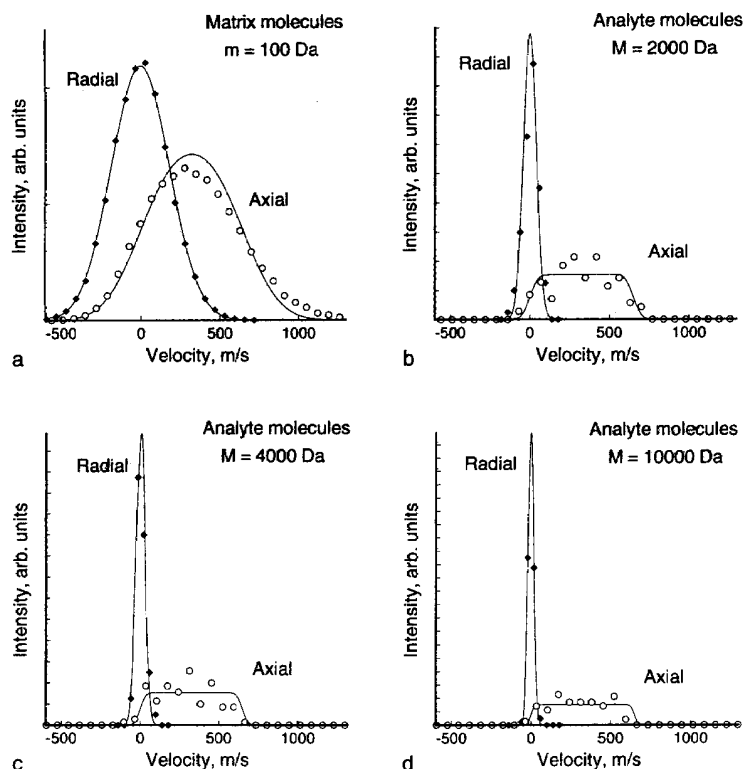
the plume in the direction out from the surface and thus appears to be associated with the thermal motion in the plume. As is shown below, the radial velocity distributions of the ejected molecules fit well to a Maxwell-Boltzmann distribution substantiating the association of the spread of the radial velocities with the thermal motion in the plume.

Considering the data shown in Fig. 1 one can expect that an approximation of a single temperature and a range of stream velocities marked by solid lines in the figure and used to derive Eqn(1), can be applied not only to matrix molecules but to analyte molecules as well. Figure 2 shows the axial and radial velocity distributions from the simulations and distribution functions given by Eqn (1) for  $T = 400$  K and  $u_{\max} = 650$  m/s. A remarkable feature of this figure is that a single equation with a single set of parameters is used to describe all eight axial and radial velocity distributions for molecules of four different masses. In the case of a radial distribution the modified distribution given by Eqn(1) becomes equivalent to an ordinary Maxwell-Boltzmann distribution. It is evident from the figure that the radial distributions are well represented by Maxwell-Boltzmann distribution at  $T = 400$  K. The good fit of the radial velocity distribution for both the matrix molecules and analytes indicates that nearly complete thermalization of translational molecular motion occurs between matrix and analytes in the ablation process.

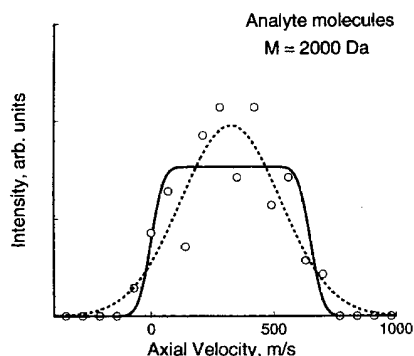
Within the limited statistics for the analyte molecules, a good representation of the axial velocities is provided by Eqn. (1) with the same temperature of 400 K and a maximum stream velocity of 650 m/s. The flat-top shape of the axial velocity distributions in Fig. 2(b)–(d) suggests that the spread in the axial velocities of analytes is due to the entrainment in the expanding matrix and the thermal motion does not significantly affect the distributions.

For entrained molecules the spread of the axial velocities is mass independent whereas the 'thermal' radial distribution becomes narrower with increasing molecular mass, Fig. 2. Thus, while both matrix and analyte molecules would have forward-peaked angular distributions, the analyte molecules of higher masses would exhibit a higher degree of forward peaking. This prediction is in accord with a recent experimental observation by Zhang and Chait, who reported a considerably higher degree of forward peaking for the peptide ions as compared to the matrix ions.<sup>7</sup>

The description of the velocities in the ablation plume based on the range of stream velocities not only provides a consistent representation of the velocity distributions for both matrix and analyte molecules but also uses the parameters that have clear physical meaning. The maximum stream velocity corresponds to the velocity of the plume propagation in the direction normal to the surface. The temperature of 400 K used in Figs 1 and 2 is only slightly above the melting temperature of the model material and has been shown to result from the fast cooling of the ejected material due to the explosive disintegration of the overheated matrix and a slower gradual cooling during the plume expansion.<sup>13</sup> An alternative analytic function commonly used to describe the experimental velocity distributions is a Maxwell-Boltzmann distribution on a stream velocity.<sup>3–5</sup> In Fig. 3 both the Maxwell-Boltzmann distribution function on a stream velocity and the modified function given by Eqn (1) are used to describe the simulation data for the axial velocities of the analytes with masses of 2000 Da. Although there is insufficient statistical significance in the data to determine which fit is better, the



**Figure 2.** Velocity distributions in the axial and radial directions for (a) the matrix molecules and (b, c, d) the analyte molecules of different masses. The points are data from the simulations and the curves are obtained using Eqn (1) with  $T = 400$  K and  $u_{\max} = 650$  m/s.



**Figure 3.** Axial velocity distribution of analyte molecules of mass 2000 Da. The points are data from the simulation, the solid curve is obtained using Eqn (1) with  $T = 400$  K and  $u_{\max} = 650$  m/s, and the dashed curve is generated using Maxwell-Boltzmann distribution function on a stream velocity with  $T = 10,000$  K and a single stream velocity of 325 m/s.

temperature of 10000 K used in the distribution with a single stream velocity can be hardly justified. Since the axial velocity spread is nearly identical for analyte molecules of different masses, the average kinetic energy and the temperatures resulting from the fits would scale with mass yielding even higher temperatures for heavier analyte molecules. It is a common result from the fits of experimental velocity distributions to the Maxwell-Boltzmann distribution function to get an unrealistically high fit temperature.<sup>3,6,7</sup> In particular, temperatures of 930 K and 5179 K have been obtained by Huth-Fehre and Becker from the axial velocities distributions for the neutral matrix

(ferric acid,  $M = 194$  Da) and analyte (Gramicidin S,  $M = 1141$  Da) molecules measured by laser post-ionization.<sup>3</sup>

## CONCLUSION

The mass dependence of the velocity distributions of analyte molecules in MALDI is analyzed based on the results of molecular dynamics simulations. We find that a nonthermal ejection mechanism driven by the relaxation of the laser induced pressure leads to a much broader distribution of the axial components of the velocity of ejected molecules as compared to the distribution of the radial components. The spread of the velocities along the direction of the flow exhibits only weak dependence on the mass of the analyte molecules and reflects the entrainment of the analyte molecules into the expanding matrix. The radial velocity distributions for analytes of different masses, on the other hand, fit well to a Maxwell-Boltzmann distribution with the same temperature, suggesting the association of the spread in the radial velocities with the thermal motion in the plume. We find that the approximation based on a range of stream velocities and a single temperature provides a consistent analytical description of the complete velocity distribution for matrix molecules and analyte molecules of different masses.

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