TA Instruments – Technical Note

Buffer Compatibility with Nano DSC

Colette Quinn, Ph.D.
Microcalorimetry, TA Instruments – Waters LLC, 890 West 410 North, Lindon, UT 84042, USA

Introduction

When using differential scanning calorimetry (DSC), a high quality buffer scan is imperative in order to accurately determine the partial specific heat capacity of a protein sample or when working with low concentrations of biological macromolecules. The reproducibility of the scans in a Nano DSC instrument is a desirable feature and well documented in the application note: *High-throughput DSC: A Comparison of the TA Instruments Nano DSC Autosampler System™ with the GE Healthcare VP-Capillary DSC™*. One of the key preparatory steps that will ensure the collection of a good background buffer scan is to condition the DSC sample and reference cells with buffer. Proper cell conditioning is accomplished by filling both cells with buffer and then scanning to the upper temperature limit anticipated for scans that will be collected with test samples in this buffer. During the initial conditioning heat scan, the surface of the cells becomes populated with ions from the buffer. These ions physically adsorb to the surface of the cells. The cells will remain conditioned as long as they are either rinsed with water or cleaned with a dilute detergent, such as 1% Contrad®, followed by a water rinse. Because most proteins are removed using these gentle conditions and the conditioning will be left intact, the cell does not need to be pre-conditioned prior to every sample scan. However, if after a sample scan, a particular biological macromolecule can only be cleaned from the sample cell by the thorough cleaning conditions of 4 M sodium hydroxide followed by 50% formic acid, as recommended in the Nano DSC Getting Started Guide, then the cells will need to be reconditioned.

The data presented in this document represents the typical kind of DSC scans that can be obtained from various buffers, including those with alkyl, morpholine and piperazine moieties. This data does not represent a comprehensive study of all biological buffers, but is a representation of several different structural components that are encountered in frequently used biological buffers.

Materials and Method

All DSC scans were obtained using a Nano DSC Autosampler System™ with a platinum (Pt(0)) capillary cell. The buffers and small molecules investigated were: ethylenediaminetetraacetic acid (EDTA), glycerol,4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 2-morpholinoethanesulfonic acid monohydrate (MES), 3-(N-morpholino)propanesufonic acid (MOPS), piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) and 2-amino-2-(hydroxymethyla-1.3-propanediol (Tris

or THAM), potassium phosphate and phosphate buffered saline (PBS). Each buffer was made at either 20 mM or 100 mM with Nanopure® water (>16 MΩ-cm^{-1}) and the ionic strength was adjusted to μ= 0.1 M with NaCl. Exceptions to this adjustment were the phosphate and glycine buffers. Glycine buffer was prepared as 20 mM without ionic strength considerations and the phosphate buffers were prepared as 20 mM potassium phosphate and 10 mM PBS made with 138 mM NaCl and 2.7 mM KCl. The pH of all of the buffers was adjusted to 7.40 ± 0.02 with HCl and NaOH as needed, the exception being MES and glycine, which was prepared at pH 6.00 ± 0.02 and 2.40 ± 0.02, respectively.

DSC scans were performed at 1 ˚C/minute from 10 to 100 ˚C with a 600 second initial equilibration period. Reproducibility was evaluated by comparing the scans collected by running multiple scans with a replacement of the buffer between scans as well as performing multiple scans without reloading the buffer in between scans. For each scan, buffer was loaded into the sample cell and Nanopure water was loaded into the reference cell. This experimental design was chosen so that any unique features of each buffer would be visible and not be cancelled out. Typically, when collecting DSC data, the sample and reference cells would both contain the same buffer solution. The unique profile of a specific buffer scan is related to the change in the pK_a of the buffer at different temperatures. For more information on the importance of this feature, please refer to the TA Instruments technical note entitled, "*Choice of Buffer for a DSC Scan"*. During data analysis, the individual buffer scans were manually shifted to visually set apart the different scans. In addition, rescans, where the same buffer remained in the sample cell for a second scan, are always designated in the final graphs as a dashed line, instead of a solid line.

Results and Discussion

Both of the alkyl buffers considered have a similar DSC profile. The characteristics of these scans, as well as all subsequent scans, result from the temperature dependence of the pK_a of the buffer. The offset of all of the results was normalized to a window of the same height (Figures 1-5), with the exception being the viscous buffer scans (Figure 6).

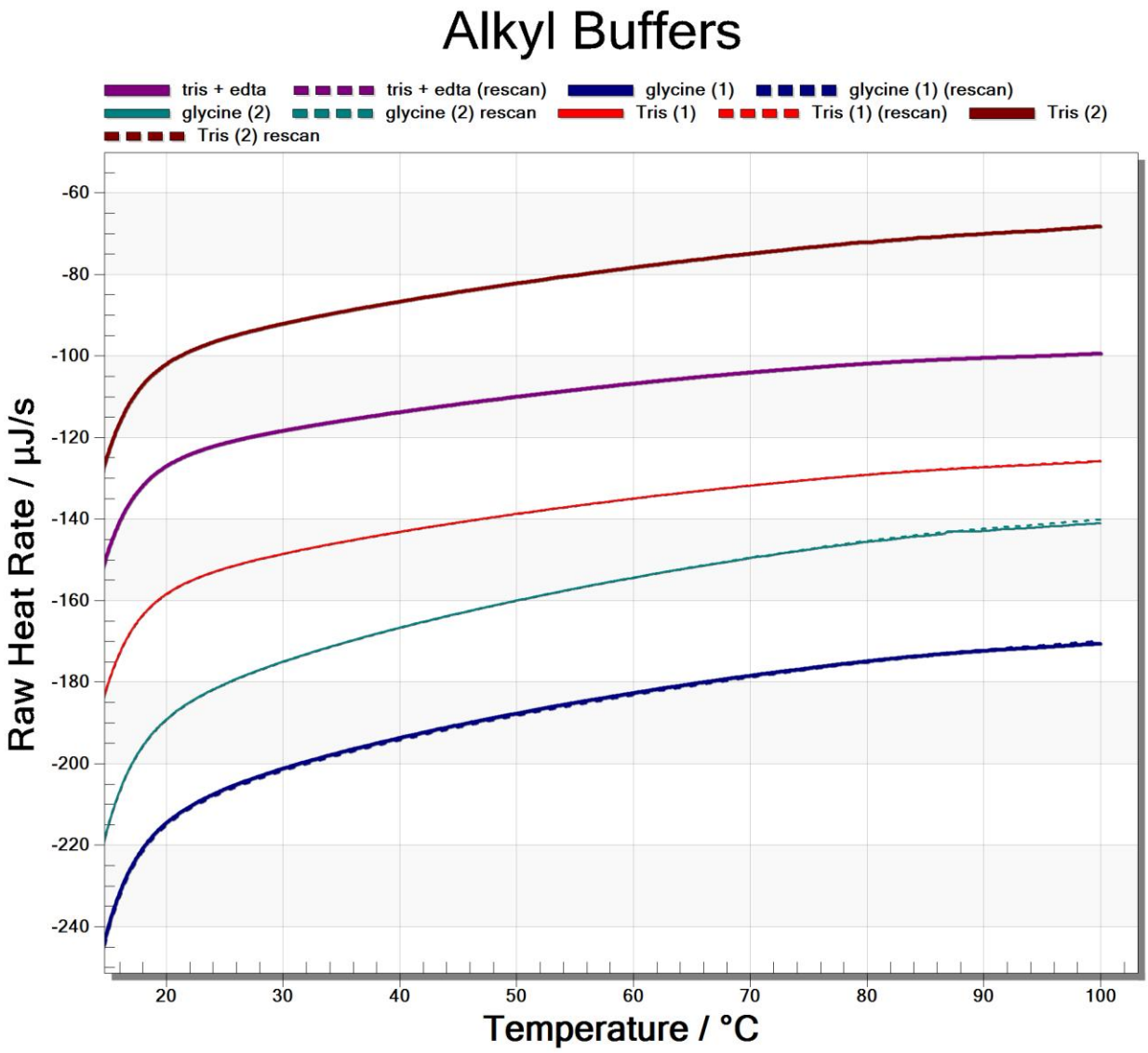

Figure 1. Nano DSC scans of alkyl buffers: glycine, Tris, and Tris + EDTA. In the legend above the number after a scan indicates the number of duplicates and the word rescan indicates that the scan was performed a second time without removing the buffer solution from the sample cell.

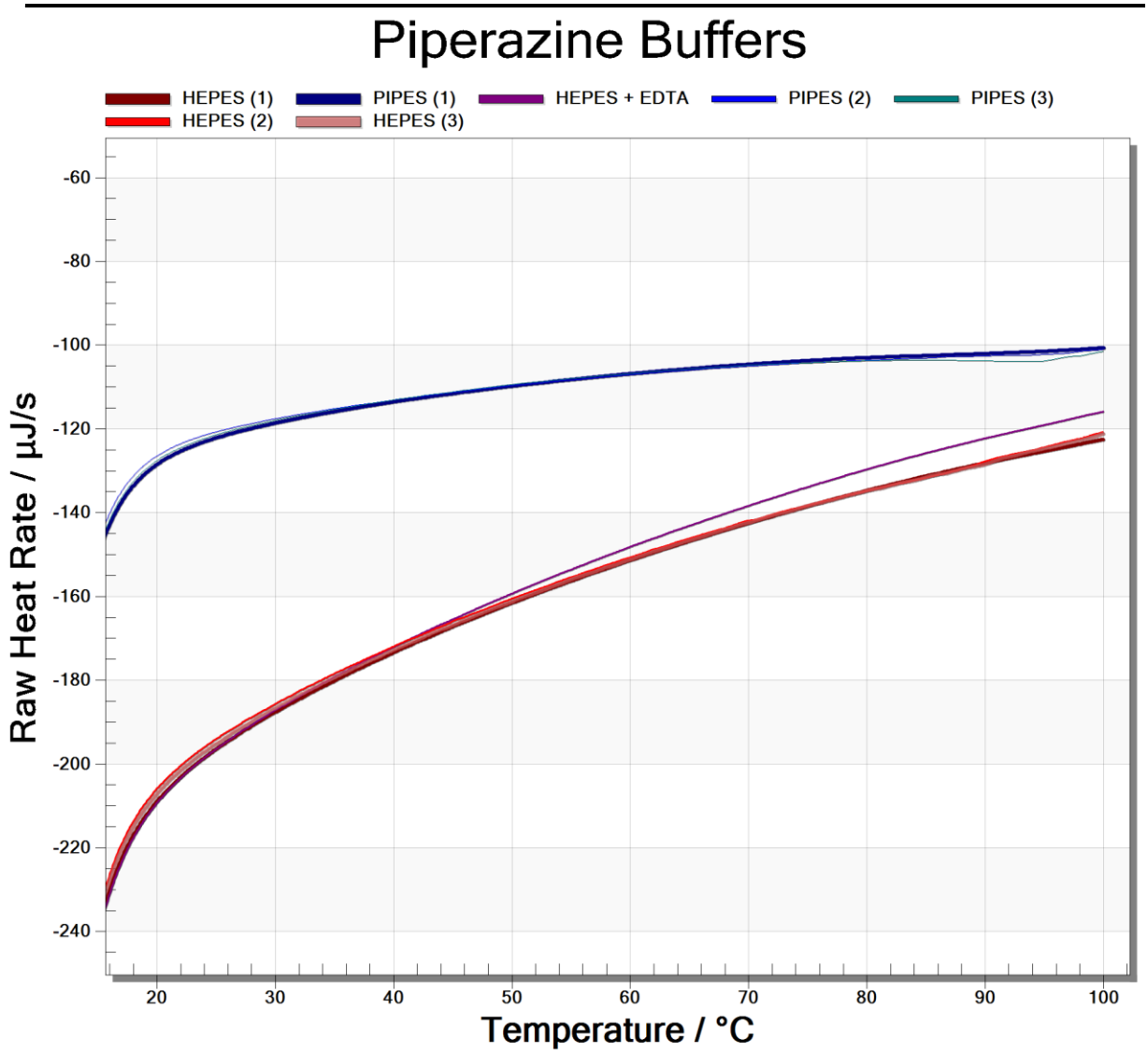

Figure 2. Nano DSC scans of piperazine buffers: PIPES, HEPES and HEPES + EDTA. The number after a scan indicates the number of duplicates.

The large change in the heat capacity of HEPES, as visualized with the more severe slope, is related to the pK_a of the buffer, which changes 1.02 log units over the temperature range shown, while the slope of the PIPES buffer indicates that pK_a of this buffer changes by only half of the HEPES value. The scans of both buffers have a consistent shape; however, there is a difference in the raw heat rate of the HEPES buffer when EDTA is added. This change in the buffer scan resulting from a component change in the composition of the sample would complicate the analysis of any unknown in the buffer and emphasizes the importance of exactly matching the buffers in each cell.

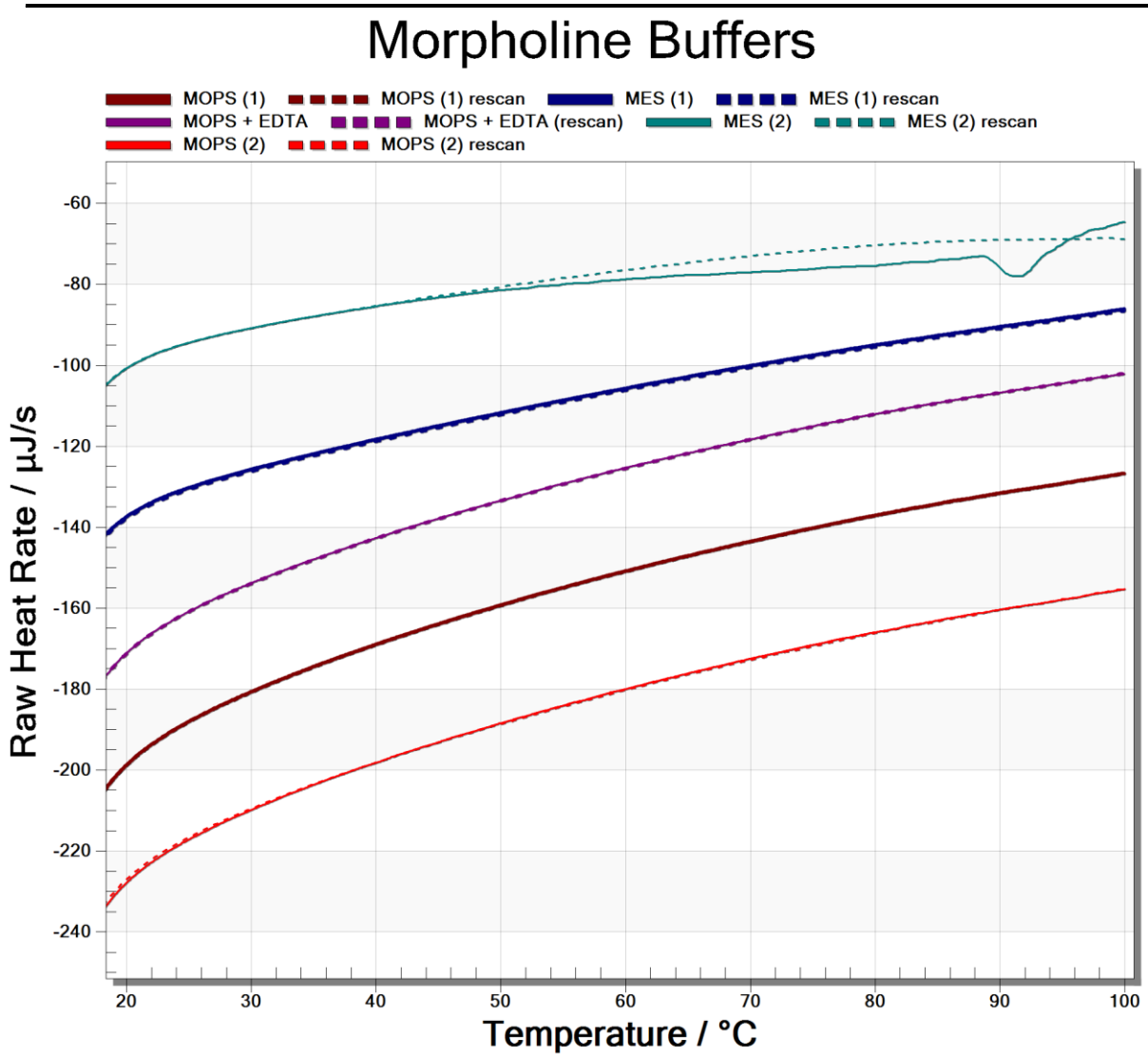

Figure 3. Nano DSC scan of morpholine buffers: MES, MOPS, and MOPS + EDTA. The number after a scan indicates the number of duplicates and the word rescan indicates that the scan was performed a second time without removing the buffer solution from the sample cell.

MOPS and MES are heterocycles with an oxygen opposite to a nitrogen in a six-membered ring. Both MES and MOPS have a similar shape, and the additional methyl in the alkly chain of MOPS does not have as large an effect on a DSC scan as the substitution of sulfate group for the alcohol, as is the case for PIPES and HEPES. The feature observed in scan MES (2) was not reproducible and could be related to an ineffective conditioning scan or an artifact created when a small air bubble was released within the sample cell. This scan was included as an example of a scan that, in most cases, would not be useful and would need to be re-run.

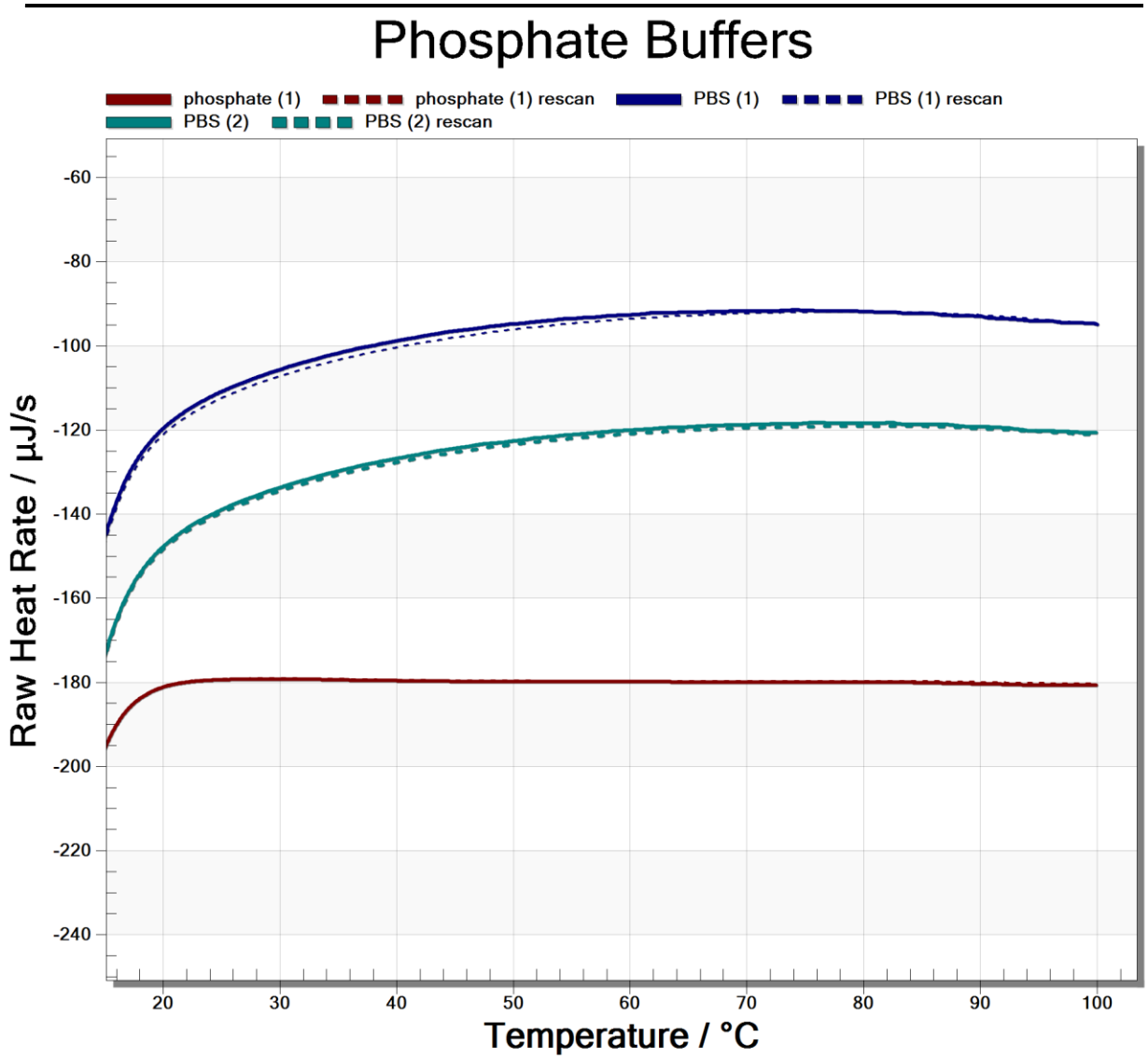

Figure 4. Nano DSC scans of phosphate buffers. The number after a scan indicates the number of duplicates and the word rescan indicates that the buffer was scanned a second time without removing the buffer solution from the sample cell.

The flat slope of the phosphate buffer is a result of the unchanging pK_a over the temperature range of interest. Since phosphate buffer does not exhibit a changing pK_a over the DSC scanning temperature range, it is usually a good buffer choice when performing DSC scans. With a stable pKa, a phosphate buffer can usually be depended on to provide an effective and stable pH environment for the macromolecule being analyzed. For buffers that exhibit a dramatically changing pK_a, such as MOPS and MES, the pH environment of the macromolecules of interest may also be changing, and thus influencing the results.

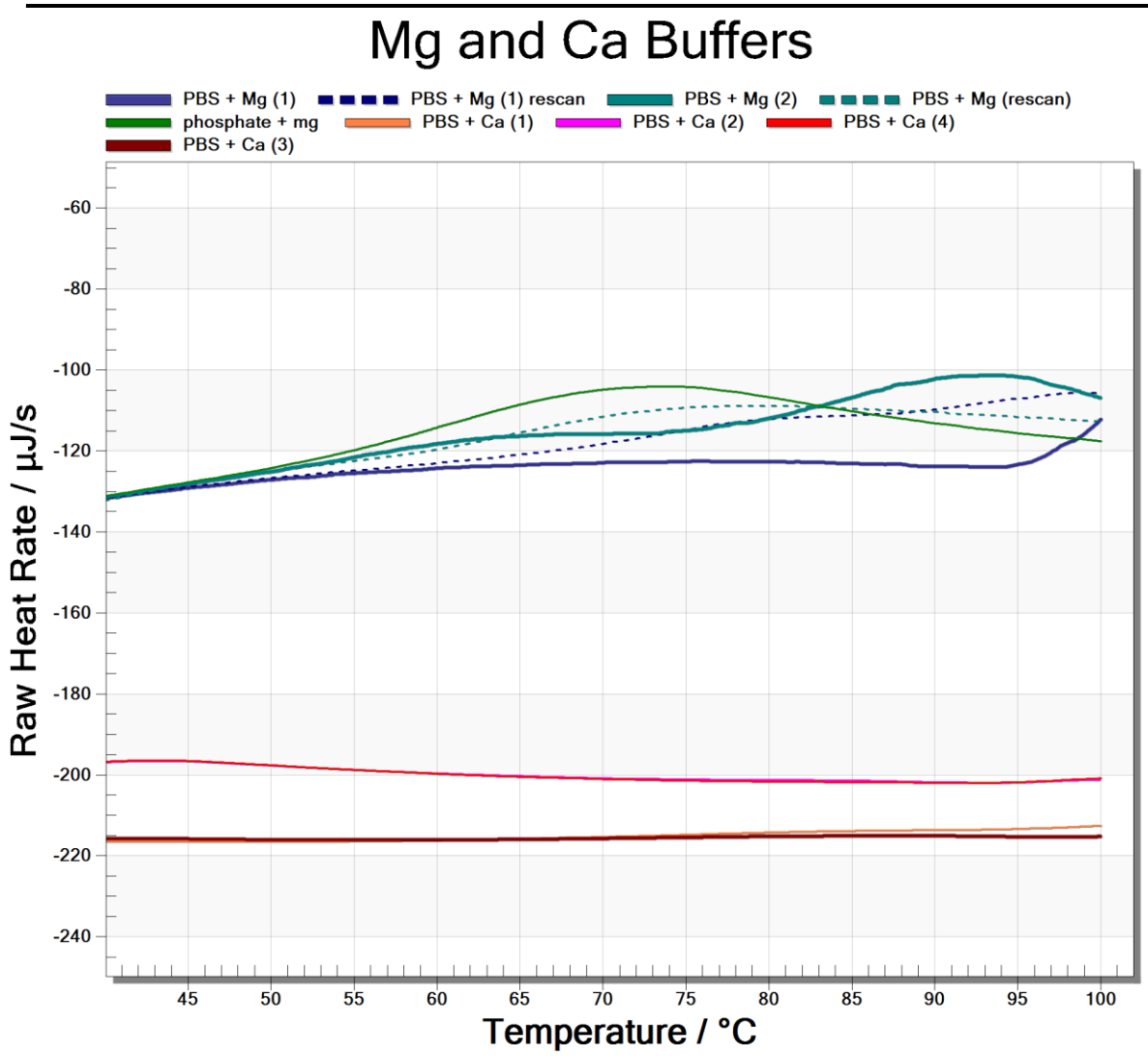

Figure 5. Nano DSC scans of phosphate buffers with Mg^{2+} and Ca^{2+}. The number after a scan indicates the number of duplicates and the word rescan indicates that the buffer was scanned a second time without removing the buffer solution from the sample cell.

The events observed in the scans for phosphate buffer containing Mg^{2+} were reproducible in the second reheat scan. This typically indicates that a chemical event is taking place during the scan and is not related to the typical conditioning event where ions adhere to the cell surface. Based on this data, it is recommended that divalent metals be avoided when using phosphate containing buffers. However, if a divalent metal is an absolute requirement to maintain molecular stability, the data from Figure 5 would indicate that Ca^{2+} added to phosphate buffer will yield a stable flat DSC scan and in some cases this divalent ion may be an acceptable replacement for Mg^{2+}.

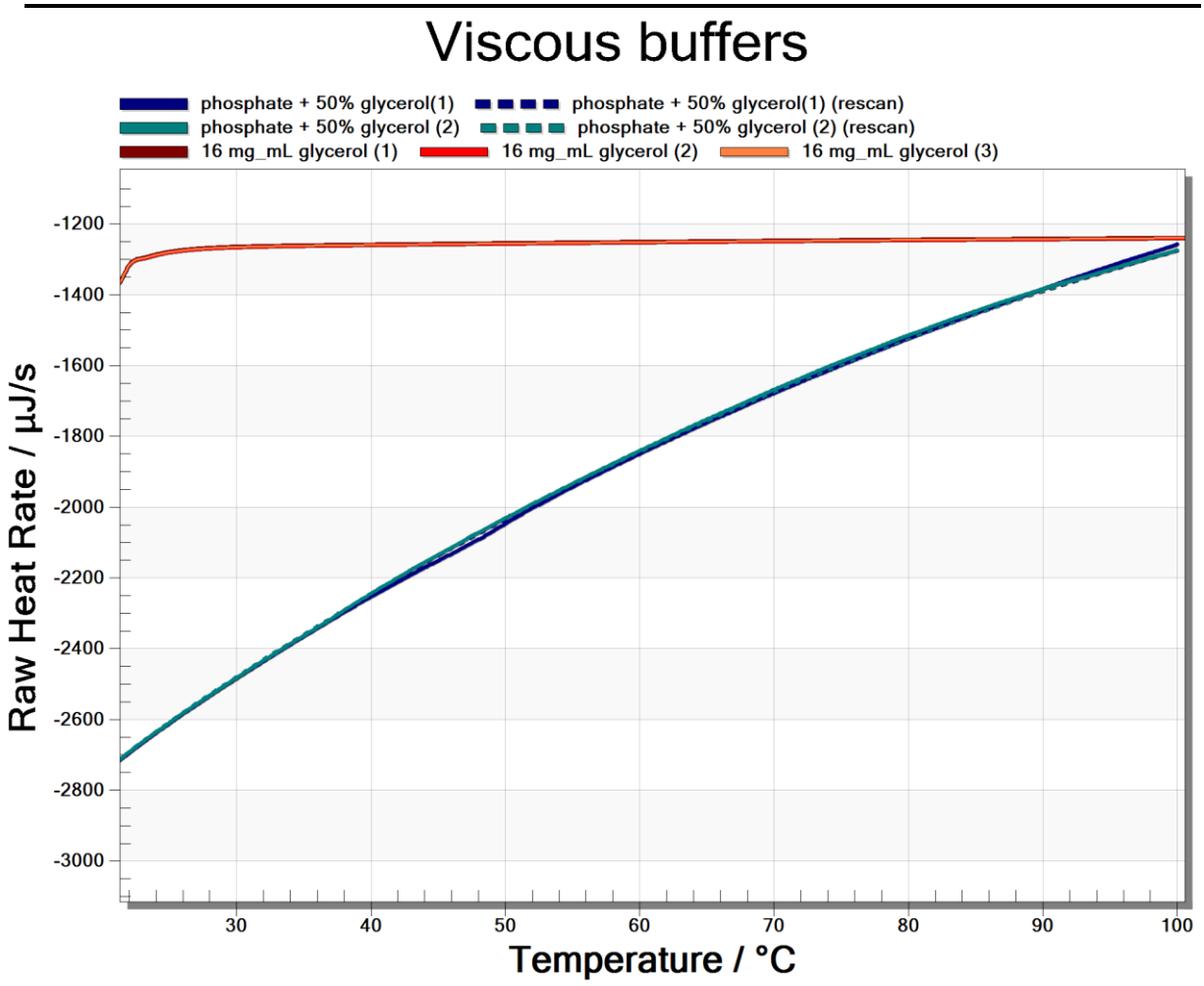

Figure 6. Nano DSC scans of viscous buffers containing up to 50% glycerol. The number after a scan indicates the number of duplicates and the word rescan indicates that the buffer was scanned a second time without removing the buffer from the sample cell.

DSC scans of buffers containing up to 50% glycerol will have this substantial thermal mass mismatch, since the reference cell contains Nanopure water. DSC scans of the buffers containing 50% glycerol have large deflection characteristics that required a larger window in order to show their complete profiles. The extreme slope, shown in Figure 6, would be minimized, if the sample and reference cell both contained the same amount of glycerol.

It is significant to note that the Nano DSC Autosampler System used in this study was very effective and reliable in handling and delivering the samples with high viscosity to the Nano DSC instrument. In this set of experiments, no special modifications were made to the autosampler for it to

easily and routinely handle the samples containing up to 50% glycerol and following the scans, effectively rinse/clean the sample cell in preparation for the next sample.

Conclusion

Accurate differential scanning thermodynamic profiles for most structured macromolecules require the generation of a reproducible baseline buffer scan. In order to obtain results similar to the baseline buffer scans presented in this study, the sample cell of the Nano DSC must be properly conditioned. The first scan in most experimental designs using the Nano DSC will be a conditioning scan. The subsequent and more important baseline buffer scans should be performed following the conditioning scan. To successfully collect a reproducible baseline buffer scan, both the reference and sample cell should be emptied of the conditioning scan buffer and then refilled with fresh buffer.

All successful experimental designs for DSC always take into consideration each component of the solvating buffer for the macromolecule being analyzed. The individual component's thermal stability and possible interactions between excipients should also be considered. In this study, the data collected on phosphate buffers with either Mg^{2+} or Ca^{2+} demonstrated the importance of understanding the thermal stability of all excipients (see Figure 5).

For typical DSC experimental designs, when both reference and sample cells are filled with the same buffer, the slopes for the resulting DSC scans are typically minimized because of matching heat capacity and thermal mass in the cells. If at any time an unexpected and significant slope persists in a baseline buffer scan, the following steps should be taken:

1) The buffer should be removed from both the reference and sample cells and the cells should be rinsed with water. Following the water rinse they should be filled with degassed, deionized water. The water should be subjected to the same scan temperature range and scan rate as used with the buffer. If a significant slope still exists,
2) The sample cell in the DSC instrument should be cleaned thoroughly and the cells, rebalanced, following the directions in the "Maintaining the Nano DSC" section of the Nano DSC Getting Started Guide.

The accuracy and precision of DSC data on any macromolecule is dependent on many factors and obtaining a reproducible buffer baseline scan is one of the more important factors. The data and conclusions in this study are from representative DSC thermograms for some of the more common buffers used when analyzing biological samples. Understanding the influence of buffer components on DSC data prior to analyzing important unknown samples will save time and effort in any project requiring an accurate, reproducible DSC thermogram.