	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ** **PRODUCTON**
Dialysis/Buffer Exchange **Module Hours: 3**	**Effective Date:10/2024** **PRQs:** **Buffers and stock solutions**	**Revision 1.0** **Author: Z. Hazlett** **Checked by** **Editor: M. Stowell**

Background

Many different types of buffers exist that can be prepared with a wide variety of buffering components. On occasion, a buffering solution needs to be exchanged with a different buffering solution to serve a specific purpose for the components contained within. This buffer exchange could be necessary for a protein solution recently in lysis buffer conditions that needs to be put in a buffer suitable for protein purification. It could be beneficial for changing conditions between purification steps, or even after purification to provide stable conditions for long term storage. Whatever the case, buffer exchange is a common laboratory technique that can be adapted for a variety of situations.

Buffer exchange can be achieved in a variety of ways. Buffers can be exchanged via repeated steps of sample dilution with a new buffer. In this case, one can place their sample in a semi-permeable dialysis membrane and place the membrane in a large volume of the new desired buffer. As the dialysis membrane containing the sample of interest soaks in the new buffer, buffering components and salts are exchanged via osmosis. Eventually the permeable components of the solution and buffer will come to an equilibrium (Figure 1). This process can be repeated for as many times as necessary to dilute out any undesirable components and place your sample in the new buffering conditions.

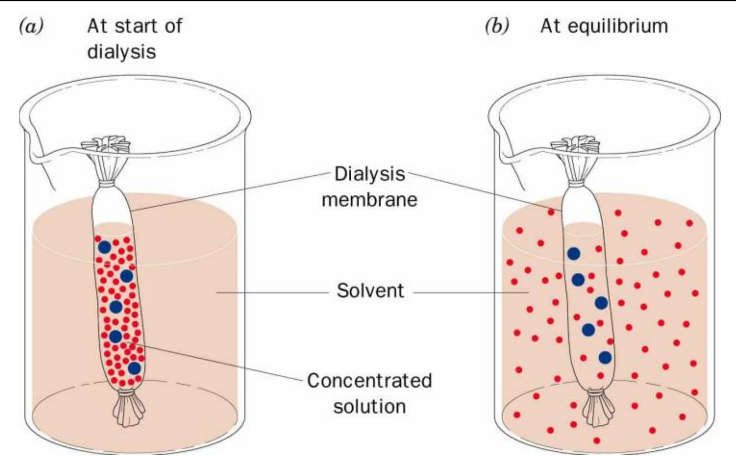

Figure 1. Dialysis of a solution contained within a semipermeable dialysis membrane. The dialyzed solution is shown before equilibrium (a) and at equilibrium (b).[1]

This principle can also be applied to buffer exchange of samples using repeated steps of sample concentration via filter centrifugation and dilution with the buffer of choice. One

downside to buffer exchange techniques that use repeated steps of concentration and dilution is that it is possible that undesirable buffering components might still be present at low concentrations if the process is not repeated enough. The benefit to these techniques is that these methods are low cost, do not require much equipment, and minimize the opportunity for sample loss during exchange.

Buffers can also be exchanged by column chromatography. For example, a high salt protein buffer can be exchanged for a low or no salt buffer by passing the sample over size exclusion chromatography. As this form of chromatography, that separates small molecules based on their size, does not depend on salt concentration, one can easily exchange buffers completely by passing through this type of column with the new buffer of choice. The downside of this technique is that column chromatography takes much more equipment, preparation, and sometimes time to achieve. There is also a much greater risk of sample loss with this method. The benefit is that the buffer of a sample can be completely exchanged into a new buffer with virtually no remaining contents of the old buffer. Choice of technique must be made based on the specific needs of the sample and experiment.

1. Purpose

The purpose of this SOP is to instruct students on the background and procedures necessary for dialysis and buffer exchange.

2. Scope

This procedure applies to qualified skills center users.

3. Responsibility

3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
3.3. It is the responsibility of the user to become trained on the procedure.

4. Definitions

4.1 Osmosis: The transfer of soluble components of a solution from higher to lower concentration across a semipermeable membrane until they reach equilibrium across both solutions.
4.2 Dialysis: The separation of particles in a liquid based on their ability to pass through or be filtered by a semipermeable membrane.
4.3 Centrifugal Filtration: The process by which a solution can be concentrated by centrifugation. A centrifugal filter is a centrifuge tube with a removable upper chamber that is lined with a filter at the bottom designed to allow molecules under a defined molecular weight to pass through but retain molecules above that molecular weight. When a solution is placed in this upper filter chamber and that filter chamber is placed in a larger collection tube, the lower chamber, this device can be centrifuged to filter the solution as it passes from the upper to the lower chamber. As described above, this device can be used to exchange buffers with repeated cycles of sample concentration and dilution with buffer of choice.
4.4 Column Chromatography: The general technique for describing the purification of small molecules by passing the solution through a column packed with a selective resin. Can also be used to exchange buffers in a solution as described above.

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5. Materials/Equipment

5.1 Solution to be dialyzed
5.2 Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassette (Fig. 2a)
Choose size of cassette based on total volume of solution to be dialyzed.
5.3 Glass beakers (for soaking dialysis cassette)
5.4 Serological pipette + sterile tips
5.5 Dialysis tubing foam float pads (Thermo Scientific Slide-A-Lyzer Cassette Float Buoys or similar device)
5.6 Dialysis buffer
5.8 2L bucket
5.9 Magnetic stir bar and stir plate
5.10 4° fridge
5.11 Amicon® Ultra-15 Centrifugal Filter (Fig. 2b)

(*a*.) (*b*.)

Figure 2. *a*) Dialysis setup using Slide-A-Lyzer™ Dialysis Cassette filled with sample, floating in dialysis buffer.[2] *b*) Amicon® Ultra-15 Centrifugal Filter to be used for buffer exchange by repeated steps of sample concentration via centrifugation and sample dilution using new buffer.[3]

6. Procedures

6.1 Consider any temperature requirements to keep your sample stable and dissolved in solution.
6.1.1 Some protein samples need to be kept on ice. If this is the case for your sample, make sure to prepare your dialysis buffer first and chill it at $4^{\circ}C$ prior to starting dialysis of your sample.
6.2 Prepare dialysis buffer.
6.2.1 Decide on the volume needed for your dialysis buffer. This all depends on the final desired conditions of your sample.

6.2.2 For example, if your sample volume is 200 ml, the sample buffer contains 1M NaCl, and your dialysis buffer does not contain any salt, dialyzing your sample in 2L of dialysis buffer to equilibrium will drop the concentration of salt in your buffer 10-fold (from 1M to 0.1M NaCl). These steps can be repeated as many times as necessary to exchange your sample buffer to the desired conditions.

Dialysis using Slide-A-Lyzer™ Dialysis Cassette

6.3 Prepare dialysis cassette (Fig. 2a)

6.3.1 Consider the molecular weight (MW) of any protein or small molecule that you do not want passing through the membrane into the dialysis buffer. Choose a dialysis cassette that has pores with a MW cut off value that is SMALLER than the MW of your small molecule of interest.

6.3.2 Consider the volume of your solution to be dialyzed. Choose a Slide-A-Lyzer™ Dialysis Cassette that will fit the full volume of your solution.

6.4 Place dialysis cassette in a glass beaker filled with ddH20 and incubate in water for 2 min to hydrate the membrane.

6.5 Carefully remove the cassette from the water and unscrew the cassette cap.

6.6 While holding the cassette securely, pipette your entire sample slowly into the dialysis cassette with a serological pipette.

6.7 Once your whole sample is transferred, gently squeeze out the remaining air from the cassette bag with a gloved hand. Screw on cap securely.

6.8 Submerge the entire prepared sample into your dialysis buffer. Add a stir bar to the bottom of your dialysis buffer, place on a stir plate, and set stir plate to gently stir your dialyzing sample.

6.8.1 If your sample needs to be chilled for it to stay stable, make sure your buffer was pre-chilled at 4ºC and place the whole stirring dialyzing apparatus in a 4ºC fridge.

6.9 Dialyze, stirring, for 4-6 hours or overnight for the sample to reach equilibrium.

6.9.1 Repeat steps 6.9-6.10 with fresh buffer each time as many times as needed to reach desired sample conditions.

6.10 When you are finished dialyzing, remove dialysis cassette, unscrew the cap and transfer your sample into a new clean container for next steps of your experiment (50 ml conicals, clean beaker, etc.)

6.11 Clean up. Record any notes along the way.

<u>Dialysis using Amicon® Ultra-15 Centrifugal Filter</u>
6.12 Prep enough buffer to reach the final desired concentration of new buffer components.
    6.12.1 For example, a buffer exchange from 1M NaCl to 1 mM NaCl (1:1000 dilution) would require 3, 1:10 dilutions. Using a centrifugal filter this could be accomplished by concentrating 15 ml of sample to 1.5 ml, diluting the solution up to 15 ml in buffer without salt (one 1:10 dilution), and repeating the process two more times.
6.13 Prepare centrifugal filter for use with your sample solution.
    6.13.1 Choose a centrifugal filter appropriate for the volume of solution to be dialyzed and with membrane pores smaller than sample to be dialyzed.
    6.13.1 Fill with ddH20 and centrifuge for 5 minutes, at 4000 g's, to hydrate the filter membrane. Discard water.
6.14 Add sample to upper chamber of centrifugal filter.
6.15 Concentrate by centrifugation at 4000 g's, 4ºC or room temp (depending on sample stability conditions), starting 5 min at a time to determine how quickly your sample is concentrating.
6.16 Concentrate till desired volume is reached. Dilute with new buffer as needed. Repeat steps 6.15-6.16 till buffer exchange has reached desired conditions.
6.17 Determine concentration of sample remaining in buffer exchanged solution.
6.18 Transfer to a new clean container for storage.
6.19 Clean up.

7. Troubleshooting

7.1 My filled flask does not float in the dialysis solution? Ensure that the flask capacity was not exceeded and that the flotation ring was attached. In some cases, the density of the solution in the flask may require reduced volumes for proper orientation during dialysis.

7.2 Contaminants are not removed completely from my dialysis experiment using the Slide-A-Lyzer™ Dialysis Flask? All molecules have different diffusion rates across membranes and may not have acted as other compounds of similar molecular weight. Increase dialysis time and/or number of buffer exchanges performed, or alternatively, use a device containing a higher molecular weight cut-off membrane**.**

7.3 After dialysis, my sample volume increased significantly. Water moves quickly and easily across the dialysis membrane. When dialyzing a high solute concentration against a dilute dialysis buffer, there will be a net movement of water (and possibly salts) into the dialysis unit through the membrane. Glycerol and some sugars are especially hygroscopic, and as rapidly as they diffuse across the membrane to reach equilibrium, they also significantly affect the osmosis of water across the membrane and so may cause a change in volume of the sample.

7.4 I lost a lot of my proteins during the dialysis. The dialysis membranes are made of regenerated cellulose, which may cause some molecules to stick nonspecifically resulting in sample loss. The percent of total protein lost is partially dependent on the protein concentration. Protein loss caused by nonspecific binding to the membrane is negligible for concentrated samples (>0.5 mg/mL) but may be significant with dilute protein samples (<0.1 mg/mL). Adding a “carrier” protein such as BSA to dilute protein sample before dialysis can help prevent this loss.

8. References

1. A. William 2020. Selective Permeability of Dialysis Tubing Lab: Explained. Retrieved from https://schoolworkhelper.net/selective-permeability-of-dialysis-tubing-lab-explained/
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3. Amicon® Ultra-15 Centrifugal Filter Units. *Millipore Sigma*. Retrieved from: https://www.emdmillipore.com/US/en/product/Amicon-Ultra-15-Centrifugal-Filter-Units,MM_NF-C7715

9. Module Mastery Task

9.1 You will be preparing and buffer exchanging a solution containing protein (BSA) and free dye (fluorescein) using the two methods described above. What is the wavelength of peak absorbance for each? What is the extinction coefficient for each sample being dialyzed at those peak absorbance wavelengths?

9.2 Prepare 3 ml of a **10 µM Fluorescein and 0.5 mM BSA** mixture. Show math.

9.3 Split the 3 ml solution into two, 1.5 ml solutions. Using the methods described above, buffer exchange each 1.5 ml solution into water using either dialysis buffer exchange or filter centrifugation. You will need dialysis membranes or filters with MW cutoff values below the MW of BSA and above the MW of fluoresceine. Buffer exchange until the Fluorescein concentration in solution is ≤ 0.1 µM.

> A) Calculate MW range of the filter you need. Which size of membrane filter did you choose for each method?
> B) Describe the steps required to accomplish the desired buffer exchange. Describe how your plan will result in a solution with ≤ 0.1 µM.

9.4 Describe expected results given the differences in buffer exchange between the two methods.

9.5 Record concentration of Fluoresceine and BSA in solution before and after buffer exchange. Do your results match the predicted results? Why or why not?

9.6 Compare the efficiency of each method in regards to both time and sample recovery.