# A comparison of performance characteristics of AP and low-pressure (SubAP) MALDI sources


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## Overview

A comparative study was conducted on the analytical performance of MALDI source operating at either low pressure (0.8-20 Torr) or atmospheric pressure. In both cases, the source was attached to LTQ mass spectrometer equipped with the ion funnel assembly.

- The detection limits achieved in the peptide MALDI MS analysis were record low at 0.9 -1.5 Torr pressure (LODs = 50-200 amol range).
- Working at ~1-Torr pressure one observes the appearance of analyte ion signals at laser fluences considerably (~1.5 fold) lower compared to those utilized in the AP MALDI source.
- The chemical noise at 1-Torr was significantly less pronounced compared to that in the AP MALDI source at the same laser fluences.
- Matrix adducts (for Arg-rich peptides) were barely detectable at 1 Torr. With the increase in pressure, the adduct peaks start to rapidly grow.

A substantial sensitivity improvement can be explained by the fact that the composition and cooling rate of the matrix clusters ablated from the MALDI samples at 1 Torr are very different from those at atmospheric conditions. It is hypothesized that at atmospheric pressure, very high cooling rate of ablated cluster impedes the temperature activated escape of protonated ions from the surface of matrix clusters (nanoparticles) thus necessitating to heat the clusters to initially higher temperature using higher laser fluences compared to those used in low-pressure or vacuum MALDI sources.

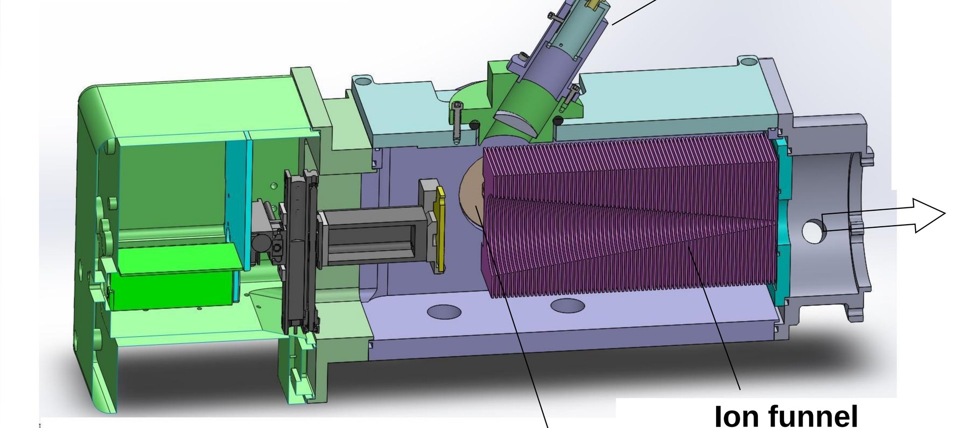
## Method

The MassTech's AP MALDI source was attached to the chamber comprising the ion funnel with the orthogonal capillary inlet. The face of 0.75 i.d. (2.0 mm o.d.) heated capillary (250°C) was placed 1.9 mm from the MALDI plate. The ion funnel assembly was mounted onto LTQ (ThermoFisher) replacing the standard nozzle-skimmer interface.

The low-pressure (SubAP) MALDI source design followed that of the MassTech's AP source, yet, a chamber accommodating the sample plate was made air-tight and evacuated using a separate forevacuum pump. The target plate in SubAP MALDI source was mounted 10 mm from the ion funnel entrance. Up to 50 volt was applied across the gap between the target plate and funnel entrance.

The desorbed ions entered the ion funnel and were transported to LTQ. The laser spot diameter was the same in both the LP and AP sources (~250 micron, flat top laser intensity distribution). Peptide/CHCA(DHB) matrix samples were prepared using standard dried-droplet method.

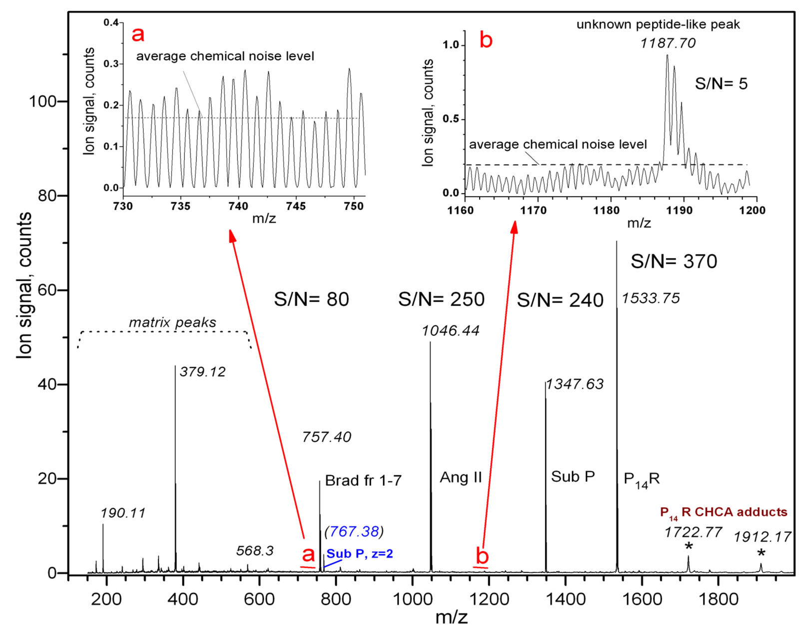
### 1-Torr MALDI source



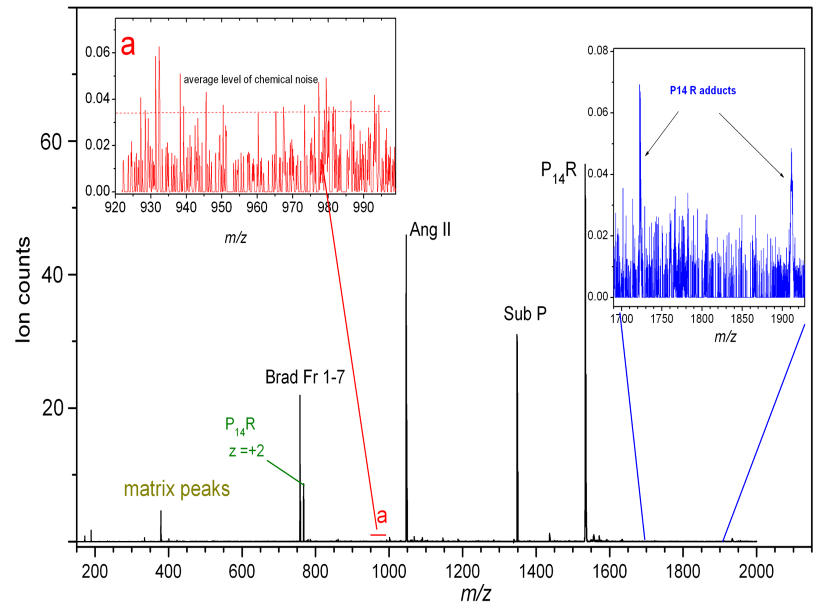

## RESULTS

### Detection limits

**AP MALDI** CHCA



**1-Torr MALDI** CHCA



Peptides: Bradykinin fr.1-7 300 fmol
Ang II 100 fmol
Sub P 50 fmol
$P_{14}R$ 100 fmol
CHCA: 0.5 mg/mL
Dried-droplet spot ~1.8 mm in diameter

Laser: **11 µJ** in the laser spot of ~250 µm diameter (the threshold energy was 7 µJ for AP MALDI source)

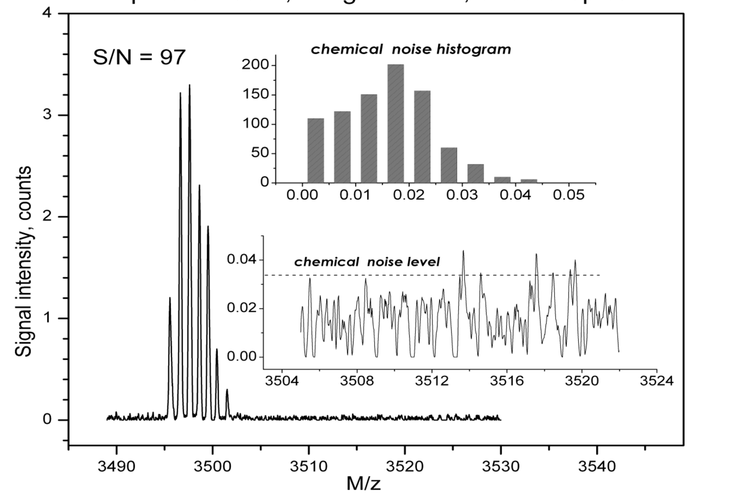
Laser: **7 µJ** in the spot of ~250 µm (the threshold energy was 4 µJ)

| | AP MALDI | SubAP MALDI |
|---|---|---|
| Brad. fr.1-7 | 80 | 700 |
| Ang II | 240 | 2,100 |
| Sub P | 250 | 2,300 |
| $P_{14}R$ | 370 | 3,500 |
| | S/N | S/N |

**AP MALDI (ZoomScan mode)**

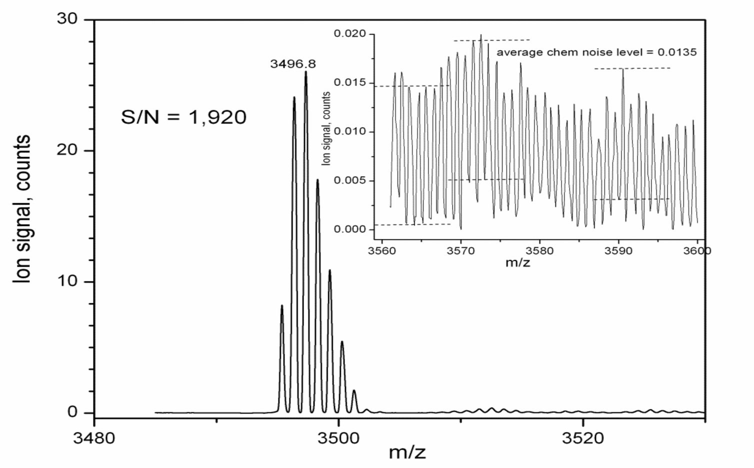
~29 µJ /laser pulse, 1-min integration of ion signals

AP MALDI, high-resolution (Zoom) mode DHB
1 pmole Insulin, 7 mg/mL DHB, dried-droplet



**1-Torr MALDI (ZoomScan mode)**

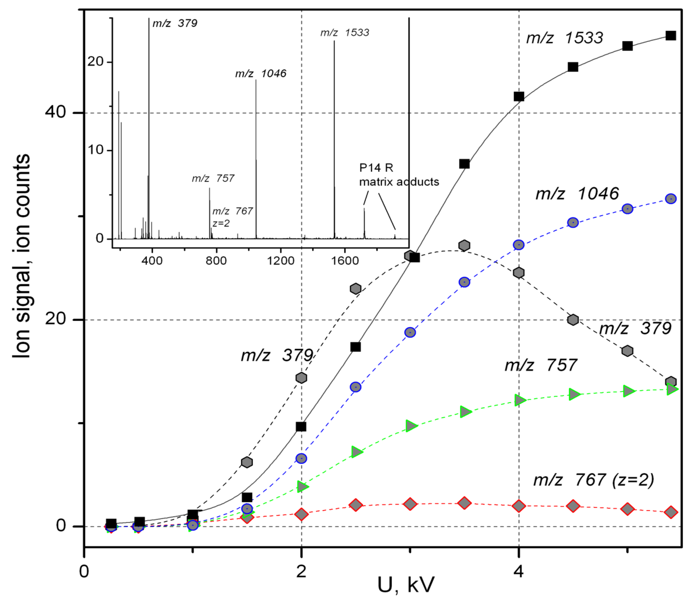
20 µJ / laser pulse, 1-min integration of ion signals DHB



**Relative intensity of Sub P (z=+2) peak increased by a factor of 2.5; matrix region signals (100 - 600 m/z) dropped ~ 10 fold at 1 Torr**

### Ion yield *vs* electric field near the MALDI plate

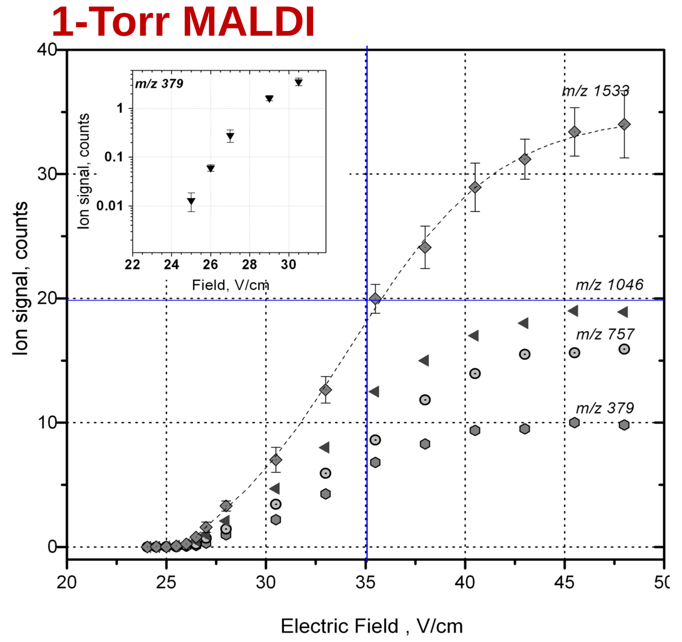
**AP MALDI**



The plate-to-inlet distance : 1.9 mm.

CHCA - 0.5 mg/mL; 100 fmol of Brad 1-7 (m/z=757), Ang II (m/z=1046), and $P_{14}R$ (m/z=1533)

**1-Torr MALDI**

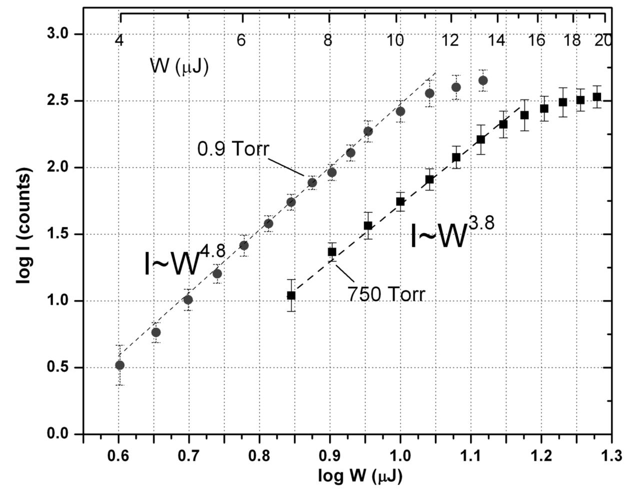


*The dependence of ion signal s on the electric field is scaled with the gas pressure:*
***AP***
*5.4kV/0.19 cm =*
***30*10³V/cm***
*vs*
***Sub AP***
*45 V/cm*750*
***=33*10³V/cm***

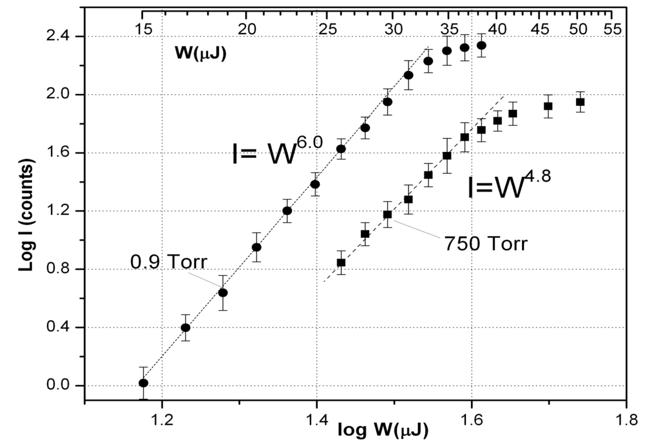
### MALDI threshold

Ion yield *vs* laser energy: Intensities of three major peptide peaks were summed up and plotted against selected level of laser energy, LTQ operated in the Normal Scan mode (low mass resolution).

CHCA




DHB

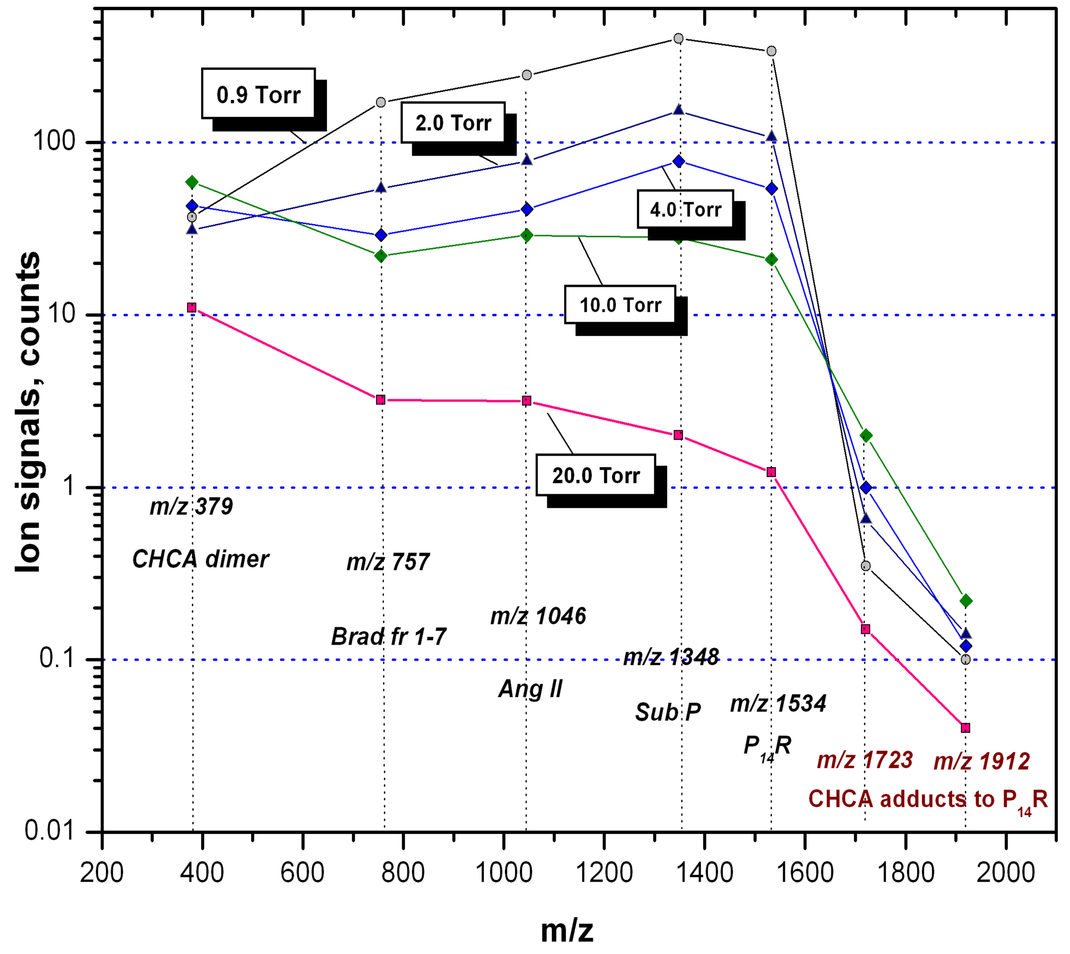



At atmospheric conditions, the ablation dynamics of matrix material is greatly different from that at 1-Torr pressure: cluster size is generally larger (Ref.1) and the cooling rate of matrix clusters is much higher. In a model where the analyte ions escape from the surface of hot matrix clusters, the escape rate *R(t)* is a steep (Arrhenius) function of cluster temperature *T(t)*. So given the limited time for the ion to escape the cluster (before the cluster becomes too cold), the initial temperature $T_0$ of the cluster at atmosphere should be much higher compared to that at 1 Torr due to significantly higher cooling rate $\tau$

$$R(t) = R_0 \cdot e^{-\frac{U_{act}}{kT(t)}}, \qquad T(t,p) = T_0 \cdot e^{-\frac{t}{\tau(p)}}$$

It was noticed in many studies that a mild thermal activation of peptide molecular ions in a presence of chemical noise background increased the decomposition rate of ions of chemical noise to a larger extent compared to decomposition of peptides. Assuming that chemical noise comprises various species of the bio-organic origin, higher initial temperature in the laser plume brings to life more fragments of those background species.

### Ion yield in **MALDI MS** at different background gas pressures




***each peptide - 1 pmol, CHCA – 0.5 mg/mL, the accelerating field E=50V/cm***

At pressure higher than 10 Torr, the ions are lost due to a sharp decrease in the ion transport efficiency through the funnel. The same is true when the gas pressure is below ~0.9 Torr.

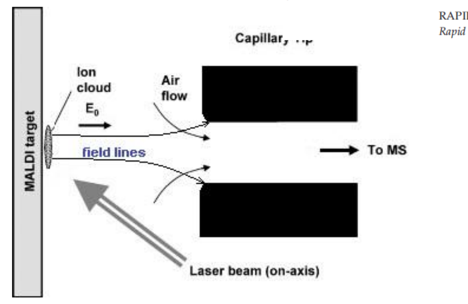
At 0.9-1.5 Torr pressure, the plume dynamics becomes more favorable for the efficient ion production while the rates of matrix adduct formation and in-source decay (Ref. 2-4) are still limited .

## *Discussion*

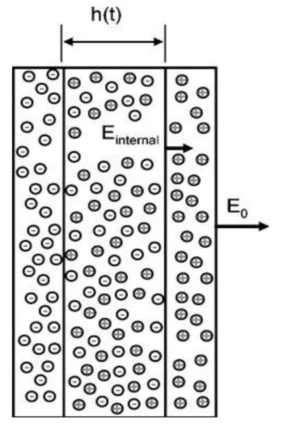
Modeling of ion processes in atmospheric pressure matrix-assisted laser desorption/ionisation

Vadym D. Berkout, Sergey I. Kryuchkov, and Vladimir M. Doroshenko

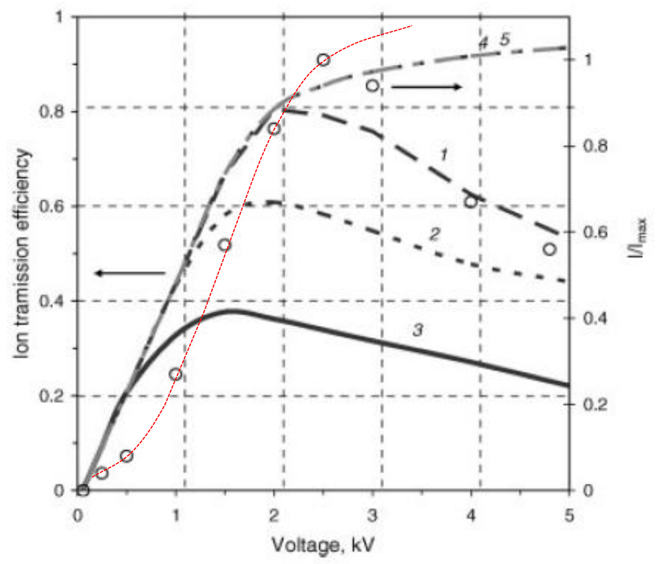
RAPID COMMUNICATIONS IN MASS SPECTROMETRY
*Rapid Commun. Mass Spectrom.* 2007; 21: 2046–2050




**Anions and cations in the initially neutral plasma plume near the MALDI plate are separated under the action of external electric field**




**In AP MALDI source, light ions are lost when they hit the capillary edge because the ions tend to follow the electric field lines. On contrary, larger ions are aerodynamically dragged by the air flow focusing those ions into the capillary inlet.**




$$E_{\mathrm{internal}} = E_0 - \frac{e\nu}{\varepsilon_0}$$

$$\frac{d\nu}{dt} = 2\mu n(t) E_{\mathrm{internal}} = 2\mu \frac{n_0}{(1+Kn_0t)}\left(E_0 - \frac{e\nu}{\varepsilon_0}\right)$$

Solving this equation one can obtain:

$$\nu(t) = \frac{\varepsilon_0 E_0}{e}\left(1 - (1+Kn_0t)^{-\frac{2\mu e}{\varepsilon_0 K}}\right)$$

Similarly one can derive the equation determining the thickness $h$ of the overlapping region:

$$\frac{dh}{dt} = -2\mu E_{\mathrm{internal}} = -2\mu\left(E_0 - \frac{e\nu}{\varepsilon_0}\right)$$

$$h(t) = h_0 - \frac{2\mu E_0}{Kn_0\left(\frac{2\mu e}{\varepsilon_0 K} - 1\right)}\left(1 - (1+Kn_0t)^{1-\frac{2\mu e}{\varepsilon_0 K}}\right)$$

**In the approach developed in the original paper, ion neutralization rate K does not depend on mutual velocity $v=\mu E$ of the ions. When such a dependence, $K = K_0+\alpha(E_0/E)$, is introduced, the model starts to fit a nonlinear character of ion survival rate $n(t)$ at small accelerating field E.**

## Conclusion

- SubAP MALDI source that utilizes the ion-funnel technology offers a 6-20 fold higher sensitivity (the gain is peptide dependent) in MALDI MS analysis of analytes of large mass range and different chemical nature.
- The gas pressure in the SubAP MALDI source can be easily changed from ~0.8 Torr to 10 Torr to study MALDI processes at different conditions. The leak valve can be connected to other source of gas (argon, nitrogen, *etc*)
- The dependence of the ion yield on the applied electric field can be described by a process of the charge neutralization/ field-driven charge separation in a gas phase

### References


1. S.S. Harital *et al* "Background gas collisional effects on expanding fs and ns laser ablation plumes", *Applied Physics A*, **117**(1): *319* (2014)
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3. Soltwisch *et al.* "Effect of gas pressure and gas type on the fragmentation of peptide and oligosaccharide ions generated in an elevated pressure UV/IR-MALDI ion source coupled to an orthogonal TOF-MS". *Anal Chem*, **81**:2921 (2009)
4. Hieke A. "Suppression of unimolecular decay of laser desorbed peptide and protein ions by entrainment in rarefied supersonic gas jets under weak electric fields." *J Chem Phys*, **140**(3): *034201* (2014 )



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