Plasma Desorption Mass Spectrometry – Achievements and Frontiers

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INTRODUCTION AND HISTORICAL NOTES

Almost twenty years ago, R.D. Macfarlane , D.F. Torgerson and others at Texas A&M discovered that fission fragments from a ^{252}Cf source can cause ejection of whole intact molecular ions from a sample consisting of a multi-layer deposit of organic molecules [1]. The secondary ions were mass analyzed with a time-of-flight (TOF) technique. The potential of the new mass spectrometric method, called Plasma Desorption Mass Spectrometry (PDMS) was demonstrated in a number of experiments by the Texas group but it was not until almost ten years later when the full potential for high mass analysis was demonstrated [2,3], in fact almost coinciding with the appearance of the FAB method [4], that the method started to be used more extensively in mass spectrometry applications. In fact the use of adsorption of sample molecules to a substrate of nitrocellulose [5] was probably the singly most important reason why PDMS started to be used as a tool in protein chemistry. In establishing the potential of the method important contributions, in addition to those at Texas A&M university were made by Field and Chait [6] at Rockefeller university and by the Uppsala-Odense collaboration [7]. Already a few years after the Texas discovery groups at Darmstadt [8], Orsay [9], Erlangen [10] and Uppsala [11] started to use accelerators, in addition to ^{252}Cf, to study various aspects of the basic mechanisms involved in PDMS.

In 1984 the first commercial PDMS mass spectrometer appeared, marketed by Bio-Ion Nordic AB. Early Bio-Ion instruments were acquired by the Odense and Johns Hopkins groups [12] and important early experiments were made in those laboratories which demonstrated the potential of PDMS. Since then, more than 30 spectrometers have been sold

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by the Uppsala company. Bio-Ion Nordic AB was bought by Applied Biosystems Inc. in 1989. In all, more than 50 PDMS spectrometers are now being used around the world.

In the last few years a number of new techniques have rivaled PDMS in terms of being able to study larger molecules. Until two years ago PDMS was the most successful mass spectrometric method for large organic molecules but since then Matrix assisted laser desorption [13] and the Electrospray [14] methods have been demonstrated to be more powerful in order to produce gas-phase ions of large organic molecules, like proteins.

The chairmen of this meeting (Ken Standing) has asked one of us (BS) to give a summary of the status of PDMS as one of the "established methods and as an introduction to the new and more exciting methods". The new methods are exciting but there are still many unsolved problems in the field of PDMS and open possibilities that are of considerable interest to study. The solutions of these unsolved problems may lead to new important knowledge and there are even new frontiers of PDMS research that should be pushed.

The illustrations given below will mainly be picked from the work of the Uppsala group but the progress in understanding the basic mechanisms underlying the PDMS technique and its applications is of course the result of a collective effort of all the groups mentioned above.

ELECTRONIC SPUTTERING OF LARGE ORGANIC MOLECULES

One of the early findings in the accelerator experiments was that the yields of secondary ions were correlated with the electronic stopping power [9,10,11] of the primary ion. Electronic stopping is the dominating energy loss mechanism for fast ions as those used in those experiments. Electronic sputtering is the expression used for ejection of material from a surface bombarded with fast particles [15]. However for a long time progress in understanding the sputtering mechanism was hindered by the lack of proper experimental sputter yields, i.e. data for ejected neutral organic molecules. Salehpour and Hedin were able to use a collector method to measure the absolute magnitude and the scaling of neutral yields with electronic stopping power (Fig. 1)[16,17]. The yields were the number of whole molecules ejected per fast ion impact on multilayer samples of the amino acids leucine. The main findings were that about 1000 molecules are ejected at the impact of a fast heavy ion, with an elctronic stopping power like a fission fragment, and that the yield scales with the third power of the electronic stopping power. PDMS-experiments on Langmuir-Blodgett films of fatty acids at Uppsala [18] and Orsay [19] did subsequently show that the large neutral yields measured by Salehpour et al. [16] are most likely associated with crater formation.

At this point one may already conclude (on the basis of the results of these experiments) that there is a physical limit to how large molecules one can hope to eject intact. This is consequently a mass which is of the order of 130 000 u (the neutral yield mentioned above multiplied with the mass of an amino acid mass). However, the maximum mass of a whole ejected molecule is probably considerably less as the infra-track of the fast ion will penetrate the sputtered volume and it is more reasonable to assume that the maximum mass of a whole molecular ion will be more like 50 000 u. How does this correspond to what has been observed experimentally so far? In fact the qualitative argument above fits surprisingly well to experiments. Already at a meeting at Texas A&M (celebrating the 50th birthday of R.D. Macfarlane) the Uppsala group showed a broad tetramer ion peak of phospholipase A2 of mass 56 000 u [20]. Furthermore using surface adsorption of ovalbumin (45 000 u) to nitrocellulose the same group have recently demonstrated a weak signal of intact multiply protonated molecular ions (See Fig. 2) [21]. We think that this is state of the art and that it is unlikely that considerably larger molecules will be studied with PDMS.

An important experiment, related to the mechanism for electronic sputtering of large molecules, was carried out at Uppsala when Werner Ens from the Manitoba group spent a "post-doc" year there. The experimental finding was that molecular ions of electronically sput-

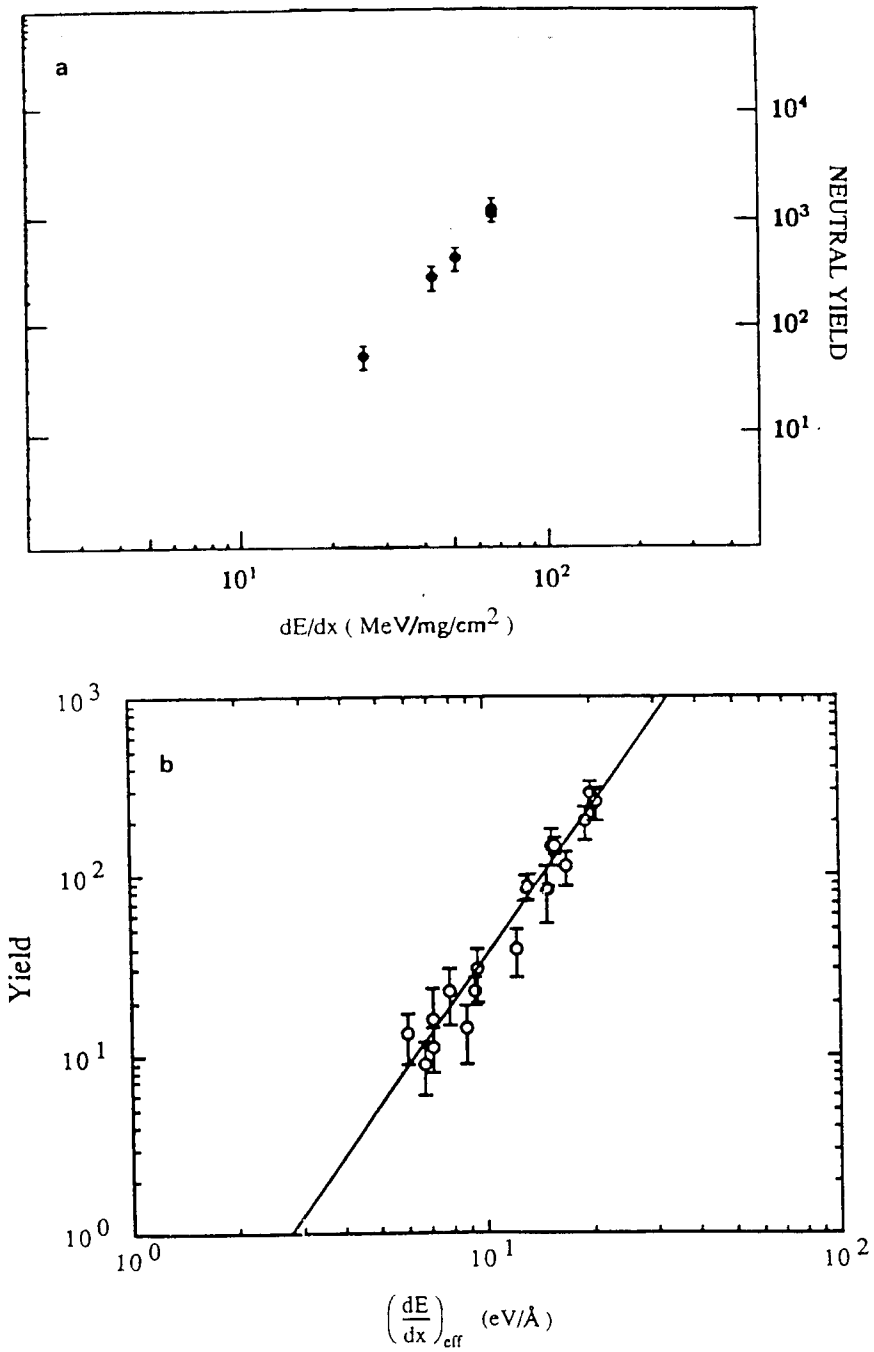

Figure 1 Neutral electronic sputtering yields of organic molecules as a function of electronic stopping power. If a [dE/dx (electronic)]^n is fitted to the distributions the result is n = 3.2 ± 0.2 for the experiment and n = 2.9 ± 0.1 for the simulation.
a. Experimental data on leucine from ref. 17.
b. Yields from a Molecular dynamics simulation of electronic sputtering of organic molecules (MW 10 000). For details see ref.28.

tered organic molecules, unlike most secondary ions in sputtering processes, show ejection angle distributions which are non-symmetric around the normal to the sample surface [22]. Those findings have recently been confirmed by the Darmstadt group [23]. More recently Fenyö and coworkers have made more detailed experimental studies of this effect [24] and in Fig. 3 a collection of data from those studies, illustrating the effect, is shown. One of the immediate conclusions from the early experimental data was that these findings finally rules out attempts to describe the ejection mechanism for large molecules based on the concept of evaporation from a hot spot. Rather the experiments support the idea of a shockwave ejection like that suggested by Parilis and coworkers [25]. At the time the idea of the shock wave mechanism was suggested,however, we were confused because the scaling with stopping power was predicted by the Tashkent group to be $(dE/dx)^{3/2}$, i.e. different from what we had measured. The experimental data did indicate that the molecular ions were ejected as if pushed out by the expanding ion-track region. The concept of track-expansion had at that time already been suggested by Peter Williams and one of us (BS) [26] and it was natural to try to simulate the experimental results in an expansion model by the Molecular Dynamics (MD) approach. In doing that we were inspired by some early attempts to use MD-techniques by the Oldenburg group [27], one of the new groups in the field. In Fig. 3 the simulations (described in detail in ref. 28) show that indeed the model can qualitatively explain experimental results on the ejection angle effect [24]. Also the simulations reproduce the scaling of neutral sputtering yields with primary ion energy deposition (See Fig. 1, lower part). During these studies we also started to use the approach of summing impulses [29] (developed by R.E Johnson) to try to describe the process [30] analytically and indeed it was found that the scaling of yield to the third power of the energy deposited could be calculated in the so called pressure pulse model.

Although the details of the mechanism for setting up the expansion is not known, the general picture of ejection of large organic molecules by electronic sputtering is now fairly well established. The frontier is now to decide which of the known (or unknown) alternatives for causing the expansion, i.e. Coulomb explosion [31], repulsive decays [32] or low energy secondary electron excitation causing soft expansion (pop-corn) [26], is mainly responsible for the expansion. As will be shown below, the ejection angle effect found also has consequences for the practical applications of PDMS.

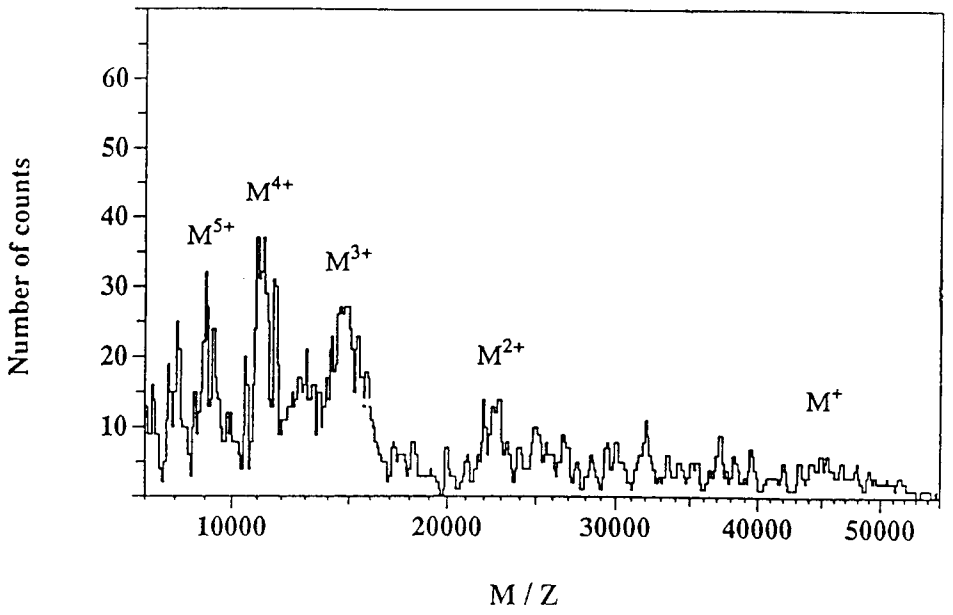

Figure 2 PDMS spectrum of positive ions from ovalbumin (45 000 u) illustrating intact ejection of whole molecular ions in PDMS [21]

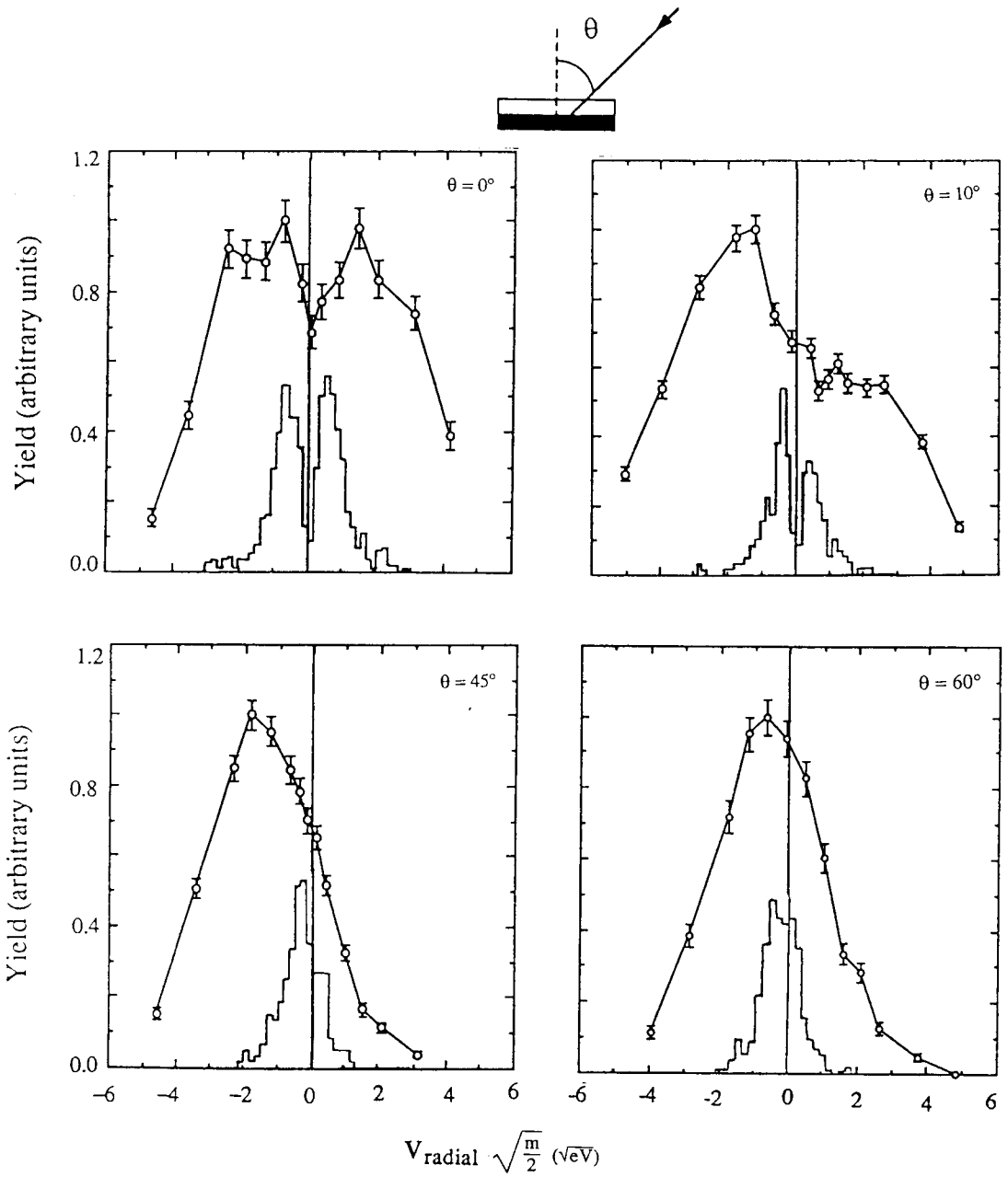

Figure 3 Experimental data on radial velocity distributions of molecular ions from renin substrate sputtered by 72.3 MeV $^{127}I^{13+}$ [24]. The lower distributions in each spectrum are simulated spectra in a model described in ref. 28.

PLASMA DESORPTION MASS SPECTROSCOPY (PDMS) - SIMS BASED ON ELECTRONIC SPUTTERING

Over the years there have been many discussions wheter the name Plasma Desorption Mass Spectromtry ia an appropriate one. This is not an important issue but the method might be described as Secondary Ion Mass Spectrometry based on electronic sputtering.

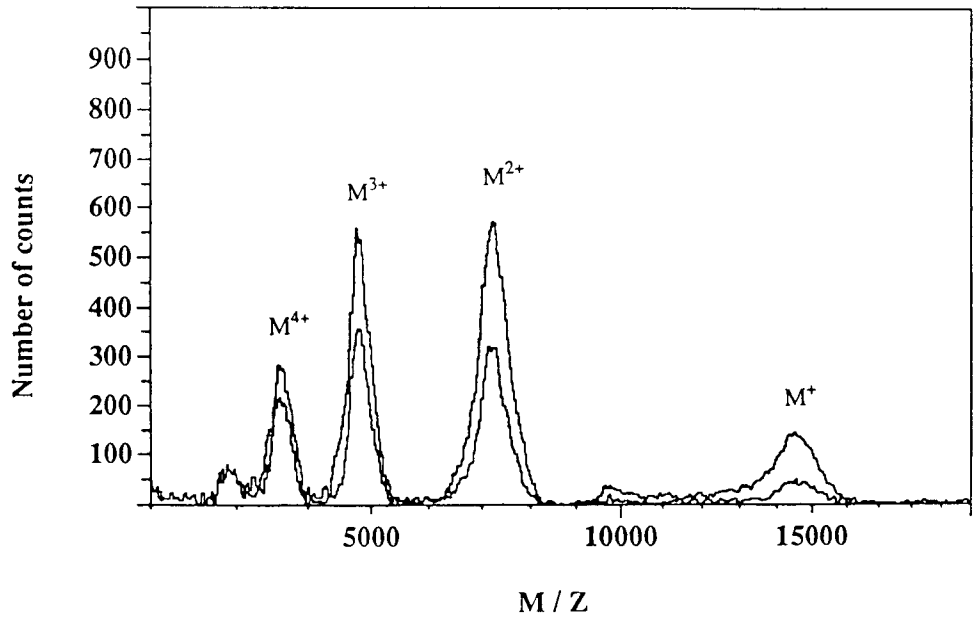

Figure 4 PDMS spectrum of hen egg lysozyme in a straight TOF-spectrometer with (upper curve) and without (lower curve) einzel lens in the field free region [21].

For years the method was only available in a small number of laboratories. Except for the Texas laboratory the only established MS laboratories which started to use the method were the ones at Rockefeller University and National Institute of Health, Bethesda (H. Fales). In fact it was not until the commercial system appeared and the method of using surface adsorption to nitrocellulose for sample preparation that the method was widely accepted as a method to consider for MS studies of proteins.

In this short review I therefore like to focus on this particular development. The use of nitrocellulose for sample preparation was discovered in an attempt at Uppsala to couple PDMS to gel-electrophoresis by blotting to a nitrocellulose membrane and thereafter analyzing the membrane with PDMS. The results were very surprising to us and the particular feature which initially caught our attention was the fact that higher charge states than before were observed. We soon learnt that the same effect had already been found by Macfarlane in using Nafion as a substrate [33]. Later when using very well-defined thin films of nitrocellulose and Ellipsometry for monitoring the thickness of the surface layers the Uppsala group established that the high charge-states are connected to proteins being bonded to the substrate rather than to other proteins [34]. The practical usefulness of nitrocellulose was demonstrated in applications of the method by the Rockefeller [35] and Odense [36] groups. Today, the procedure of using a combination of proteolytic enzymes to successively break down a protein to pieces and to monitor each step by PDMS can be of considerable help in determining the structure of a protein. This procedure is in my view the most successful application of the PDMS method so far. The fact that only a small part (less than a permille) is consumed in one PDMS analysis makes it possible to use a whole spectrum of wet-chemical fragmenting reactions on the sample foil and to monitor the results of each step in a subsequent mass analysis.

However it is not clear that this advantage of PDMS will survive the attack of the new methods because the same technique can be used in solution and the new methods are fast and sensitive. The practitioners of the methods will finally decide which method is preferable. The simplicity of the PDMS spectrometer with no ion source to tune, in comparison with other techniques, is still for many users a great advantage. In addition, it is clear that at present the

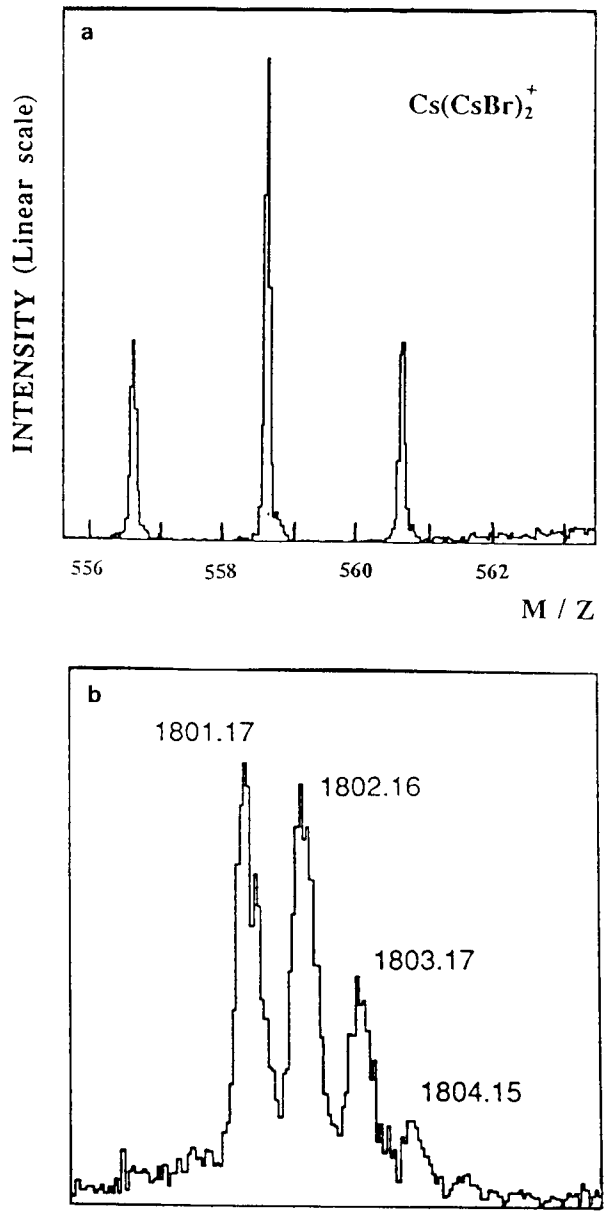

Figure 5 Data from experiments [38] with an electrostatic mirror TOF-experiment on a. positive ions of CsBr b. renin substrate

PDMS method and the keV-TOF-SIMS methods are the most powerful of the MS-techniques to study surface adsorption phenomena for macromolecules.

In the former section it was described that the research on the mechanism for ejection in PDMS has indicated that the simple straight TOF-PDMS spectrometer may have to be modified to get better mass spectrometric performance for large molecular ions. The first example is the ejection angle effect which means that if one uses a long drift region in the mass spectrometer one will loose intensity of molecular ions because of the ejection angle effect. This is demonstrated in Fig. 4 where an einzel lens was installed to compensate for the effect [21]. An alternative way to deal with this problem is to install an ion guide [6] in the field free region. The ejection angle effect is probably also of relevance when one uses various electrostatic mirrors to compensate for differences in axial initial velocity distributions and get a better mass resolution in a PDMS-TOF-spectrometer. We have used the design of the Manitoba group [37] and coupled such an electrostatic mirror TOF spectrometer to a beam of the Uppsala tandem accelerator. Indeed we have found that we can reproduce state of the art mass resolution of a mirror with grids and in Fig. 5, a mass resolution on a CsBr cluster-ion of 8000 (M/ΔM(FWHM)) is demonstrated [38]. However if we apply the mirror to an organic like renin substrate (1800 u) now ejected in an electronic sputtering process (45 degree angle of incidence) we find that only about 4000 (M/ΔM) can be achieved. This is also in agreement with what other groups have found for keV ion bombardment secondary ion production. Our interpretation is that the angular distributions of ejecta of organic molecular ions ejected by ion induced pressure pulses, give in general larger initial velocity radial components and such secondary ions tend to use much more of the non-central parts of the mirrors used. That may very well lead to the lower mass resolutions observed for the organic ions.

FRONTIERS IN PDMS RESEARCH

In the fields of applications of PDMS the search for new substrates for surface adsorption and desorption will continue. Macfarlanes group have developed very promising substrates of immobilized surfactants and there are many other attempts in this direction [39]. An attractive and often discussed possibility is to study a substrate of antibodies to a particular antigen. Such a substrate would of course form a natural coupling to the analysis for the particular antigen in a biological fluid.

In mechanism studies, a basic problem (as mentioned above) is to identify the main source for the coupling between electronic and nuclear (molecular) motion. Experimental data on total sputtering yields (including the major component, namely ejected neutral molecules and clusters of molecules) are scarce. So far there is only one measurement (based on a collector method) of neutral sputtering yields for electronic sputtering of neutrals. An important new direction is to try to study neutral sputter yields with other methods like post ionization by laser light.

One of the least studied problems in PDMS research so far is that of ionization mechanisms. Further work in this area is essential. In fact such studies are of great general interest because results from such studies are of interest also in other ionization techniques discussed at this meeting. In Fig. 6 a collection of mass spectra for three different techniques discussed at this meeting [40,41,42] are given. The spectra shown are for bovine insulin and illustrate that in a method like PDMS the charge state distribution can be influenced by changing the concentration of molecules on the sample-surface. In Matrix assisted laser desorption the charge-state distribution seems very stable and mostly the lower charge states of even quite large proteins are observed. Finally an electrospray spectrum illustrates the very high charge states favoured in this technique. In our opinion the charge states observed in PDMS are illustrating the amount of protein-protein interaction in the sample. Less interaction favours the higher charge states.

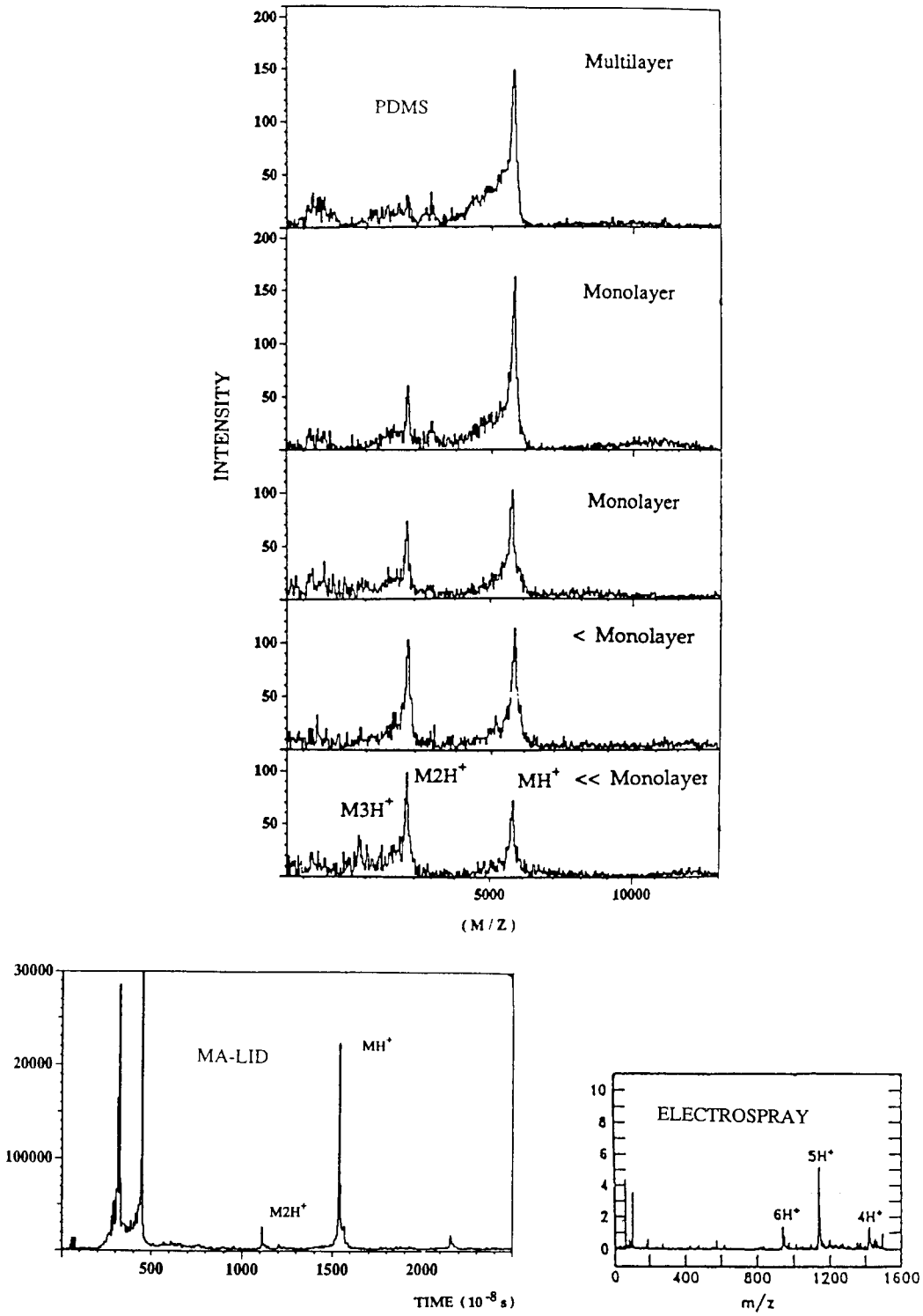

Figure 6 Spectra of positive ions of bovine insulin illustrating molecular ion charge-states observed in different MS-techniques, PDMS [40], Matrix assisted laser desorption TOF [41] and Electrospray-MS [42].

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