SAMPLE PREPARATION FOR BOTTOM-UP PROTEOMICS USING A DISPOSABLE CARTRIDGE (PROTRAP XG™) EQUIPPED WITH CHITOSAN-IMMOBILIZED TRYPSIN

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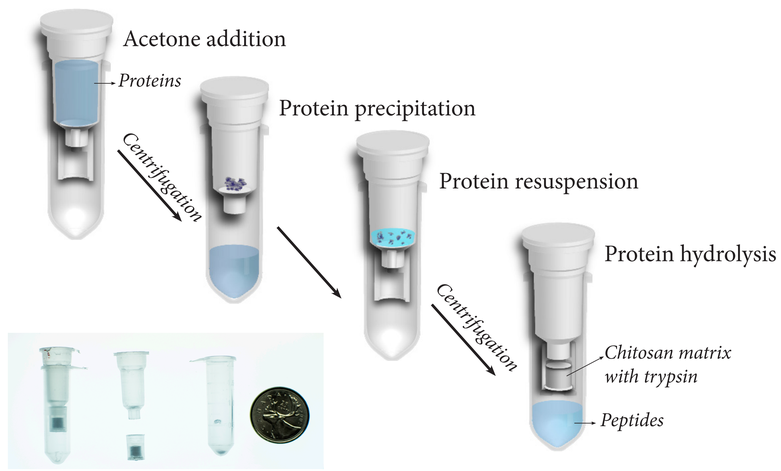
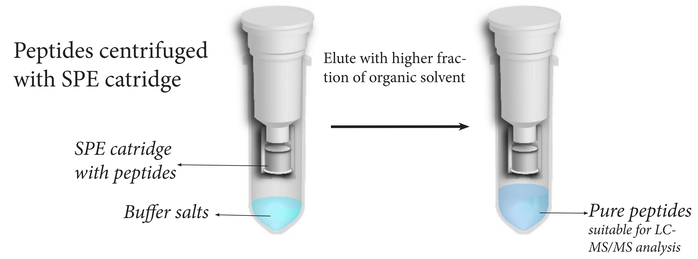
Introduction

- Conventional protein precipitation is slow & erratic as a front-end method for bottom-up proteomics.
- A two-step cartridge system was developed by our group previously (Crowell et al., 2015) for effective sample prep., facilitating the effective removal of SDS while maintaining high protein recovery.
- Chitosan matrix with immobilized trypsin is used here, to enhance, automate and facilitate rapid hydrolysis of proteins recovered by ProTrap XG™.

Methods





Results

Estimation of immobilized trypsin activity

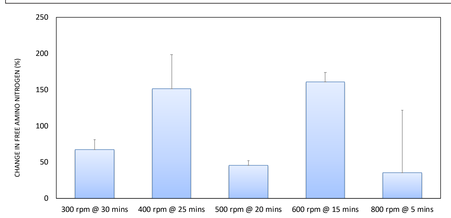


Figure 1: Effect of centrifugation speed and time on free amino nitrogen (FAN) content of BSA solutions (0.1 g/L) passed through catridges with chitosan matrix with immobilized trypsin

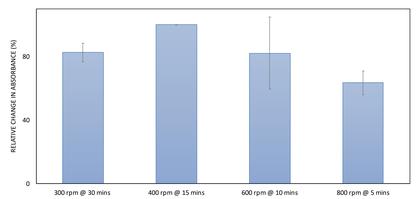


Figure 2: Effect of centrifugation speed and time on trypsin activity evaluated on Nα-Benzoyl-L-arginine ethyl ester hydrochloride (BAEE) passed through catridges with trypsin immobilized in chitosan matrix

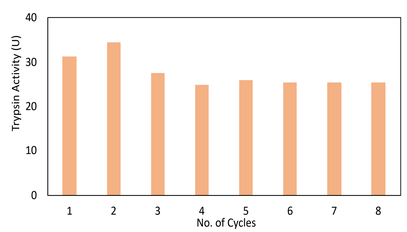


Figure 3: Effect of multiple use cycles on immobilized trypsin activity measured using BAEE

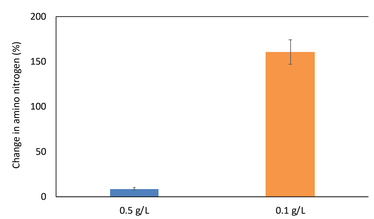


Figure 4: Effect of substrate concentration on trypsin activity measured using FAN

Comparison of immobilized and in solution trypsin hydrolysis

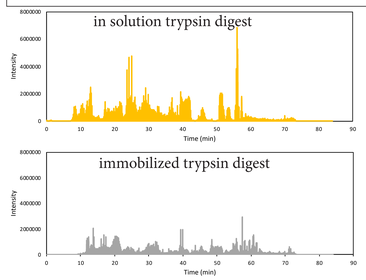


Figure 5: Base peak chromatograms obtained from LC/MS of bovine serum albumin digest prepared from in solution and immobilized trypsin

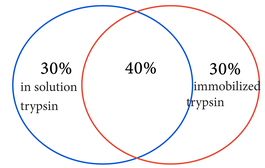


Figure 6: Venn diagram comparing peptides identified from BSA digests prepared from in solution and immobilized trypsin

Conclusions

- Chitosan immobilized trypsin presents itself as a valuable addition to ProTrap XG™ for rapid sample preparation for proteomics.
- Proteolytic activity is higher at low spin speeds (such as 400 rpm) and remains mostly conserved through multiple cycles.
- While similar number of peptides were identified with both immobilized and conventional trypsin, peptides identification requires further characterization using more heterogenous samples.

Acknowledgements

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www.proteoform.com

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Reference: Crowell, et al. (2015). A two-stage spin cartridge for integrated protein precipitation, digestion and SDS removal in a comparative bottom-up proteomics workflow. Journal of Proteomics, 118, 140–150.