NT-Dialya

Dialysis

Technical tips: Principle of dialysis | Advantages and main applications
Choosing the right membrane:
Cut-Off MWCO | Membrane type | Porosity | Permeation rate | Membrane compatibility chart

Applications: Desalting / Buffer exchange
Sterile dialysis
Dialyse à l'équilibre - Fraction liée/libre : information | Equilibrium Dialysis products

FAQ – choosing the right membrane

What dialysis membrane should I choose?(type, MWCO, size/format)
Which membranes minimize protein binding the best?
What is the shelf life for dialysis membranes?
Are dialysis membranes endotoxin free?
What size of tubing size to choose / or dialysis device?
What MWCO of tubing size to choose?
How accurate are the membrane pore sizes and what is MWCO?
What are Daltons (MW) –why not microns unit?- and how do you convert Da/µm?
What is the difference between Spectra/Por 2 and 4 dialysis membranes?
Membrane Porosity – convertion Dalton / Microns?
and see Tech tip: choosing the right membrane(Cut-Off, Porosity, Membrane type)

FAQ – using dialysis membranes

How do you prepare dialysis membrane tubing?
How long should I cut the dialysis tubing?
Should I close the dialysis tube using closures or tyieng knots?
How to select the right closure and closure size for membrane tubing?
Is there a "rule of thumb" regarding membrane surface area to sample volume?
How much volume of dialysate is needed to dialyze a sample and how often does the dialysate need to be changed?
How how long does dialysis take to complete -does dialysis work-?
How how long does dialysis take to complete -does dialysis work-?
Can dialysis membranes be re-used for the same protein samples?
Can dialysis membranes be chemically or heat sealed?
Which dialysates (buffers) are commonly used in dialysis?
How to sterilize dialysis membranes / devices
How much pressure can a dialysis membrane withstand if used for ultrafiltration?
Why is the volume of my sample increasing during dialysis / my dialysis has leaked or has burst!
and see Tech tip: using dialysis membranes | Sterile dialysis

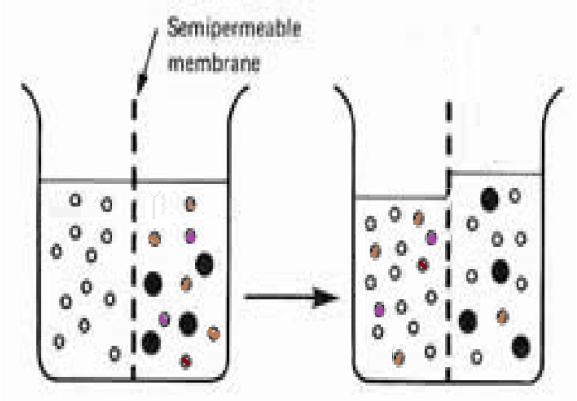
FAQ – deeper insight in dialysis

What is dialysis versus osmosis?
How good is the mass transfer across the membrane if the osmolarity is equal on both sides but concentration gradients still exist?
and see Tech tips: Principle of dialysis | Permeation rate | Membrane compatibility chart

Dialysis principle and operating

Dialysis principle

The dialysis process of a solute occurs when its concentration differs between each side of an hemipermeable membrane surface. This process is affected by the variables of temperature, viscosity and mixing rate of a solution.



The movement of a solute across a semipermeable membrane is the result of random molecular motion. As the solute molecules in a solution move, they will collide from time to time with the membrane until they diffuse.
Diagram: The permeation of a given small solute (•) from a solution on the right of the membrane to the left, and back again, will depend upon the frequency of collisions between the molecules on either side of the membrane. Larger molecules (●) will not cross the membrane.

For example, if the concentration of solute •, in a s&ample solution (right) is 100 mM, and solution left is 10 mM, the probability of Y-solute • colliding and crossing the membrane is much higher than in opposite sens. Therefore, the net rate of transfer of a given solute (at a certain temperature, viscosity and mixing rate) will increase with greater concentration differences between the two solutions.

FAQ: What is dialysis versus osmosis?

Dialysis is the passage of molecules with molecular/size across an hemipermable membrane, from a compartment with a higher solute content to the lower concentration compartment (see above).
Osmosis is similarly, the passage of water (or other solvent) across an hemipermeable membrane from a compartment with a higher water content to the lower content compartment. In fact, one should consider the difference of "oncotic pressure", that is driven by solutes dissolved in the buffer in each compartment.
other said, the osmosis process is linked to dialysis one, being a diffusion of molecules through an hemipermeable membrane, but applies to the solvent (water) and not to the small samples compound to be removed by dialysis.
Dialysis usually restrains osmosis, because it participates to equilibrate small hydrophilic compounds in the 2 compartments. It can be more rapid or, most often, longer to take place.
+
Theses consideration are generally with few importance when performing dialysis, provided one know that the sample volume may inflate 1-20% and consequently have forseen the dialysis chamber volume to avoid burst and leaking of sample. See FAQ

FAQ: How good is the mass transfer across the membrane if the osmolarity is equal on both sides but concentration gradients still exist?

Most dialysis is done with no osmotic pressure across the membrane. The dialysis process is driven by the concentration gradient from the inside and outside of the dialysis tubing. If there is a large difference in osmotic pressure, water will move across the membrane. If too much water migrates across the membrane, the dialysis tubing can potentially burst or collapse, depending upon the direction of water movement.

Advantages and applications

ADVANTAGES OF DIALYSIS

- Very Gentle Conditions
- Easy Operation
- Wide Range of Sample Volumes
- Many Membrane Types & MWCO's
- Inexpensive Materials
- Disposable Membranes & Devices

DIALYSIS APPLICATIONS

- Macromolecular Purification
- Protein Concentration
- Solute Fractionation
- Contaminant Removal
- pH Change
- Desalting
- Buffer Exchange
- Binding Studies
- Electro-elution
- Sample preparation analysis (MS, electrophoresis, Immuno- or Cell- assays)
- Cell culture

Selecting "the Right Membrane"

FAQ: What type of dialysis membrane should I choose?

The key to a successful dialysis separation is using the RIGHT MEMBRANE under the right conditions.

In most applications, the choice amongst available membrane types is not difficult. It is driven essentially by the MW of molecules to keep and MW of molecules to remove to define membrane MWCO, typically 10KDa with RC type membraens. Additionally, special membranes may be preferred for demanding application, when considering undesired adsorption of biomolecules of interest (i.e. proteins), the compatibility (resistance) toward solvents, requirement of strongest dialysis rate or mechanic strength, ...

On can follow below steps to select a membrane:

Select **membrane type/grade** (CE, RC or PVDF) based on application: Choose typically

- CE membranes for standard applications
- RC membranes for more accurate molecular separation and compatibility with organic solvents
  - Standard RC membranes (CelluSep T1) are ideal for routine dialysis (economic), or S/P 1 to 7
- Biotech CE,RC (CelluSep H1, S/P Biotech) when no cleaning required (purer synthetic membranes without metals traces).
- PVDF or PE or PES membranes for large MW molecules, and harsh conditions
  (compatibility with acids, bases, high temperature/autoclave...) – available now only in HC or RTU formats

⇨ See more insight below at 'choice of membrane type'.

⇨ See also if you need sterile dialysis: sterile membranes are available now only in

Select **membrane MWCO** based on solutes sizes (Molecular Weight),
the one(s) to be retained iin dialysis compartement, and (often less critical) the one that should be removed.

- Choose typically a MWCO at mid-range between the MW of solutes to remove and analytes to keep.
  - 10KDa suits mots applications, provide MS of samples >15KDa and compounds to remove are <8Da

⇨ See below Choice of Cut Off, Membrane Porosity/MWCO chart.

Select **membrane configuration/format/size** based on sample volume and number:

- Tubings for routine and economic dialysis such as CelluSep –
- Disposable ready-to use devices such as FloatALyser & GebaFlex for small samples and limited series, and convenient use
- Sacks for large volumes, Flat sheets for dialysis systems
- Microfibers for process dialysis and filtration systems (easy scaling, higher through flow).
- Tubing for medium to large volumes, RTU devices for (very) small volumes, fiber for Liters

See suitable sample volumes in each product line presentation.

Please see at Dialysis selection guide.
Please ask interbiotech@interchim.com if you don't find enough help in below technical notice.

Choice of Membrane type – Nature, Compatibility

● *Quel type de membrane choisir ?*Les membranes sont proposée en 2 matériaux principaux, l'acétate de cellulose (CE) et la cellulose régénérée (RC). Elles se distinguent par leur résistance physique et chimique. Les matériaux plus résistants (PVDF, Nylon) ne sont désormais disponibles qu'en microfibres.

Biotech Cellulose Ester (CE)	wide range of selectivity & purity
Biotech Regenerated Cellulose (RC)	combined selectivity, purity & organic resistance. Available in RTU devices (FAL, TAL)
Standard Regenerated Cellulose (RC)	" same. + broader option: MWCO, flat sheet.. Dialysis sacks. lower cost lab applications.
CelluSep Regenerated Cellulose (RC)	" same. + More economic. But less MW options.

Résistance à la température:
RC (autoclavable) > Biotech RC > CE
Résistance aux solvents acides, alcalins :
PVDF > RC ≥ CE.
Fixations non spécifiques: (protéines globulaires):
CE < RC < PVDF.

See also the detailled chemical compatibility chart below.

Chemical nature and structure of membranes

Cellulose Ester (CE) is the first intention choice for biochemistry works (proteins), but may have limitation for diluted proteins because of low resistance to some organic solvents, and even alcohols or other compounds*.
CE is a generic term, including **Cellulose Acetate (CA)** –its main component-, and **Mixed Cellulose (ME)**, depending on the CA content.

Regenerated Cellulose (RC) provides more accurate molecular separation and better compatibility with organic solvents: RC is more resistant to chemicals*. It is also purer and has narrower pore size.
RC is thus preferred for nucleic acids and for proteins, when they should be precipitated with organic solvents, or which structure or state can be affected by traces of metals. They absorption of protein is low or higher than CE (depends on protein nature. Binding of globular protein is slightly higher).
Limitations may come from the fact they are a little more expensive, and may not be available with low or large MWCO (<3000Da, >50 000Da), and they need to be soaked and rinsed before use (except the products offered already cleaned and wetted (S/P6; FAL.) or specially pure grades (CelluSep H1; S/P7, Biotech RC).

The polymers in the cellulose ester (CE) membrane cross-link to form a more rigid molecular lattice.. The **opaqueness** comes from the pores in a more rigid frame. The larger the pore size, the more opaque the membrane. In comparison, regenerated cellulose (RC) polymers form a more flexible lattice structure, hence translucid.

PVDF, PE, PES, Nylon and PC are for dialysis of large MW compounds, and harsh conditions (compatibility with acids, bases, high temperature/autoclave...), and are available now only in HC or RU formats. They have higher protein adsorption level than CE and RC. PVDF material can be sealed by heating.

see compatibility chart for compatibility level of thedifferent membranes toward different substances.

Grades of membranes
Standard membranes (CE, RC: CelluSep T1, S/P 1 to 7) for routine applications
Biotech CE,RC and PVDF (CelluSep H1, S/P Biotech) for more pure membranes (synthetic membranes without metals traces: no cleaning
Please ask interbiotech@interchim.com for more information.

FAQ: Are dialysis membranes endotoxin free?
Most membranes are intended for laboratory use, not checked for endotoxin. Please inquire +

FAQ: Which membranes minimize protein binding the best?
Each type of membrane displays a different affinity for various molecules. For globular proteins, the relative binding affinity is CE < RC < PVDF.

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.

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!

FAQ: What size fof tubing size to choose?

● *Quel dimension de tube de dialyse choisir ?*
Il s'agit de choisir un diamètre adapté à la taille de l'échantillon (tenir compte du volume contenu par une longueur de 1cm de tube de dialyse indiquées dans les tableaux de référence) pour avoir une hauteur (et surface d'échange) suffisante, plus une longueur en bas et en haut pour fermer le tube (clamp ou nœud)

Les tableaux de référence indiquent le volume contenu par une longueur de 1cm de tube de dialyse. Choisir le diamètre du tube pour avoir 1-3cm de hauteur d'échantillon pour les microvolumes et diamètres, et jusqu'à 50cm maximum (ou la hauteur de vos récipients pour le bain de dialyse). Prévoir que l'échantillon occupe en fait plus que cette longueur théorique, et notamment après dialyse (osmose : jusqu'à 10-30% de volume), et qu'il faut une longueur en bas et en haut pour fermer le tube (2-3cm pour un clamp, 3-7cm pour un nœud)

Le choix dépend aussi éventuellement d'options liées aux besoins du labo,
-opter pour du **tubing**, en rouleau de 10m ou plus, est interessant pour une consommation régulière. Prendre plus un diamètre correspondant aux volumes supérieurs moyens traités.
-opter pour des **dispositifs de dialyse** permet plus de flexibilité selon les besoins du labo, sur les tailles d'échantillons traités (et les MWCO + coté prêt à l'emploi).

Choice of MW Cut Off

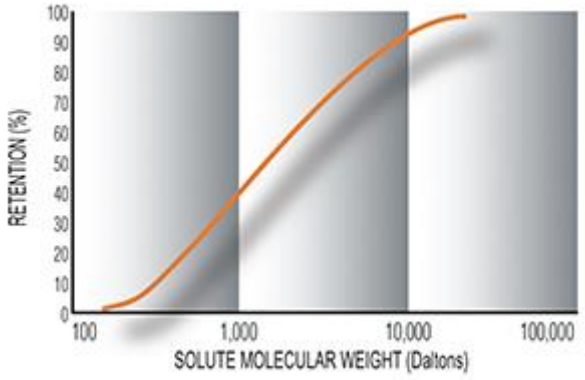
FAQ: What MWCO of tubing size to choose?

As a **general advice**, choose a MWCO intermediate between the MW of the compound(s) to eliminate, and the compound(s) to retain.
I.e. choose a 10KDa membrane to separate salts (<1000Da, and even peptides (2-6kDa) from large proteins (>20KDa). But a 10KDa membrane will give poor dialysis rate if too close from the MW of compound to eliminate, and mediocrous yield if too close ftom the MW of the compound to retain.

Ideally, the MWCO should be 10-fold that of the solute to eliminate, and at least 2-fold less that the molecule to retain.

For **large compounds or particules**, choose a MWCO depending on rather the MW of the substance to be removed.
I.e. one typicall y choose a 10 000Da MCO to remove salts, whatever the size of large compound is. But a MW of 100 KDa(ca0.01µm) up to 1 000 KDa(ca 0.1µm) may be preferable if you should remove efficiently bulky salts (500-2000Da) or peptides (1-10KDa), or even proteins (10-50KDa).

More: fundamentals:
Since the dialysis membrane consists of a spongy matrix of crosslinked polymers, the pore rating referred to as Molecular Weight Cut Off (MWCO), is an indirect measure of the retention performance. More precisely, the membrane MWCO is determined as the solute size that is retained by at least 90%. However, since a solute's permeability is also dependent upon molecular shape, degree of hydration, ionic charge and polarity, it is recommended to select a MWCO that is half the size of the MW of the species to be retained and/or twice the size of the MW of the species intended to pass through.



The MWCO should be chosen as high as possible in order to maximize the dialysis rate. However, in order to achieve a higher sample recovery you can select the MWCO that is about half of the molecular weight of the macromolecules that need to be retained. Alternatively, choice a MWCO intermediate between large molecules to be retained , and smaller molecules to be removed.
For Applications in which separation of molecules is required, there must be at least a 5x difference between the molecular weight of both species for membrane dialysis to be effective. Otherwise, you may require other separation techniques such as chromatography or TFF filtration.

FAQ: How accurate are the membrane pore sizes and what is MWCO?
Since dialysis membrane consists of a spongy matrix, it is more appropriate and practical to measure the "pore size" indirectly by rating its retention performance characterized by its "Molecular Weight Cut Off" (MWCO). The MWCO is defined by the molecular weight solute that is 90% retained by the membrane during a 17 hour period. For this reason, you should select a MWCO that is just smaller than the size of the solutes you want to retain.

FAQ: What are Daltons (MW) –why not microns unit?- and how do you convert Da/µm?
While the size of dissolved molecules is defined typically by molecular weight (MW) units in Daltons (but alos molecular shape, that depends on its structure and on environment), th size of particles and cells is defined by metric diameter because the MW units become impractical and do not account for shape in the microscopic range. Since microns are a measure of a 2-dimensional distance and Daltons are a measure of 3-dimensional size based on atomic weight units, there is no direct conversion from one to the other.
For this reason, many common biological materials were characterized for dialysis, ultrafiltration and microfiltration purposes and plotted on a conversion chart to correlate the approximate scales as a reference for estimating conversions. Above Pore Size Chart may helps to converting between Daltons and metric units.
See Membrane Porosity – convertion Dalton to Microns

FAQ: What is the difference between Spectra/Por 2 and 4 dialysis membranes?
Both Spectra/Por 2 and 4 have a MWCO of 12-14 kD. While Spectra/Por is more suited for general dialysis, Spectra/Por 2 offers special higher and lower FW's and/or higher permeability; i.e. water permeability of Spectra/Por 2 is superior to that of Spectra/Por 4.

Porosity and Permeation rate

The permeation/exclusion of a molecule across/from a semipermeable membrane depends mainly on the shape, charge, and size of the solute, driving the dialysis performence i.e. speed and size exlusion. Typically with globular biomolecules, i.e. most proteins, the size of a solute correlates highly with the molecular weight. The flux, or rate of transport across a semipermeable membrane of solutes in solution, is then inversely related to the molecular weight. As the molecular size approaches and exceeds the size of the membrane pores (MWCO), passage of solutes will completely or partially be prevented.

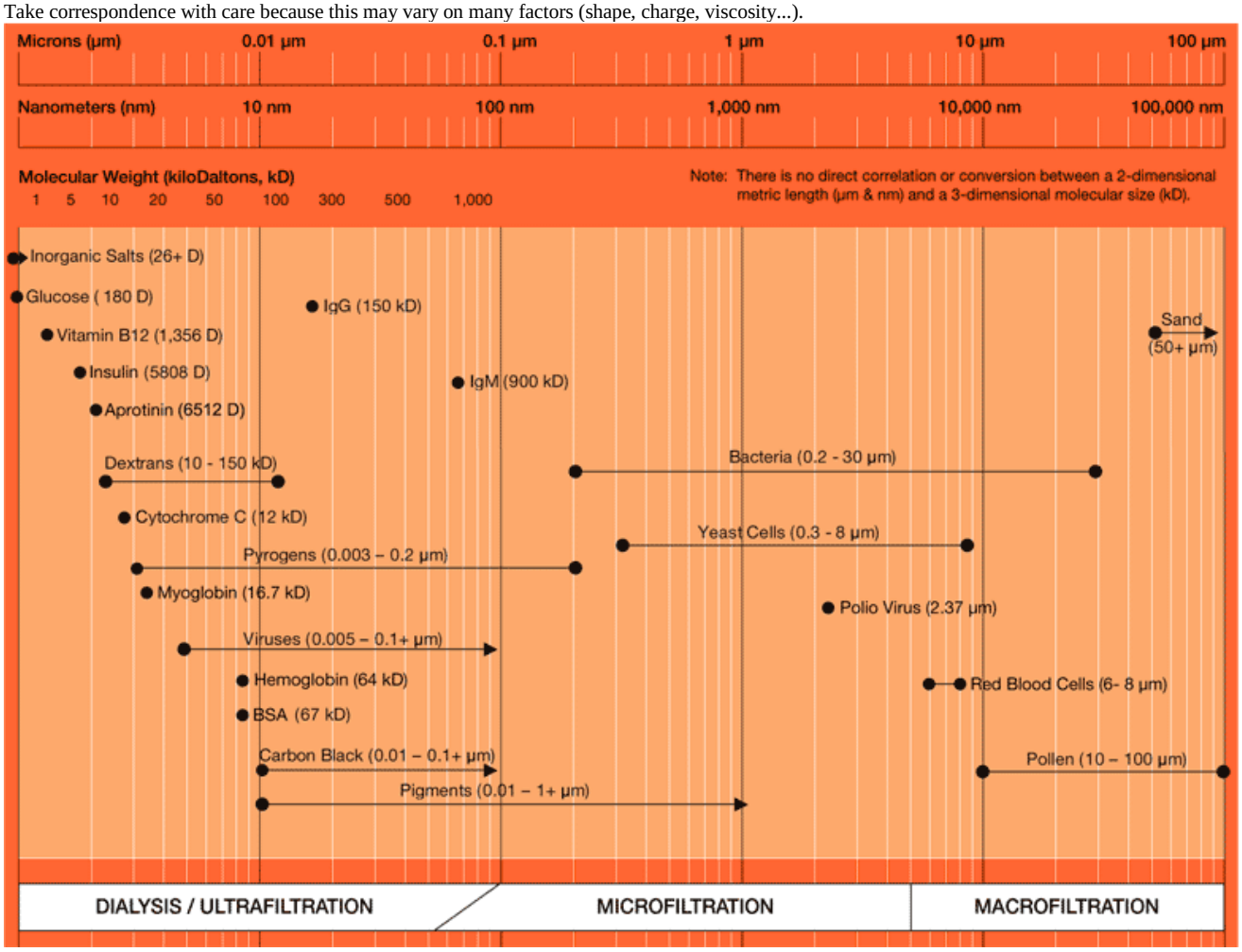
Small molecules collide more often with the membrane, thus, their rate of molecular migration through the membrane will be high. Large molecules, moving at low velocities collide infrequently with the membrane. Therefore, their rate of migration through the semi-permeable membrane will be low (even those that fit through the membrane pores).

The **permeation rate** is increased with :
-larger difference of solute concentrations between dialysis compartments
-smaller and more spheric molecules
-higher temperatures
-thiner membrane thickness
-larger membrane surface area over sample volume.

Membrane Porosity – convertion Dalton to Microns

See FAQ: What are Daltons (MW) –why not microns unit?

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...).



Note: RC membranes have more uniform and pores, and are thus recommended for more precise separations. See above.

Membrane compatibility chart

This chemical resistance chart is intended for use as a guide, not as a guarantee of chemical compatibility. Variations in temperature, concentrations, durations of exposure and other factors may affect the performance of the product. It is recommended to test under your own conditions.

The following codes are used to rate chemical resistance:

R = Recommended **L = Limited Exposure** **NR = Not Recommended** **U = Unknown**

Substance	Cellulose Ester (CE) / Mixed Cellulose (ME)	Regenerated Cellulose (RC)	Polysulfone (PS) / PolyetherSulfone (PES)	Polypropylene (PP)	PolyVinylidene DiFluoride (PVDF)	Nylon (N)	Stainless Steel (SS)	Polyester (P)	Fluorocarbon (F)
Acetic acid (diluted-5%)	L	R	R	R	R	NR	L	L	R
Acetic acid (med conc-25%)	NR	R	R	R	R	NR	L	NR	R
Acetic acid (glacial)	NR	R	R	R	R	L	L	NR	R
Acetone	NR	R	NR	R	L	R	R	R	R
Acetonitrile	NR	R	NR	R	L	U	U	U	U
Ammonium hydroxide (diluted)	NR	R	R	R	R	R	R	U	R
Ammonium hydroxide (med conc)	NR	L	R	R	R	R	R	U	R
Amyl acetate	NR	R	NR	R	R	L	R	L	R
Amyl alcohol	L	R	L	R	R	R	R	R	R
Aniline	NR	R	NR	R	R	R	R	U	R
Benzene	NR	R	L	R	R	R	L	R	R
Benzyl alcohol	NR	R	NR	R	L	U	L	NR	R
Boric acid	R	R	R	R	R	L	L	R	R
Brine	R	R	R	R	R	R	R	R	R
Bromoform	NR	R	NR	R	R	U	U	U	U
Butyl acetate	NR	R	NR	R	R	R	L	R	R
Butyl alcohol	L	R	R	R	R	L	R	R	U
Butyl cellosolve	NR	L	NR	U	R	U	U	U	U
Butylaldehyde	NR	R	NR	R	R	U	U	U	R
Carbon tetrachloride	NR	R	NR	R	R	NR	L	R	U
Cellosolve	NR	L	R	R	R	U	U	U	R
Chloroacetic acid	NR	R	NR	R	R	NR	L	U	R
Chloroform	L	R	L	R	R	R	R	R	R
Chromic acid	NR	NR	NR	L	R	NR	L	U	U
Cresol	NR	R	NR	R	NR	NR	R	U	R
Cyclohexane	L	R	L	R	R	R	R	U	R
Cyclohexanone	NR	R	NR	R	L	R	R	R	R
Diacetone alcohol	NR	R	NR	R	R	R	L	U	R
Dichloromethane	L	R	L	R	R	L	L	NR	R
Dimethyl formamide	NR	L	NR	R	NR	R	R	NR	U
Dimethylsulfoxide	NR	R	NR	R	L	U	U	U	U
1,4 Dioxane	NR	L	L	R	R	U	U	R	R
Ethyl acetate	NR	R	NR	R	R	R	L	U	R
Ethyl Alcohol	L	R	R	R	R	R	R	R	R
Ethyl alcohol (15%)	R	R	R	R	R	R	R	R	R
	Cellulose Ester (CE)	Regenerated	Polysulfone (PS) /	Polypropylene (PP)	PolyVinylidene	Nylon (N)	Stainless Steel (SS)	Polyester (P)	Fluorocarbon (F)

	/ Mixed Cellulose (ME)	Cellulose (RC)	PolyetherSulfone (PES)		DiFluoride (PVDF)				
Ethyl alcohol (95%)	L	R	L	R	R	R	R	R	R
Ethylene dichloride	NR	R	NR	L	R	R	L	U	R
Ethylene glycol	L	R	R	R	R	R	L	R	R
Ethylene oxide	NR	L	R	R	R	R	L	U	R
Formaldehyde (2%)	L	R	R	R	R	R	R	R	R
Formaldehyde (30%)	L	R	R	R	R	R	R	R	R
Formic acid (25%)	NR	R	R	R	R	NR	L	NR	R
Formic Acid (50%)	NR	R	R	R	R	NR	L	NR	R
Freon®	R	R	R	R	R	NR	R	R	R
Gasoline	R	R	L	R	R	R	R	R	R
Glycerine	R	R	R	R	R	R	R	R	R
Glycerol	R	R	R	R	R	R	R	R	R
Hexane	R	R	R	R	R	L	R	R	R
Hexanol	L	R	R	R	R	R	R	R	R
Hydrochloric acid (diluted-5%)	R	R	R	R	R	L	NR	R	R
Hydrochloric a. (25%)	NR	NR	R	R	R	NR	NR	R	R
Hydrochloric a. (37%)	NR	NR	R	L	R	NR	NR	R	R
Hydrofluoric a. (25%)	NR	L	L	NR	R	L	NR	NR	R
Hydrogen peroxide (30%)	R	R	R	R	R	NR	L	R	R
Iodine solutions	NR	NR	NR	R	R	L	NR	U	R
Isobutyl alcohol	R	R	R	R	R	NR	R	U	R
Isopropanol	L	R	R	R	R	NR	L	R	R
Isopropyl acetate	NR	R	NR	R	R	L	L	R	R
Isopropyl alcohol	L	R	R	R	R	NR	L	R	R
Isopropyl ether	L	R	R	L	R	R	R	U	R
Jet Fuel 640A	R	R	R	R	R	L	R	U	R
Kerosene	R	R	R	R	R	R	R	L	R
Lactic acid	R	R	R	R	R	L	L	R	R
Methyl acetate	NR	R	NR	R	R	R	R	L	R
Methyl alcohol	L	R	L	R	R	L	R	U	R
Methyl alcohol (98%)	L	R	R	R	R	L	R	U	R
Methyl cellosolve	L	L	R	R	R	L	L	U	R
Methyl chloride	NR	R	NR	R	L	L	R	U	R
Methyl ethyl ketone	NR	R	NR	R	L	R	R	U	R
Methyl formate	NR	L	NR	R	R	U	U	U	U
Methyl isobutyl ketone	NR	R	NR	R	L	L	L	R	R
Methylene chloride	L	R	L	R	R	L	L	NR	R
N-methyl-2-pyrrolidone	NR	R	NR	R	R	U	L	U	U
Mineral spirits	R	R	R	R	R	R	R	U	R
Monochlorobenzene	L	R	NR	L	R	U	U	U	U
Nitric acid (diluted-5%)	L	R	R	R	NR	NR	R	R	R
Nitric acid(mi-conc.25%)	NR	NR	R	R	NR	NR	R	L	R
Nitric acid (6N)	NR	N	R	L	R	NR	R	R	R
	Cellulose Ester (CE)	Regenerated	Polysulfone (PS) /	Polypropylene (PP)	PolyVinylidene	Nylon (N)	Stainless Steel (SS)	Polyester (P)	Fluorocarbon (F)

	/ Mixed Cellulose (ME)	Cellulose (RC)	PolyetherSulfone (PES)		DiFluoride (PVDF)				
Nitric acid (conc-70%)	NR	NR	NR	NR	NR	NR	R	NR	R
Nitric acid (concentrated)	NR	NR	R	NR	L	NR	R	NR	R
Nitrobenzene	NR	L	NR	NR	R	L	L	NR	R
Nitropropane	NR	L	NR	L	R	U	U	U	U
Oils, mineral	R	R	R	R	R	R	R	U	R
Pentane	R	R	R	R	R	R	L	R	R
Perchloric acid (25%)	NR	L	NR	NR	R	NR	L	U	R
Perchloroethylene	NR	R	NR	L	R	L	L	U	R
Petroleum based oils	R	R	R	R	R	R	R	R	R
Petroleum ether	R	R	R	R	R	U	U	R	U
Phenol (0.5%)	R	R	R	R	R	NR	L	L	R
Phenol (10%)	NR	R	L	R	R	NR	L	NR	R
Phosphoric acid (25%)	NR	L	R	R	R	L	NR	U	R
Potassium hydroxide (1N)	L	L	NR	R	R	L	L	R	R
Potassium hydrox(25%)	NR	R	R	R	R	L	L	R	R
Potassium hydrox(50%)	NR	NR	R	R	R	L	L	L	R
Propanol	R	R	R	R	R	NR	R	R	R
Pyridine	NR	R	NR	R	L	L	R	NR	R
Silicone oil	R	R	R	R	R	R	R	U	R
Sodium hydroxide (0.1N)	L	R	R	R	R	R	L	R	R
Sodium hydroxide (diluted-5%)	NR	L	R	R	R	R	L	L	R
Sodium hydroxide (25%)	NR	L	R	R	R	R	L	NR	R
Sodium hydrox (50%)	NR	NR	R	R	R	R	L	NR	R
Sodium Hydrox.(conc)	NR	NR	R	R	R	L	L	NR	R
Sodium hypochlorite	R	R	R	L	R	NR	NR	U	R
Sulfuric acid (dil.-5%)	L	R	R	R	R	L	NR	NR	R
Sulfuric acid (mico.25%)	NR	L	R	R	R	NR	NR	NR	R
Sulfuric acid (6N)	NR	L	R	R	R	NR	NR	NR	R
Sulfuric Acid (conc)	NR	NR	R	NR	L	NR	NR	NR	R
Tetrahydrofuran	NR	R	NR	R	R	R	R	R	R
Toluene	R	R	L	R	R	R	R	U	R
Trichloroacetic acid (25%)	NR	NR	R	R	R	L	NR	NR	R
Trichlorobenzene	NR	R	NR	R	R	U	U	U	U
Trichloroethane	L	R	L	R	R	L	L	L	R
Trichloroethylene	R	R	R	R	NR	L	L	R	R
Triethylamine	NR	R	NR	L	R	R	R	U	R
Turpentine	NR	R	NR	R	R	L	R	U	R
Urea	R	R	R	R	R	R	L	R	R
Urea (6N)	NR	R	NR	R	R	R	L	R	R
Water	R	R	R	R	R	R	R	R	R
Xylene	NR	R	NR	R	R	R	L	NR	R
	Cellulose Ester (CE) /	Regenerated Cellulose	Polysulfone (PS) / PolyetherS	Polypropylene (PP)	PolyVinylidene DiFluoride	Nylon (N)	Stainless Steel (SS)	Polyester (P)	Fluorocarbon (F)

Dialysis operating – using dialysis membranes

FAQ: How do you prepare dialysis membrane tubing?

Preparation instructions will vary based on the membrane type as follows. Note that boiling membrane is not recommended as it can damage the membrane and alter the pore rating.

A) CelluSep, Biotech RC, CE, and PVDF membranes should be rinsed in DI water for 15 to 30 minutes to remove sodium azide preservative.

B) Spectra/Por® 7 Standard RC has been pretreated to remove the trace levels of heavy metals and sulfides and only requires a 15 to 30 minute soak in DI water to remove the sodium azide preservative.

C) Spectra/Por 1 through 6 Standard RC membranes may require some extra preparation. While rinsing Spectra/Por 1 through 6 in water is typically sufficient to remove glycerin or preservative, Spectrum offers two membrane pre-treatment solution kits for the removal of the trace levels of heavy metals and sulfides introduced during manufacturing. Heavy Metal Cleaning Solution and Sulfide Removal Solution Kits are recommended for ultra-sensitive dialysis applications like binding studies or when low level presence of these contaminants may interfere with downstream analysis of the dialysis sample. Refer to the Membrane Dialysis Accessories webpage for more product information.

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:
Dont hesitate to ask at interbiotech@interchim.com if you need help!

FAQ: Should I close the dialysis tube using closures or tyieng knots?

Tying knots with the tubing is for many an easy and economic way to close the dialysis chamber. Knots however may lead to sample leakage if not properly done. Additionally, one might feel it is not convenient to make knots, there is a risk to leave the tubing fall and loose precious sample. After dialysis, opening tied tubing need scissors that may contaminate the opening, hence the sample when filling it out. Sample recovery may also be lowered because folded tubing keep more sample on its surface.
To these points, tubing clamps are recommended for more convenient operating.
Yet, people familiar with dialysis can see no real advantages using clamps, and prefer doing knots.

FAQ: How to select the right closure and closure size for membrane tubing?

Standard RC membrane (CelluSep, Spectra/Por 1-7) and Biotech RC is constructed of flexible regenerated cellulose polymers and can be sealed using any of the dialysis tubing closures. You just have to choose the right clamp width depending on the flat width of you tubing. Large clamps can be used for smaller tube width, but with caution because the tubing may be no so good maintained properly.
Biotech CE and PVDF are constructed of a more rigid polymer requiring gentler Universal closures.
Since these work well for all dialysis tubing; when in doubt, use Universal Closures. Standard Closures should ONLY be used with Standard RC tubing.

It is recommended to use a closure with a sealing width of 4-10 mm longer than the flat width of the dialysis tubing. The smallest Universal Closure has a sealing width of 50 mm. This will seal all flat widths .

FAQ: Is there a "rule of thumb" regarding membrane surface area to sample volume?

The surface area to volume ratio is a function of the tubing flat width. If you have a two equal length pieces of tubing with two different flat widths, the smaller flat width piece possesses a higher surface area to volume ratio and dialyzes quicker while the larger flat width piece possesses a lower surface area to volume ratio and dialyzes slower. The smaller flat width has a shorter distance for diffusion and less solute "competition" through the membrane pores. Larger flat widths have a longer distance to the membrane and more solute competition through the pores. In general, the greater the surface-area-to-volume ratio, the quicker the dialysis.
The membrane volumes, the dialysis bath volume to sample volume should be considered.

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

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.

You may consider a final/total volume of bath of at least 100 to 1000 and even 10 000 over the volume sample, in similar proportion of the desired dilution you expect for undesired substance(s) removed by dialyzis.
Typically, it is more economic in buffer to perform a shorter dialysis steps and and at least a second dialysis step for a longer period to reach an equilibrium in a larger bath (overnight). I.e. a fist dialysis with a volume ratio 250 : 1 of buffer/sample and a secund one of 1000:1 would ideally dilute removed salts by 1:250 000 if both dialysis were complete. Even with a 50% efficiency during the first dialysis step, this is generally sufficient in most applications. But by precaution one may dialyze even more, because it is so easy to increase volume, duration and even add a 3rd step!

FAQ: How how long does dialysis take to complete -does dialysis work-?

Dialysis is the diffusion of dissolved solutes across a selectively permeable membrane against a concentration gradient in an effort to achieve equilibrium. While small solutes pass through the membrane larger solutes are trapped on one side.
By exchanging the dialysate buffer on the outside side of the membrane, you can continually pull away the smaller solutes to purify the trapped larger molecules. In general, dialysis will be most effective when the buffer is replaced a few times over the course of a day and then left overnight at room temperature on a stir plate. A standard protocol for dialysis is 16 to 24 hours. Many factors affect the rate dialysis, including: diffusion coefficients, pH, temperature, time, concentration of species, sample volume, dialysate (buffer) volume, number of dialysate changes, membrane surface area, membrane thickness, molecular charges and dialysate agitation (stirring).

FAQ: Can I still use the membrane if it dries out or freezes?
Should I be concerned about the creases, roller marks, or fold lines on membranes?

The roller marks or fold lines along the delivered membranes will not affect the diffusion properties as long as the membrane is integral. However, attention should be payed when unproper storage:

If wetted membrane **dries** out, the pore size is adversely affected and the membrane becomes brittle and will likely leak. The membrane should be discarded.

If the membrane **freezes**, the ice crystals may rupture the membrane and also cause leaking. It is recommended not to use the membrane. However, you can try slowly increasing the temperature until the storage solution completely melts. The possibility remains that the membrane is not longer integral.

FAQ: Can dialysis membranes be re-used for the same protein samples?

We do not recommend re-using dialysis membranes since they can be contaminated through handling and dialysis conditions (pH, temperature, chemical exposure, etc.) can alter the membrane integrity and/or cause leaking, especially when removing and reapplying closures. Dialysis membranes are designed for single use.

FAQ: Can dialysis membranes be chemically or heat sealed?

The CE and RC dialysis membranes can only be mechanically sealed.

NT-Dialya
However, the PVDF dialysis membranes can be mechanically sealed or heat sealed and is often used in this manner for the purposes of sample "encapsulation".

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: How to sterilize dialysis membranes / devices
You can order sterilized dialysis membranes or devices – available in certain formats-. Please inquire.

You can sterilize dialysis membranes or devices
By irradiation (the mostly recommanded method)
Chemically. See 3 protocols in FT-CelluSep. *
By autoclaving (not possible for CE membranes; not recommended with RC membranes) *
On custom: please inquire
* Chemical sanitization and autoclaving may affects slightly the cut off.

FAQ: How much pressure can a dialysis membrane withstand if used for ultrafiltration?
Dialysis membranes are not designed for pressure filtration. The maximum recommended pressure is 1.5 psi without affecting the MWCO.
Alternatively to supported (reinforced) dialyiss membranes, one may overlay it with a paper filter.

More / trouble shhoting

FAQ: Why is the volume of my sample increasing during dialysis / my dialysis has leaked or has burst!
A sample often contains more solutes than the dialysis buffer. This drives the solvent (water) to cross the membrane in order to equilibrate the concentration in the 2 compartment, and so the sample volume to increase. This phenomenon is called Osmosis (see FAQ What is dialysis versus osmosis?), and normal. It is generally limited, causing a few% to 20% increase in sample volume, up 20% with very concentrated samples like 3/M glycine, urea or glycerol, and SulfateAmmonium precipitated globulins). That's why it is recommended to always leave unfilled tubing (see FAQ How long to cut the dialysis tubing?)

Dialysis Applications in R&D

Desalting / Buffer exchange

Desalting / Buffer exchange is the main application of dialysis, to keep purified protein free of undesired small compounds that are present in biological samples, or added during upstream purifications steps.

Sterile dialysis

Same samples require dialysis to be performed in sterile conditions, notably with cells to be cultured free of some componends form medium of that are released.

There are very few commercial sterile dialysis membranes and devices (please inquire), but lab-made sterilization is possible. See FAQ: How to sterilize dialysis membranes / devices

Dialyse à l'équilibre - Fraction liée/libre

Une application importante de la dialyse correspond à l'étude de la répartition de petits composés entre compartiments, et notamment entre la fraction qui reste libre en solution et la fraction qui se lié aux molécules ou particules du milieu. La méthode présentée ci après correspond à l'application typique de quantification de la fraction d'un médicament liée aux protéines plasmatiques.

1) Méthode approximative par ultrafiltration/dessalage

Ultrafiltrer un échantillon (plasma+médicament) permet de séparer rapidement le médicament en solution. C'est une méthode rapide pour apprécier la quantité de médicament lié, approximativement (Clin Chem. 1985 Jan;31(1):60-4): car cette méthode ne mesure pas forcément toute la fraction de médicament liée, pour différentes raisons: l'équilibre (et taux de médicament lié) est modifié par la concentration réalisée lors de l'UF; tout le médicament libre n'est pas éliminé en une étape d'UF; le biais n'est pas gommé si vous faites une 2eme séparation UF après ajout de plasma sans médicament (et pire si ajout de PBS): au contraire le médicament peut se décrocher en partie durant le process d'UF; ...
Regardez nos dispositifs d'UF centrifugeables VivaSpin[]:

2) Méthode par dialyse à l'équilibre.

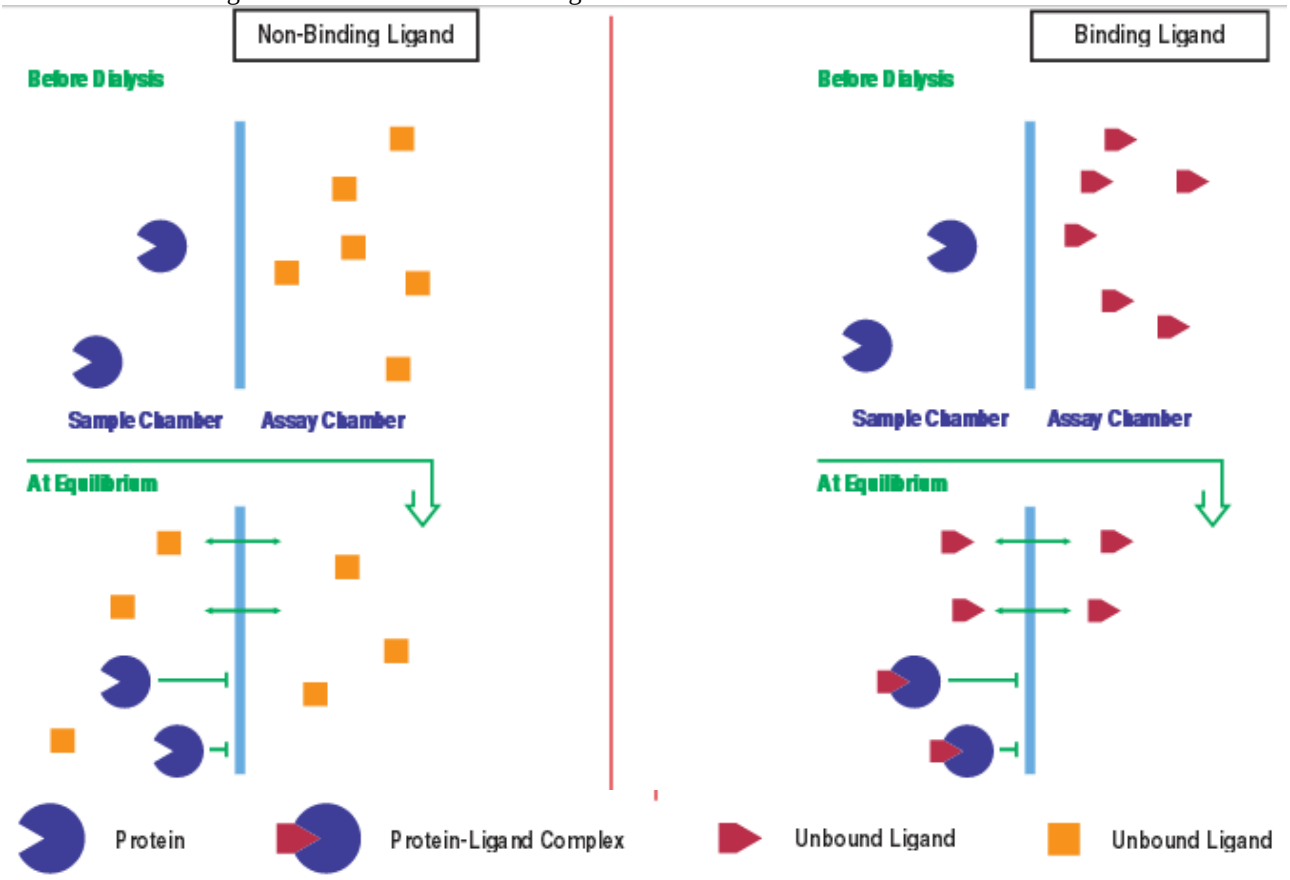
La dialyse à l'équilibre permet de mesurer précisément la fraction lié, au moyen d'une durée de manip plus longue et de quelques calculs (équation avec lles concentrations [liée] et [dialysée] -méthode préférée en pharma-:

Equilibrium Dialysis products

Dispositifs de dialyse pour ces études (& voir 'Dialysis Products Guide)[]:
RED (rapid Equilibrium Device)[]:
DispoDialyser devices (equilibrium version)[]:
HTD Equilibrium Dialysis System(96 samples)[]:
96-Well DispoDialyser plate(equilibrium version)[[]]:

Equilibrium Dialysis information

Equilibrium dialysis is a specific application of the general phenomenon of dialysis that is important for the study of the binding of small molecules and ions by proteins. It is one of several methods currently available but its attractive feature continues to be its physical simplicity.
The objective of an equilibrium dialysis experiment is usually to measure the amount of a ligand bound to a macro-molecule. This is typically done through an indirect method because in any mixture of the ligand and macro-molecule, it is difficult to distinguish between bound and free ligand.



If, however, the free ligand can be dialyzed through a membrane, until its concentration across the membrane is at equilibrium, free ligand concentration CL(f) and the following data can be measured:

Temperature (absolute)	T
Concentration of binding component, e.g. protein	$C_{P(o)}$
Starting concentration of ligand	$C_{L(o)}$
Final concentration of free ligand	$C_{L(f)}$

From which the following parameters can be derived directly:

Concentration of bound ligand	$C_{L(b)}$
Free fraction (of ligand)	*f*
Bound fraction (of ligand)	b
Degree of binding or saturation fraction	r

Data obtained from several experiments at a range of temperatures and with varying initial concentration of ligand can provide other binding parameters:

Association constant	K
Number of binding sites	n
Binding capacity	N

Further, the thermodynamics of the binding reaction can be derived:

Change of free energy	ΔG
Enthalpy change	ΔH
Entropy change	ΔS

Since equilibrium exists, the value $C_{L(f)}$ is the same on both sides of the membrane.
(Note: where charged species are involved the Gibbs-Donnan effect can upset the equilibrium unless moderately concentrated salts are in solution; say 0.6% NaCl).

Hence: $C_{L(o)} = C\,L\,(f) + C_{L(f)} + C_{L(b)}$ *

*It is essential to correct this equation to take account of any ligand which might be bound to the membrane.

$C_{L(b)} = C_{L(o)} - 2 \times C_{L(f)}$

The free fraction f is given by: $f = C_{L(f)} / [\, C_{L(o)} - C_{L(f)} \,]$

The bound fraction b is: $b = 1 - f$

The degree of binding or saturation fraction r is: $r = C_{L(b)} / C_{P(o}$

If the protein concentration is known, the Scatched plot can be used to determine binding constants and the number of binding sites. If the protein concentration is unknown, the absolute number of binding sites is replaced by binding capacity N .

In the former case, values of r would be plotted on the abscissa against $r/C_{L(f)}$ on the ordinate. If only one class of binding sites is present, the Scatched plot results in a straight line with slope equal to –K see Fig. 1.

The intercept on the abscissa give the value n . If two classes of binding sites are involved, the plot takes the form of an hyberbola. In this case, the asymptotes have slopes equal to –K for each class of site, and their intercepts on the abscissa give the two values for n . The intercept between the curve and the abscissa is equal to the sum of the two values for n , see Fig. 2.

The free energy change is obtained simply by substituting the appropriate values in the following equation:

$\Delta G = - RT \ln K$ where R is the gas constant.

ΔH can be obtained from a graph based upon an integrated form of the van/t Hoff equation.

$\ln K = - \Delta H/RT + C$

In this case a plot In K versus 1 /T has a slope of $- \Delta H/R$. Once a value for ΔH has been found it can be substituted into: $\Delta G = s\,H - T\,\Delta S$ to obtain a result for the entropy change ΔS.

Fig. 1.

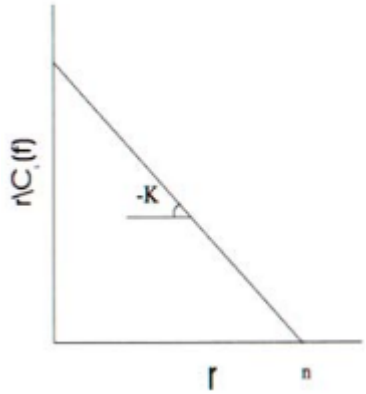
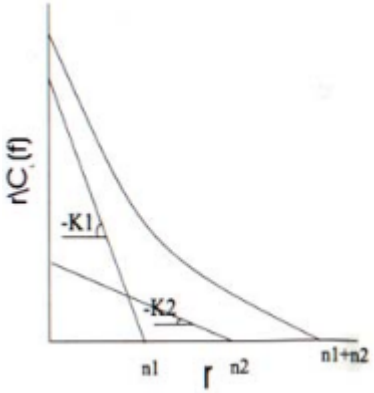


Fig. 2.



References:

Scatchard, G., Am. N.Y. Acad. Sci. 51, 660-672 (1949)
Rosenthal, H.E., Anal. Biochem. 20, 525-532 (1967)
Weder, H.G., Schildknecht, J., Lutz, R.A. and Kesselring, P., Eur. L. Biochem. 42, 475-481 (1974)

(a) *Clin Chem. 1985 Jan;31(1):60-4.* Equilibrium dialysis, ultrafiltration, and ultracentrifugation compared for determining the plasma-protein-binding characteristics of valproic acid. Barré J, Chamouard JM, Houin G, Tillement JP.

Abstract: Equilibrium dialysis, ultrafiltration, and ultracentrifugation were compared to determine their reliability and applicability in the study of binding of an anticonvulsant drug, valproic acid, by plasma proteins. We studied drug binding with pooled serum and with solutions of human serum albumin at physiological concentrations. We compared binding characteristics such as number of binding sites, affinity constants, and percent of binding as measured by each method in the therapeutic range for valproic acid. Results by ultracentrifugation differed from those by equilibrium dialysis and ultrafiltration, which agreed reasonably well with each other.

Process Dialysis – Applications & Tips

Large-volume Process Dialysis is an efficient and economic technology driven by the increasing demand for gentle and consistent multi-batch purification at the production scale. While Laboratory Dialysis for research and analytical testing typically involves static stirring of small volumes for sample prep or solute release studies, **process dialysis facilitates dynamic buffer flow** around the membrane-encased sample to increase purification efficiency and improve large buffer handling for the production of purified or synthetic compounds or particucles. Dialysis is notably a nice choice for **fragile proteins, viscous fluids and polymer gels**, such as hyaluronic acid.

Spectra/Por® Products Designed for Process Dialysis
Large Volume Dynamic Buffer Flow
Largest Membrane Selection *Smart Dialysis*
Customized Dialysis Tubing & Membrane
Applications, tips

=> see Process Dialysis catalog [BB111p]

Contact Interchim for any question

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