

detected ion current for higher concentrations are lower  $m/z$  related and not solvent related ions and/or charged droplets.

The effects of pressure were explored by partially closing a valve located in between the ion funnel and the first stage mechanical pump. As the pressure in the ion funnel was raised, a higher RF amplitude was required to achieve similar ion transmission than when measured at lower relative pressure as shown in FIG. 8A. For the 1–10 Torr range, as measured using the convection gauge, maximum ion currents were achieved for the 1–5 Torr range but above this the required RF amplitude needed to maximize ion transmission was above the RF breakdown threshold (i.e. 400–500  $V_{pp}$ ) of the ion funnel. Increasing the size of the capillary inlet from 510 to 760 micrometer inner diameter accommodated more ions, as evidenced by the higher ion current for the DC-only mode for the 760 micrometer i.d. capillary inlet as shown in FIG. 8B. However, the larger capillary consequently resulted in a higher operating pressure (7.1 Torr) and thus resulted in a larger RF requirement to focus the available ions. Note that the appearance of this curve is similar to the curve measured at 7.8 Torr with the 510 micrometer i.d. capillary. Therefore, there exists a useful operating pressure range for the ion funnel operating at a given RF frequency and this operating range in practice is determined on the low end by the size of the inlet capillary and the pumping speed applied to the ion funnel region and on the high end by the RF breakdown threshold for the ion funnel.

Ion current transmitted to the octapole ion guide was measured using aluminum foil covering its entrance. The ion currents detected for 29 and a 2.9 M solutions of horse heart myoglobin for the 0–350  $V_{pp}$  RF amplitude range are shown in FIG. 8C. Similar to the results obtained with ubiquitin, the maximum ion current displays a 2 order of magnitude increase compared to the ion funnel operating in the DC-only mode. An important figure of merit for the ion funnel is the fraction of total current entering the interface that is effectively transmitted. The ion current entering the vacuum chamber and directed towards the entrance to the ion funnel was measured using a plate at ground immediately following the exit of the capillary inlet (~5 mm). Table 2 gives the currents measured for myoglobin, cytochrome c, and gramicidin S solutions.

TABLE 2

Ion Current Measured on Octapole Ion Guide Using the: Standard Ion Source (A), Ion Funnel (B)*; Ion Current Measured Entering the Ion Funnel (C), Ratio of B/A and Ratio B/C ( $\times 100$ ).					
	A	B	C	B/A	B/C ( $\times 100$ )
<b>Myoglobin</b>					
29 M	77 pA	1.5 nA	6.0 nA	19	25%
2.9 M	18 pA	.75 nA	3.2 nA	42	23%
<b>Cytochrome c</b>					
40 M	57 pA	1.4 nA	5.8 nA	25	24%
4.0 M	20 pA	.84 nA	4.0 nA	42	21%
<b>Gramicidin S</b>					
3.0 M	15 pA	.13 nA	2.7 nA	9	5%

\*Measured at 700 kHz with 98  $V_{pp}$  except gramicidin S which used 75  $V_{pp}$ .

These values allow a low end transmission estimate for the ion funnel of approximately 21–25% for the proteins. The actual transmission of the ion funnel is certainly higher since the current includes both low  $m/z$  (solvent related) and high  $m/z$  droplet components. The low  $m/z$  ions will not be transmitted (due to instabilities in the applied RF fields) while the high  $m/z$  ions will not be focused at the applied RF

amplitude and will be transmitted with very low efficiency. Thus, the overall efficiency of protein ion transmission through the ion funnel for the analytically significant portions of the ion current transmitted through the capillary inlet is likely 50% or greater. The transmission efficiency for the peptide, however, is lower by a factor of ~5. This stems from the fact that there is a low  $m/z$  cut-off for the ion funnel, i.e. a low mass limit to which ions are not efficiently transmitted through the interface.

Ion current transmitted to the octapole ion guide was also taken for the standard Finnigan ESI ion source for selected concentrations of myoglobin, cytochrome c, and gramicidin S (Table 2).

The ratio of the ion current measured with the ion funnel over the ion current measured with the standard ESI ion source can be used to estimate the effectiveness or overall sensitivity gain using the present ion funnel design. For the proteins studied, the ratios indicate that the ion funnel delivers a 20 to 40 times greater ion current to the octapole ion guide (and eventually the mass analyzer) than the standard ESI ion source. The peptide gave a ratio of 9 times the ion current over that of the standard ESI ion source.

Mass Spectra. Mass spectra for selected protein and peptide solutions were acquired with the prototype ion funnel mounted directly in front of the octapole ion guide using a Finnigan TSQ 7000 triple quadrupole mass spectrometer. The relative ion current (RIC), detected by the mass spectrometer, was then compared to the RIC obtained with the standard ESI ion source under identical multiplier and other operating conditions. An example of such a comparison for a 4.0  $\mu$ M solution of horse heart cytochrome c is shown in FIGS. 9A and 9B. The spectrum obtained using the ion funnel displays 10 times the RIC and over 20 times the base peak intensity compared to the spectrum with the standard ESI source.

By interfacing the ion funnel directly to the octapole ion guide it was not necessary to use a skimmer. In fact, replacement of the skimmer by a simple conductance limiting aperture (i.e. final orifice electrode) led to a factor of 2 to 3 increase in the RIC measured for all of the protein solutions studied. Hence, in this new design, the ions are more efficiently transmitted to the octapole ion guide which enables a lower potential gradient to be used between the final orifice electrode and octapole ion guide. This characteristic is generally desirable since it minimizes the likelihood of undesired collisional activation in this region, which may induce dissociation or preclude detection of non-covalent complexes.

Ratios of relative ion current were derived from mass spectra for solutions of myoglobin, cytochrome c, and gramicidin S (Table 3).

TABLE 3

Ratio of Relative Ion Current (RIC) Obtained from Mass Spectra Measured with the Ion Funnel Prototype Divided by that Measured with the Standard Ion Source.*	
	Ratio
29 M Myoglobin	12
2.9 M Myoglobin	12
40 M Cytochrome c	12
4.0 M Cytochrome c	14
3.0 M Gramicidin S	3

\*Ion funnel operated at 700 kHz (98  $V_{pp}$  except for gramicidin S which used 75  $V_{pp}$ ). Ratios based on RIC for the proteins and peak intensity for the 2+ charge state ( $m/z$  572) for gramicidin S.

When comparing the RIC measured using the ion funnel to the standard ESI ion source, the ion funnel yielded a 12–14 times improvement over the standard ESI ion source