Optimization of Sample Loading Capacity For Peptide Quantification by Nanobore LC-MS/MS

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Introduction
A dominant workflow for qualitative proteomics has been “GeLC-MS,” a combination of 1- (or 2-D) gel electrophoresis with reverse-phase nanoflow liquid chromatography mass spectrometry (nLC-MS/MS). The limited protein quantity isolated from a single gel band and column loading capacity necessitate the use of 75 um inside diameter (ID) packed columns for optimal sensitivity. However, limitations on sample injection volume, gradient and flow characteristics, and excessive delay volume hinder quantitative applications. Novel solution phase tube-gel fractionation yields a 5-10 fold increase in gel capacity. This increase permits the effective use of a larger column (150 to 200 um) range. These larger columns prove effective for absolute quantification method development, reducing analytical cycle time by 4-fold.

Methods
Peptide standards, single protein digests and whole yeast digests were prepared to test LC system performance. Samples (2-20 uL) were loaded via autosampler onto C18 packed-emitter columns of 75, 150, or 200 um ID. Gradient elution was generated by direct flow nanobore pumps. Columns were mounted on nanospray equipped conventional 3D ion trap, or triple quadrupole, MS/MS instrument. Electrospray high voltage was applied using a Pt wire junction contact. For the operation of 150 um ID columns at flow rates of 1-2 ul/min sheath gas assistance was employed. Complex samples were fractionated using SDS polyacrylamide tube gel electrophoresis into fractions ranging from 3.5 to 150 kDa. Samples were processed to remove SDS, reduced, alkylated, and digested.

Preliminary Data
Varying concentrations of BSA, cytochrome C, insulin, carbonic anhydrase, and B-lactoblobulin, as well as yeast lysate were fractionated using a novel tube gel electrophoresis system and using standard 1D gel electrophoresis. Fractions containing the standards were eluted from the tube gel into a trapped liquid volume, collected and resolved on a 1D gel for determination of load capacity and recovery. Results indicate that tube gel fractionation and elution provided >5X the load capacity of a standard 1D gel for all standards tested, as well as yeast lysate. Recoveries for protein standards eluted from the tube gel were >70%. Individual fractions were collected reduced, alkylated, and digested with trypsin under standard conditions. Sample aliquots were injected (full loop at 2, 5, 10 uL) from the autosampler to 75 or 150 um ID (10 cm) nanobore C18 packed-emitter columns. Standard gradient elution (20 min to 50% B) was performed at flow rates of 300 and 1500 nl/min for 75 and 150 um ID columns respectively. Column loading capacity was determined by increasing total injection mass on-column and monitoring subsequent peak capacity; peak capacity was calculated using a method based on peak width and symmetry. Larger bore 150 um ID columns were found to maintain resolution and peak capacity at sample loads ≥ 4x that of 75 um columns, making 150 um ID columns a good combination with tube gel fractions. Peptide column capacity for (150 um ID) was estimated to be approx. 0.2 to 0.4 ug. Because system volume was nearly identical for the two column formats, gradient delay was reduced (from approx. 10 min for 75 um columns) for 150 um columns by more than 70%. The robustness of the 150 um ID format will be exploited in quantitative analysis by migrating methods to a QQQ-MS.