USES OF DIALYSIS IN PURIFICATION OF PROTEIN

DR . DHIA . F AL-FEKAIKI

FOOD SCIENCES- BIOCHEMISTRY ENZYMES

الأستاذ المساعد الدكتور ضياء فالح الفكيكي

كيمياء الحيوية – انزيمات

قسم علوم الأغذية – كلية الزراعة

جامعة البصرة

2019

DIALYSIS

ديلزة (كيمياء حيوية) Dialysis (biochemistry)طريقة لفصل المواد الكيميائية بالانتشار عبر غشاء نصف نفوذ semitransparent.يقوم الغشاء بدور منخل ذي ثقوب صغيرة تسمح بمرور الجزيئات والشوارد الصغيرة، وتحول دون مرور الكبيرة منها. استعمل مصطلح الدَّيلَزة لأول مرة من قبل الكيميائي الاسكتلندي توماس گراهام Thomas Graham، الذي استخدم عام ١٨٦٦ غشاء لفصل السكر عن الصمغ العربي

تستخدم عملية الدَّيلَزة لفصل الملح عن البروتين، فيوضع محلول البروتين المشوب بالملح في كيس قماشي يقوم بدور الغشاء، ثم يغمس الكيس في الماء النقي فينتشر الملح إلى الماء عبر الكيس، ويبقى البروتين داخله.

تتأثر عملية الدَّيلَزة بنوع الغشاء تأثراً كبيراً. فمثلاً لا يَسمح غشاء من المطاط بمرور الماء، لكنه يسمح بمرور سائل البنزن C6H6 على الرغم من أن جزيئاته أكبر من جزيئات الماء. ويعود السبب إلى أن جزيئات البنزن تنجذب نحو سلاسل المطاط التي ترتبط فيما بينها بطريقة الفلكنة Vulcanization.

General Protocol

A typical dialysis procedure for protein samples is as follows:

Prepare the membrane according to instructions

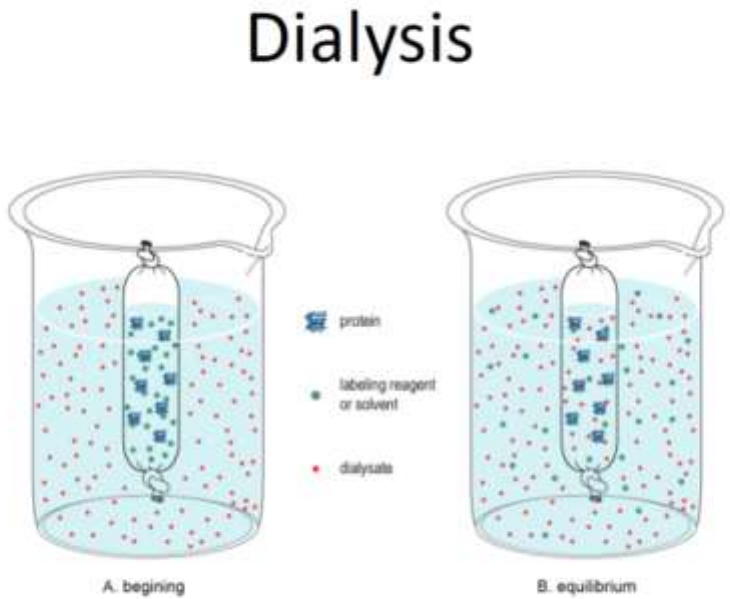
Load the sample into dialysis tubing, cassette or device

Place sample into an external chamber of dialysis buffer (with gentle stirring of the buffer)

Dialyze for 2 hours (at room temperature or 4 °C)

Change the dialysis buffer and dialyze for another 2 hours

Change the dialysis buffer and dialyze for 2 hours or overnight



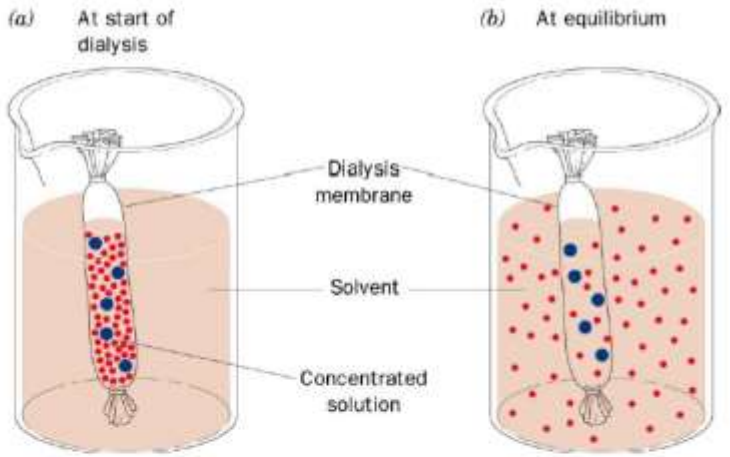
The total volume of sample and dialysate determine the final equilibrium concentration of the small molecules on both sides of the membrane. By using the appropriate volume of dialysate and multiple exchanges of the buffer, the concentration of small contaminants within the sample can be decreased to acceptable or negligible levels. For example, when dialyzing 1mL of sample against 200mL of dialysate, the concentration of unwanted dialyzable substances will be decreased 200-fold when equilibrium is attained. Following two additional buffer changes of 200mL each, the contaminant level in the sample will be reduced by a factor of 8 x 106 (200 x 200 x 200).

Uses of dialysis

- To remove unwanted small molecules from a protein solution

  - DNA
  - salts
  - detergents
  - small proteins

- For buffer exchange

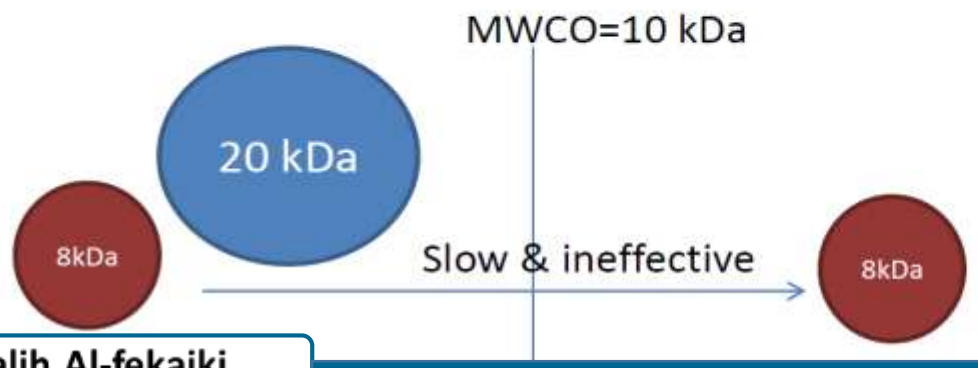


The dialysis membrane

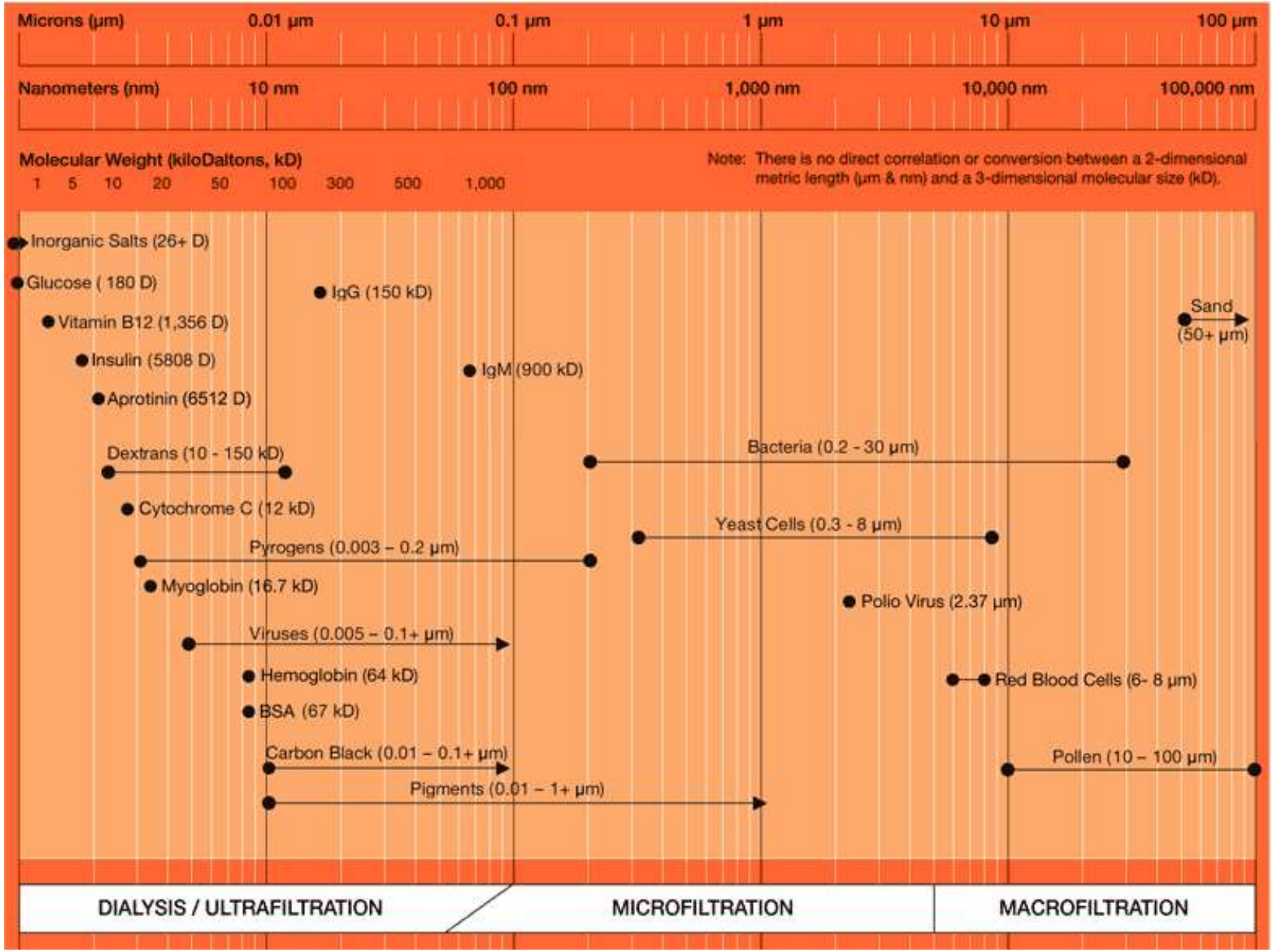
- Contains pores at different sizes
- Molecular weight cutoff (MWCO)- the average pores size

  MW>MWCO - molecule will not transfer

  MW<MWCO – molecule will transfer
- MW<<MWCO transfer faster than MW<MWCO



This table gives indicative correspondence of MWCO and membrane porosity or molecular size for globular molecules. Take correspondence with care because this may vary on many factors (shape, charge, viscosity...).



DIALYSIS MEMBRANES

Dhia Falih Al-fekaiki

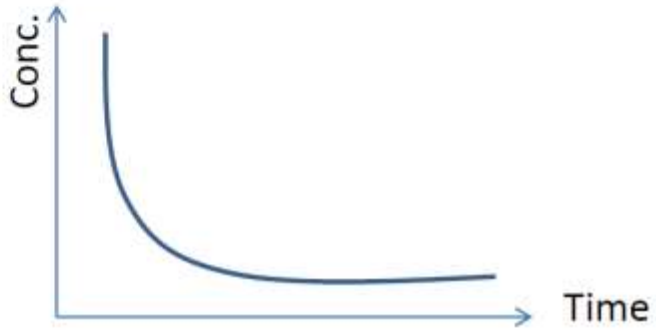
General Protocol:

- Choose the membrane due to protein size.
- The “old” membranes are with cut-off of 13 kDa
- Load the sample into dialysis tubing.
- Place sample into an external chamber of dialysis buffer (with gentle stirring of the buffer).
- Dialyze for 2-4 hours
- Change the dialysis buffer and dialyze for another 2-4 hours
- Change the dialysis buffer and dialyze for 2 hours - ON.

Volume:

- For 10ml sample of 1M in 10L buffer – sample will reach to 1mM at equilibrium (~4h)
- Same sample in 1L – 10mM after 4h

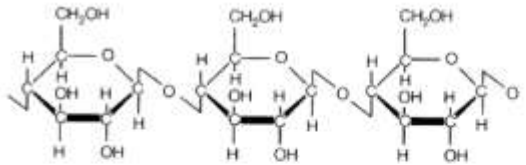
  Then replace buffer 1L – 0.1mM after 4h.
- Time vs. buffer



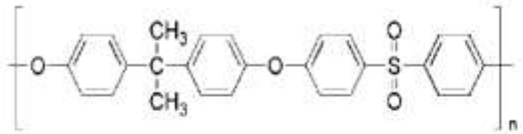
Types of membrane

- There are more then 30 types of synthetic and natural dialysis membranes

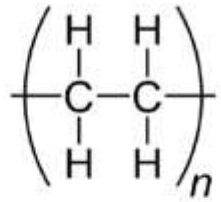
Cellulose



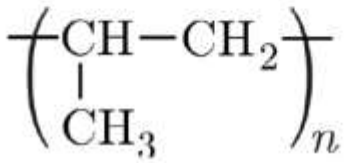
Polysulfone



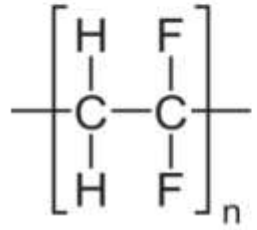
Polyethylene

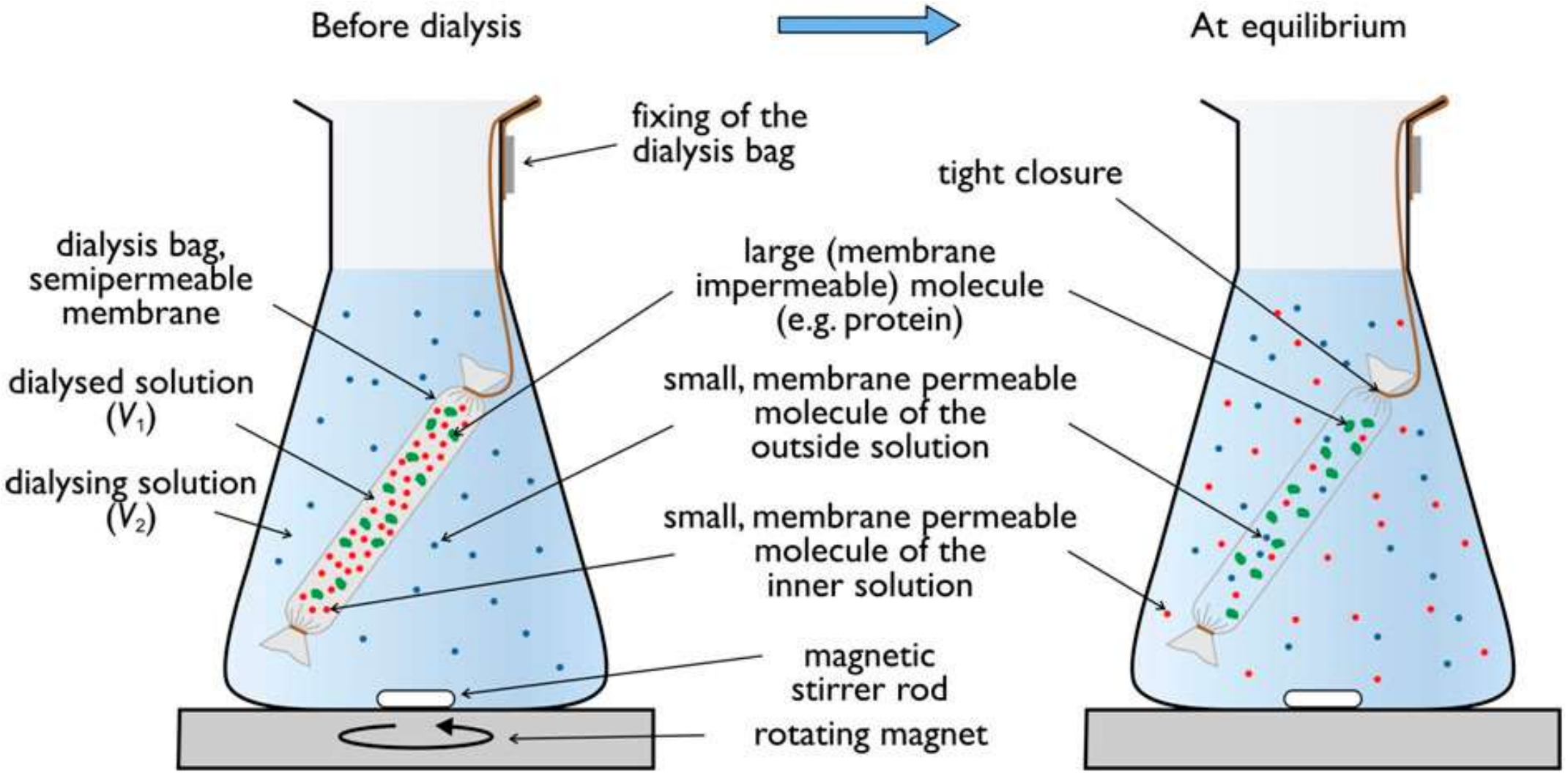


Polypropylene



Polyvinylidene fluoride




Before dialysis
At equilibrium
fixing of the dialysis bag
tight closure
dialysis bag, semipermeable membrane
dialysed solution (V_1)
dialysing solution (V_2)
large (membrane impermeable) molecule (e.g. protein)
small, membrane permeable molecule of the outside solution
small, membrane permeable molecule of the inner solution
magnetic stirrer rod
rotating magnet

FAQ: How long should I cut the dialysis tubing?

Total length = (sample volume) / (vol/length) + (additional 10-20%) + 4 cm (for the knot or clamp)

Along with each tubing flat width, the correlating volume/length ratio are generally indicated, and so can be used to calculate how much length is required to contain your sample volume. For example if the Flat Width is 16 mm, the volume/length ratio is 0.79 ml/cm. To contain a sample of 5 ml, you will need a length of approximately 6.5 cm. However, you also need to add about 10 to 20% more length ato prevent osmosis, and as head space
(an air buble willto keep your sample buoyant). Lastly you need to add enough about 2 cm at each end to allow for applying two closures. The total tubing length would be at least 11.5 cm. The simple equation to calculate total required tubing length is as follows:

Q: How much volume of dialysate is needed to dialyze a sample and how often does the dialysate need to be changed?

A:

Dialysis buffer volume depends on the number of dialysis steps you are doing, and their duration!
The larger the dialysate volume, the greater the driving force for diffusion of small molecules.
We generally recommend a +100:1 buffer to sample volume ratio. By replacing the buffer just as the rate of diffusion slows down and the solutions are approaching equilibrium, you can maintain the driving force and the rate of dialysis

We generally recommend two or three buffer changes over the period of 12 - 24 hrs as follows:

First buffer change: volume 100:1 during 0.5-3 hours
Second buffer change: volume 100-300:1 during 4-5 hours
Last buffer change: volume 500:1 overnight

FAQ: Which dialysates (buffers) are commonly used in dialysis?

Biomolecules must be maintained under strict pH control to stabilize their molecular properties. The typical pH range for dialysis buffers is 6 to 8. The following are some of the common solutions/buffers found in biochemical solutions:
Water

PBS: Phosphate buffer saline
TBS: Tris buffered saline
HEPES
Amino Acid Buffers

FAQ: What is the shelf life for dialysis membranes?

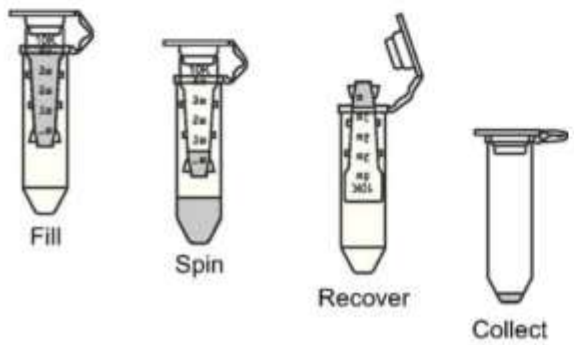
The dry packaged dialysis membranes have a shelf-life of 5 years. The wet packaged (0.05% sodium azide solution) membranes have a shelf-life of 3 years. The irradiated membranes have a shelf life of 1.5 years.

FAQ: Choice of dialysis tubing size /format

The size of the tubing or dialysis device depends directly on your sample size, and eventually of general consumption in your lab. Dialysis devices are a more flexible choice in labs with lower et more various applications, and also more convenient: ready to use!

Ultrafiltration or dialysis

- Ultrafiltration is faster than dialysis and requires less buffer
- Protein will be concentrated during ultrafiltration



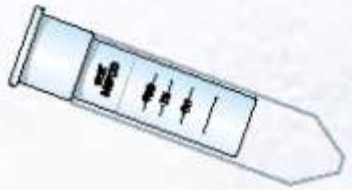
Ultrafiltration

- A process that uses semi-permeable membranes to separate macromolecular species on the basis of size.
- It is particularly appropriate for the concentration of macromolecules, partial purification or for buffer exchange.
- Is a gentle and non denaturing method.
- The ultrafiltrate is cleared of macromolecules which are significantly larger than the cutoff of the filter
- The buffer concentration in the ultrafiltrate will be exactly the same as in the concentrate
- Do not replace GF, although the principles are the same: separation according to ratio of the molecule
- Proteins with MW lower than the cut-off, will be retained in the concentrate if they aggregate, or are part of a complex
- Cut-off at least two or three times of the protein size
- Some proteins can stick to the membranes

Ultrafiltration

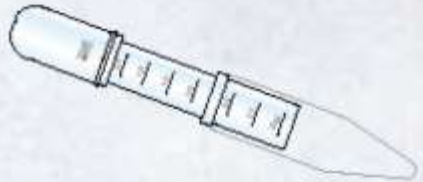
VIVASPIN 500

Process Volume:	500µl to 5µl
Operating Mode:	Centrifuge
Pages:	6 - 9



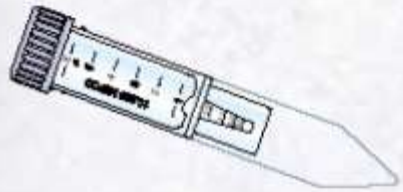
VIVASPIN 2

Process Volume:	2ml to 8µl
Operating Mode:	Centrifuge
Pages:	6 - 9



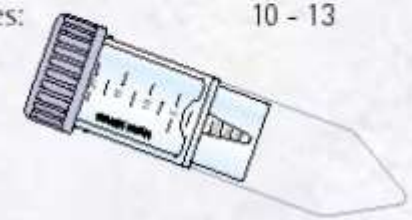
VIVASPIN 6

Process Volume:	6ml to 30µl
Operating Mode:	Centrifuge
Pages:	10 - 13



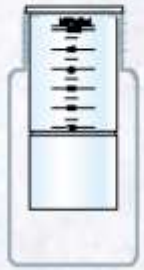
VIVASPIN 20

Process Volume:	20ml to 50µl
Operating Mode:	Centrifuge Gas Pressure
Pages:	10 - 13



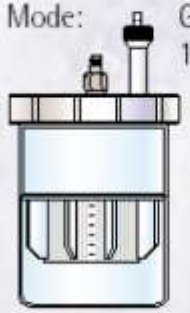
VIVACELL 70

Process Volume:	70ml to 150µl
Operating Mode:	Centrifuge Gas Pressure
Pages:	14 - 15



VIVACELL 250

Process Volume:	250ml to 600µl
Operating Mode:	Gas Pressure
Pages:	16 - 17



Selecting Hollow Fiber Cartridges and Systems

According to GE Healthcare

Application	Ultrafiltration (NMWC)	Microfiltration (µm)
Bacterial/pyrogen removal	10,000	
Protein concentration	3,000, 5,000, 10,000, 30,000	
Enzyme concentration	10,000, 30,000, 50,000	
Virus concentration/purification/removal	100,000, 300,000, 500,000, 750,000	
Protein/antigen recovery from fermentation broth	500,000, 750,000	0.1, 0.2, 0.45, 0.65
Bacterial cell concentration	500,000	0.1, 0.2
Insect cell concentration		0.1, 0.2
Mammalian cell concentration		0.2, 0.45, 0.65
Yeast concentration		0.1, 0.2, 0.45
Continuous cell culture perfusion		0.1, 0.2, 0.45
Red blood cell washing		0.45, 0.65
Red blood cell stroma removal	500,000	0.1
Hemoglobin concentration	5,000, 10,000	
Peptide concentration	1,000, 3,000	

Table 2. Recommended pore sizes for select applications

Ultrafiltration

According to Vivascience

Solute Fractionation or Clarification

- Membrane cut-off: 5000 -10000 - 30000 - 50000 & 100000 MWCO
- During filtration, the permeating solute remains at its initial concentration whilst the retained macromolecules will be enriched.

Protein Desalting or Buffer Exchange

- The protein solution may be purified from salts, non-aqueous solvents and generally from low molecular weight materials.
- Multiple solvent exchanges, will progressively purify macromolecules from contaminating solutes.
- Diafiltration: Microsolutes are removed most efficiently by adding buffer to the solution being ultrafiltered at a rate equal to the speed of filtration.

https://scholar.google.com/citations?user=EFkC7y4AAAAJ&hl=en

http://www.researcherid.com/rid/F-1710-2019

https://independent.academia.edu/Dhiaalfekaiki

https://www.mendeley.com/profiles/dhia-falih-al-fekaiki3/

https://www.researchgate.net/profile/Dhia_Al-Fekaiki

https://orcid.org/0000-0002-7510-5881

https://www.linkedin.com/in/dr-dhia-al-fekaiki-60265186/

https://publons.com/researcher/1718509/dhia-al-fekaiki/

Dhia Falih Al-fekaiki

Food sciences- Biochemistry /enzymes