JPP JOURNAL OF PROTEINS AND PROTEOMICS
5(4), December 2014, pp. 151-161

Research Article

COMPARISON OF COMMERCIAL LC MS/MS COMPATIBLE DETERGENTS WITH SODIUM DEOXYCHOLATE FOR SHOTGUN PROTEOMICS

Caleb J. Porter[1] and Michael S. Bereman[1,2,*]

[1]*Department of Chemistry, North Carolina State University, Raleigh, NC 27695, USA*
[2]*Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695, USA*

Abstract: In this study, we compared the performance of sodium deoxycholate (SDC) with several commercially available LC MS/MS compatible detergents for digestion of complex proteomic mixtures. First, the parameters affecting in-solution digestion using SDC were investigated with a full factorial experimental design. Metrics explored were trypsin ratio, digestion time, and concentration of SDC. These parameters were not found to be statistically associated with total peptide identifications in the experimental space investigated. However, in terms of digestion efficiency, digestion time was highly significant ($p = 0.0095$) as determined by the percent of peptides identified with missed cleavages. The optimized protocol for peptide identification and throughput was used to compare the performance of SDC with various commercially available LC MS/MS compatible surfactants namely Invitrosol, RapiGest, and PPS Silent Surfactant. The detergents were found to be similar through comparisons of the total identified peptides and the hydrophobicity of recovered peptides. We found suitable recovery across a large range of SDC concentrations determined from a bicinchoninic acid (BCA) assay. In a spike down experiment, no distinct differences in total number of peptide identifications were discovered when comparing PPS (Silent Surfactant) and SDC for preparation of peptide samples derived from low protein amounts (< 20 μg). Combined, these results indicate that SDC is a cost effective alternative to other commonly used LC MS/MS compatible surfactants.

Keywords: Detergent; sodium deoxycholate; shotgun proteomics; mass spectrometry; sample preparation

Introduction

A continued goal in both discovery and targeted bottom-up proteomics is the development of sample preparation procedures that maximize cost effectiveness, reproducibility, and throughput without sacrificing performance (James, 1997; McDonald and Yates, 2002; Wolters et al., 2001; Wu, 2002). The success of any shotgun proteomics experiment is dependent on the robustness (i.e., reproducibility, recovery) of sample preparation from protein solubilization, stabilization, digestion, and sample clean-up. Different procedures exist for preparation of complex mixtures in shotgun/bottom-up proteomics. Two main methods involve in-solution digestion and filter aided digestion (Manza et al., 2005; Wisniewski et al., 2009) both of which are being continually improved upon (Bereman et al., 2011). Sample preparation in proteomics experiments via LC MS/MS is an area where exact procedures, including reagents, vary widely amongst laboratories and sample type. Protein identification is initially predicated on the degree of protein solubilization and thus detergent selection is an important component in sample preparation procedures. Sodium dodecyl sulfate (SDS) is the "gold standard" detergent used ubiquitously in biological research However, SDS is incompatible with LC MS/MS and requires

Corresponding Author: **Michael S. Bereman**
E-mail: michaelbereman@ncsu.edu

Received: November 10, 2014
Accepted: December 23, 2014
Published: December 30, 2014

specific procedures for near-complete removal (Bereman et al., 2011; Wisniewski et al., 2009).

Acid cleavable commercial LC MS/MS detergents have been developed as an alternative to SDS for sample preparation in proteomics (Norris et al., 2003). They fill a niche of increasing the solubility of hydrophobic/membrane proteins and peptides without the need for specific steps for removal. Acid cleavable surfactants can be easily removed prior to LC MS/MS analysis by lowering the pH of solution—a necessary step regardless following trypsin digestion. Early work with acid cleavable detergents in proteomics was done comparing acid labile surfactant performance to SDS in 2002 by Meng et al. (Meng et al., 2002). Other studies tested the use of multiple detergents simultaneously and differential detergent fractionation for enhancement of protein digestion and peptide recovery (Guerrera et al., 2005; Lu and Zhu, 2005; McCarthy et al., 2005; McCarthy et al., 2009; van den Berg et al., 2007). Yates and coworkers compared the peptide recovery of tryptic digestions with Invitrosol, RapiGest, and PPS Silent Surfactant. Their study showed MS compatible detergents increase the number of unique peptides and proteins observed in mammalian tissues (Chen et al., 2007; Chen et al., 2008). Waas et al. expanded studies to include Progenta in their testing and found increases in peptide identifications with the patented detergents (Waas et al., 2014). However, these commercial detergents are relatively expensive which can limit resources especially for large biomarker and system biology studies.

Deoxycholate is an anionic water soluble acid produced in the liver and poses a less expensive alternative to commercially available LC MS/MS compatible detergents. Acidification of solutions containing sodium deoxycholate causes the formation of a white deoxycholic acid precipitate. This enables easy and rapid removal of the detergent prior to LC MS/MS. One of the early reported proteomic workflows using SDC was by Zhou et al. where they concluded SDC enhanced the solubility of peptides and proteins. They observed an increase in peptide and membrane protein identifications using SDC compared to SDS (Zhou et al., 2006). A study comparing chaotropic agents and surfactants by Proc et al. used urea, SDS, and SDC. Their study verified that SDC had higher digestion efficiency, peptide recovery, and did not foul the instrument (Proc et al., 2010). Further work by Yong et al. assessed trypsin activity at various concentrations of SDC to ascertain optimal conditions for digestion compared to urea, methanol, and SDS. They found trypsin activity is compromised significantly at concentrations higher than 5% SDC using a standard five protein mix (Lin et al., 2008). Recent work has been reported comparing SDC to other detergents in eFASP (Erde et al., 2014) in addition to optimizing peptide recovery from SDC precipitates using the phase transfer or acid wash mechanisms (Valente et al., 2014). However, it is still unknown the degree at which common parameters affect in-solution digestion of complex mixtures using SDC and how the peptide recovery of SDC compares to other commercially available LC MS/MS compatible detergents.

Herein, we investigated various conditions (e.g., digestion length, trypsin ratio, SDC concentration) that affect in-solution digestion using SDC with a full factorial experimental design. We then compared the performance of SDC to commercially available mass spectrometry compatible detergents. Based upon our findings and comparisons using these metrics, SDC is a cost effective alternative to LC MS/MS commercial detergents even at low protein starting amounts < 20 µg.

Materials and Methods

Materials

Formic acid (FA), ammonium bicarbonate, ammonium hydroxide, dithiothreitol (DTT), SDC, hydrochloric acid (HCl), bovine serum albumin (BSA), and iodoacetamide, were obtained from Sigma Aldrich (St. Louis, MO). Proteomics grade trypsin was purchased from Promega (Madison, WI). Invitrosol was purchased from Invitrogen (Carlsbad, CA), RapiGest from Waters (Milford, MA), and PPS Silent Surfactant from Expedeon (San Diego, CA). HPLC grade acetonitrile, methanol, and water were purchased from Burdick & Jackson (Muskegon, MI). The Micro

BCA Protein Assay Kit (Part # 23235) was purchased from Thermo Fisher (San Jose, CA).

Methods

Sample preparation– Design of Experiment with SDC

The 293 kidney cell line was grown and harvested following the procedure described in Supplemental under *Cell Culture*. The cells were split into 50 µL aliquots containing ~ 10*e6 cells. Samples were lysed with an ultra-sonicator model #CL-34 (Thermo Fisher) in three 20 second bursts at 20% power in 50 mM AB with 1% or 3% SDC. Protein concentrations were determined via absorbance at 280 nm on a Nanodrop 2000 spectrophotometer (Thermo Fisher). Aliquots of 100 µg of protein were taken and were diluted to 100 µL with 50 mM AB with 1% or 3% SDC. DTT was added to final concentration of 5 mM and the samples were incubated for 30 minutes at 60 °C. Samples were alkylated with iodoacetamide added to achieve a final concentration of 15 mM and samples were incubated at room temperature for 20 minutes in the dark. Trypsin was added to the samples in a ratio of 1 µg of trypsin: 100 µg of total protein or of 1 µg of trypsin: 50 µg of total protein. Samples were incubated for 4 or 12 hours at 37 °C. To quench tryptic digestion and remove SDC, HCl was added to the samples to achieve a final concentration of 250 mM. After precipitation of deoxycholic acid samples were centrifuged at 14 x g for two minutes. The supernatant was collected for further processing. To improve peptide recovery deoxycholic acid precipitates were acid washed (Zhou et al., 2006). The acid wash was performed with 100 µL of 250 mM HCl. The samples were briefly vortexed and were centrifuged at 14 x g for two minutes. The acid wash supernatant was also collected for further processing. Solid phase extraction (SPE) columns were used to remove neutrals and salts from the samples. MCX SPE columns (30mg LP extraction cartridges part# 186000782) from Oasis (Milford, MA) were mounted onto a MCX manifold (Waters). Columns were conditioned with one mL of methanol, one mL of 10% NH_4OH in water, two mL of methanol, and three mL of 0.1% FA in water. Samples were added to SPE columns and washed with 0.1% FA to remove salts. Neutral species were removed with one mL 0.1% FA in methanol. Peptides were eluted and collected in one mL of 10% NH_4OH in methanol. Samples were lyophilized with a Savant SPD313DDA speed vacuum (Thermo Fisher). Peptides were reconstituted with mobile phase A (98 % water, 2% acetonitrile, and 0.1 % formic acid) to achieve a final concentration of 0.25 µg/µL of digested peptide in solution.

Sample Preparation – Detergent Comparison

For the detergent analysis, samples were prepared in PPS, RapiGest, Invitrosol, SDC, and a control containing no detergent. Three technical replicates were taken through the procedure for each detergent tested (including the control). For samples containing RapiGest, Invitrosol, and PPS the manufacturers' suggested concentrations were used (0.1% w/v, diluted 1:5, & 0.1% w/v), whereas for samples containing SDC 1% w/v was the concentration used. The samples were digested for 4 hours at a ratio of 1 µg of trypsin: 50 µg of total protein. After tryptic digestion and acidification, the samples containing commercial detergents were allowed to incubate for 45 minutes at room temperature.

SDC Peptide Recovery

SDC peptide recovery was tested at various concentrations using a BCA assay. Six aliquots of ~ 25 µg of peptide material were taken from digested kidney cell lysate. Aliquots were diluted to 60 µL of total volume with varied concentrations of SDC spiked into each aliquot. Mixtures contained (final concentration) 0, 0.5, 1, 2, 3, and 5% SDC. These solutions were acidified with HCl added to a final concentration of 250 mM. The solutions were centrifuged at 14 x g for two minutes and the supernatant was collected. The pellets were acid washed as previously described. However, the volume of 250 mM HCl used in the wash was 40 µL instead of 100 µL. Peptide recovery was evaluated using the manufacturers' protocol for the BCA assay and absorbance was measured at 562 nm on a BioMate 3S (Thermo Fisher).

Detergent Comparison from Low Protein Starting Material

For the detergent comparison using low starting amounts of material, protein concentrations were

determined via absorbance at 280 nm. Aliquots were taken of 20, 10, 5, and 1 µg of total protein and aliquots were diluted to 50 µL with 50 mM AB with detergent (1% SDC or 0.1% w/v for PPS). The samples were taken through the same reduction, alkylation, digestion, and clean up steps as used in the detergent study. After lyophilization, samples were reconstituted with appropriate volumes such that each sample was in theory at a final concentration of 0.2 µg/µL of digested peptide in mobile phase A. This allowed the same amount of protein to be injected on column (assuming 100% peptide recovery) for each sample.

Quality Control

To ensure optimal data quality, a simple BSA digest (i.e., quality control standard) was analyzed every fifth injection. The QC runs were collected and were imported into Skyline (UW Seattle, WA) to check retention times, peak area ratio for precursors and products, and mass accuracy using Statistical Process Control in Proteomics (Bereman et al., 2014).

NanoLC-MS/MS

A sample volume of two µL was injected and analyzed with an Easy nanoLC 1000 coupled to a Q Exactive Plus mass spectrometer (Thermo Fisher Bremen, Germany). PicoFrit columns (New Objective Woburn, MA) were packed with 3 µm C18 silica particles (Dr. Maisch, Germany) to a length of 25 cm in house. A four cm trap was packed in house and was placed prior to the column. The composition of mobile phases were 99.9% acetonitrile with 0.1% FA for mobile phase B and 98% water, 2% acetonitrile, and 0.1% FA for mobile phase A. LC MS/MS methods consisted of either a 60 minute (SDC DOE) or a 180 minute (detergent comparisons) linear gradient from 0-40% B followed by a ramp to 80% B in one minute and a column was at 80% B for ten minutes. The column was regenerated at 0% B for ten minutes. A data dependent acquisition method was used for both gradients in which a full mass spectrum was acquired from m/z 400 to 1400 at an automatic gain control (AGC) of 1*e6. Following each full scan, twelve data dependent scans were acquired at an AGC of 5*e4 in which the top twelve most abundant precursors were selected for isolation and fragmentation. Dynamic exclusion was set for thirty seconds to limit interrogation of abundant peptide species. Instrument peptide match was set to *preferred*.

Peptide Identification

The database searches were conducted using Proteome Discoverer (PD) version 1.4 (Thermo Fisher) and the Sequest hyper-threaded algorithm. Data were searched against the *Homo sapiens* Swiss Prot protein database (number of sequences: 26,148, date accessed: 10/17/2013). The peptide spectrum matches were post processed using percolator to enforce a peptide spectral match threshold with a q value < 0.01 (Kall et al., 2007). The following modifications were included: carbamidomethylation of cysteine as a static modification, and dynamic modifications including methionine oxidation and de-amidation of glutamine and asparagine. The SDC DOE samples were searched allowing identification of peptides containing up to two missed cleavages and a second time allowing identification of peptides containing zero missed cleavages. The number of peptides, number of missed cleavages, peptide sequences, and precursor charge of identified peptides were obtained from PD output files.

Statistical Analysis

For the SDC DOE, data containing the number of identified peptides were analyzed in JMP Pro 10.0.0 from SAS (Cary, NC). JMP was used to design a two-level three factor full factorial which enabled interactions to be determined. The trypsin ratio (1:100, 1:50), the digestion time (4, 12 hours), and the concentration of SDC (1, 3%) were tested at the given levels. The DOE study sequence was randomized and the detergent study was block randomized. In a spike down study samples were analyzed from the lowest amount of starting material to highest amount of starting material to minimize any biases associated with peptide carry-over. For the detergent comparison, one-way analysis of variance (ANOVA) and least significant difference (LSD) were used to assess the significance of parameters at $\alpha = 0.05$.

Results and Discussion

Figure 1 describes the experimental workflows used for this study. First, designed experiments explored the experimental space of the parameters that affect in-solution digestion with SDC (Figure 1A). Next, the optimized total peptide identifications protocol for the digestion with SDC was compared to three commercially available LC MS/MS compatible surfactants (Figure 1B). A BCA assay was used to evaluate SDC peptide recovery for samples prepared from low starting protein amounts (Figure 1C).

Experiments were statistically designed to investigate and optimize the parameters that affect in-solution digestion with SDC. Table 1 summarizes the experimental parameters and levels tested. Additionally, the table offers the motivation for each parameter investigated. The parameters tested in the full factorial DOE included trypsin ratio, detergent concentration, and digestion time. The combination of these factors could affect peptide recovery and digestion efficiency, thus affecting the total number of peptides identified by LC MS/MS. The parameter levels chosen sought to cover a range of common trypsin to protein ratios and digestion times utilized in typical bottom-up proteomic experiments. The primary goal for the SDC concentration optimization was to find an optimal range where tryptic activity was not compromised and to enable maximum peptide recovery.

Figure 2 illustrates half normal plots which describe the impact of a parameter on response (i.e., total peptide identifications). In half normal plots, the vertical axis (absolute contrast) represents the difference in the response of the means at high and low levels for the various parameters. The horizontal axis shows the corresponding normal quantile for each factor and interaction coordinate. Parameters which are on or near the diagonal line represent effects that would be observed based on a normal distribution and are not significant. As the distance from the line increases, the significance of the parameter does as well. Figure 2A-B summarizes the results obtained from the SDC DOE experiment. The raw data is found in Supplemental Table 1. According to the DOE analysis none of the parameters were significant. There were trends of higher trypsin ratio (14% increased peptide identifications), shorter digestion time (18% increased peptide identifications), and lower percentage of SDC (9.4% increased peptide identifications), for augmented numbers of peptide identifications. The percent increase was calculated from the difference in average peptide identifications for each condition (e.g. 4 hour vs 12 hour digestions). It is possible the study was conducted near the optimum for this experimental space, thus the parameter levels did not yield a significant difference relative to each other. However, these results do indicate that SDC is a robust detergent and operates well over a broad range of preparation conditions. Additionally, the percent of peptides identified containing missed cleavages were analyzed as a metric for assessing digestion efficiency. The only factor found to be statistically significant was digestion time ($p = 0.0095$) (Figure 2C-D). Longer digestion times with SDC showed higher digestion efficiency (i.e. lower percent of peptides identified containing missed cleavages). Longer digestion time was also implicated with a lower number of peptide

Table 1
The parameters, levels and motivations for each factor utilized for the SDC DOE optimization

Parameter	*Low*	*High*	*Primary Motivation*	*Motivation of Interactions*
SDC Concentration	1 %	3 %	Peptide recovery at different SDC concentrations can vary	Does high SDC concentration yield higher or lower trypsin activity?
Trypsin Ratio	1 : 100	1 : 50	More or less trypsin can impact digestion efficiency	Does longer trypsin digestion allow for lower amount of enzyme? Possible reduction of cost/sample by lowering enzyme concentration
Digestion Time	4 hrs	12 hrs	Digestion times can affect digestion efficiency	Does optimal SDC concentration vary with time?

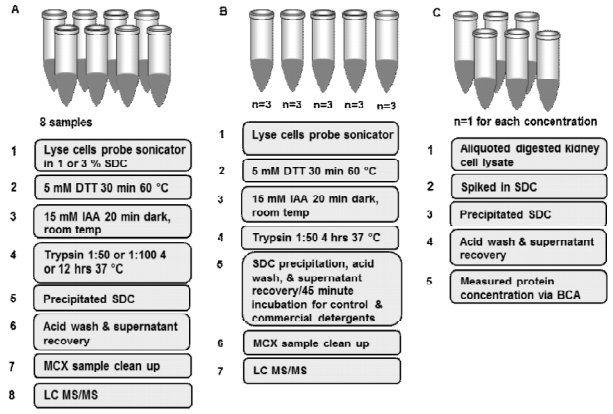

Figure 1: **The experimental workflow for the:** (A) **SDC DOE,** (B) **Detergent comparison, and** (C) **SDC peptide recovery using a BCA assay.**

A Peptide Identifications

Parameter	P-value
SDC %	0.94
Trypsin Ratio	0.258
Digestion Time	0.135
DT*Trypsin	0.773
TD*SDC	0.742
Trypsin*SDC	0.500
DT*SDC*Trypsin	0.773

C Percent Missed Cleavages

Parameter	P-value
SDC %	0.815
Trypsin Ratio	0.229
Digestion Time	0.0095*
DT*Trypsin	0.227
TD*SDC	0.718
Trypsin*SDC	0.396
DT*SDC*Trypsin	0.625

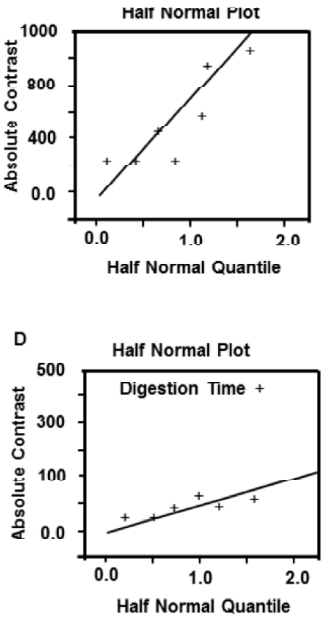

Figure 2: (A) **Results from the DOE factorial study for maximization of the number of peptides identified.** (B) **Half normal plot for peptides identified. Half normal plots describe the impact of each parameter and interaction tested on the response. The vertical axis (absolute contrast) represents the difference in response of the mean of the high level against the mean of the low level for a parameter. The horizontal axis shows the corresponding normal quantile for each factor and interaction coordinate.** (C) **Results for minimization of the percent of recovered peptides identified containing missed cleavages.** (D) **Half normal plot for minimization of the percent of recovered peptides identified containing missed cleavages.**

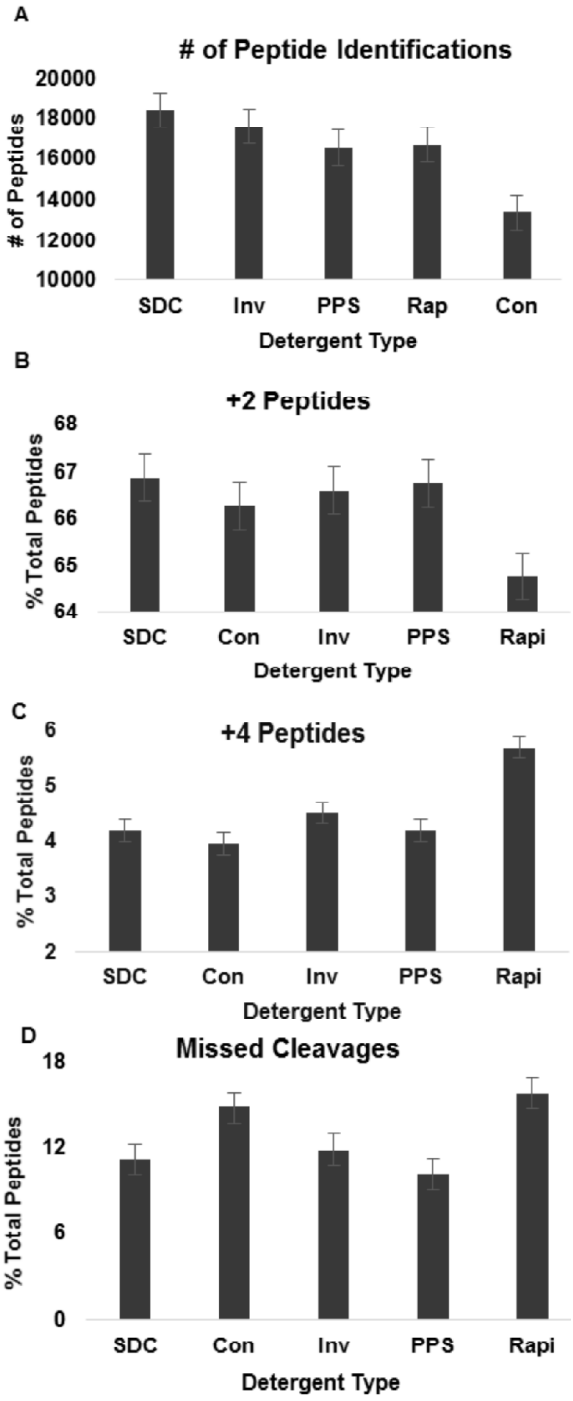

Figure 3: (A) **The average number of identified peptides based upon detergent type. The ANOVA and LSD calculations show that the control was significantly lower for number of peptides detected (p = 0.024).** B-C) **Percentage of charge state ions for each detergent was compared and RapiGest was significantly lower (p = 0.005) in the percentage of 2+ charged peptides identified, but significantly higher in the 4+ charged peptides (p = 7.7*e-7).** D) **The percentage of missed cleavages is plotted against detergent condition. The control and RapiGest have significantly higher percentage of missed cleavages (p < 0.05). Error bars represent standard error in each sample.**

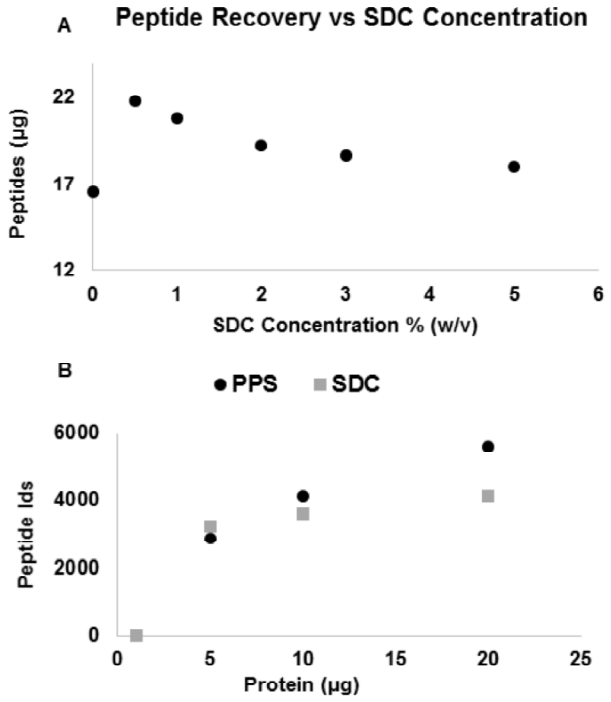

Figure 4: (A) **Results from the bicinchonic acid (BCA) assay performed on a complex lysate after precipitation of SDC. Results show peptide recovery is greater with SDC present and increases slightly with lower concentrations of SDC. However, without SDC present peptide recovery is lowest.** (B) **Performance of PPS and SDC at recovering peptides with low levels of starting protein material ranging from 1 µg - 20 µg of protein. No peptides for either detergent were identified from starting materials of less than 1 µg of protein.**

identifications – although this did not reach significance ($p = 0.18$). One potential explanation for this observation is that the identification of more peptides in samples digested during the shorter time period correlates to the identification of a higher number of peptides containing missed cleavages. To investigate whether the total number of peptides identified containing missed cleavages was a function of the difference in the total number of peptide identifications as related to digestion time, the files were searched with zero missed cleavages. The results showed the higher number of peptide identifications still correlated to the shorter digestion times. However, this observation did not reach significance ($p= 0.20$). As a result, increased peptide identifications with shorter digestions times were not simply due to a larger number of peptide identifications with missed cleavages.

Interestingly, these results are suggestive of the need for different digestion procedures dependent on the experimental goals. If one is performing a global discovery experiment, one would want to maximize peptide identifications and throughput by choosing a shorter digestion time. If one is performing absolute quantification of targeted peptides using protein cleavage isotope dilution mass spectrometry (Barr et al., 1996) one would want to minimize the number of targeted peptides containing missed cleavages. The reduction of peptides containing missed cleavages could be accomplished by selecting a longer digestion time. Recent work by Chiva et al. corroborates these results, indicating that different techniques and digestion enzymes can be customized to specific studies and that a one size fits all digestion approach for both discovery and targeted proteomic studies is not ideal (Chiva et al., 2014).

Using the optimized procedure for global peptide identifications and throughput, we compared SDC with various commercially available LC MS/MS compatible surfactants. Three technical replicates were prepared for a control (i.e. no detergent was used) and for each detergent tested. It was important to have the lysis step executed multiple times using the same detergent, as we wanted to compare the variability in the whole procedure. The total number of peptide identifications obtained from the output files was compared amongst the detergents as illustrated in Figure 3A. One-way analysis of variance (ANOVA) followed by least significant difference (LSD) testing were used to determine significant methods (Fisher, 1951). The ANOVA results showed significant difference among the means for the different experimental conditions. As one would expect, there was a significant increase in the number of peptides identified due to the presence of a detergent ($p < 0.025$). The number of peptides identified was not significantly different across the detergents. However, the percentage of identifications of peptides with missed cleavages was significantly higher ($p < 0.05$) in the control samples and samples digested with RapiGest. Similar trends to those observed for the number of total peptide identifications were reflected in the total number of protein groups identified and the total number of PSMs as shown in Supplemental Table 2.

Additionally, the charge states of peptides identified were analyzed to compare the digestion

efficiency of each detergent. A sample with optimal digestion efficiency would show a higher percentage of doubly and triply charged peptides. However, a less efficient digestion would result in the identification of peptides in higher charged states and less doubly and triply charged peptides. We examined the charge state distribution of the total identified peptides from each detergent. In the samples digested with RapiGest, the proportion of doubly charged peptides identified was significantly lower ($p = 0.0055$) than the others and was significantly higher for the 4+ ($p = 7.7*e-7$) as shown in Figure 3 B-C which may indicate less efficient digestion.

Additionally, we compared the distribution of gravy scores (Grand Average Hydropathicity Index) for the total number of peptides identified for each detergent tested (Kyte and Doolittle, 1982). Gravy scores were calculated using S. Fuch's website (Fuchs, 2011 *http://www.gravy-calculator.de/index.php*). The gravy scores of each replicate were combined yielding five distributions containing between 40,000-55,000 gravy scores. The raw gravy scores were formatted as boxplots showing the distributions and median gravy scores as displayed in Supplemental Figure 1. The distributions of gravy scores for all samples were similar; a bias was observed toward peptides that were slightly hydrophilic as previously reported (Bereman et al., 2011) for digestion of complex mixtures. Larger numbers of hydrophobic peptides were identified in samples prepared with detergent compared to the control. No distinct differences were observed for hydrophobic peptides identified using these three detergents.

Increases in instrument sensitivity combined with targeted biological questions have led to a significant trend in proteomics towards the preparation of samples with low amounts of starting material (e.g., Co-IP, LCM) (Gorini et al., 2010; Neubauer et al., 2006; Osman and van Loveren, 2012, 2014; Simone et al., 2000; Xu, 2010). A concern with using SDC for in-solution digestion with low protein starting amounts is the possibility of peptides being lost in the precipitation which are not recovered in the acid wash or ethyl acetate transfer step (Zhou et al., 2006). In the previous experiments, high amounts (100 µg) of starting material were used. The goal of this experiment was to assess the peptide recovery of SDC from low amounts of starting material in order to identify whether peptides were lost due to precipitation of deoxycholic acid. We tested the performance of the SDC in recovery of peptides spanning an order of magnitude (0.5-5% SDC) with a BCA assay. Suitable recovery of peptides was found across a wide range of SDC concentrations. Interestingly, it was observed that peptide recovery was lowest in the absence of SDC as shown in Figure 4A. This observation may be due to peptides which had greater affinity for the plastics used during sample manipulation (e.g., pipet tips, Eppendorf tubes) in the absence of a detergent (i.e., carrier).

To verify the performance of SDC peptide recovery with low amounts of starting material further experimentation was performed which compared PPS and SDC. PPS is an acid cleavable detergent that does not precipitate from solution which makes it suitable for comparing peptide recovery that utilizes samples with low amounts of starting material (i.e. 1-20 µg of protein). In Figure 4B total number of identified peptides was plotted against starting amount of protein in samples. The data illustrate the observed number of peptide identifications using PPS were similar to those using SDC at every protein amount tested. This observation indicates that there was efficient peptide recovery from the SDC pellet. It is interesting to note that 1 µg of protein was deemed insufficient to recover peptides with this particular procedure. The comparable performance of SDC with PPS demonstrates that SDC is a robust detergent for preparation of peptide samples derived from high and low protein starting amounts.

Conclusions

We have reported a study comparing peptide identifications, peptide characteristics, and digestion efficiency amongst SDC and other commonly used commercial LC MS/MS compatible detergents. DOE was used to map the experimental space of parameters affecting in-solution digestion with SDC through a full factorial approach. The peptide recovery was comparable in the head-to-head comparison of detergents to SDC. Further, two experiments which utilized independent detection techniques,

LC MS/MS and absorbance with a BCA assay, were used to assess SDC peptide recovery. In conclusion, SDC is a cost efficient LC MS/MS compatible reagent and it performs similarly to other more expensive detergents with low and high amounts of protein starting material.

Acknowledgements

The authors acknowledge support from the Pilot Project Award from the Center for Human Health and the Environment (850 Main Campus Dr. Raleigh, NC 27606). We also appreciate the help of Drs. Jonathan Hall and Rob Smart with preparation of the HEK 293 cell line.

Abbreviations

SDC, sodium deoxycholate; BCA, bicinchoninic acid; SDS, sodium dodecyl sulfate; FA, formic acid; DTT, dithiothreitol; HCl, hydrochloric acid; SPE, solid phase extraction; DOE, design of experiment; AGC, automatic gain control; PSM, peptide spectral match; ANOVA, analysis of variance; LSD, least significant difference

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Supplemental

Cell Culture

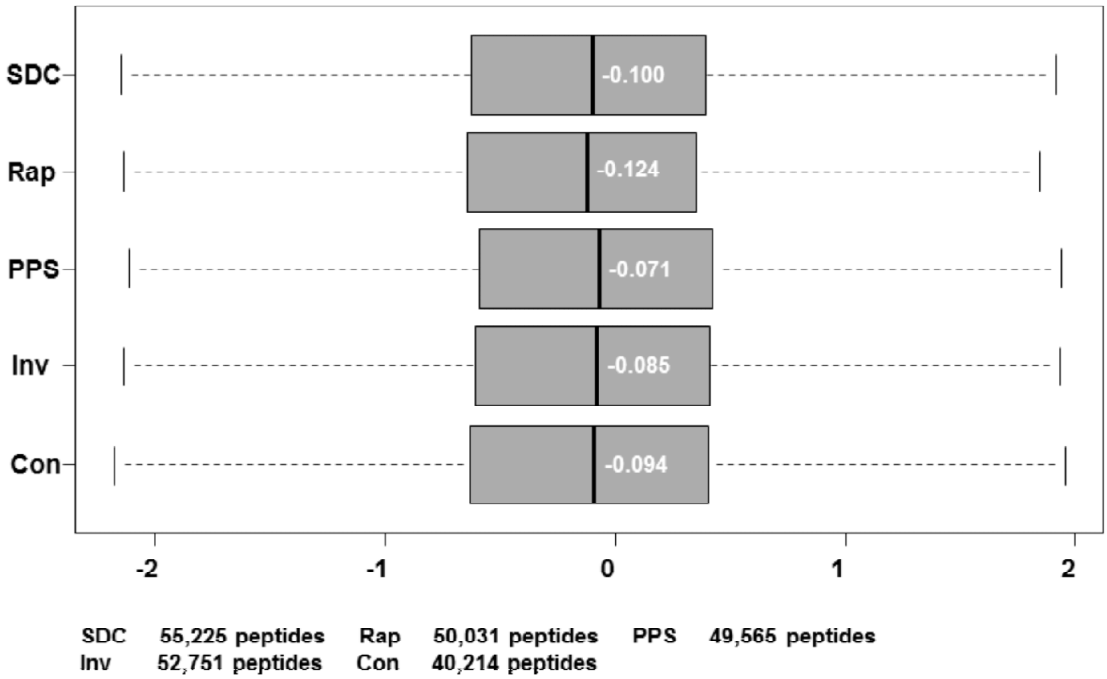
Cells provided from North Carolina State University were frozen in a pellet. Cell culture was performed utilizing a HEPA lab Conco purifier class 2 biosafety cabinet delta series. Cells were thawed and plated in a T-75 flask from Fisher Scientific with DMEM from Invitrogen Life Technology (Carlsbad, CA). 10% Fetal Bovine Serum (FBS) from Life Technology was added to flasks and cells were incubated in a Napco Model 6200 incubator (Thermo) at 37 °C with 3% carbon dioxide. After three days cells were split, passaged, and monitored daily to check confluence microscopically with an Olympus CK2 microscope (Lake Success, NY). Once cells were ~ 90% confluent they were passaged by aspirating old media and washing with 10 mL of PBS solution (Life Technology) aspirating PBS solution, and trypsinizing cells with 1 mL of trypsin (Life Technology) then adding 9 mL of DMEM. Trypsinized cells and new media were aspirated and combined with 10 mL of DMEM to yield a cell solution of 30 mL. In 15 new T-75 flasks 16 mLs of DMEM, 2 mL of FBS, and 2 mL of the cell solution were added. Incubation of cells was accomplished using the aforementioned conditions for three days. Cells were harvested by aspirating media and rinsing cells with 10 mL of DMEM. Cells were centrifuged into a pellet, and supernatant was decanted. Cells were counted using a hemocytometer Cole-Parmer (Vernon Hills, IL). The pellet was estimated to contain ~ 2*e8 cells. Pellet was flash frozen in liquid nitrogen then re-suspended in 10 mL of 50 mM ammonium bicarbonate. Ten one mL aliquots containing 20*e6 were taken and were split into 50 µL aliquots containing 1*e6 cells. These aliquots of cells were used in the analysis.

Supplemental Table 1
The results of the SDC DOE experiment searched allowing identified peptides with up to two missed cleavages

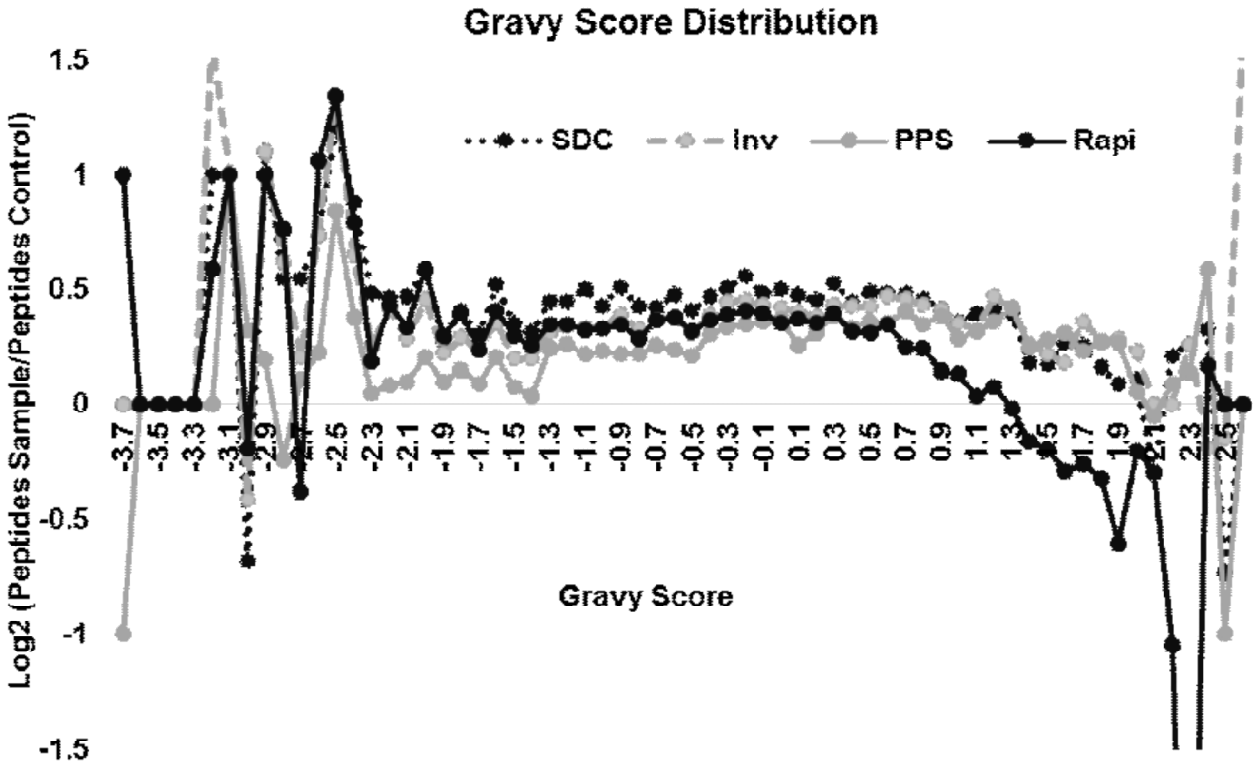
Run #	*Peptide Identifications*	*% SDC (1,3)*	*Digestion time (4,12)*	*Trypsin ratio(1:100, 1:50)*
1	9559	3	12	1:50
2	9222	1	12	1:100
3	10364	1	4	1:100
4	8188	3	12	1:100
5	11413	1	12	1:50
6	12722	1	4	1:50
7	11306	3	4	1:100
8	10912	3	4	1:50

Supplemental Table 2
The average performance of detergents based upon PSMs, total number of identified peptides, and total number of protein groups. The control is significantly lower for each metric

Detergent	*Peptides*	*PSMs*	*Proteins*
Invitrosol	17583 ± 1424	26374 ± 2772	3475 ± 164
PPS	16521 ± 996	24302 ± 1145	3412 ± 100
RapiGest	16677 ± 847	24932 ± 1394	3374 ± 104
SDC	18408 ± 1063	27380 ± 2110	3550 ± 129
Control	13404 ± 1154	20064 ± 1800	2882 ± 181



Supplemental Figure 1: **The box plots of the gravy score distribution for control and detergents. The median gravy score is shown in white. The total number of peptides recovered, from each set of replicates, is listed in the legend.**



Supplemental Figure 2: **Comparison of distributions of gravy scores for each of detergent. The distribution of the detergents was compared after normalizing to the control. This was accomplished by dividing the range of gravy scores into 0.1 sized bins from -3.5 to +2.5, and taking the quotient of total peptides for each detergent divided by those of the control in the corresponding bin.**