Offline Nano-ESI Phosphopeptide Analysis with Carbon, TiO$_2$ and ZrO$_2$ Wall-Coated Trap'nTips™
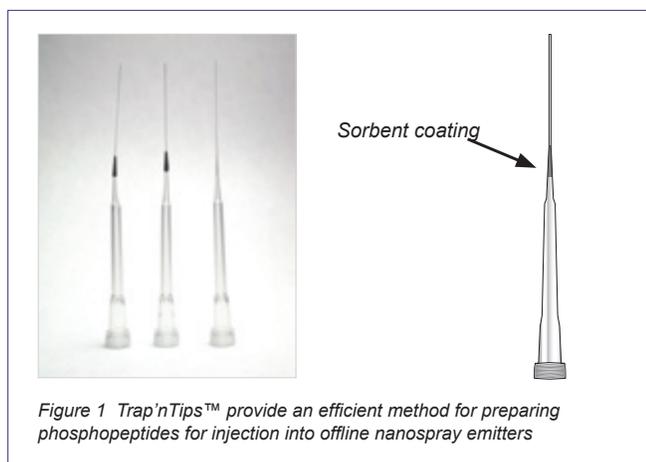
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Introduction

Until recently, in-pipette sample preparation for nanobore LC-MS has been impeded by geometric constraints of sample preparation and transfer. Conventional practice has involved sample delivery from a pipette tip to an emitter, pressurized back-loading into a pipette, or coupling the pipette tip to a larger pipette tip[1].

The design of carbon, TiO$_2$ and ZrO$_2$ wall-coated pipette tips (Trap'nTips™, New Objective) facilitates phosphopeptide enrichment and manual aspiration through the pipette tip followed by expulsion of purified solution for immediate nanobore column injection or direct loading into an offline nanospray emitter. In addition to concentration, desalting, and MS signal enhancement, Trap'nTips coated with these analyte-specific substrates facilitate offline nanospray analysis by separating phosphopeptides from a tryptic ß-casein digest.

Sorbent coating

Figure 1 Trap'nTips™ provide an efficient method for preparing phosphopeptides for injection into offline nanospray emitters

Figure 2 Borosilicate glass GlassTip™ with multi-layer platinum coating

Methods & Materials

Instrumentation and Components

- Ion-trap mass spectrometer (LCQ Deca™, Themo Electron)
- Nanospray source (Digital PicoView® 150, New Objective, modified for offline analysis)
- A 0.5-10 µL Eppendorf® Single-Channel Research Pipette
- Sorbent-coated Trap'nTips™ containing carbon, TiO$_2$, and ZrO$_2$ sorbents (New Objective)
- GlassTips™ (BG12-94-4-CE) offline nanospray emitters (New Objective)
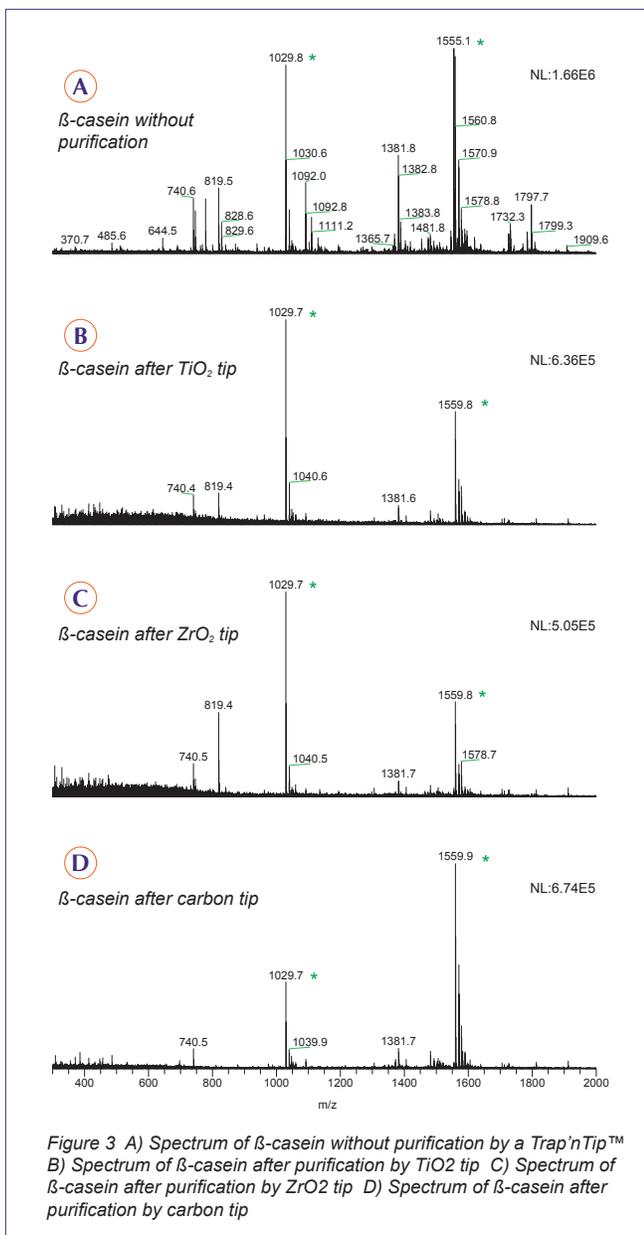
Sample Preparation

- Phosphopeptide Positive Control Set (Set P9615, Sigma) was prepared by diluting each vial with 1 mL HPLC-grade water / 0.1% formic acid.
- For each phosphopeptide, a 2 ng / µL solution was generated by diluting 10 µL of vial contents with 480 µL water / 0.1% formic acid solution.
- An overnight trypic ß-casein digest was performed, and the sample was diluted with water / 0.1% formic acid solution to generate a 1 pmol / µL solution.

Trap'nTip™ Conditioning and Sample Loading

- Trap'nTips were initially conditioned with 5 aspiration / expulsion cycles of HPLC-grade water.
- Analytes were loaded onto the Trap'nTip wall-coating through 10 aspiration / expulsion cycles of 10 µL ea
- Loaded samples were washed by 10 aspiration / expulsion cycles using 10 µL HPLC-grade water.
- Analytes were eluted from the Trap'nTip into a clean vial using 2 µL aqueous solution containing 50 mM NH$_4$HCO$_3$ and 50 mM TEA.

Offline NanoESI-MS Preparation

- 2 µL 50 mM TEA in methanol were added to the sample followed by immediate mixing and centrifugation.
- Each sample was loaded into the distal end of a GlassTip™ nanospray emitter (New Objective).
- All samples were analyzed via ESI-MS in negative-ion mode.

Figure 3 A) Spectrum of ß-casein without purification by a Trap'nTip™ B) Spectrum of ß-casein after purification by TiO2 tip C) Spectrum of ß-casein after purification by ZrO2 tip D) Spectrum of ß-casein after purification by carbon tip

Results

GlassTips™ (New Objective) with a multi-layer platinum tip coating performed extremely well under high-pH conditions. A phosphopeptide standard was used to tune MS and to confirm peaks separated in the digest were, indeed, phosphopeptides. Carbon-, TiO_2-, and ZrO_2-coated Trap'nTips™ successfully separated phosphopeptides contained in the ß-casein digest and phosphopeptide standard (Figure 3).

Conclusions

- For complex digest solutions, Carbon, TiO_2, and ZrO_2 wall-coated Trap'nTips™ successfully remove phosphopeptides for analysis via offline nanospray

- GlassTips™ with a multi-layer platinum-coated tip demonstrate robust performance in negative-ion mode

- Mass spectra of digest samples purified via carbon, TiO_2, and ZrO_2 Trap'nTips display exceptional signal-to-noise for phosphopeptide analysis

Figure 4 *Spectrum of phosphopeptide positive control standard*
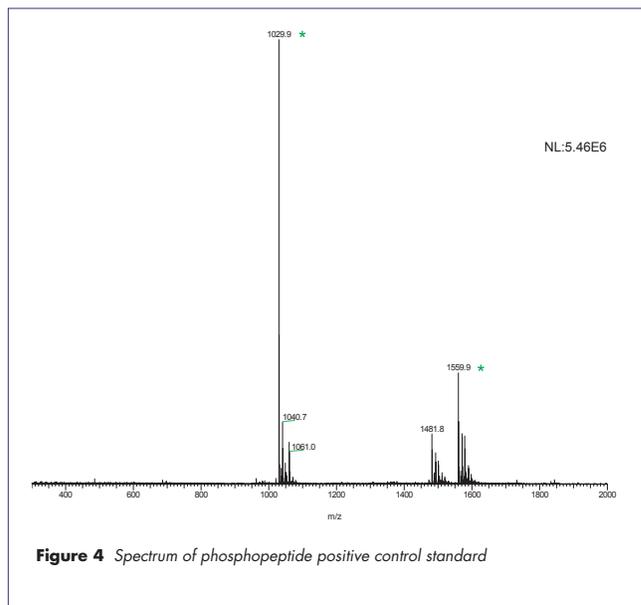
1. Toher, C.J.;, Perala, A.W., Shukla, A.K., Valaskovic, G.A. "Sample Purification for Static Nanospray MS Using Wall-Coated Pipette Trap'nTips™,". Poster presented at the American Society for Mass Spectrometry, San Antonio, 2005.