TA Instruments – Application Note

High-throughput DSC: A Comparison of the TA Instruments Nano DSC Autosampler System™ with the GE Healthcare VP-Capillary DSC™

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Purpose: The purpose of this study was to directly compare the performance of the new *Nano DSC Autosampler System*™ from TA Instruments and the *VP-Capillary DSC*™ from GE Healthcare (MicroCal, LLC). Data were collected for both platforms in the same laboratory by the same operator using identical reagents and closely matched protocols. The quality of the raw data collected under a variety of conditions and the resulting transition temperatures (T_{max}) and enthalpies (ΔH) were evaluated and compared.

Background: All differential scanning calorimetry (DSC) scans in this study were performed using the protein lysozyme and standard solution conditions and protocols as recommended by the International Union of Pure and Applied Chemistry (IUPAC) (1). Results reported by Hinz and Schwarz for the recommended test materials and protocols were validated by collecting data sets on identical reagents in six different laboratories around the world using six different DSC instruments. The protein used by Hinz and Schwarz and the current study, **lysozyme,** is a 14.3 kDa enzyme that catalyzes the hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins. This well-characterized enzyme is recognized as the standard testing material for DSC by organizations such as IUPAC and NIST (National Institute of Standards and Technology) (2).

TA Instruments recently introduced the *Nano DSC Autosampler System*, a high sensitivity microcalorimeter that features a liquid handling system for automated operation. Test samples and matching reference buffer solutions were prepared and loaded into separate thermostated 96-well plates located within the liquid handling system. The DSC sample and reference cells were automatically filled with the solutions from the 96-well plates, and following the calorimetric run and data acquisition, were then rinsed and cleaned with a preset cleaning protocol prior to the next sample run. Once test samples and their matching buffer solutions are prepared and loaded into the plate, the *Nano DSC Autosampler System* operated unattended under computer control. Table 1 compares the specifications of the TA Instruments *Nano DSC Autosampler System* to those of the MicroCal *VP-Capillary DSC* system.

Table 1. Comparison of Specification of Calorimeter Systems

	Nano DSC Autosampler	***VP-Capillary DSC***
Short-term noise (RMS Average)	0.015 µW	0.14 µW (2 µcal/min)
Baseline repeatability	0.028 µW	0.35 µW (5 µcal/min)
Experimental Temperature Range	-10 to 130 °C	-10 to 130 °C
Minimum response time	7 s	4 s
Cell volume/design	300 µL/capillary	135 µL/capillary
Sample plate volume	1.0 mL/well	0.4 mL/well
Liquid handling	Tubing, pumps	Syringe

Experimental Design: Each calorimeter system was operated using closely matched protocols (see Appendix A). For each system, run cycle times are specified by the user. Each cycle features equilibration times following cell loading, the actual data collection run, cooling, sample removal, washing and rinsing, and loading of the new samples.

The two calorimeter systems were compared by running identical lysozyme samples (see Appendix B) at two different scanning rates, 1 and 2 °C min^{-1}, and protein concentrations ranged from 0.05 - 2.22 mg mL^{-1}. A total of seventy-eight thermograms were collected from triplicate measurements of protein samples at the various conditions. Sixty-four buffer reference scans were also collected. The buffer scans were collected before and after each concentration series of five to eight sample scans. DSC data for the lysozyme concentration series of 0.05, 0.10, 0.50, 1.00 and 2.00 mg mL^{-1} were collected on both instruments. Data for additional lysozyme concentrations of 0.22, 1.11 and 2.22 mg mL^{-1} were collected on only the MicroCal *VP-Capillary DSC* to provide data for a direct comparison of identical sample mass (see Figure 1). All thermograms were baseline corrected and normalized using the manufacturer's software provided with each system.

Results: Two key performance criteria most researchers are concerned with include total sample consumption and instrument sensitivity. Figure 1 shows representative results obtained for lysozyme thermal denaturation using the two systems. For these thermograms, data were selected for comparison for which the *number of moles* of protein used in the active sample cell volume of each instrument was identical. The number of moles of protein is the product of the sample concentration and the cell volume. While the TA Instruments *Nano DSC Autosampler* sample cell volume is 2.22 times larger than the MicroCal *VP-Capillary DSC* cell volume, to

analyze identical moles of protein, sample concentrations can be reduced by the same factor to provide data of similar or superior quality.

For all cases shown, both calorimeters provided excellent thermograms and upon analysis yielded T_{max} and ΔH values fully consistent with IUPAC standards (1). Panel A shows the thermogram for a dilute sample (0.11 mg mL^{-1}) run on the *Nano DSC Autosampler* system. Panel B shows the thermogram of comparable moles of protein (required concentration of 0.23 mg mL^{-1}) run on the *VP-Capillary DSC* system. At lower protein concentrations the *Nano DSC Autosampler system* provided superior data with less noise in the signal. Panels C and D show thermograms at much higher protein concentrations for which each DSC system provided data of equivalent quality.

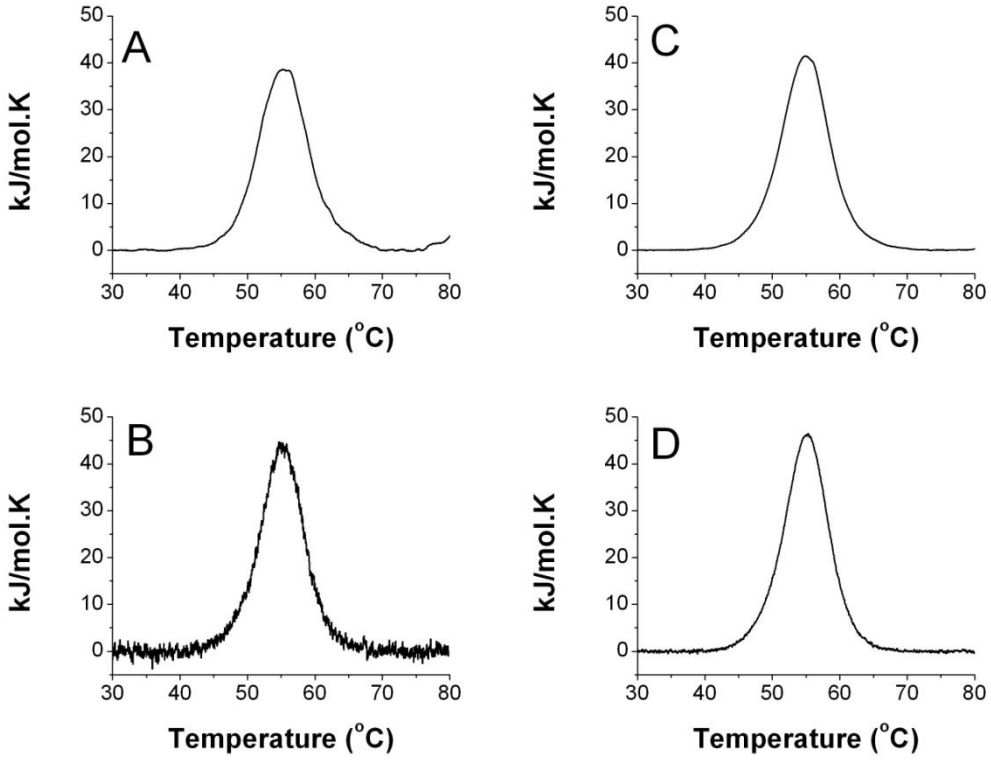

Figure 1. Thermograms for the thermal denaturation of lysozyme solutions. Comparison is made for data obtained using the TA Instruments *Nano DSC Autosampler* system (A, C) and the MicroCal *VP-Capillary DSC* system (B, D). Sample concentrations were chosen such that the total mass of protein in the calorimeter sample cell was equivalent for proper comparison. Sample concentrations (in mg mL^{-1}) used were: A, 0.11; B, 0.23; C, 0.54; D, 1.19. Instrument scan rates were 1 °C min^{-1}.

For accurate and precise protein analysis with DSC, the baseline reproducibility and stability are the most important performance specifications of the instrument. When comparing the buffer baselines that were generated on both instruments, two important observations could be made. First, the *Nano DSC Autosampler* had superior baseline reproducibility with no baseline scans excluded from the data set because of artifacts or other variations that would make them unusable in the analyses. Second, the stability of the baseline scans for the *Nano DSC Autosampler* was also superior to those generated on the *VP-Capillary DSC*.

Figure 2 shows buffer-buffer scans collected on both instruments under the same scanning conditions over the course of approximately 3 days. The buffer scans collected on the *Nano DSC Autosampler* were highly reproducible with consistent shape and similar offset. In contrast, the buffer scans collected on the *VP-Capillary DSC* were much less reproducible with a number of buffer scans exhibiting significant changes in shape and offset, and erratic high temperature noise observed in two of the scans.

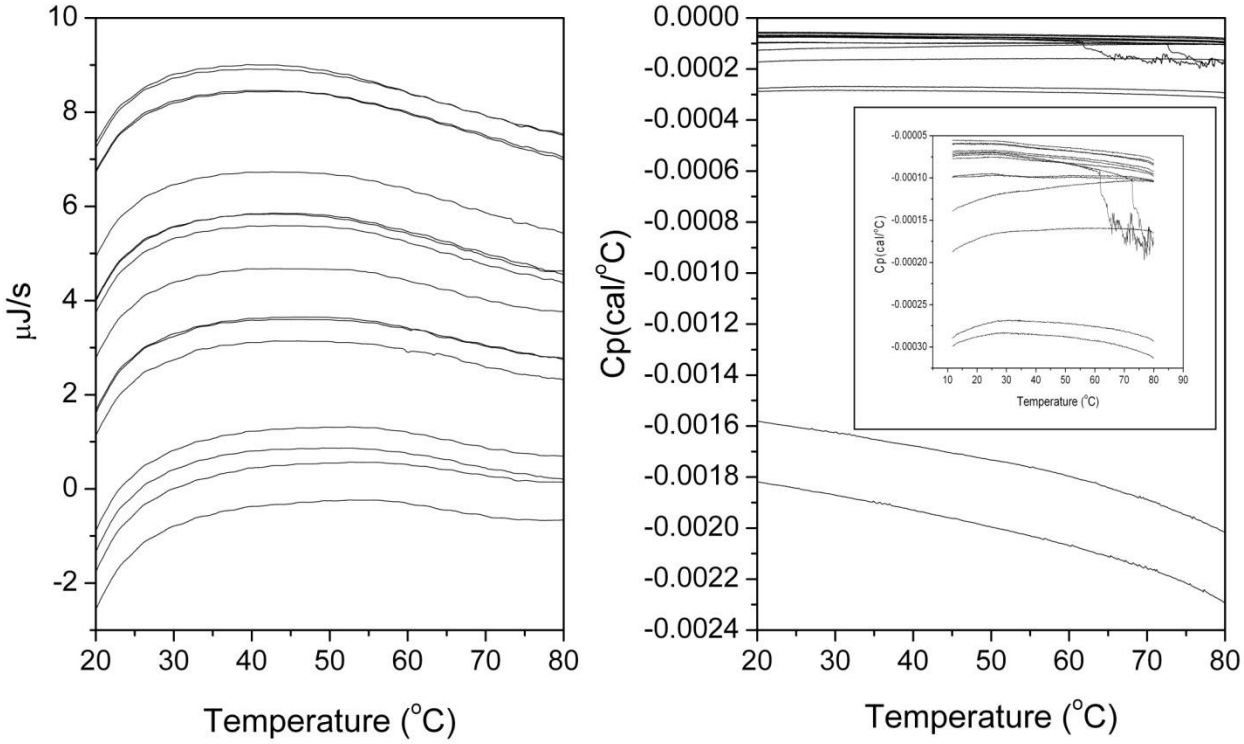

Figure 2. Buffer versus buffer reference scans collected on both instruments. Primary data for 16 buffer-buffer scans collected at 1 °C min^{-1} on the TA Instruments *Nano DSC Autosampler* system (left panel) and the MicroCal *VP-Capillary DSC* system (right panel) over the course of approximately 3 days. The inset on the right panel shows an expanded scale after exclusion of the two buffer-buffer scans centered at -0.0016 and -0.0018 cal/°C.

Several thermodynamic parameters are calculated from DSC scans. T_{max} and ΔH values are routinely used in characterizing protein stability. Figure 3 shows the results obtained in this comparative study, along with the published data from the IUPAC recommendation study. Both the *Nano DSC Autosampler* and the *VP-Capillary DSC* compare favorably. Both instruments allow data to be collected at lower protein concentrations than were used in the earlier IUPAC study with commercial calorimeters of earlier generations. T_{max} values calculated for both instruments in this study were slightly lower than those reported previously (1). Although T_{max} values were slightly lower, many factors including sample preparation methods, sample concentrations and exact sample buffer composition could explain the variation seen with both instruments.

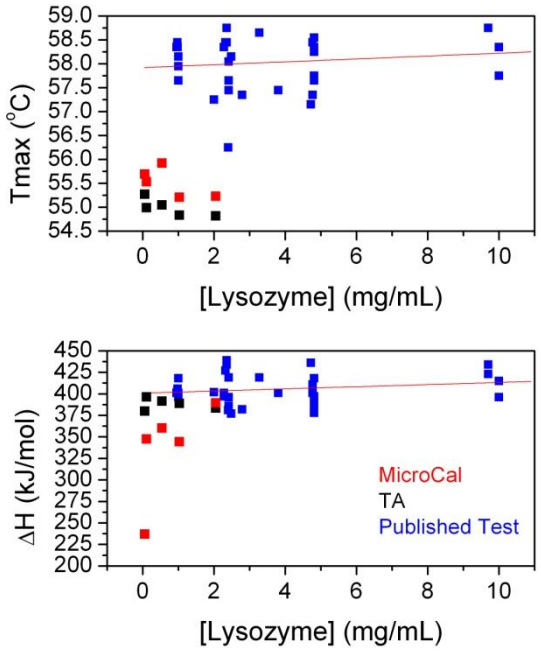

Figure 3. Comparison of transition temperature (T_{max}) and enthalpy values (ΔH) for the thermal denaturation of lysozyme. The blue squares are data taken from the published study (1). Black squares are data obtained using the TA Instruments *Nano DSC Autosampler* system. Red squares are data obtained using the MicroCal *VP-*

The ΔH values generated by both instruments were very comparable to ΔH values reported previously (1). At the lower concentrations the variance in ΔH from the expected value was much larger using the *VP-Capillary DSC* than that observed with the *Nano DSC Autosampler*. This discrepancy is most likely associated with inaccuracies in the estimation of peak areas because of greater uncertainties in baseline correction for samples of lower signal-to-noise ratios.

Summary

- The *Nano DSC Autosampler* system produced thermograms with superior baseline stability and reproducibility.
- The *Nano DSC Autosampler* system produced thermograms with superior signal-to-noise ratios at lower protein concentrations.
- At higher sample concentrations, the *Nano DSC Autosampler* and the *VP-Capillary DSC* produced thermograms with equivalent signal-to-noise ratios.
- Sample consumption was equivalent for the *Nano DSC Autosampler* and the *VP-Capillary DSC* platforms. The larger cell volume required for the *Nano DSC Autosampler* can be compensated for by the use of samples with lower concentrations. The superior signal-to-noise ratio of the *Nano DSC Autosampler* allows the routine use of lower sample concentrations.
- Both the TA Instruments *Nano DSC Autosampler* and the MicroCal *VP-Capillary DSC* are high-quality systems that perform very well and permit the highest possible sample throughput with automated, unattended sample handling.
- For each automated DSC system, the rate of sample throughput depends on user's choices of scan rate, temperature range and cycle settings (washes, equilibrations, etc.)

References

1. Hinz H-J & Schwarz FP (2001) Measurement and analysis of results obtained on biological substances with differential scanning calorimetry. *Pure Appl. Chem.* **73(4)**:745-759.

2. Gatta GDG, Richardson MJ, Sarge SM, & Stolen S (2006) Standards, Calibration and Guidelines in Microcalorimetry. Part 2. Calibration Standards for Differential Scanning Calorimetry (IUPAC Technical Report). *Pure Appl. Chem.* **78(7)**:1455-1476.

Appendix A

Details of Instrument Protocols

TA Instruments *Nano DSC Autosampler System*

Start temperature: 10 °C
Final temperature: 80 °C
Scan rate: 1 or 2 °C min^{-1}
Pre-scan equilibration time: 10 min
Cleaning start temperature: 25 °C
Cleaning routine: 2 x 10 mL deionized water each capillary; soak time 0 s; initial & final air purge
Loading window: 10 °C
Idling temperature: 20 °C
96-well tray thermostat temperature: 5 °C

GE Healthcare *VP-Capillary DSC System*

Start temperature: 10 °C
Final temperature: 80 °C
Scan rate: 1 or 2 °C min^{-1}
Pre-scan equilibration time: 10 min
Feedback mode/gain: Mid
Filtering period: 2 s
Cleaning start temperature: 25 °C
Cleaning routine: 4 x 2.5 mL deionized water each capillary; 4 x 2.5 mL deionized water, syringe
Cell filing volume: 300 µL
Speed of injection: 50 µL/s
Number of filing strokes: 4
Idling temperature: 20 °C
96-well tray thermostat temperature: 5 °C

Appendix B

Details of Sample Preparation

Materials

Glycine, ≥ 99 % (SIGMA, Catalog # G7126)
Hydrochloric acid, 37 % (SIGMA, Catalog # 258148)
Lysozyme, ≥ 98 % (SIGMA, Catalog # L4919; lot # 088K13582) *(N.B. Lysozyme, ≥ 90 %) (SIGMA, Catalog # L6876; lot # 117K1547 used for initial studies)*

Preparation of buffer: 0.1 M Glycine-HCl, pH 2.4

Glycine (15.014 g) was dissolved in freshly deionized water (1.8 L) with stirring for 15 min. The pH was adjusted to 2.4 using concentrated hydrochloric acid and the solution transferred to a 2 L volumetric flask with exhaustive rinsing of vessels during transfer. The volume was adjusted to 2 L using freshly deionized water and the solution filtered (PALL Supor-200 membrane filter, Catalog # 63025) before use. A total of 4 L of buffer was prepared.

Preparation of lysozyme solutions

Lysozyme (60 mg) was dissolved in 0.1 M Glycine-HCl buffer (5 mL) overnight at 4 ^{o}C on a rotary mixer. The lysozyme stock solution was then transferred to a dialysis cassette (Pierce, Catalog # 66110; 3-12 mL; 3.5 K MWCO) and dialyzed against 0.1 M Glycine-HCl buffer for a period of 24 h at 4 ^{o}C with four buffer changes of 1 L at intervals of 3 h, 4 h, 4 h and overnight. The lysozyme stock solution was recovered from overnight dialysis and filtered (National Scientific 0.45 µm syringe filter, Catalog # F2513-14). Dialysis buffer was filtered into a 1 L screw top buffer bottle (PALL Supor-200 membrane filter, Catalog # 63025). The concentration of the lysozyme stock solution was determined spectrophotometrically using an extinction coefficient of 2.65 L g^{-1} cm^{-1} at 280 nm (1). Working solutions at 0.05, 0.10, 0.22, 0.50, 1.00, 1.11, 2.00 and 2.22 mg mL^{-1} were prepared from the stock solution and dialysis buffer and the precise concentrations determined spectrophotometrically.