Determining optimal buffer concentration and compatibility for high-throughput intact protein analysis

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This technical note demonstrates a rapid method to determine the optimal buffer conditions for the intact protein analysis of myoglobin. Choosing the correct buffer is crucial to maintaining the activity and folding of a protein. To preserve the desired activity, scientists must prepare their protein in traditional, biochemically relevant buffers (Good's buffers), regardless of the endpoint analysis.[1] However, while Good's buffers are primarily used for biological research, utilizing these buffers for mass spectrometry applications often causes poor ionization of the target analytes due to their high salt concentration. However, it has been previously shown that Acoustic Ejection Mass Spectrometry (AEMS) can limit, or prevent, ion suppression by ejecting nanoliter volumes of sample into the carrier solvent.

Here, rapid analysis of myoglobin in 16 different buffers at 9 different buffer concentrations was performed on the Echo® MS+ system with ZenoTOF 7600 system (Figure 1). Rapid method development was performed using standard peak mode (1 second per sample) with an additional delay of 3 seconds per sample to account for changes in buffers and buffer concentrations. This added delay between ejections is not required for general analytical analysis. In approximately 34 minutes, the optimal buffer and concentration were determined for apo- and holo-myoglobin with triplicate analysis for increased confidence. This method is not limited to myoglobin and can be applied to other analytes of interest.

Key features of high-throughput intact protein analysis using the Echo® MS+ system with ZenoTOF 7600 system

- **High-throughput method development:** Determine optimal buffer concentration at a rate of 4 seconds per sample
- **Platform sample preparation:** Easily apply the sample preparation method to small and large molecules
- **Multiplexed analysis:** Analyze multiple signal responses in 1 ejection using the MS1 scans on the ZenoTOF 7600 system
- **Increased data confidence:** Perform customized replicate analysis using technical or analytical replicates (n=3) of 100 nL per ejection
- **Streamlined data management:** Utilize the mass reconstruction workflow to automate the results review process using SCIEX OS software

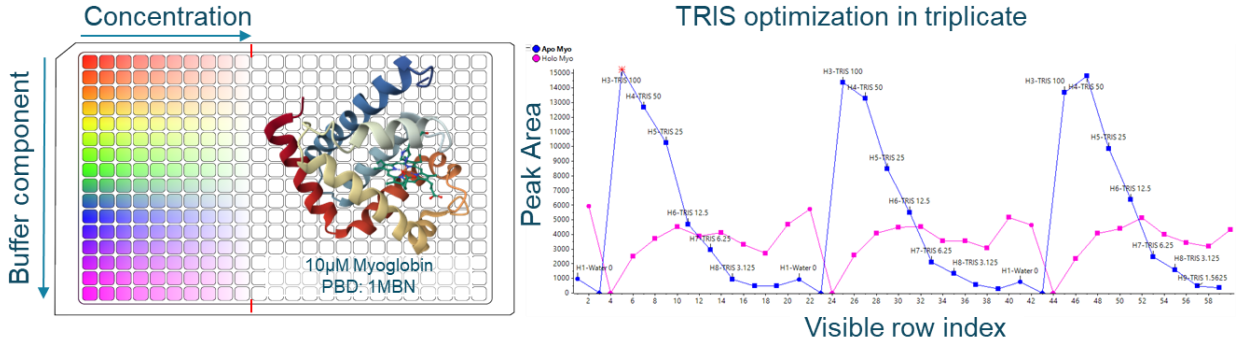

Figure 1. Rapid optimization of sample matrix using the Echo® MS+ system with ZenoTOF 7600 system. Sixteen biologically relevant buffers were added to a 384-well microtiter plate. The buffers were then 2x serially diluted horizontally across the plate until row 10. Following dilution, 10µM myoglobin was added to each well to monitor the response of the apo- (blue trace) and holo- (pink trace) myoglobin under the different buffer conditions. Triplicate analysis of various concentrations of TRIS was used to determine its optimal concentration using SCIEX OS software. The PBD structure is shown from 1MBN.[2,3]

Introduction

Determining the optimal buffer for protein mass spectrometry experiments can be challenging since many buffers are available for such studies.[4] Furthermore, choosing the optimal buffer at the optimal concentration adds complexity to the experiment.[5] Method development for selecting the appropriate buffer and correct concentration is time-intensive and can delay final assay experimentation without a high-throughput solution.

AEMS offers label-free sampling at rates of up to 1 sample per second. The high-throughput nature of AEMS enables the rapid determination of optimal buffer conditions for 384 samples in approximately 10 minutes, compared to the 32 hours that would be required for a conventional method utilizing LC-MS at a rate of 5 minutes per sample.

The Echo® MS+ system with the ZenoTOF 7600 system offers a high-throughput solution for determining the optimal protein buffer concentration. In this study, apo- and holo-myoglobin in varying buffer concentrations were acoustically ejected using an Echo® MS+ system with the ZenoTOF 7600 system. The data were then reconstructed using the mass reconstruction workflow in SCIEX OS software to monitor the intact mass of apo- and holo-myoglobin. Average peak areas of the reconstructed proteins were calculated and plotted to visually determine the optimal buffer concentration for a particular protein in a particular buffer.

Methods

Sample preparation: An array of 16 traditional, biologically relevant buffers were selected and plated onto a 384-well microtiter plate. The buffers were 2x serially diluted horizontally across the plate until row 10. A 10µM myoglobin sample was then added to each well to monitor the response of the apo- and holo-myoglobin under the different buffer conditions. Samples were analyzed following the acoustic ejection and mass spectrometry methods (Tables 1-2).

Plate map of the buffers used in the study is described in Table 3.

Table 1: Acoustic ejection method.

Carrier solvent	DI water
Carrier solvent flow rate	400 µL/min
Fluid class	SP
Ejection volume	100 nL
Rep rate	400 Hz
Interval	4000 ms

Table 2: MS analysis on the ZenoTOF 7600 system.

Parameter	Value
Polarity	Positive
Ion source gas 1	90 psi
Ion source gas 2	50 psi
Curtain gas	35 psi
Source temperature	400°C
Ion spray voltage	5500 V
CAD gas	10
MS method	TOF MS
Time bins to sum	40

Data processing: SCIEX OS software was used to process the data qualitatively and quantitatively. The mass reconstruction workflow found in SCIEX OS software was used for the peak area determination of proteins.

Table 3. Plate map of the buffers used in the study and their respective concentrations. Concentrations are expressed in mM or % v/v. Column 1 (green) indicates protein with no buffer (positive control) and column 2 (orange) indicates buffer without protein (negative control).

		Water Blank	Buffer blank								
	mM	1	2	3	4	5	6	7	8	9	10
Water	A	0	0	0	0	0	0	0	0	0	0
TCEP	B	0	5	5.00	2.50	1.25	0.63	0.31	0.16	0.08	0.04
EDTA	C	0	5	5.00	2.50	1.25	0.63	0.31	0.16	0.08	0.04
NaCl	D	0	500	500.00	250.00	125.00	62.50	31.25	15.63	7.81	3.91
MgCl2	E	0	5	5.00	2.50	1.25	0.63	0.31	0.16	0.08	0.04
KCl	F	0	5	5.00	2.50	1.25	0.63	0.31	0.16	0.08	0.04
10X PBS	G	0	10X	5X	2.5X	1.25X	0.625X	0.31X	0.16X	0.08X	0.04X
TRIS	H	0	100	100	50	25.00	12.50	6.25	3.13	1.56	0.78
TRIS acetate	I	0	100	100	50	25.00	12.50	6.25	3.13	1.56	0.78
HEPES	J	0	100	100	50	25.00	12.50	6.25	3.13	1.56	0.78
MOPS	K	0	100	100	50	25.00	12.50	6.25	3.13	1.56	0.78
PPB	L	0	100	100	50	25.00	12.50	6.25	3.13	1.56	0.78
Glycerol	M	0	20	20.00	10.00	5.00	2.50	1.25	0.63	0.31	0.16
TritonX	N	0	0.50%	0.500%	0.250%	0.125%	0.063%	0.031%	0.016%	0.008%	0.004%
SDS	O	0	10%	10%	5.0%	2.5%	1.3%	0.6%	0.3%	0.2%	0.1%
TWEEN	P	0	0.10%	0.100%	0.050%	0.025%	0.013%	0.006%	0.003%	0.002%	0.001%

Rapid analysis

A total of 480 samples (160 samples in triplicate) and batch-marking barcodes were analyzed in 34 minutes (Figure 2), compared to the 40 hours that would be needed to acquire triplicate analyses using LC-MS.

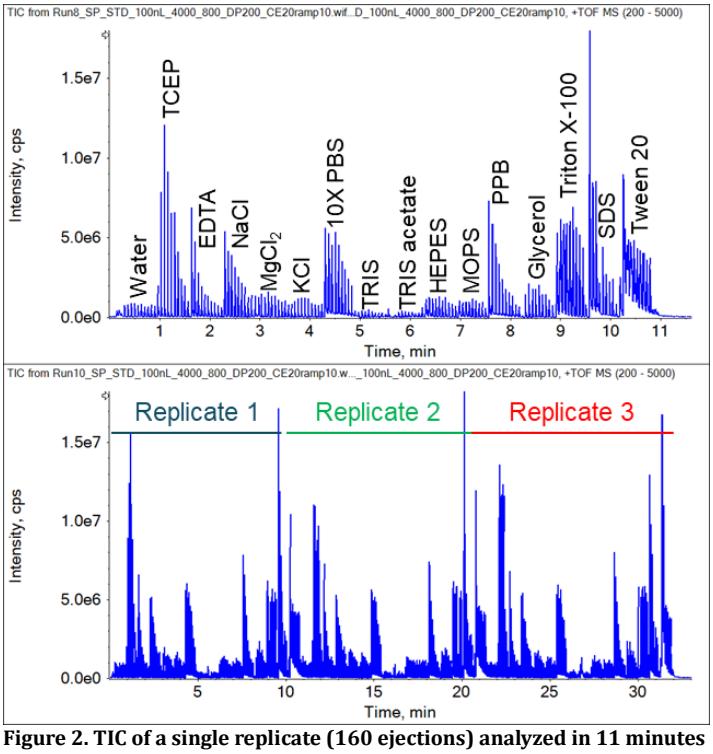

Figure 2. TIC of a single replicate (160 ejections) analyzed in 11 minutes (top) and 480 ejections acquired in 34 minutes (bottom).

Mass reconstruction

Both apo- and holo-myoglobin were analyzed simultaneously in each of the buffers at all concentrations (Figure 3). SCIEX OS software allows for targeted analysis of ejections on a per-well basis based on the total ion chromatogram (TIC), extracted ion chromatogram (XIC) and reconstructed mass. Once a given processing method is defined, the results file can be generated via the batch submission post-acquisition.

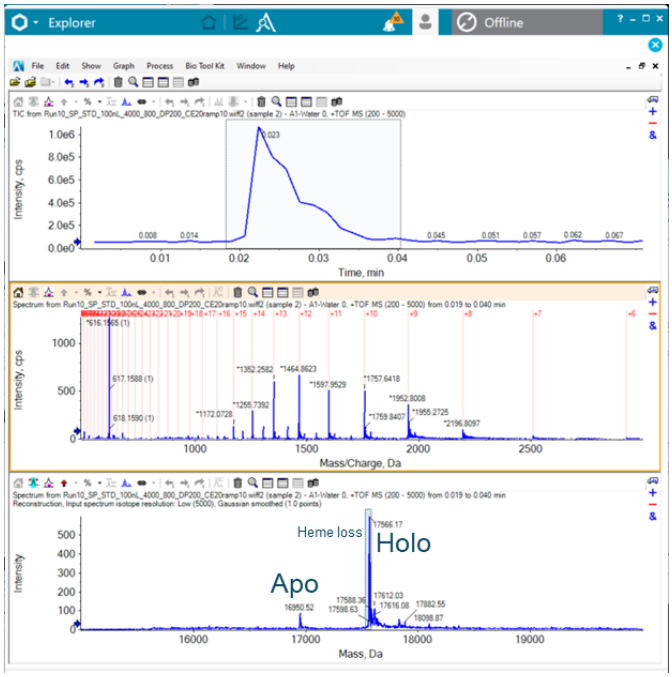

Figure 3. Mass reconstruction of the simultaneous analysis of apo- and holo-myoglobin.

The Analytics module in SCIEX OS software can deconvolute targeted large molecules in addition to its core quantitative workflow. All 480 replicate samples could be processed for apo- and holo-myoglobin (960 samples in total) at a reconstruction resolution of 5000. The reconstruction mass output range was set between 15 kDa and 20 kDa. The reconstructed masses of apo- and holo-myoglobin were found at 16,951 and 17,565 Da, respectively, matching literature values (Figure 4).[6]

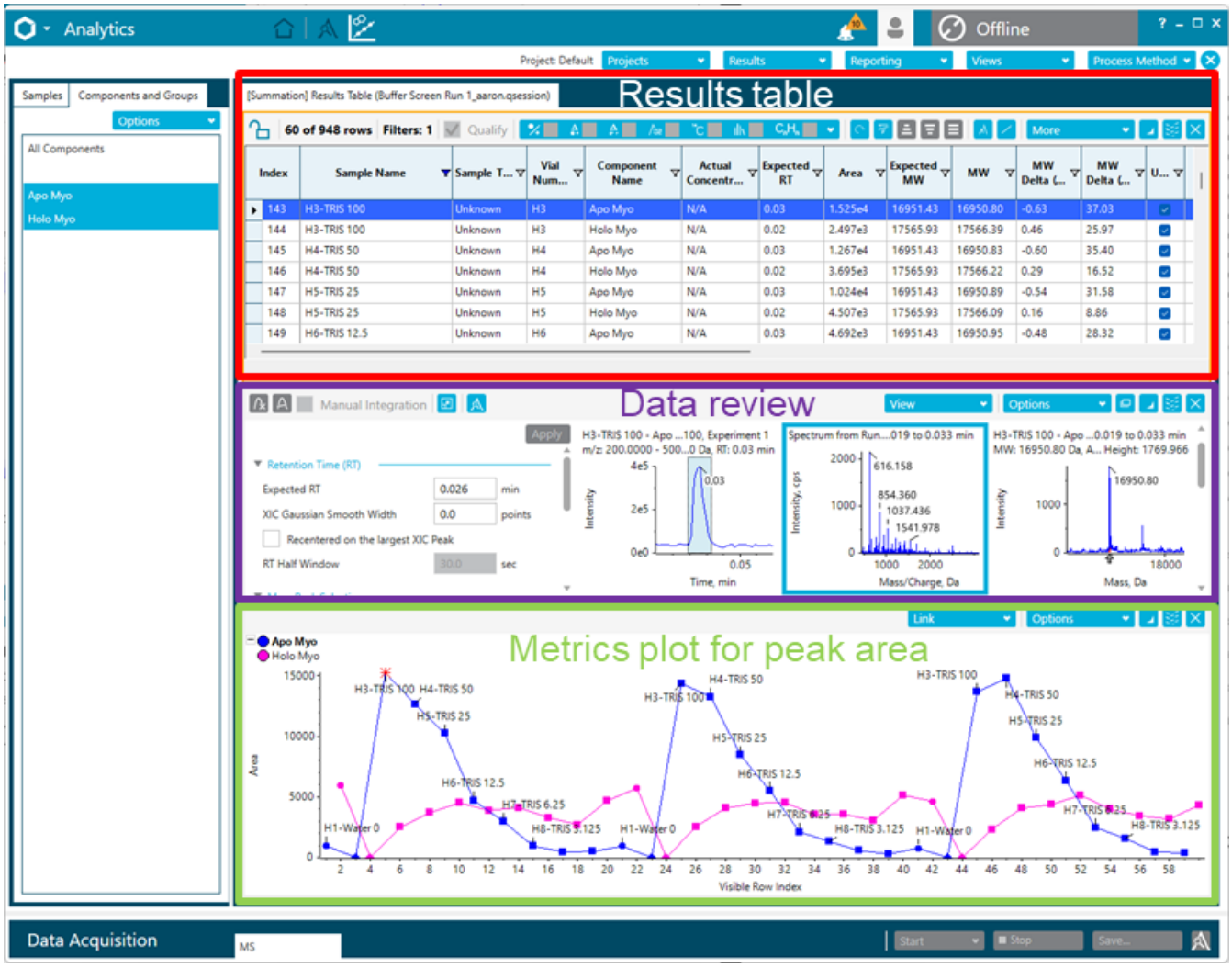

Figure 4. Mass reconstruction workflow in SCIEX OS software for the quantitation of the optimal TRIS concentration. The results table, data review and metrics plots can be tailored to the user's needs.

Optimal buffer concentrations

The optimal buffer concentration was calculated (Figure 5) and the highest peak average area shows the optimal buffer concentration. The optimal buffer concentration for apo-myoglobin often differed from the optimal concentration for holo-myoglobin. However, in cases in which it is necessary to measure both apo- and holo-myoglobin, the data shown in Figure 5 could be used to determine the optimal buffer concentration for both apo- and holo-myoglobin.

Figure 5. Average reconstructed area values for apo- and holo-myoglobin in buffers with varying TRIS, $MgCl_2$ and TCEP concentrations. Average area response is shown in light blue for apo-myoglobin and dark blue for holo-myoglobin. Error bars represent 1 standard error of the mean.

Conclusions

- The optimal concentrations were determined across buffer conditions for apo- and holo-myoglobin
- Sixteen buffers were screened at 10 concentrations in approximately 11 minutes
- Low sample consumption of 100 nL per ejection allowed for technical replicate analysis
- The mass reconstruction workflow calculated zero-charge peak areas in each buffer at each buffer concentration

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