

Comparison of Commercial Protein Concentrators Using

Bovine Serum Albumin

Gabrielle Boyd², Alena Calm¹, Kelley Betts³

¹US Army Edgewood Chemical Biological Center, APG, MD ²Excet. Springfield, VA ³Leidos Inc., Reston, VA



Abstract

Reliance on a single vendor for a particular reagent can bring risk to a research project's cost, schedule and performance. Evaluating alternate vendor sources for a reagent helps reduce this risk and ensures that work can continue should a particular reagent source stop production. Here, six commercially-available protein concentrating methods were compared for efficiency and consistency in concentrating purified Bovine serum albumin (BSA), a serum albumin protein which is often used as a protein concentration standard in many laboratory experiments. Methods used in this study employed either ultracentrifugation or a dialysis concentrating solution to concentrate the BSA. Of these six devices, the Pierce 10K ultrafiltration concentrator was favored due to its simplicity, time efficiency and high percent recovery.

Background

The ability to quickly and efficiently concentrate purified protein is of considerable value to both commercial and research communities. Protein concentrating methods are often used to increase concentration for downstream purification strategies or crystallization. Concentrating methods used must also ensure that proteins are not denatured, or lose activity or binding function. Whether it be resolving protein loss during purification, concentrating enzymes or antibodies in solution, or providing other down-stream protein analysis, it is vital that the methods used be both reliable and efficacious. When selecting a proper protein concentration method, two of the critical aspects to consider are the original volume of sample, and the desired final concentrated volume. Ideal concentration methods should be relatively quick, consistent in results, and capable of producing high percent yields.

Bovine serum albumin (BSA): BSA was chosen as it has a molecular weight of 66.4kDa which is within the suggested size of being at least 50% larger than the Molecular weight cut-off (MWCO).

Dialysis Cassette: Uses diffusion to trap proteins within a semi-permeable membrane while excess buffer solution exits the cassette. PEG-20 is typically used on the outside of the cassette in order to draw out the buffer. This method is dependent on time (figure 2.). To achieve a higher concentration, the incubation time is increased. There is no dead stop with this method.

Ultracentrifugation Filter: Uses centripetal force to draw the sample through a semi-permeable membrane that holds proteins back while letting the buffer solution pass through. Has a dead-stop volume to assure that some buffer remains, therefore preventing concentrated protein from getting stuck inside the dried membrane.

Materials & Methods

Ultracentrifugation and dialysis cassette kits are shown in Fig. 1. PEG-20 for the dialysis cassettes was made in-house, to replace the Pierce "concentrating solution" which is no longer commercially available. Ultrafiltration filters were pre-rinsed with sterile distilled water. The maximum allowed volume was used for each method. Percent Recovery was evaluated by the following calculation: % Recovery = (recovered material/starting material * 100). BSA concentrations were measured using a NanoDrop ND-1000 Spectrophotometer. Trials resulting in a percent yield over 100% were performed a second time.



Figure 1. Concentration Techniques
 1a. Pierce 10K MWCO 0.5mL concentrator; 1b. Pierce 9K MWCO 20mL concentrator; 1c. Spin-X UF 10K MWCO 6mL concentrator; 1d. Amicon Ultra-2 10K MWCO 2mL centrifugal filter; 1e. Vivaspin 2 10K MWCO 2mL concentrator; 1f. Pierce 7K MWCO 3mL dialysis cassette (3K cassette shown here).

Vendor	Product	Material	Time
Pierce	10K concentrator	PES	5 min
Pierce	9K concentrator	Cellulose	25 min
Pierce	7K concentrator	Cellulose	35 min
Corning	SpinX UF 10K	PES	10 min
Millipore	Amicon Ultra-2	Cellulose	20 min
Sartorius	Vivaspin2 10K	PES	8 min

Table 1. Vendors, Material and time to completion

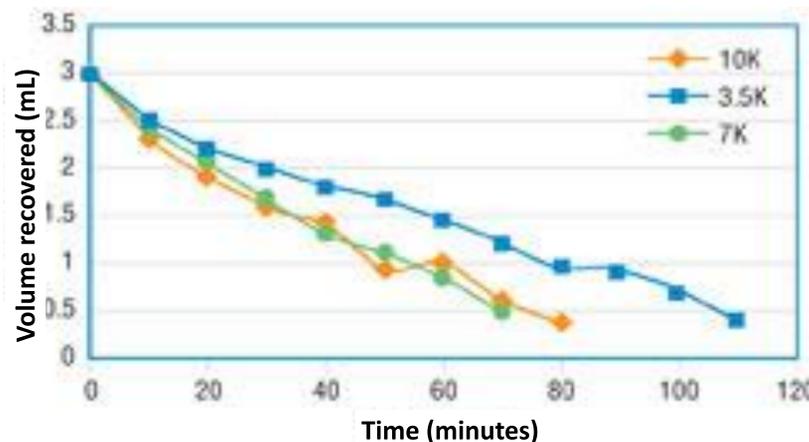


Figure 2. Concentration over time for Pierce concentrating solution

Results

Of the six devices tested, four provided logical percent recovery. The Viva spin (Sartorius) and Spin X (Corning) each produced percent recoveries greater than 100%. Three of the products (Pierce 10K, Amicon Ultra, Pierce 7K dialysis cassette) showed percent yields that were desirable (>90%). The Pierce 9K concentrator gave a poor recovery of 67.81%. The standard deviation of three runs for each device was calculated. From this, it was found that the Pierce 9K concentrator and Pierce 7K dialysis cassette provided the most consistent results.

Approved for Public Release

Concentration Method	Average % Recovery
Pierce 10K MWCO 0.5mL	97.7%
Pierce 9K MWCO 20mL	67.81%
Spin-X UF 10K MWCO 6mL	116.41%
Amicon Ultra 10K MWCO 2mL	94.81%
Vivaspin 10K MWCO 2mL	115.35%
Pierce Dialysis Cassette 7K MWCO 3mL	99.6%

Table 2. Percent Recovery

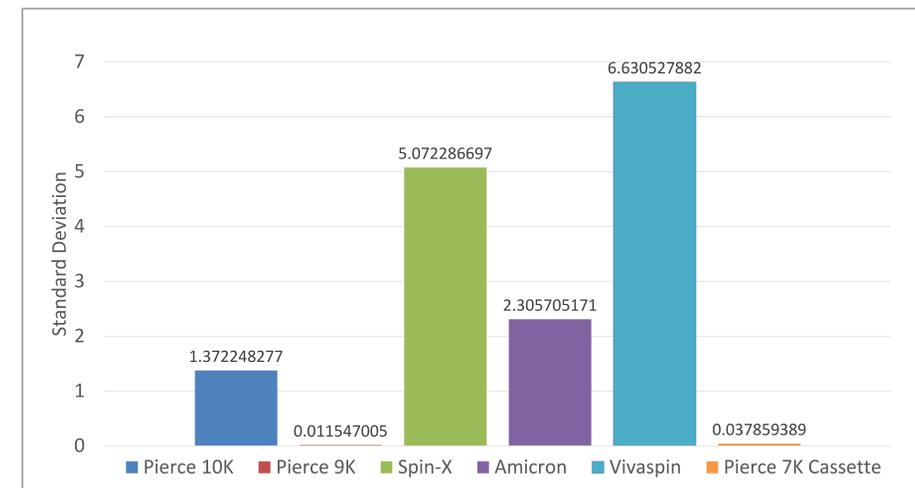


Figure 3. Consistency graph: This graph of the standard deviation of all concentration measurements shows that the Pierce 7K and Pierce 9K were the most consistent in terms of device reproducibility.

Discussion

The results of this project showed that, of the six concentration devices, the Pierce 10K, the Amicon Ultra, and the Pierce 7K dialysis cassette were most successful in providing ideal percent recovery. The Pierce 9K concentrator and Pierce 7K dialysis cassette obtained the most consistent results, however the Pierce 9K concentrator consistently underperformed, losing about 30% of the material during concentration. This was likely due to BSA binding to the membrane since the flow through was void of protein. The Viva spin (Sartorius) and Spin X (Corning) each produced percent recoveries greater than 100%. After working with tech support and following their advice to pre-rinse the membranes the yield was still over 100%; this could be due to leaching from the membrane. There were no correlations between performance and membrane material (Table 1.).

Conclusion

After evaluating six different concentration devices for use in the lab, the two that are recommended for use are the Pierce 10K ultrafiltration concentrator for its simplicity, time efficiency and high percent recovery. The Pierce 7K dialysis cassette while not as easy to use and not as fast as the ultrafiltration device, is as reliable with a great % recovery. It is more flexible with no dead stop so the user has the ability to push the limit of the concentration; and it is scalable up to 30ml.

TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.