Protein stability assessment after automated buffer exchange

Introduction

Buffer preparation, exchange and sample concentration for a formulation screen can take 2–4 days of a scientist's time. While many labs have developed strategies to streamline formulation development, it's still relatively manual and requires significant resources which can limit the number of formulations evaluated. Unchained Labs has eliminated this bottleneck with a new, easy-to-use system that automates formulation preparation, buffer exchange and sample concentration. GRUNT (Figure 1) enables scientists to easily, rapidly and automatically generate up to 12 different protein formulations at the 1–8 mL scale with minimal hands-on time.

GRUNT starts by automatically preparing buffers from stock solutions and, if necessary, titrating the buffers with acids or bases. Next, GRUNT buffer exchanges a protein into each buffer to make a final liquid protein formulation by using an innovative ultrafiltration/diafiltration (UF/DF) method that is well suited for buffer exchanging and concentrating biopharmaceuticals. After buffer exchange is complete, GRUNT automatically concentrates the protein in each final formulation based on user input. Through the user-friendly software interface all technical staff can prepare protein formulations without any specialized training, resulting in an increase in formulation preparation throughput.

Figure 1: GRUNT prepares 12 protein solutions automatically.

Automated buffer exchange of therapeutic proteins using GRUNT

GRUNT dispenses protein into funls, which are single-use filtration cups that measure the volume, and then GRUNT uses pressure to perform ultrafiltration (Figure 2). funls are gently mixed during ultrafiltration to avoid clogging the membrane. After filtration, the volume of each funl

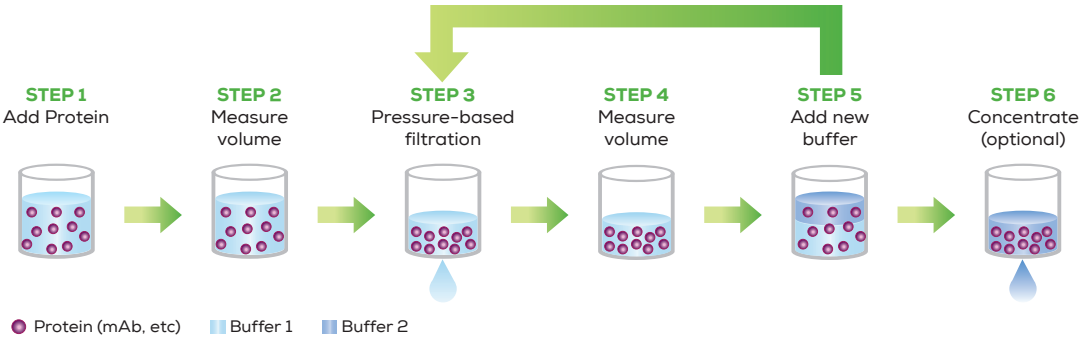

Figure 2: Protein is added to each funl and then volumes are measured. Pressure is applied to the funl along with orbital mixing to concentrate the sample. GRUNT automatically measures solution volumes in each funl, calculates the volume that was removed, refills with new formulation buffer and calculates percent exchange. The system repeats filtration, volume measurement and refill cycles until each sample reaches greater than 96% buffer exchange.

is measured and the amount of buffer removed is calculated. The system refills each funl with the desired formulation buffer and calculates the percent (%) exchange. GRUNT repeats this process until each protein formulation is at least 96% buffer exchanged.

Comparison of automated and traditional buffer exchange methods

A key question about automated processes is their comparability to traditional methods. For protein formulation, the buffer exchange process must provide excellent material recovery and not impact short term or long term protein stability. To answer these questions, we compared GRUNT's automated buffer exchange process to traditional methods by testing multiple proteins immediately after buffer exchange and on accelerated stability. The overall study design is shown in Figure 3.

Four confidential therapeutic proteins were buffer exchanged using GRUNT, Amicon® centrifugal UF devices and dialysis cassettes. Three of the proteins were monoclonal antibodies (mAbs α, β and γ) and one was a therapeutic enzyme. GRUNT, Amicon centrifugal devices and dialysis were used to buffer exchange each protein into a target formulation by replacing 99% of the original buffer. The protein concentrations for the formulations were: mAb α at 1 mg/mL, mAb β at 50 mg/mL, mAb γ at 5 mg/mL and a therapeutic enzyme at 20 mg/mL. After buffer exchange, Unchained Labs' scientists determined protein recovery, the pH of each formulation and compared the stability of the proteins after incubation at 40 °C over the course of six weeks. Protein formulation stability was monitored by size-exclusion chromatography (SEC), dynamic light scattering (DLS), pH and micro-flow imaging (MFI). Immediately after buffer exchange (T_0), each protein formulation was tested by SEC, DLS, pH and MFI. An aliquot from each sample (GRUNT, Amicon and dialysis) was removed, vialed in duplicate and then stored at 40 °C. Each vialed aliquot was analyzed by SEC and DLS at 0, 2, 4 and 6 weeks, while MFI measurements were taken at 0 and 4 weeks. For this study, GRUNT was used only to buffer exchange proteins. Buffer preparation, protein concentration and additional capabilities of GRUNT are covered in other application notes available from Unchained Labs.

Recovery

Protein recovery was calculated by comparing the mass of protein before and after buffer exchange (Table 1). Protein recovery after buffer exchange by GRUNT ranged from 95.8–99.6% of expected mass. Mass recovery for the same protein formulations buffer exchanged with Amicon

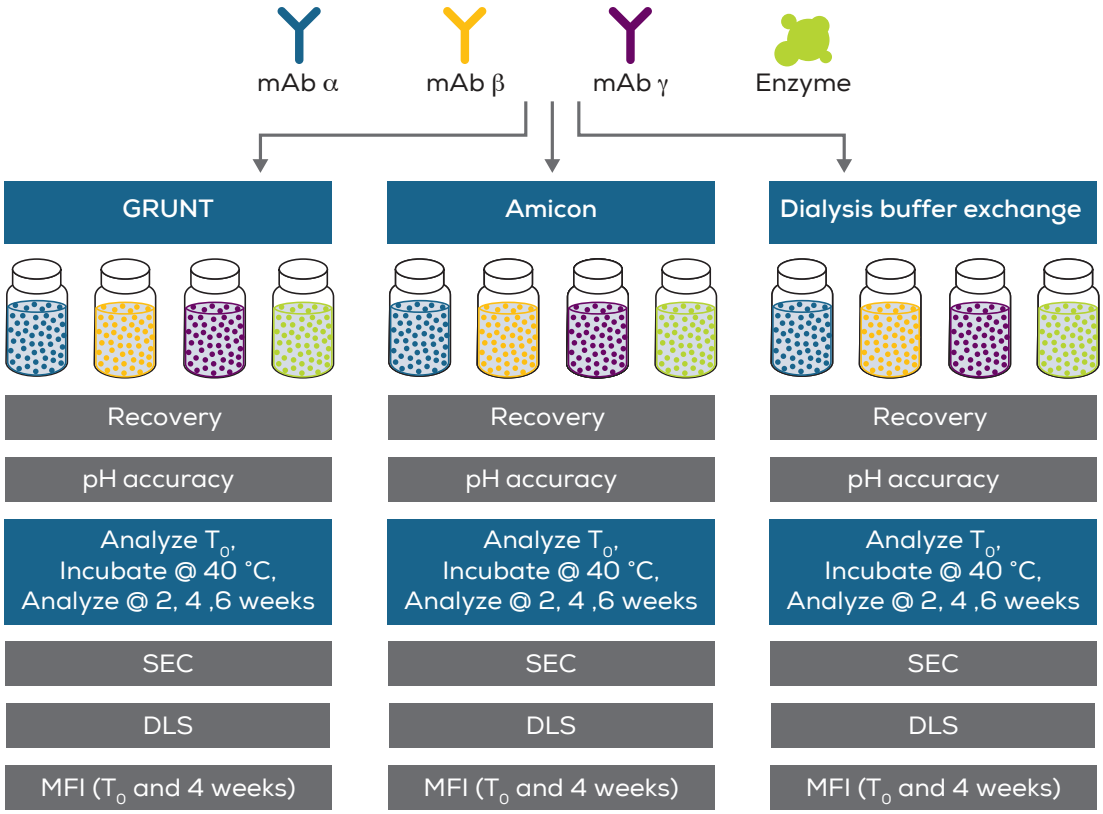

Figure 3: Stability study design for comparison of buffer exchange methods.

centrifugal concentrators and dialysis ranged from 91.1–94.3% and 91.6–96.4%, respectively. Protein recoveries for proteins buffer exchanged by GRUNT are highly comparable to Amicon and dialysis recoveries and, as seen in Table 1, GRUNT can outperform both.

pH accuracy

The pH of each protein formulation post-processing was compared to the target pH of the formulation buffer for all three buffer exchange techniques (Table 2). For all buffer exchange methods evaluated, ΔpH values of final protein formulations were within 0.09 pH units of targets. In contrast, when the two higher concentration protein formulations (mAb β at 50 mg/mL and enzyme at 20 mg/mL) were buffer exchanged using dialysis cassettes, the pH values of the final formulation were 0.28–0.37 pH units from target. These results show that GRUNT is capable of meeting strict pH requirements for protein formulations after buffer exchange. GRUNT produced superior results in achieving pH targets for the final solution compared to dialysis for formulations with ≥20 mg/mL protein. No significant changes in pH values were observed for any of the buffer exchanged proteins during the stability study (data available upon request).

Accelerated stability study results

Size-Exclusion Chromatography (SEC) results

To investigate the stability of each protein formulation after buffer exchange, mAb α, mAb β, mAb γ and a therapeutic enzyme were incubated at 40 °C for six weeks. SEC was used to determine the content of monomer, aggregates and low molecular weight species (fragments) for each protein. Monomer, aggregate and fragment content of mAb α, mAb β, mAb γ and the enzyme are highly similar for all time points and buffer exchange methods (Figures 4-7). The high degree of similarity indicates that all buffer exchange methods do not significantly influence the stability of the monomer of mAb α (Figure 4), mAb β (Figure 5), mAb γ

Protein	Concentration	Recovery		
		GRUNT	Amicon	Dialysis
mAb α	1 mg/mL	96.1%	94.3%	91.6%
mAb β	50 mg/mL	99.6%	92.3%	96.4%
mAb γ	5 mg/mL	95.8%	91.4%	96.0%
Enzyme	20 mg/mL	96.1%	91.1%	93.1%

Table 1: GRUNT provides excellent protein recovery and is comparable to Amicon and dialysis.

Protein	Concentration	pH			
		Target	GRUNT Δ	Amicon Δ	Dialysis Δ
mAb α	1 mg/mL	5.90	-0.01	0.01	-0.11
mAb β	50 mg/mL	5.88	0.09	0.06	0.28
mAb γ	5 mg/mL	6.81	-0.05	-0.03	0.00
Enzyme	20 mg/mL	6.02	0.00	0.00	0.37

Table 2: Summary of the pH results post-processing for the three mAbs and one enzyme after buffer exchange by GRUNT, Amicon and dialysis.

(Figure 6) and the enzyme (Figure 7), even after incubation for six weeks at 40 °C. Aggregate or low molecular weight species content of each protein did not significantly differ across all methods of buffer exchange. Note that no fragments were detected in any of the enzyme samples.

Accelerated stability – Dynamic Light Scattering (DLS) results

DLS experiments were performed to monitor the hydrodynamic monomer radii of four proteins after buffer exchange by GRUNT, Amicon and dialysis. Figures 8–11 present DLS results for each protein and buffer exchange method Unchained Labs tested at T_0 and after six weeks at 40 °C. DLS results indicate that no significant changes in monomer radii occur after buffer exchange any of the proteins or after six weeks of incubation at 40 °C.

Accelerated stability – Micro-Flow Imaging (MFI) results

MFI measurements allowed for the determination of particle counts for each of the therapeutic protein formulations at T_0 and after four weeks of incubation at 40 °C. MFI results of particle concentrations (number of particles/mL of sample) for all four proteins that were buffer exchanged using GRUNT, Amicon and dialysis are shown in Figures 12–15. An unprocessed control that was stored at 40 °C is included for comparison. These results clearly indicate that protein solutions that were buffer exchanged using GRUNT had comparable numbers of particles (2–>100 µm) to formulations that were buffer exchanged by Amicon, dialysis or the unprocessed control.

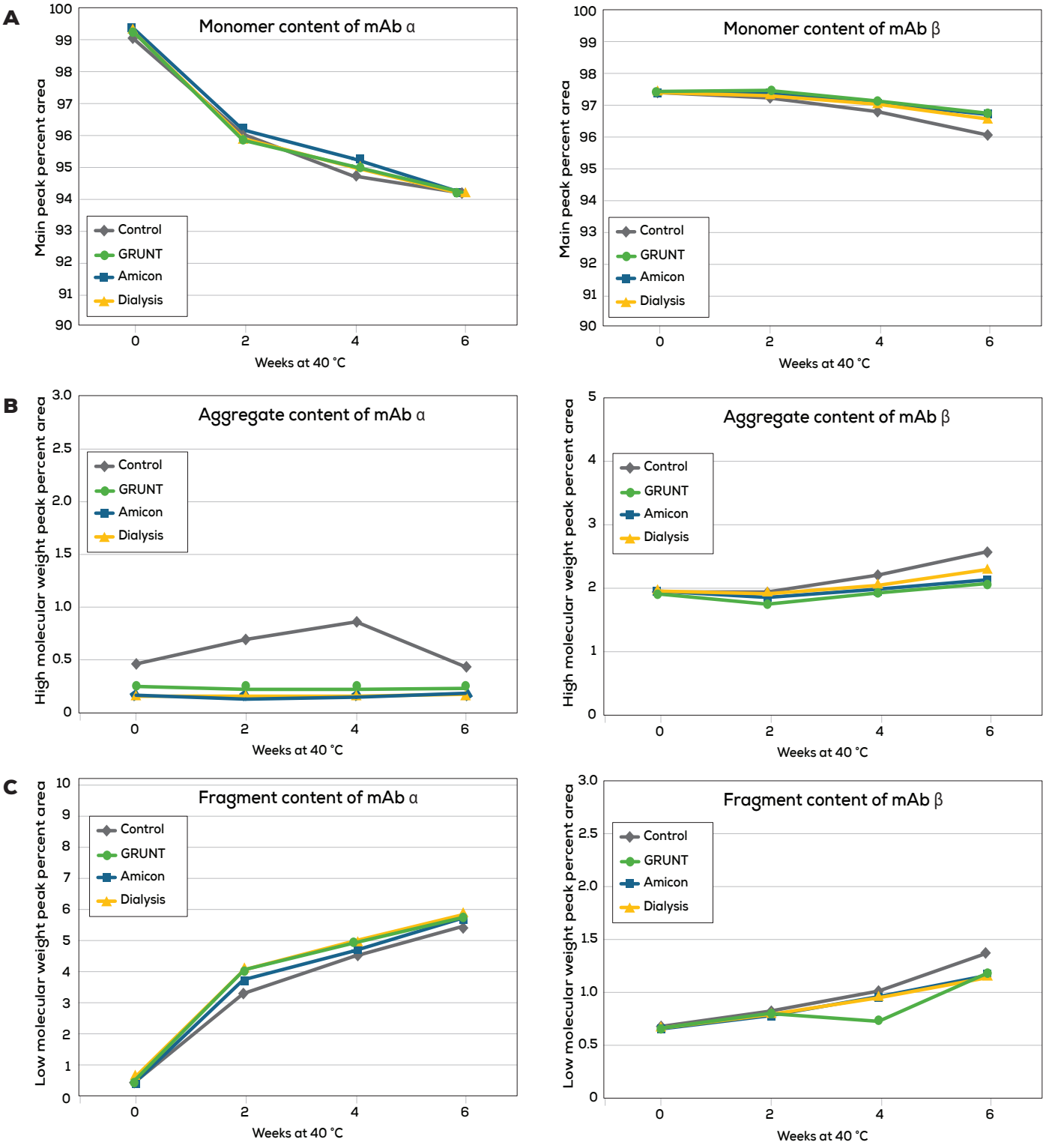

Figure 4: Buffer exchange methods did not significantly influence monomer, aggregate and fragment contents of 1 mg/mL mAb α as evidenced by SEC results. **A**: Percent area of protein monomer. **B**: Percent area of protein aggregates (high molecular weight species or HMW). **C**: Percent area of low molecular weight species (LMW or fragments).

Figure 5: Each buffer exchange method did not significantly influence monomer, aggregate and fragment contents of 50 mg/mL mAb β as evidenced by SEC results. **A**: Percent area of protein monomer. **B**: Percent area of protein aggregates (high molecular weight species or HMW). **C**: Percent area of low molecular weight species (LMW or fragments).

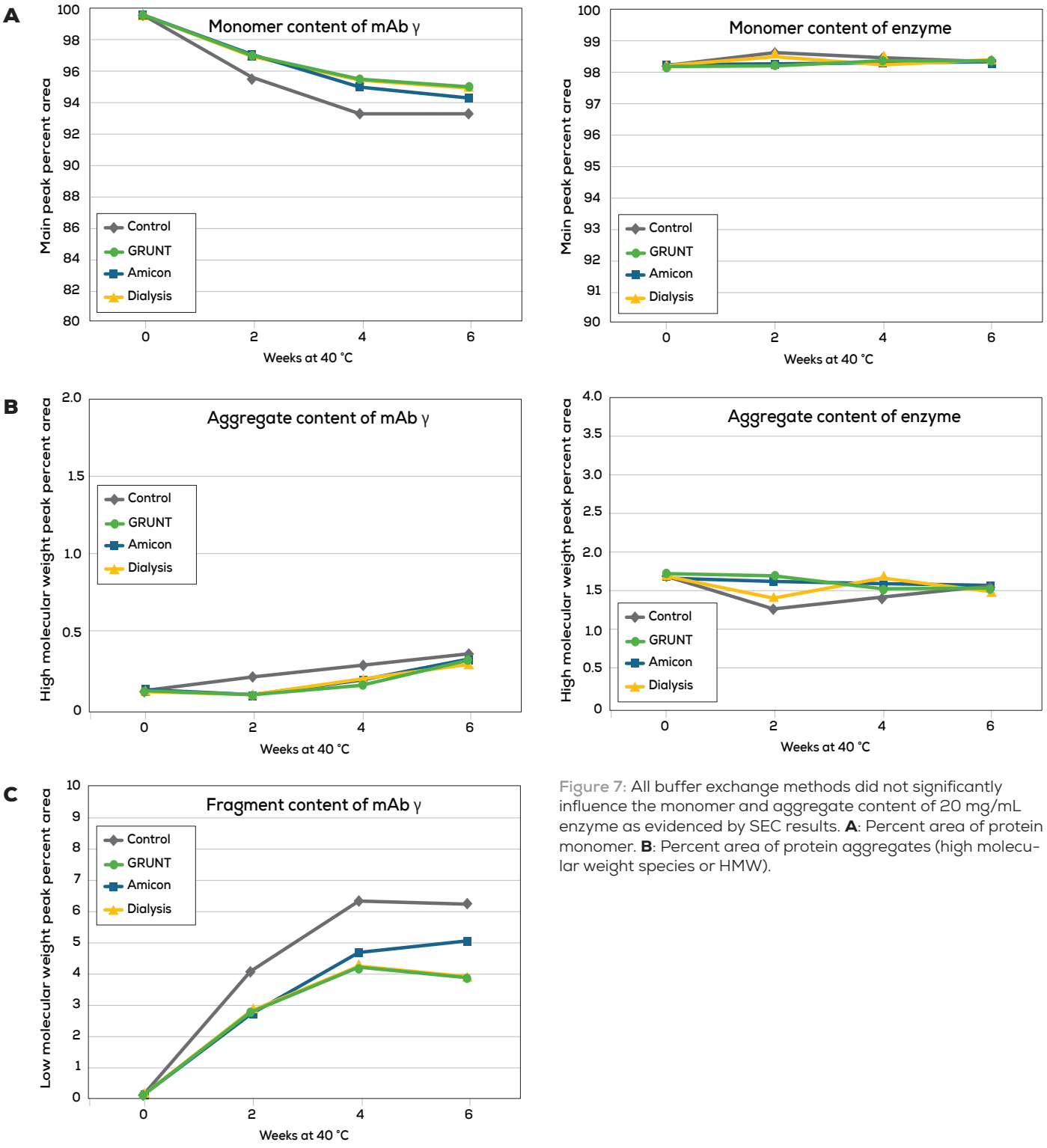

Figure 6: All buffer exchange methods did not significantly influence monomer, aggregate and fragment contents of 5 mg/mL mAb γ as evidenced by SEC results. **A**: Percent area of protein monomer. **B**: Percent area of protein aggregates (high molecular weight species or HMW). **C**: Percent area of low molecular weight species (fragments).

Figure 7: All buffer exchange methods did not significantly influence the monomer and aggregate content of 20 mg/mL enzyme as evidenced by SEC results. **A**: Percent area of protein monomer. **B**: Percent area of protein aggregates (high molecular weight species or HMW).

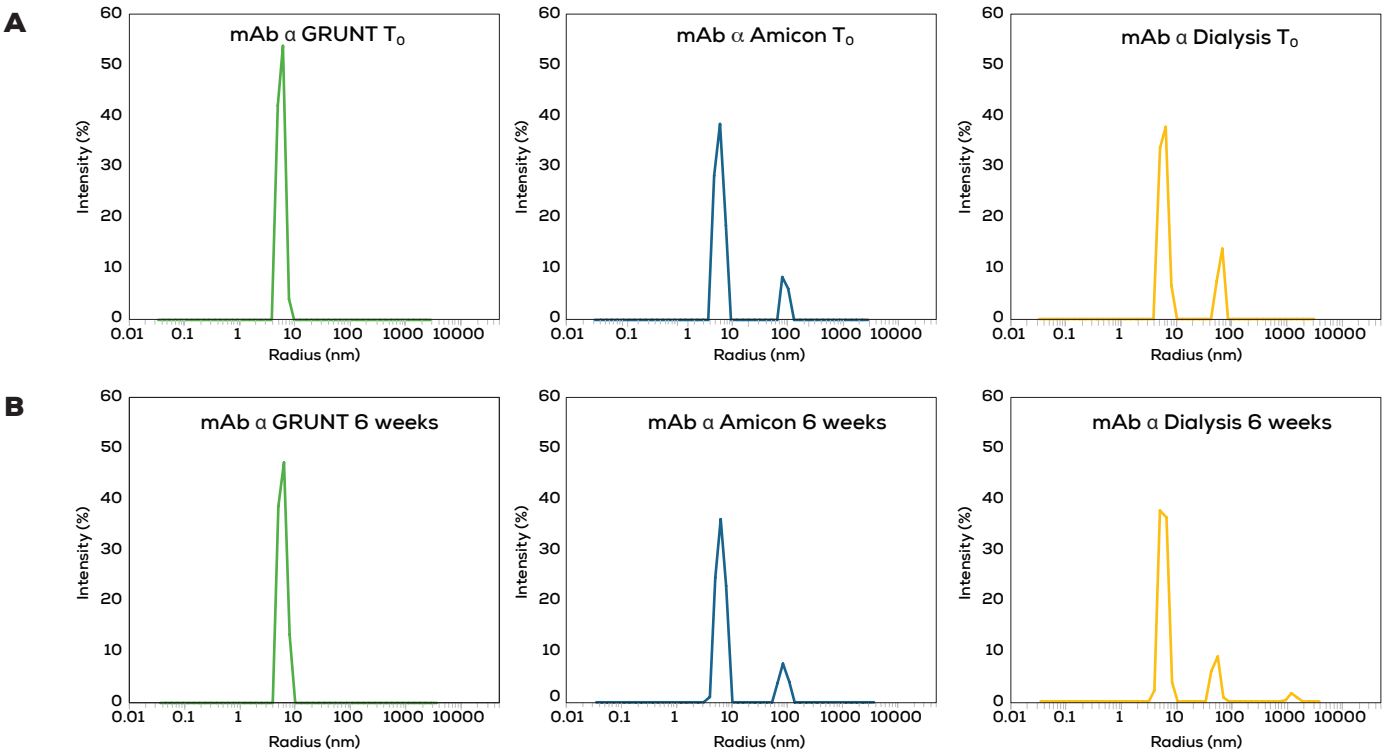

Figure 8: DLS results for 1 mg/mL mAb α buffer exchanged using GRUNT, Amicon devices and dialysis cassettes.
A: T_0. **B**: Six weeks at 40 °C.

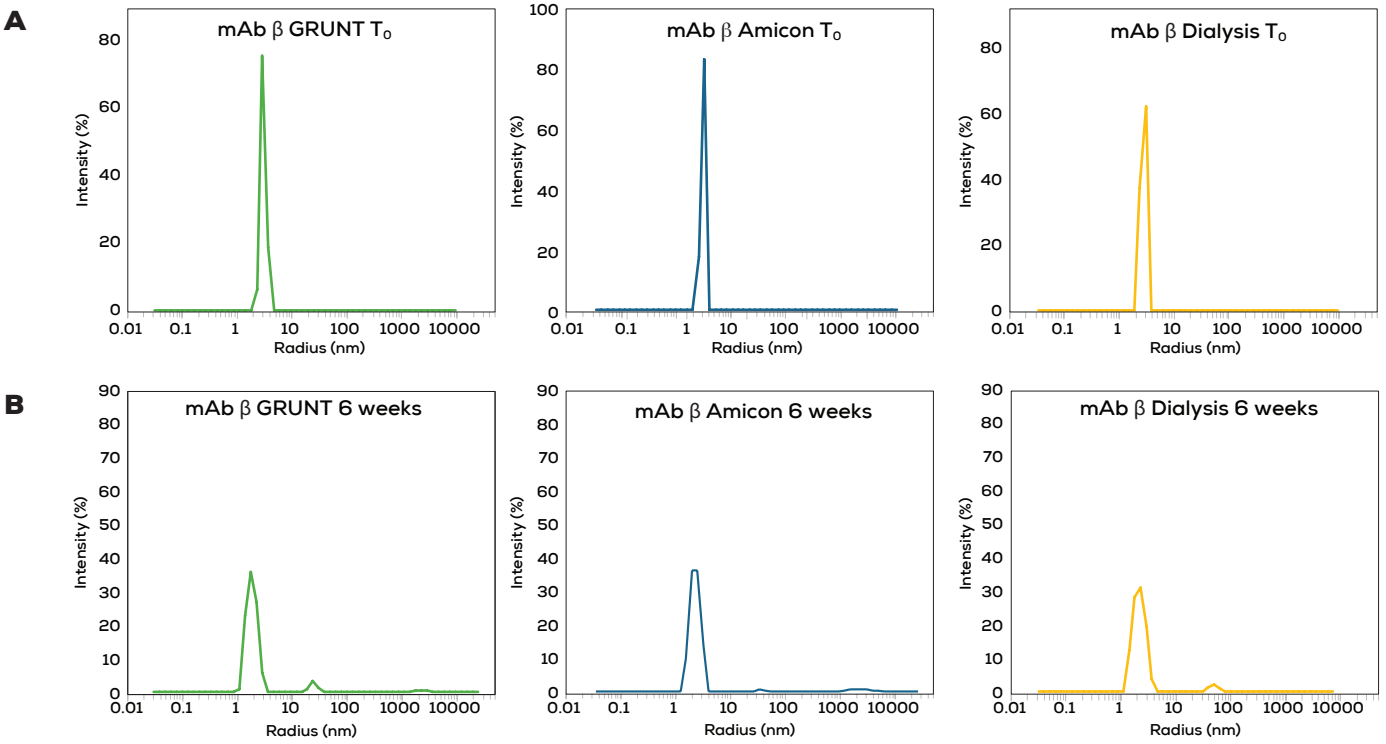

Figure 9: DLS results for 50 mg/mL mAb β buffer exchanged using GRUNT, Amicon devices and dialysis cassettes.
A: T_0. **B**: Six weeks at 40 °C.

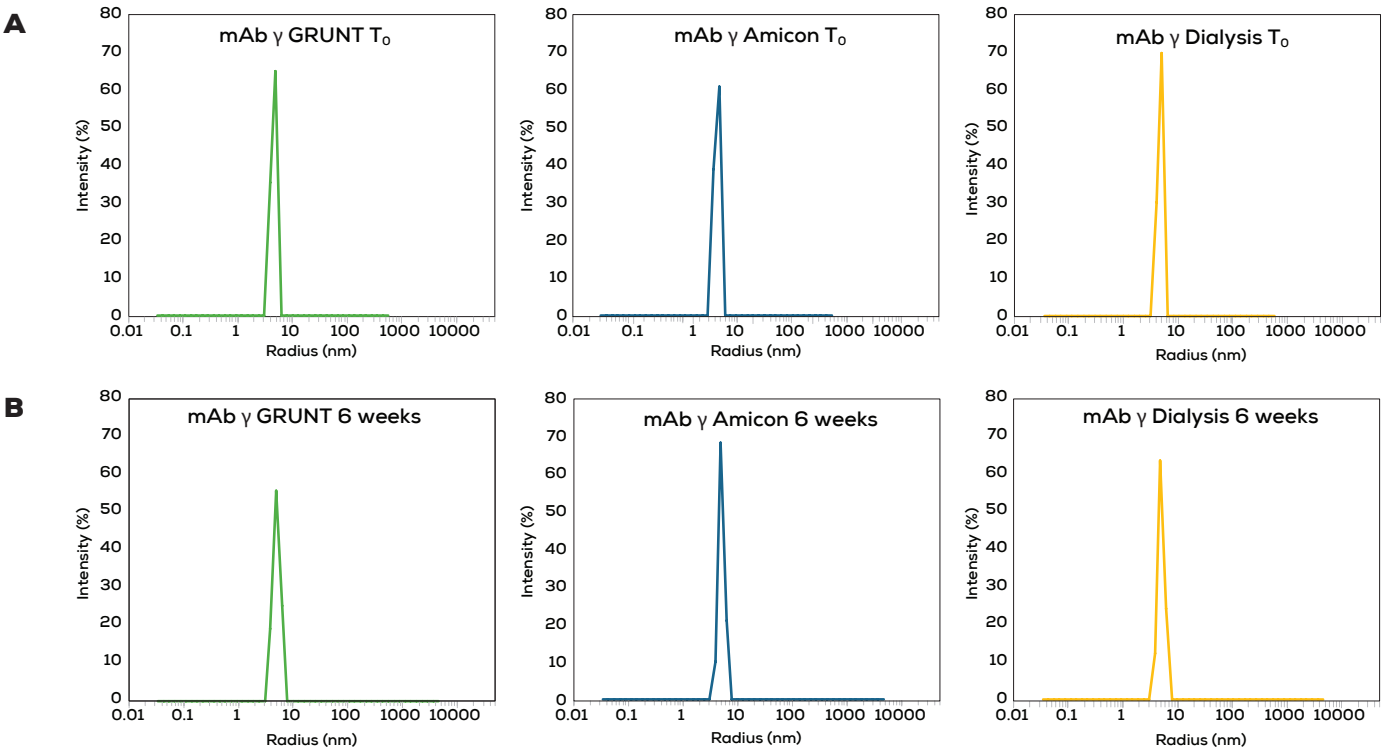

Figure 10: DLS results for 5 mg/mL mAb γ buffer exchanged using GRUNT, Amicon devices and dialysis cassettes.
A: T_0. **B**: Six weeks at 40 °C.

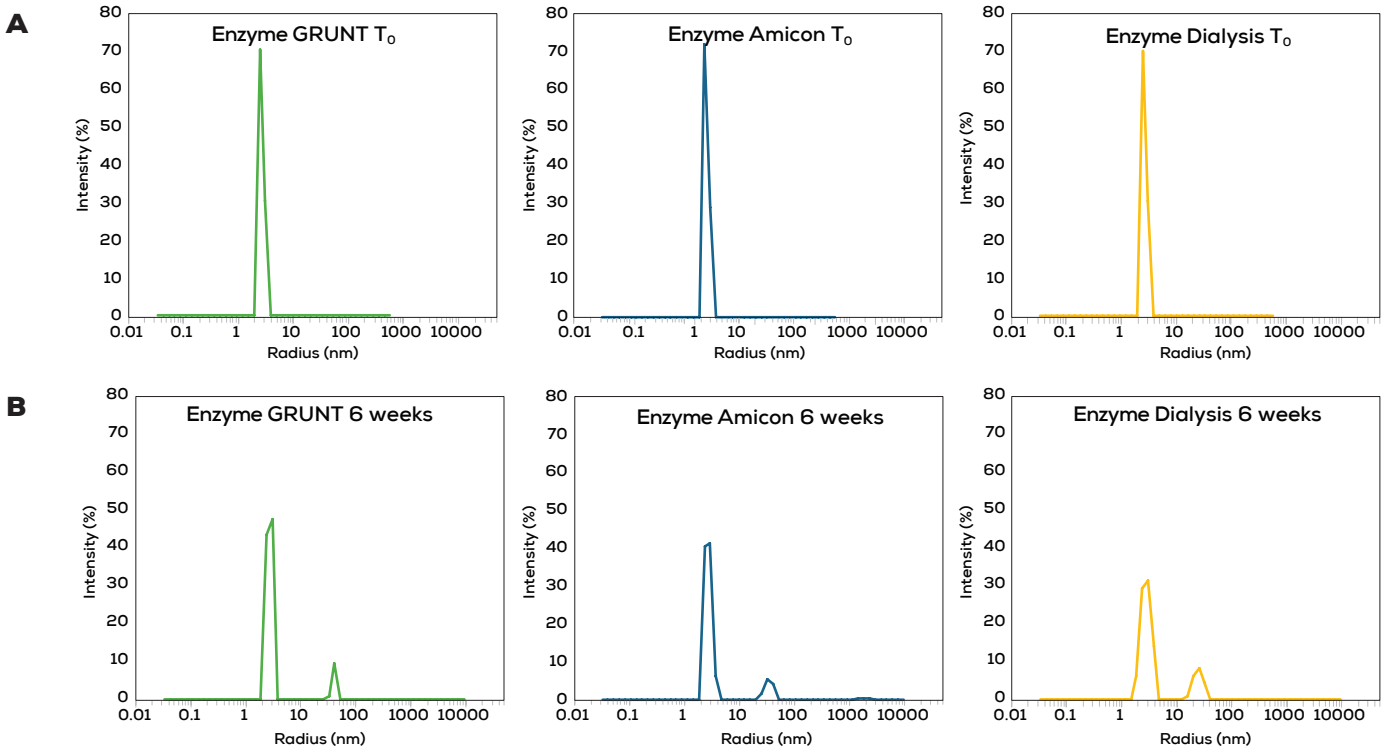

Figure 11: DLS results for 20 mg/mL enzyme buffer exchanged using GRUNT, Amicon devices and dialysis cassettes.
A: T_0. **B**: Six weeks at 40 °C.

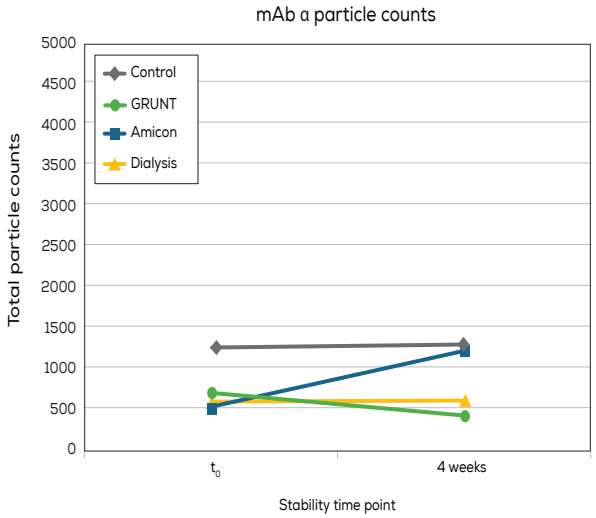

Figure 12: MFI results for 1 mg/mL mAb α buffer exchanged using GRUNT, Amicon devices and dialysis cassettes.

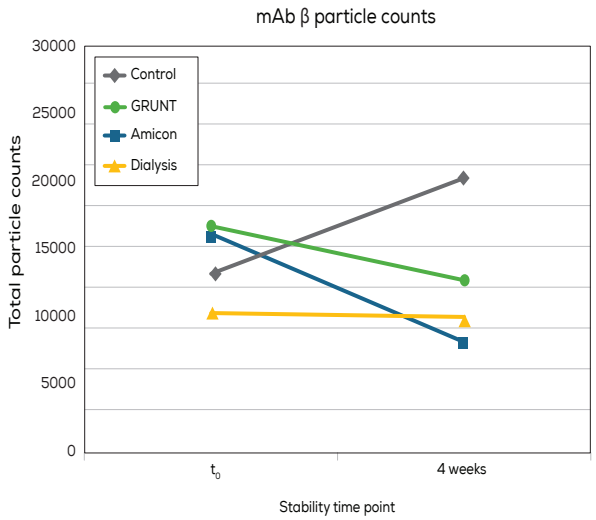

Figure 13: MFI results for 50 mg/mL mAb β buffer exchanged using GRUNT, Amicon devices and dialysis cassettes.

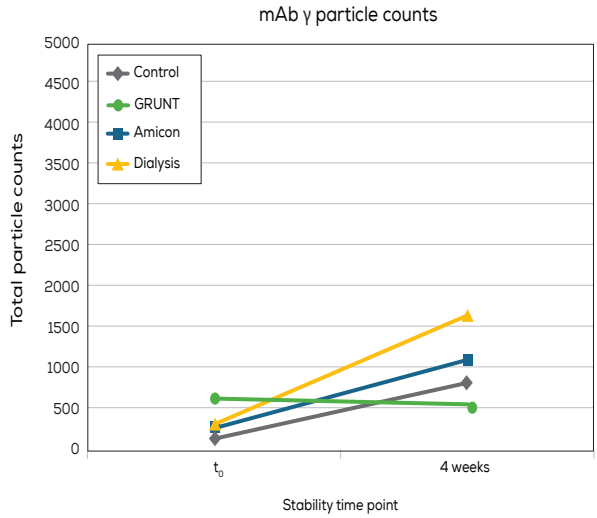

Figure 14: MFI results for 5 mg/mL mAb γ buffer exchanged using GRUNT, Amicon devices and dialysis cassettes.

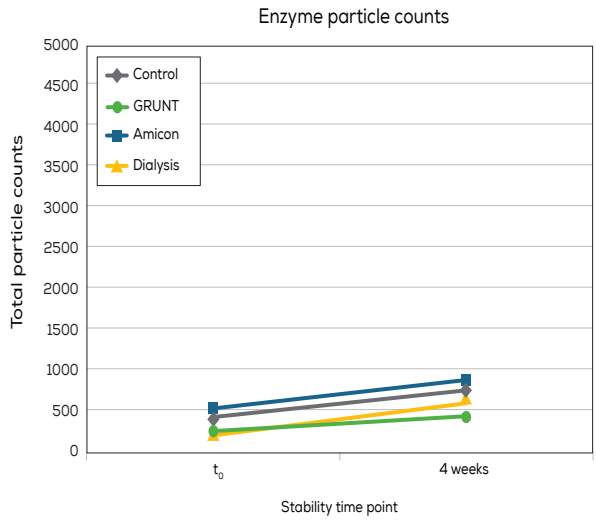

Figure 15: MFI results for 20 mg/mL enzyme buffer exchanged using GRUNT, Amicon devices and dialysis cassettes.

Conclusion

This study has shown that an automated buffer exchange system, GRUNT, provides comparable results to Amicon centrifugal UF/DF filters and dialysis. After comparing results from several biotherapeutic molecules, GRUNT clearly meets or exceeds the same standards of protein recovery, pH accuracy and protein stability at T_0 and after storage for six weeks at 40 °C as compared to centrifugal UF/DF filter and dialysis methods. Typically, formulation preparation, buffer exchange and sample concentration require 2–4 days of a scientist's time. Automating this process with GRUNT increases the number of formulations and protein concentrations they can evaluate within project timelines. The ability to prepare 12 formulations at high concentrations at a small scale also allows scientists to evaluate high protein concentrations earlier in development to assess developability and manufacturability. GRUNT's automated workflow can increase the repeatability and reproducibility of formulation preparation across projects, departments and campaigns.

Unchained Labs' GRUNT:

- Does not significantly alter protein stability.
- Is compatible with a wide variety of proteins.
- Achieves ≥96% exchange for most proteins.

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