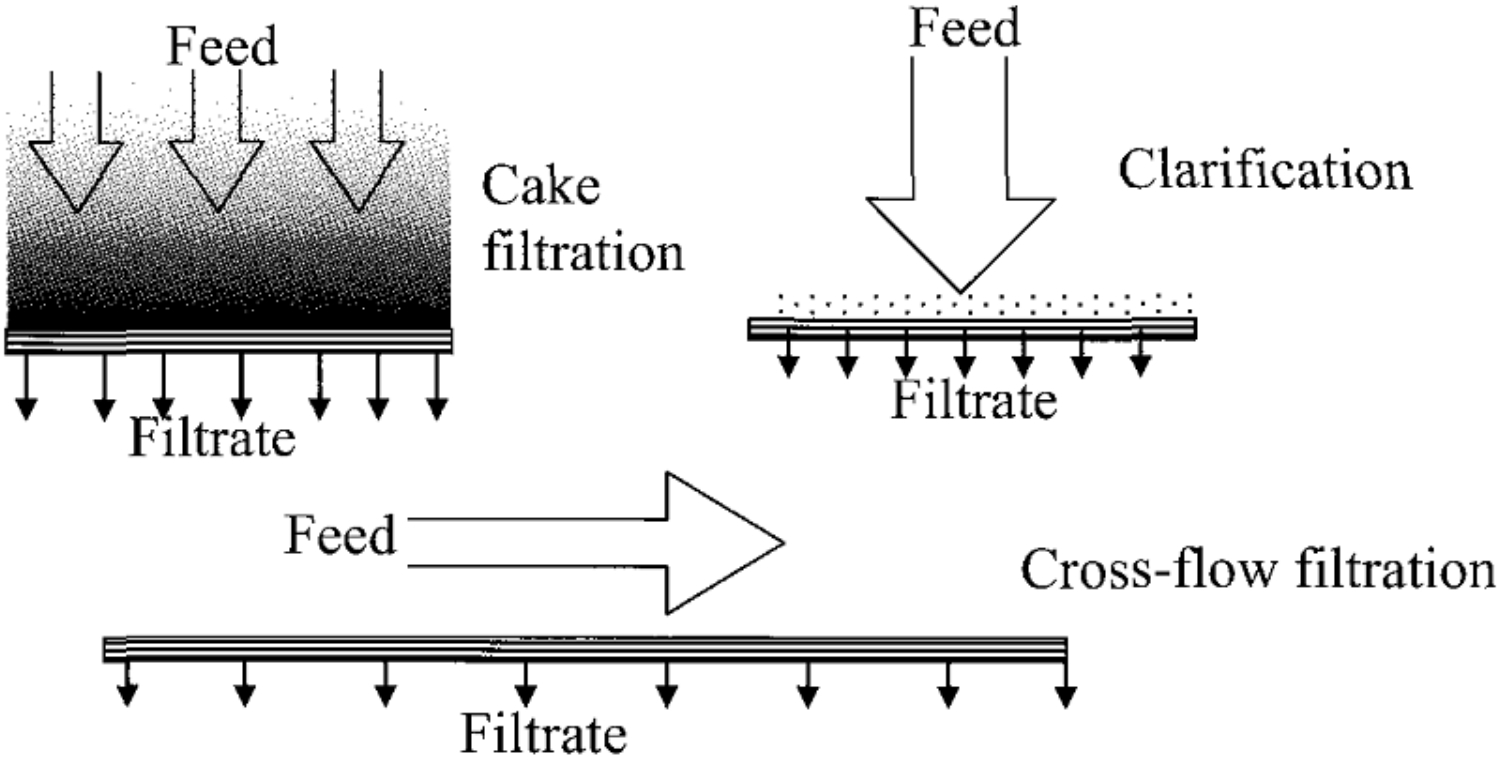
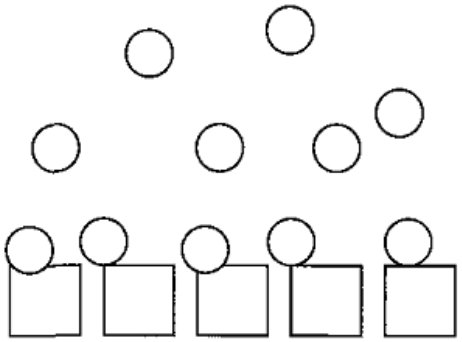
Filter

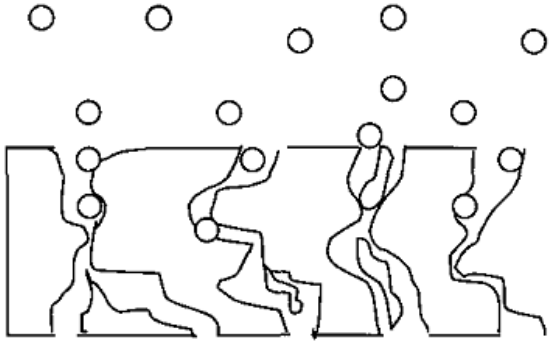
Filtration is a separation process in which a solid-liquid mixture called the feed (or the suspension) is forced through a porous medium on which the solids are deposited or in which they are entrapped. The porous medium which allows the liquid to go through while retaining the solids is called the filter. The retained solid is called "the residue" or "the cake". The clarified liquid is called "the effluent" or the "filtrate".



Types of filtration

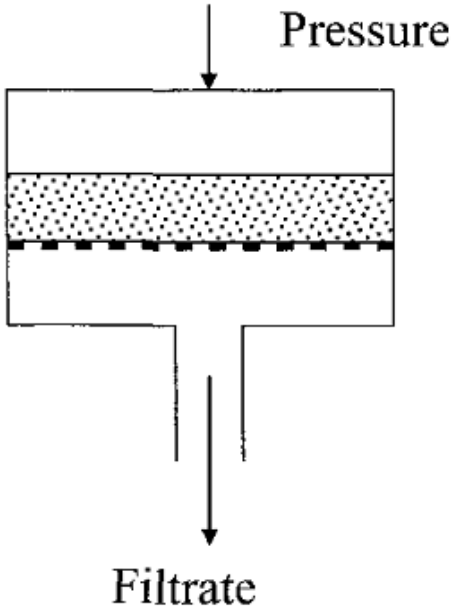


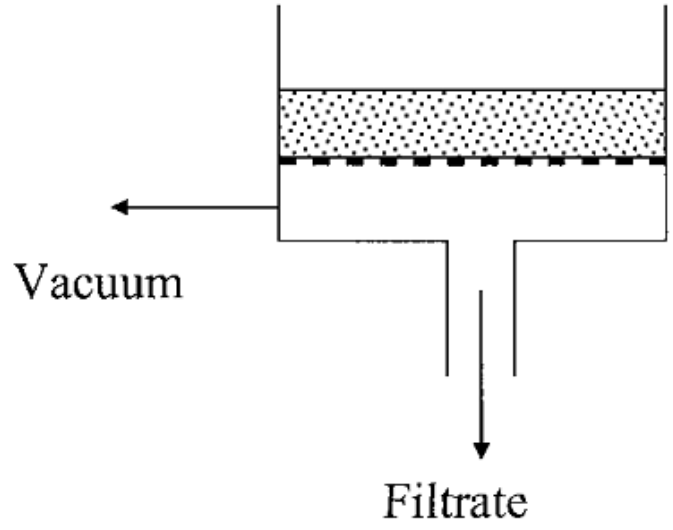
Surface filtration



Depth filtration

Mechanisms of filtration

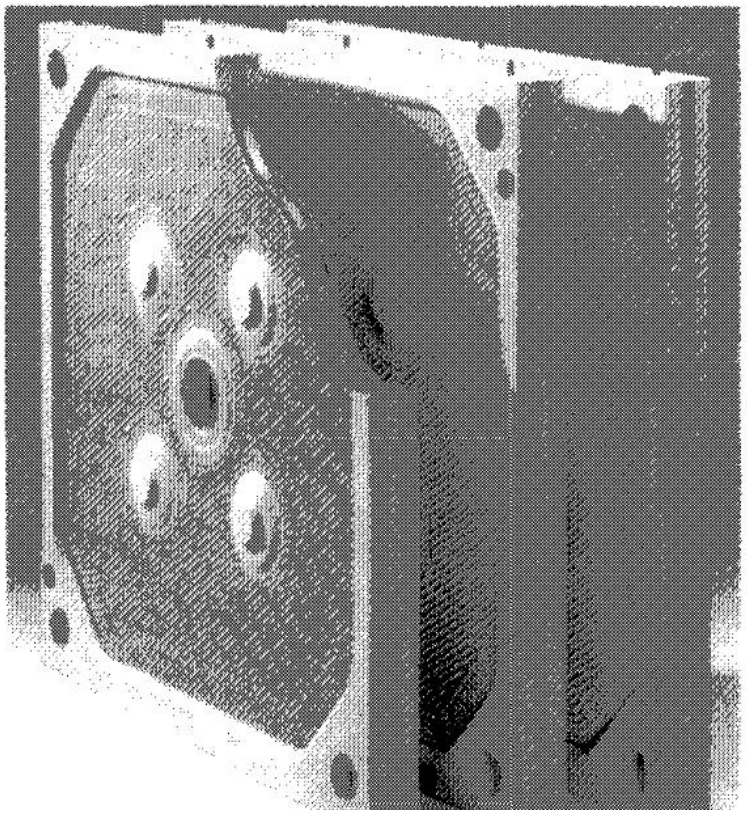


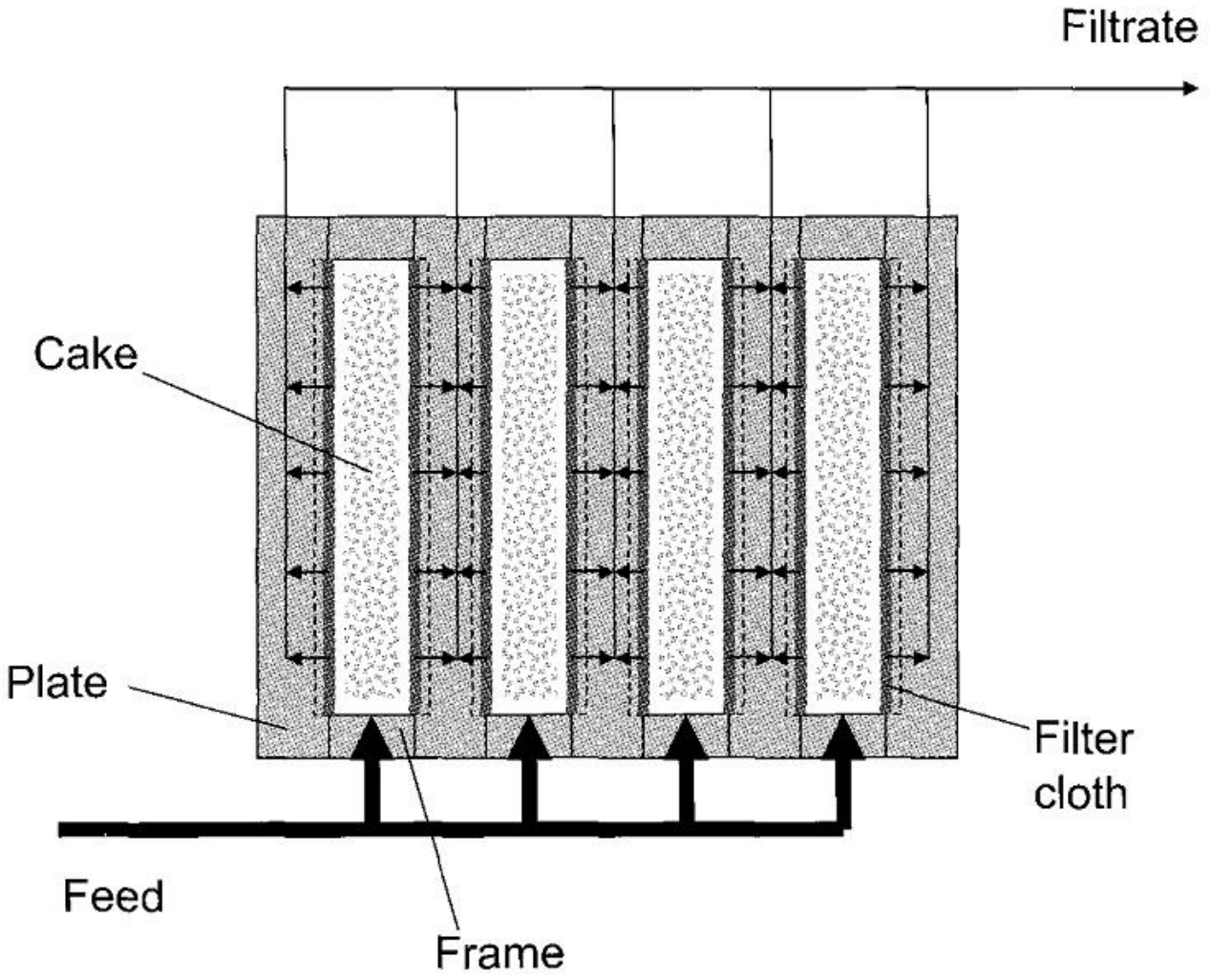


Pressure and vacuum filtration

Filter Press

A filter press consists of a series of horizontally arranged vertical filter elements, each consisting of a frame within which the cake can be accumulated sandwiched between filter medium on either side. Each filter medium is supported on a plate which has grooves to allow easy collection of the filtrate. The "press" refers to the external structure which provides the necessary force to seal each filter element and supports the plate and frame assembly.





Operation of plate and frame filter press

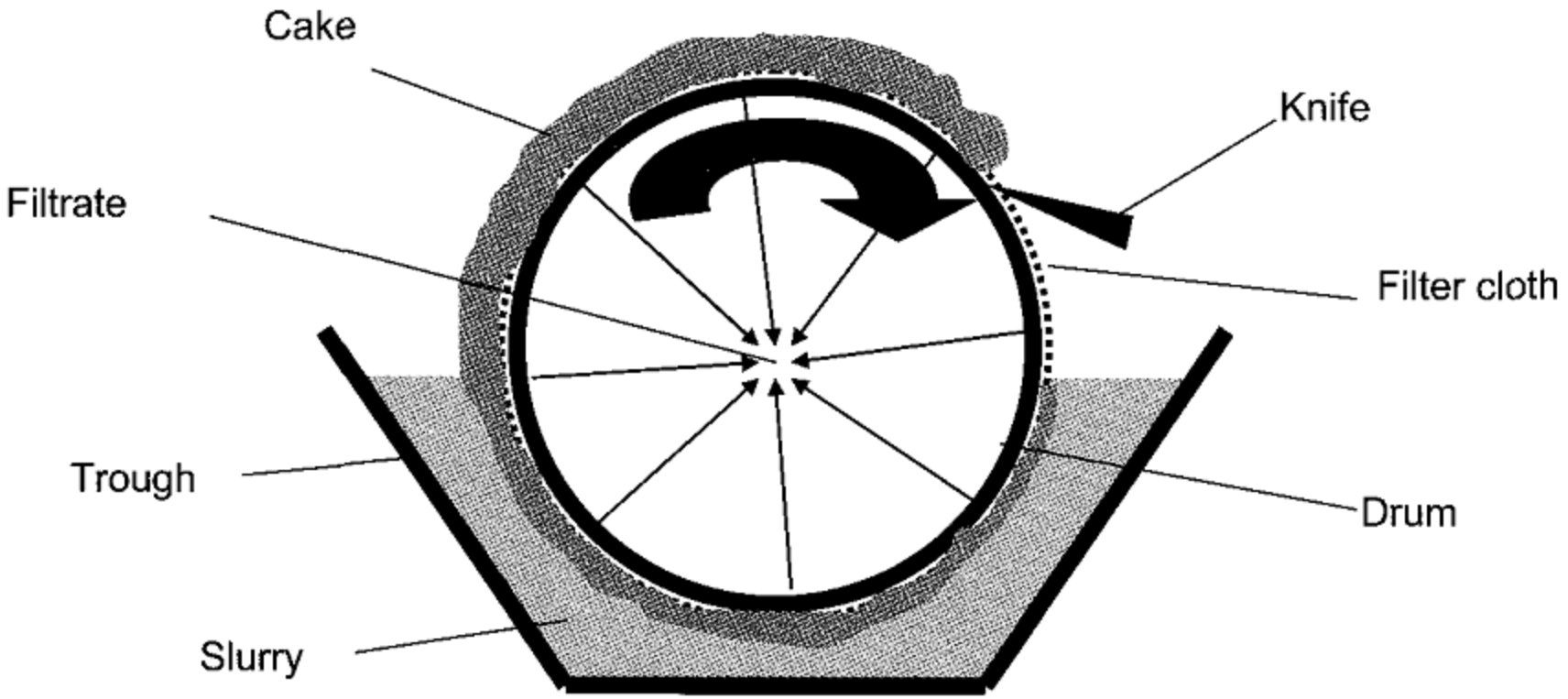
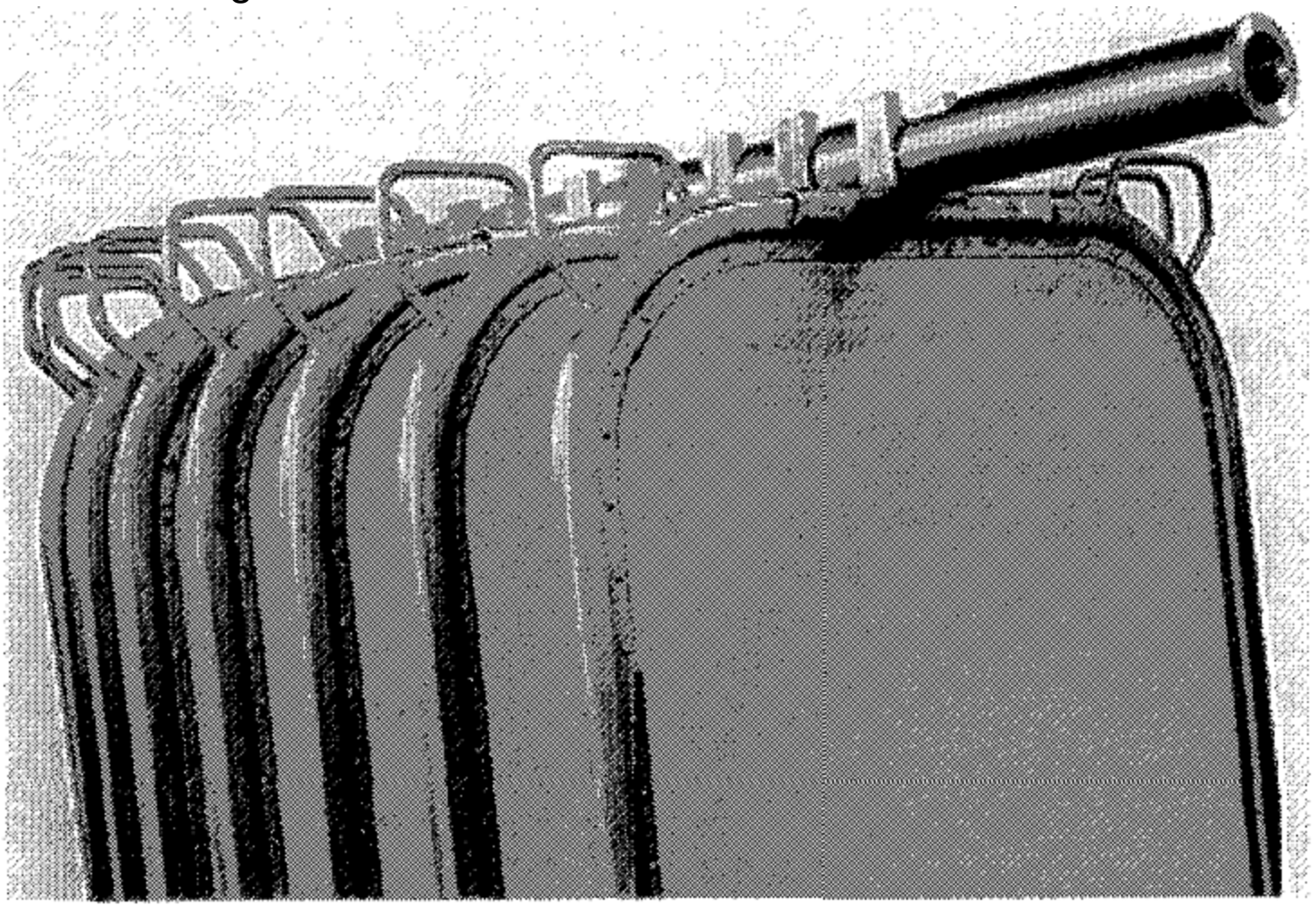


Fig. 10.9 The working principle of a rotary drum filter

Pressure leaf filter

A pressure leaf filter consists of a number of rectangular basic filtration units (also called leaves) connected together in parallel by means of flexible hose or a rigid tube manifold



Membrane

- In 1963, the first application of FS membranes in analytical chemistry was successfully described by Hoch and Kok who used semipermeable membranes made by polyethylene and Teflon as interface (inlet) to mass spectrometer for continuous sampling of gases dissolved in liquid samples

A membrane is a thin semi-permeable barrier which can be used for the following types of separation:

1. Particle-liquid separation
2. Particle-solute separation
3. Solute-solvent separation
4. Solute-solute separation

Membranes are available in three basic forms:

1. Flat sheet membrane
2. Tubular membrane
3. Hollow fibre membrane

Symmetric membranes

Dense membrane

Porous membrane

Asymmetric membranes

Dense membrane fused on porous membrane

Porous membrane fused on another porous membrane

Symmetric and asymmetric membranes

In a FS- or HF-MMLLE (microporous membrane liquid–liquid extraction), system, the membrane is used as a **miniaturised barrier** between two phases, one of them organic, filling the membrane pores (thus making it nonporous) and one side of the membrane, and the other phase is the aqueous sample on the other side of the membrane.

The types of synthetic organic membranes:

a. porous polypropylene (PP) and

b. nonporous silicone rubbers (normally composed of polydimethylsiloxane (PDMS))

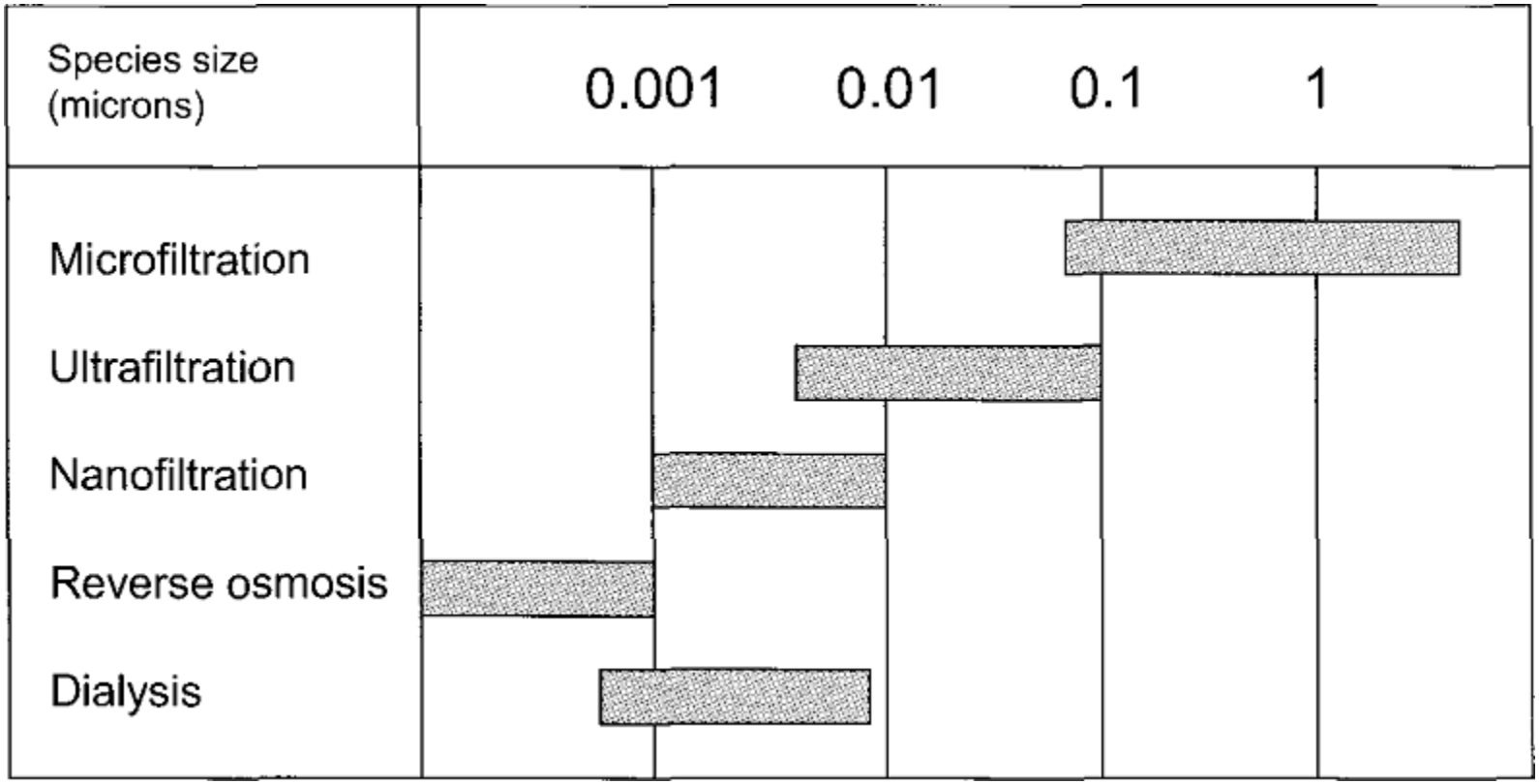
- Due to its multidisciplinary character of membranes, this technology, employing

a. Flat Sheet (FS)

b. Hollow Fibre (HF)

Porous Membranes

- unique suitability and performance for analyte extraction,
- in terms of high porosity that can enhance mass transfer,
- compatibility and
- stability when used with wide range of organic solvents



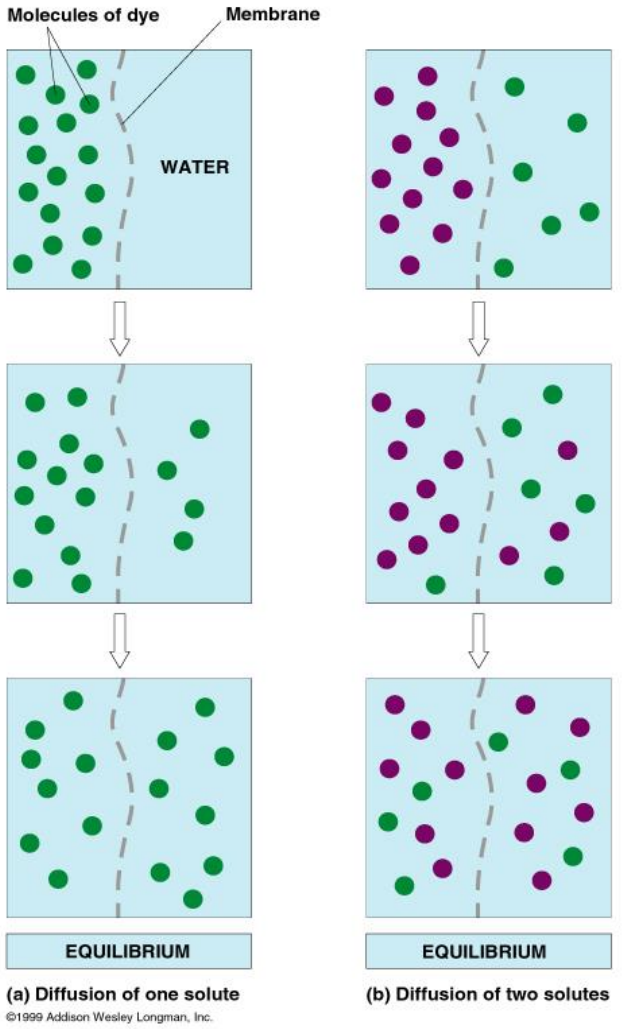
Classification of membrane processes

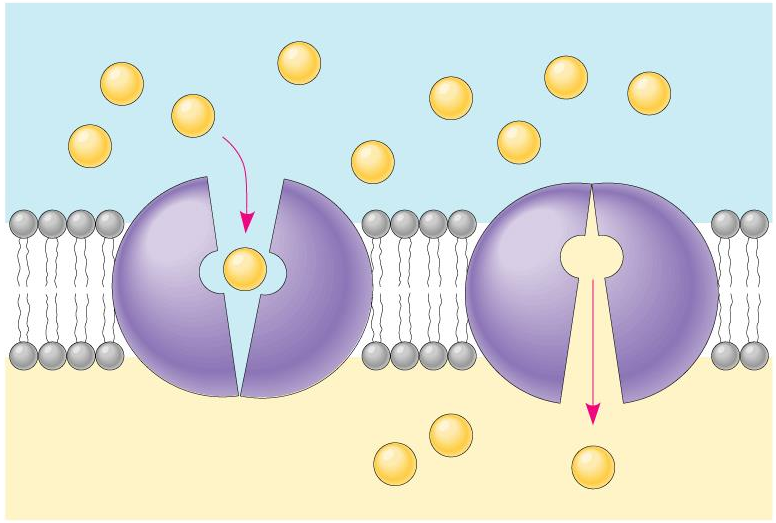
Transport Mechanisms

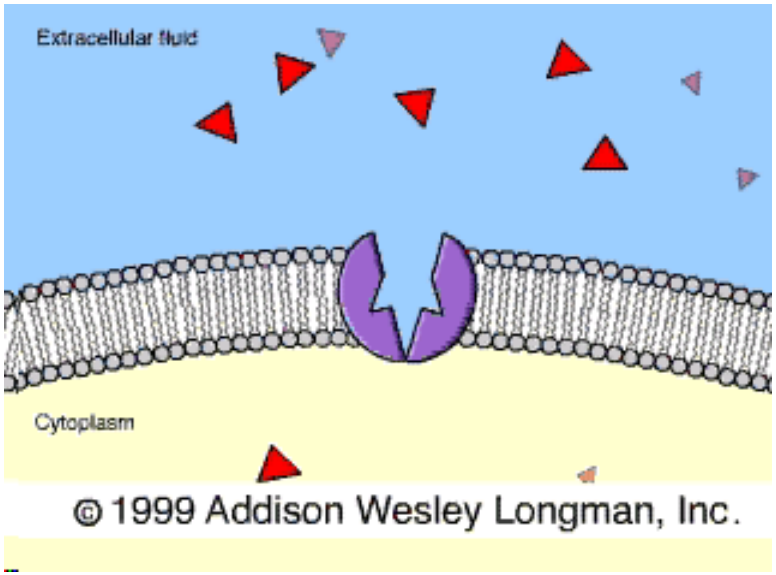
- Passive Transport
  - moves molecules along a concentration gradient
  - no cellular energy required
- Active transport
  - moves molecules against a concentration gradient
  - requires cellular energy

Passive Transport Mechanisms

- **Diffusion**: the movement of a substance from higher concentration to lesser concentration
- **Osmosis**: the diffusion of water (solvent) across a membrane
  - influenced by total solute concentration



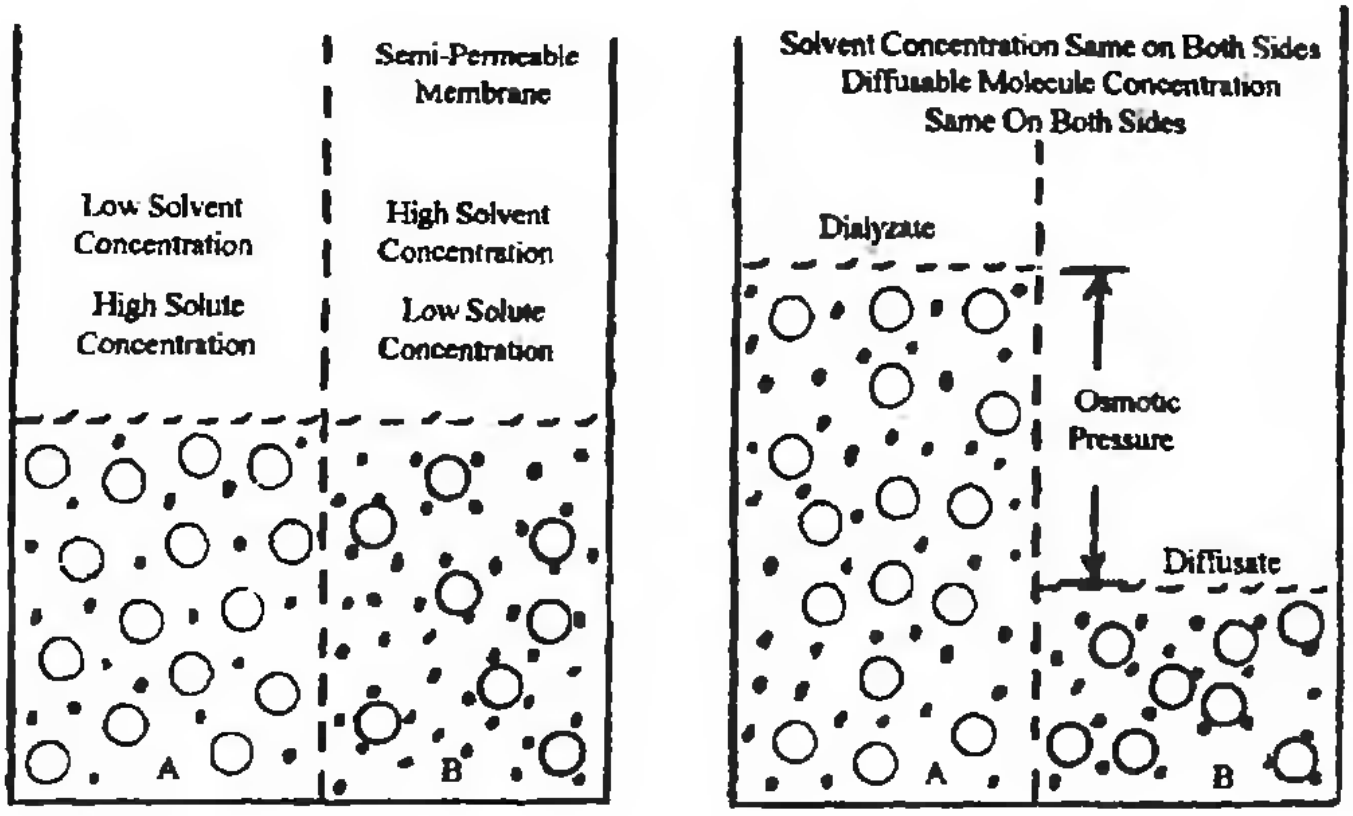




Facilitated Diffusion

- Involves transport proteins moving a solute along a concentration gradient
- May be specific
- May be saturated (can only work so fast) or inhibited
- Assist the physical process of diffusion

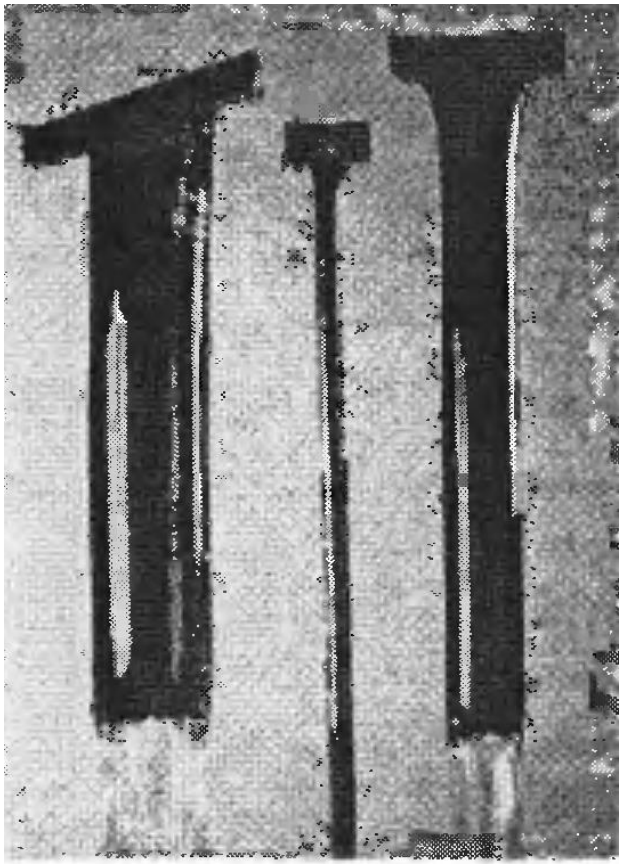
Diagram of the principle of osmosis. Left: before osmosis; right: after osmosis.



- Dialysis is the removal of low molecular weight solute molecules or ions from a solution by their passage through a semipermeable membrane driven by a concentration gradient. The same process of osmosis can take place with solute molecules or ions and it is called dialysis.

- Many commercial membranes are available. Visking (sausage casing), collodion, cellophane, and cellulose are common. Spectra/Por uses cellulose esters or regenerated cellulose. These are prepared to have a wide range of cutoff values from 1,000 to 300,000. Commercial dialysis tubing is not always pure and should be cleaned for exacting work.

Dialyzer tubing



Active Transport: Pumps

- Moves solute uphill and requires energy
- Always requires transport proteins
- Major factor that allows the cell to regulate the concentration of solute within the cell
- May result in an **imbalance of solute** across a membrane that the cell can utilize

Electrochemical Gradient

- The difference in voltage across a membrane resulting from electrogenic pumps is called **membrane potential**
- This electrical force affects the transport of charged solutes
- Cations are favored because interior of cell is usually negative compared to the outside
- Resulting **Electrochemical Gradient** affects ion transport:
  - electrical = membrane potential
  - chemical = concentration gradient

Electrodialysis

Although both neutral molecules and ions pass through a membrane based on concentration differences, it may be possible to hasten the process for ions by applying a voltage to the system, using a cathode on one side of the membrane and an anode on the other. In 1869 Maignot and Sabetes used a three-compartment system with the sample in the center compartment and membranes serving to make the inner compartment. This was found to work fairly well and was and is used to desalt biological preparations.

Liquid membrane processes

Liquid membrane processes involve the transport of solutes across a thin layer of a third liquid interposed between two miscible liquids. There are two types of liquid membranes:

1. Emulsion liquid membranes (ELM)
2. Supported liquid membranes (SLM)

They are conceptually similar but different in their engineering.

Emulsion liquid membranes are multiple emulsions of the water/oil/water type or the oil/water/oil type

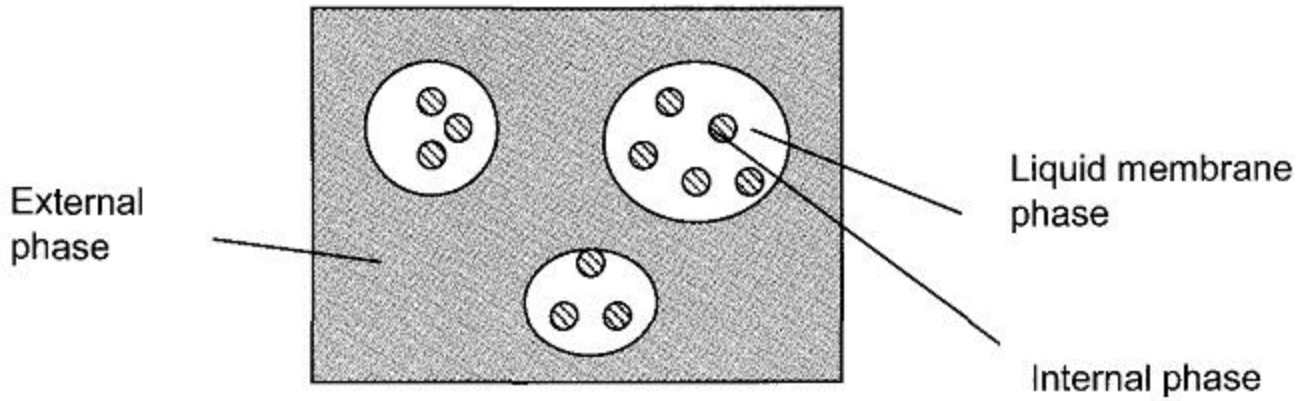


Fig. 11.34 Emulsion liquid membrane

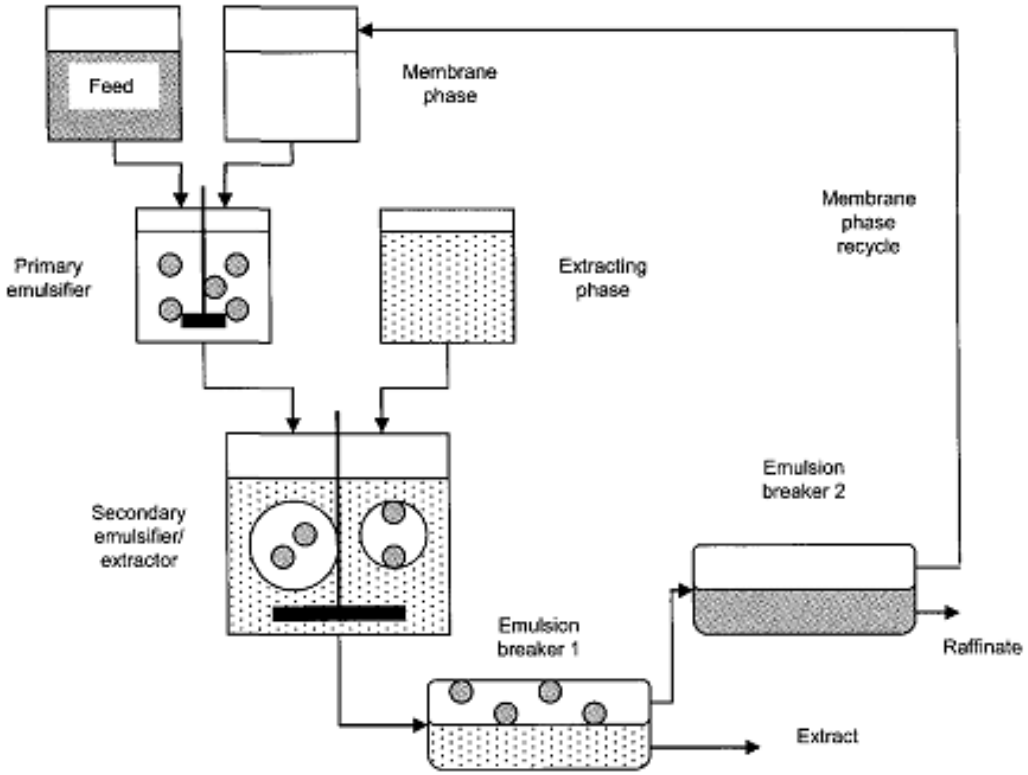


Fig. 11.35 Emulsion liquid membrane process

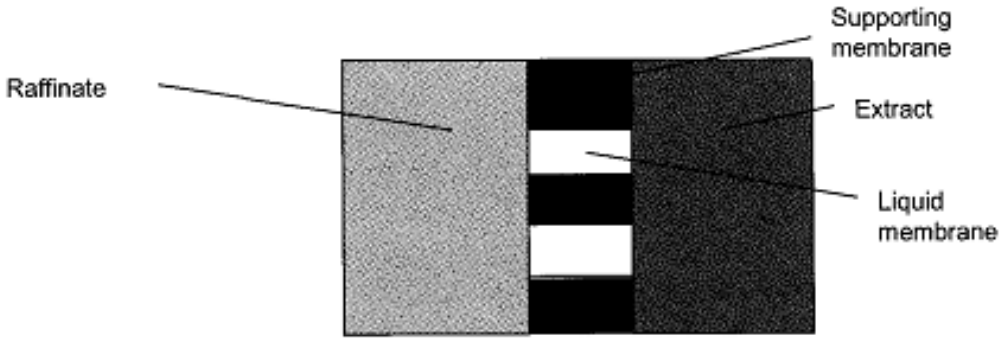
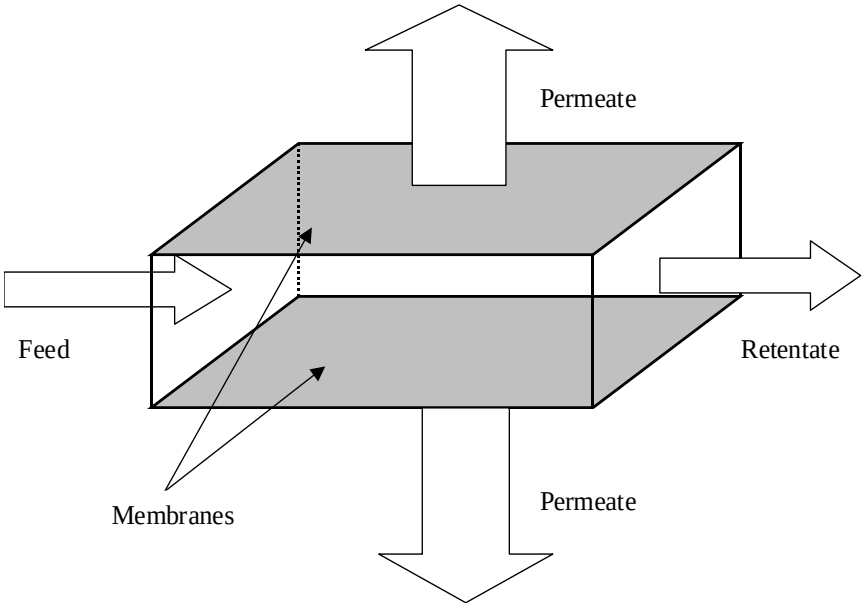
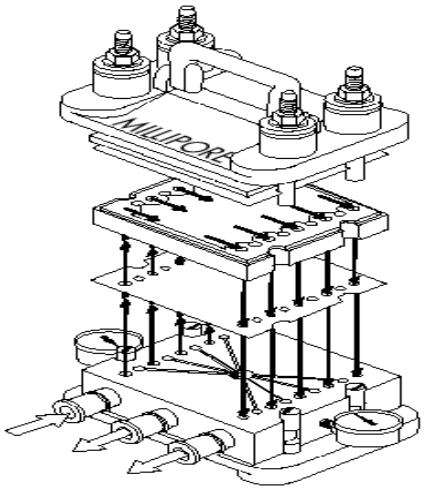


Fig. 11.36 Supported liquid membrane

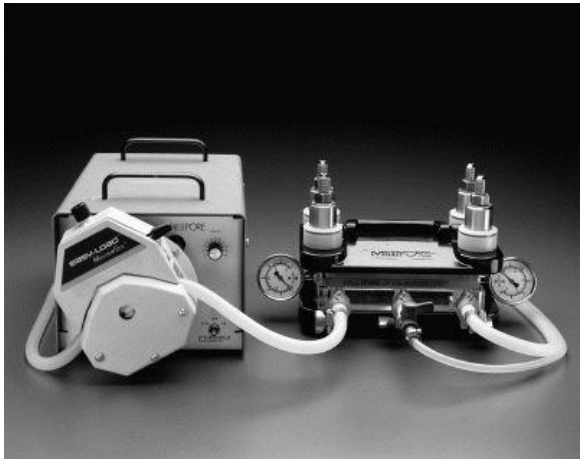
Instrumentation

Flat sheet tangential flow module

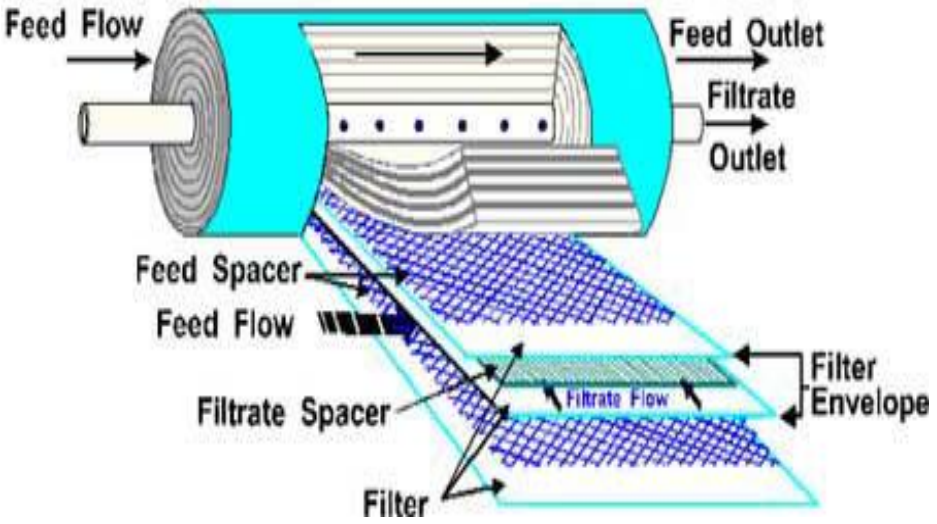




- Similar plate and frame filter press
- Alternate layers of membranes, support screens and distribution chambers
- Used for microfiltration and ultrafiltration

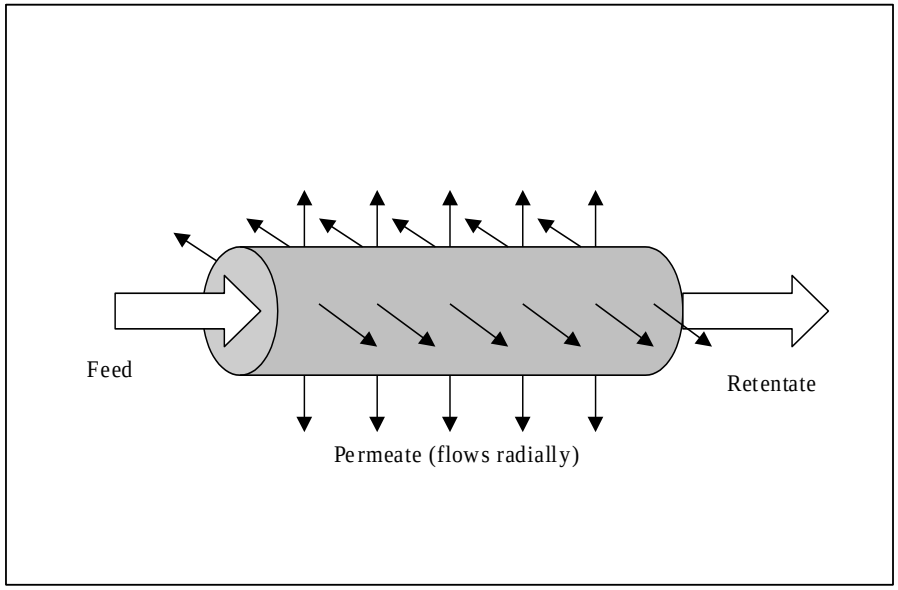


Spiral flow membrane module



- Flat sheet membranes are fused to form an envelop
- Membrane envelop is spirally wound along with a feed spacer
- Filtrate is collected within the envelop and piped out

Tubular membrane module



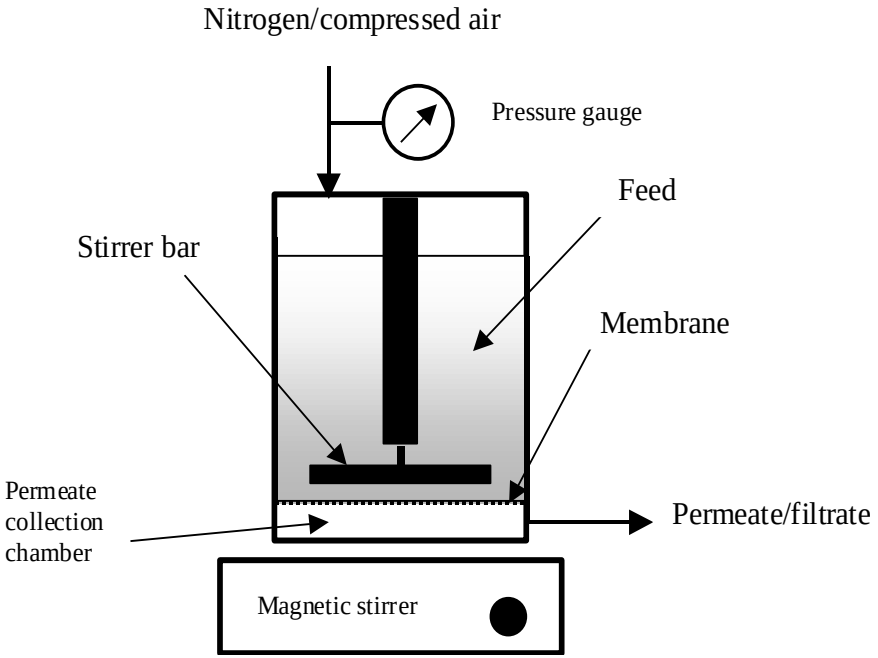
- Cylindrical geometry; wall acts as the membrane
- Tubes are generally greater than 3 mm in diameter
- Shell and tube type arrangement is preferred
- Flow behaviour is easy to characterise

Hollow fibre membrane module

- Similar to tubular membrane module
- Tubes or fibres are 0.25 - 2.5 mm in diameter
- Fibres are prepared by spinning and are potted within the module
- Straight through or U configuration possible
- Typically several fibres per module

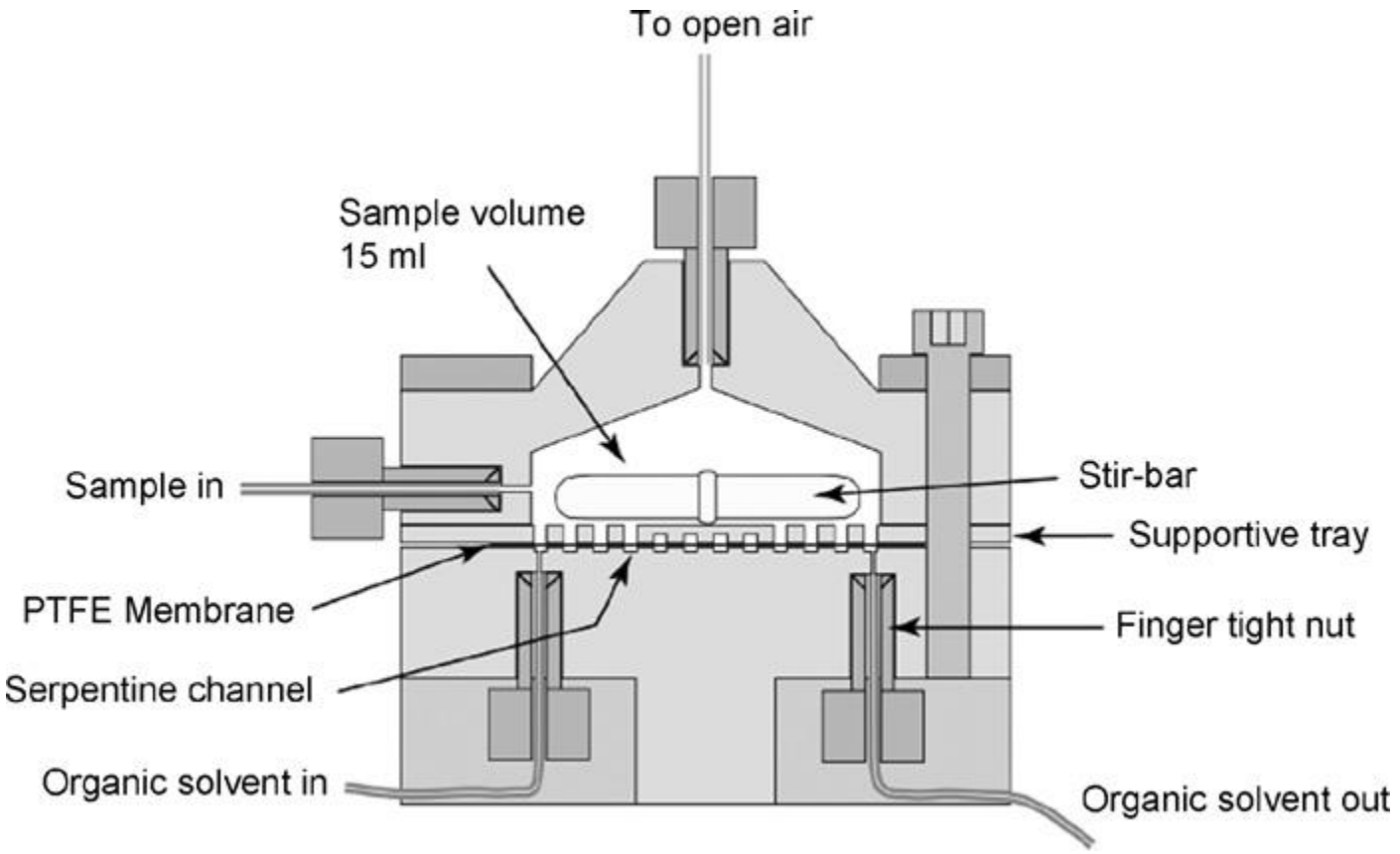
Applications

Stirred cell

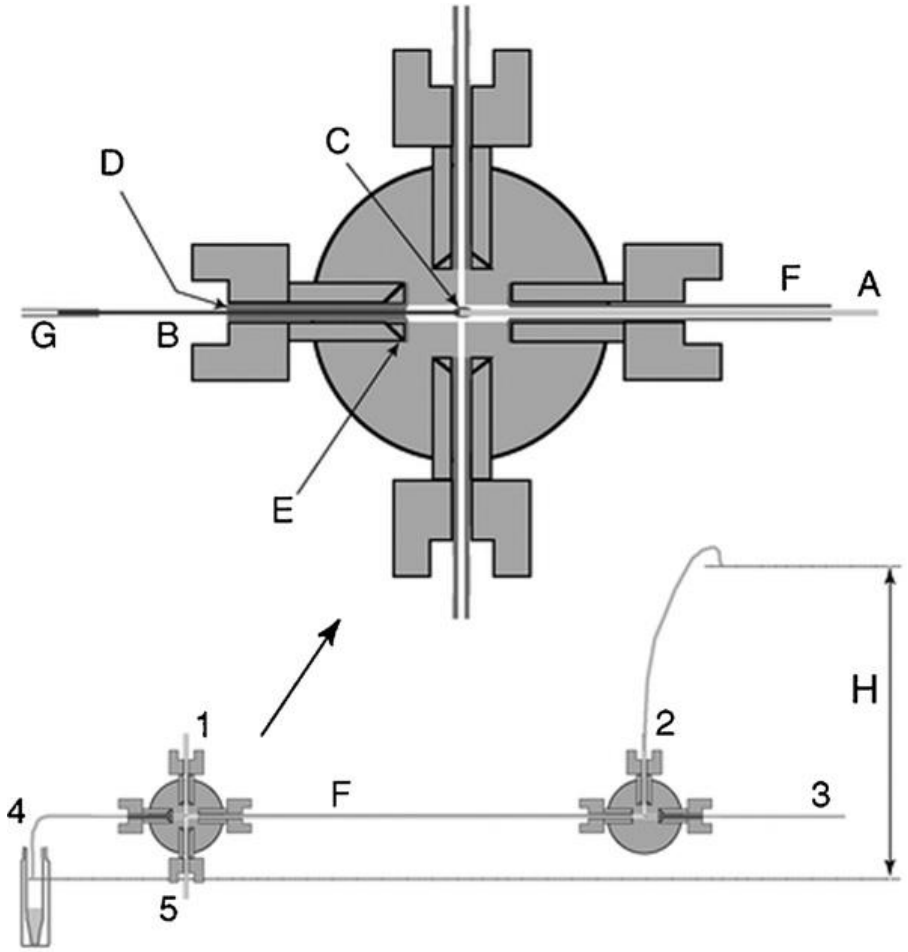


- Research and small-scale manufacturing
- Used for microfiltration and ultrafiltration
- Excellently suited for process development work

The stir bar-assisted MMLLE device



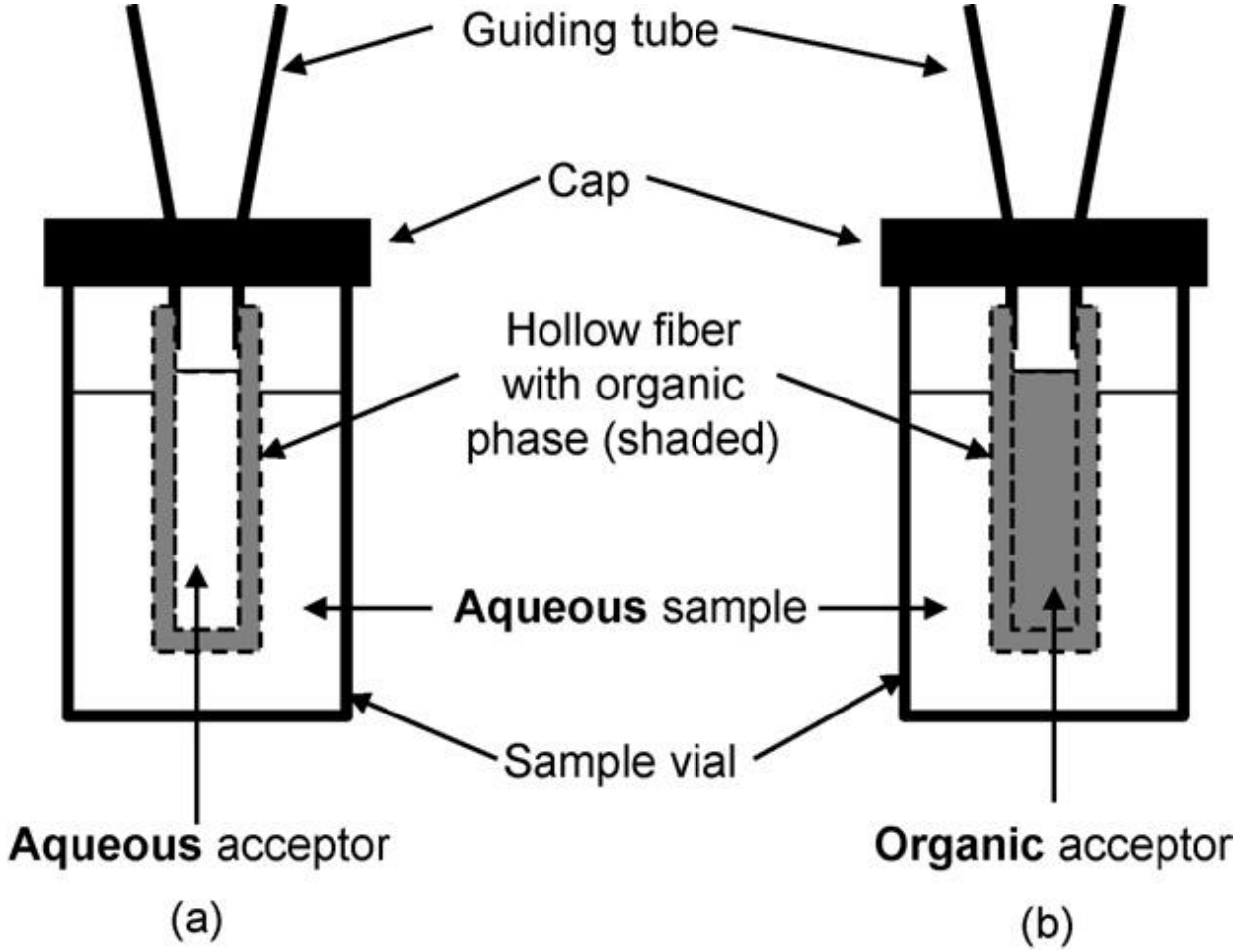
Porous PP HF (A), silica capillary (B), cyanoacrylate glue (C), short piece of PTFE tubing (D), Tefzel ferrule (E), a 1/16 i.d. 0.75-mm PTFE tube (F), and 0.33-mm i.d. PTFE tubing (G).



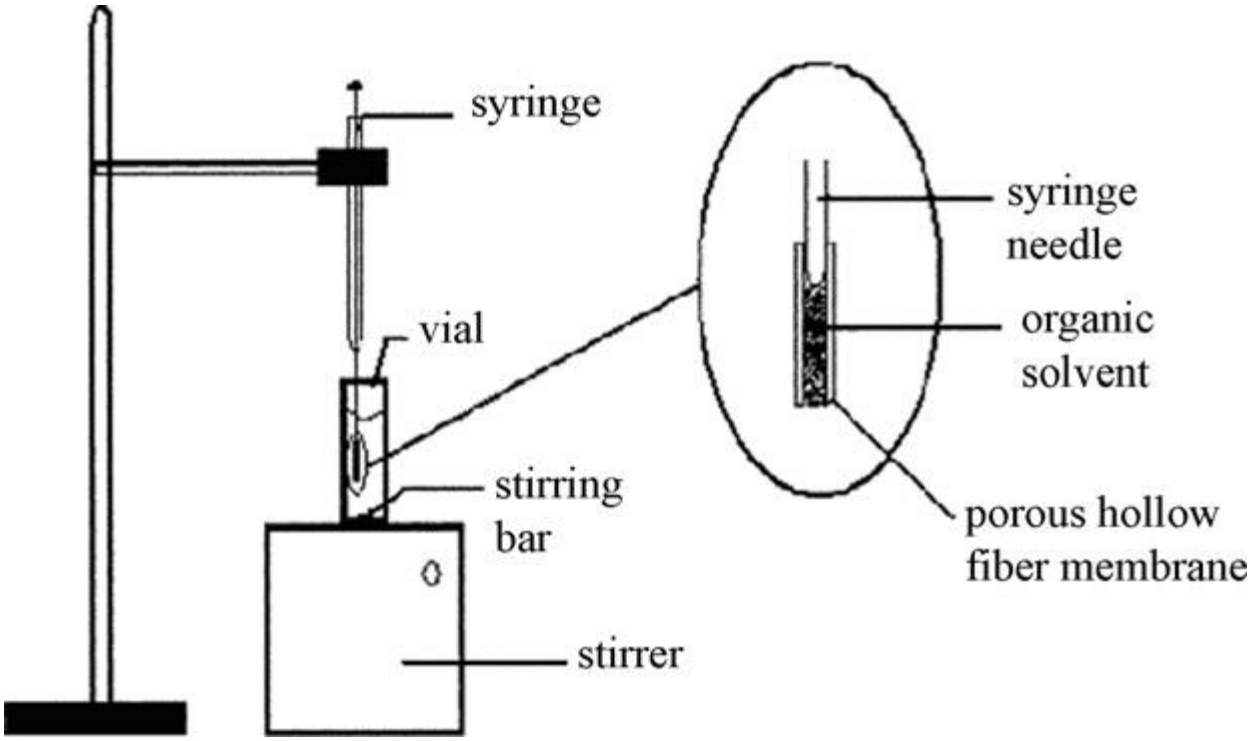
- An alternative miniaturized solvent-minimized sample preparation approach to complement SPME appeared in the middle-to-late 1990s; liquid-phase microextraction (LPME) utilizes only a small amount of solvent (low microliter range) for concentrating analytes from aqueous samples.

- In LPME, extraction normally takes place into a small amount of a water-immiscible solvent (sometimes referred to as the acceptor phase) from an aqueous sample containing analytes (donor phase). In the simplest form, the acceptor drop is held at the tip of a microsyringe needle and is directly

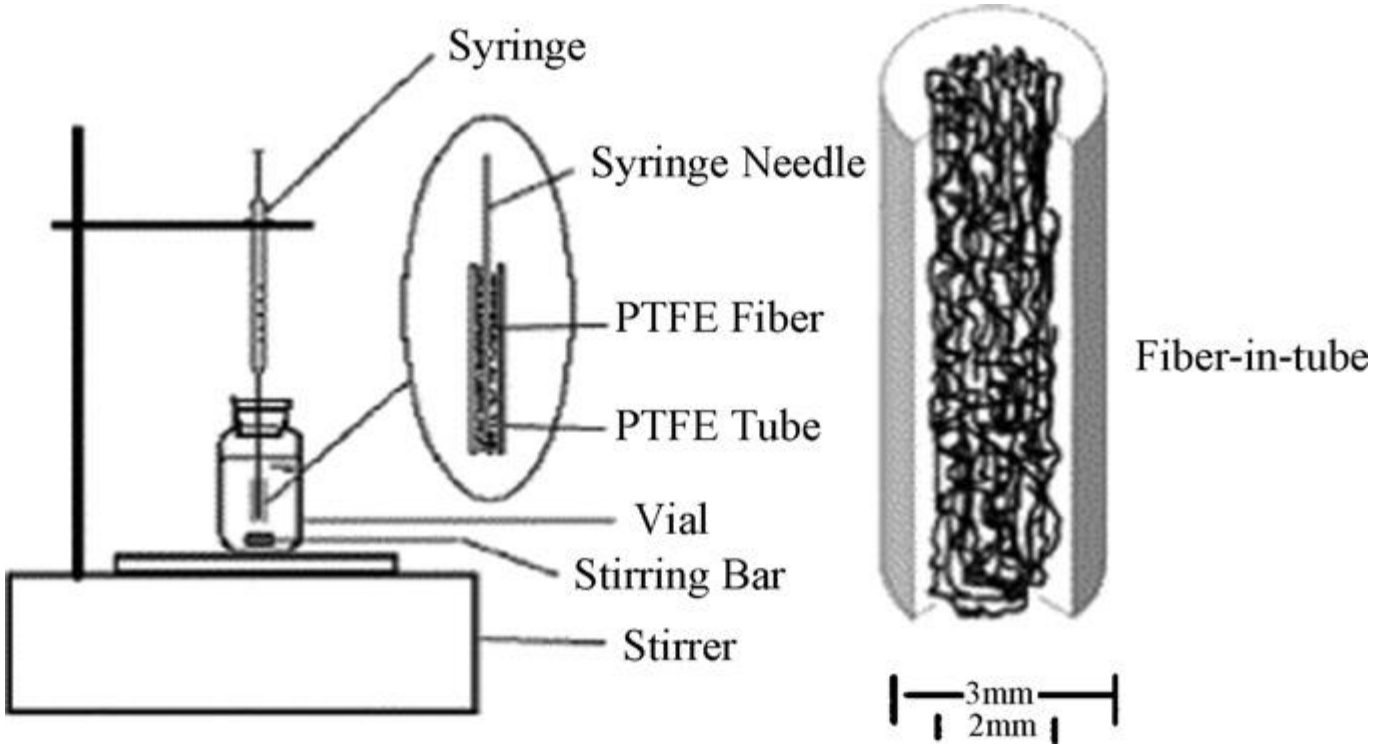
Principle of (a) three- and (b) two-phase LPME.



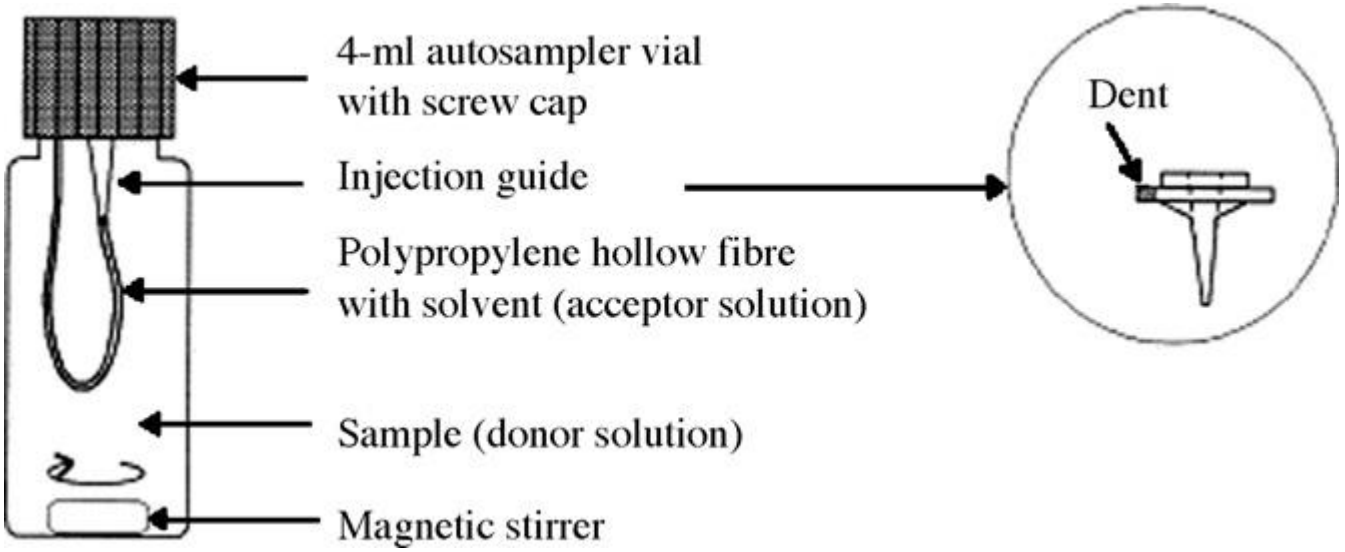
A hollow-fibre liquid-phase microextraction system



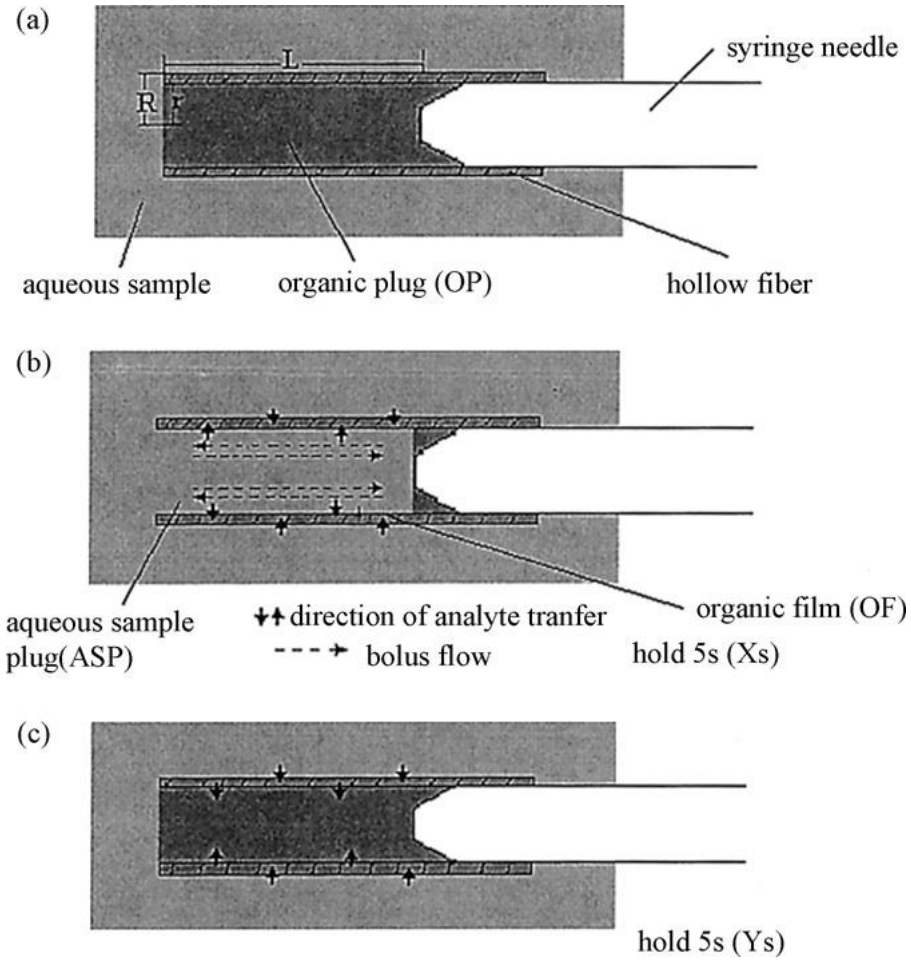
The fibre-in-tube liquid-phase microextraction device



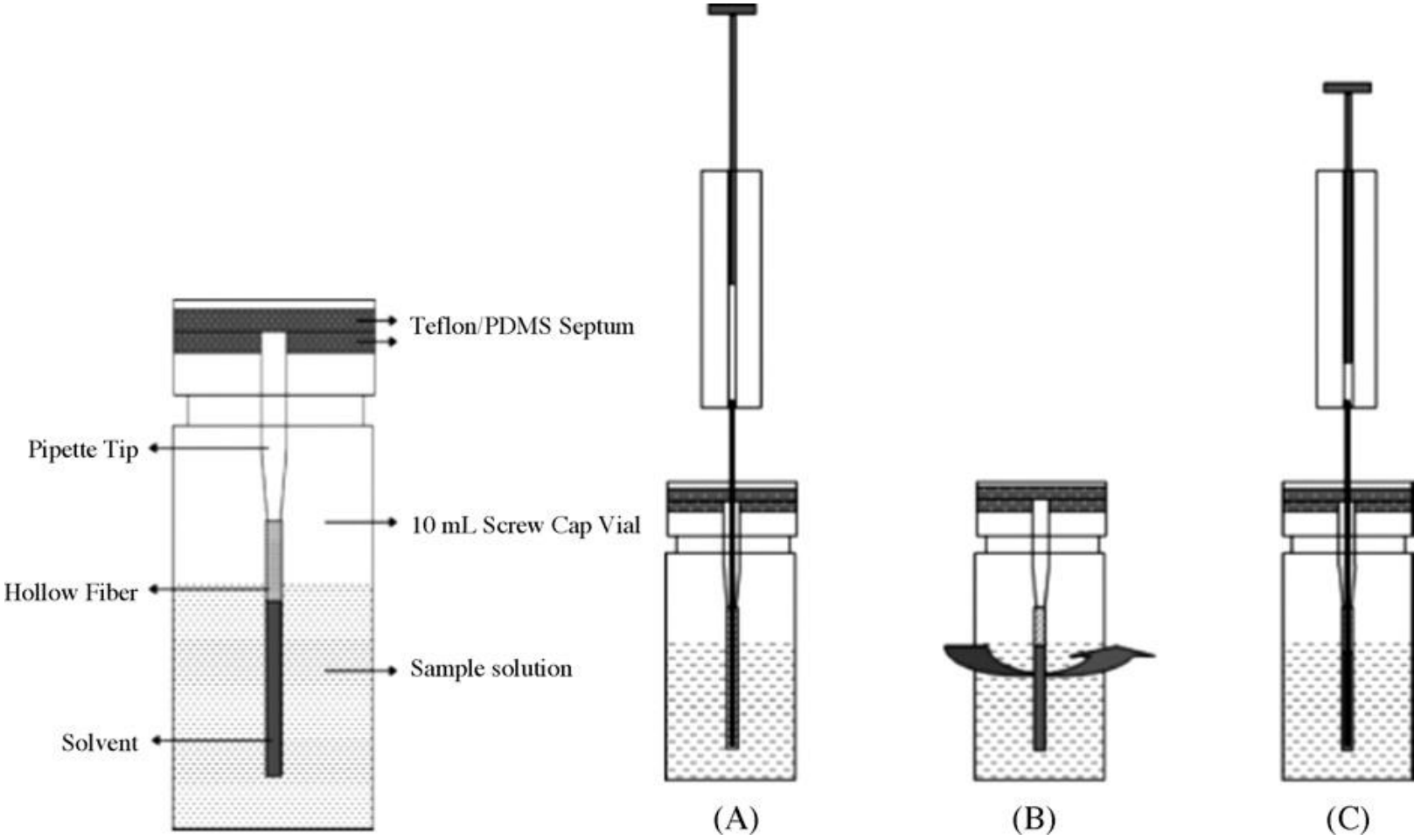
A semi-automated set-up of the hollow-fibre liquid-phase microextraction



An illustration of the dynamic hollow-fibre liquid-phase microextraction steps. (a) HF filled with organic solvent is immersed in aqueous sample, (b) the organic solvent is withdrawn inside the syringe needle, where the solvent is not shown in it and (c) the organic solvent inside the needle is released into the HF.



Left side (first vial): a schematic diagram of the hollow-fibre liquid-phase microextraction (HF-LPME) device. Right side (vials A, B and C): a schematic diagram of the automated HF-LPME steps. (A) Filling of extraction solvent; (B) agitation; (C) withdrawing the solvent into syringe and injection to GC



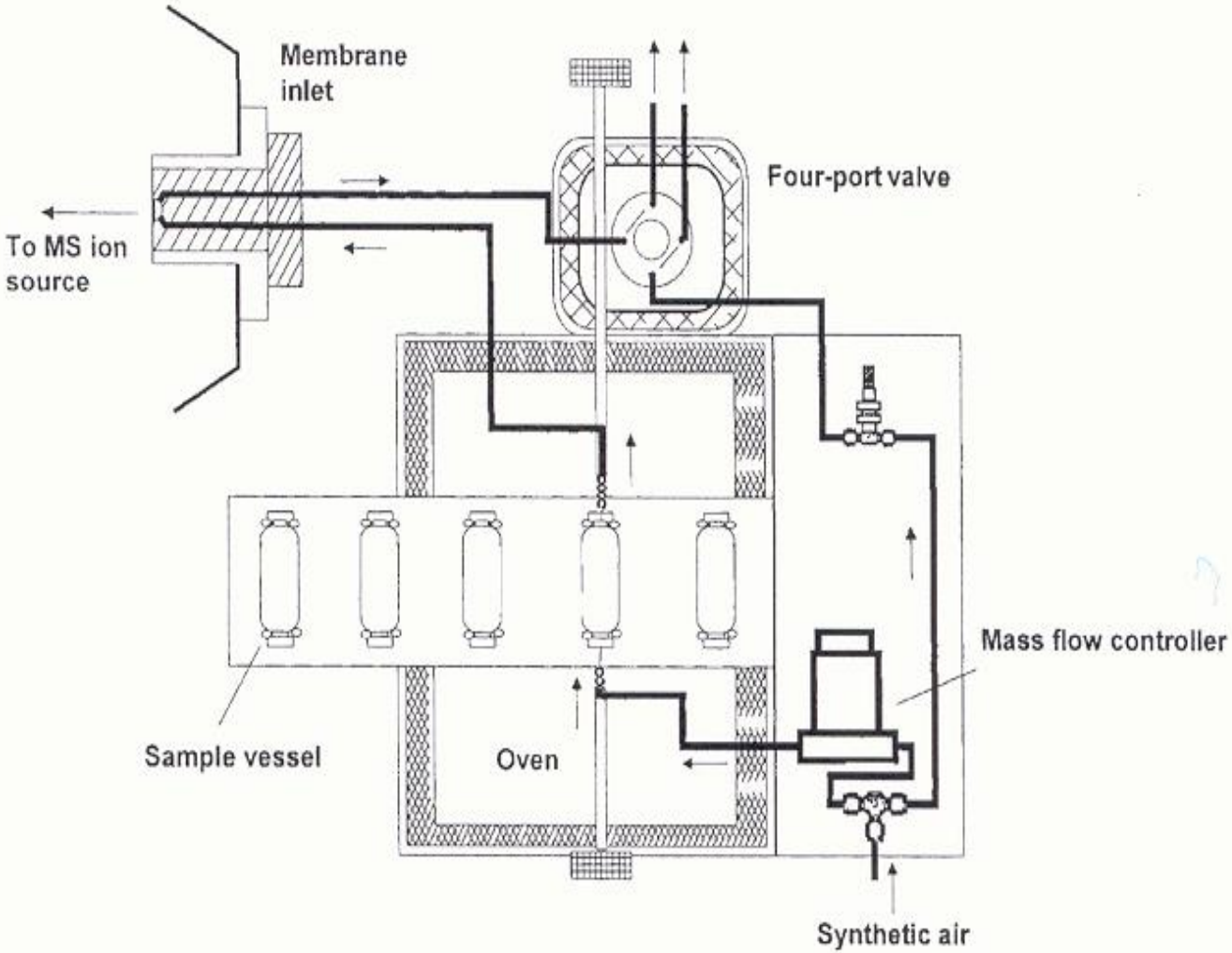
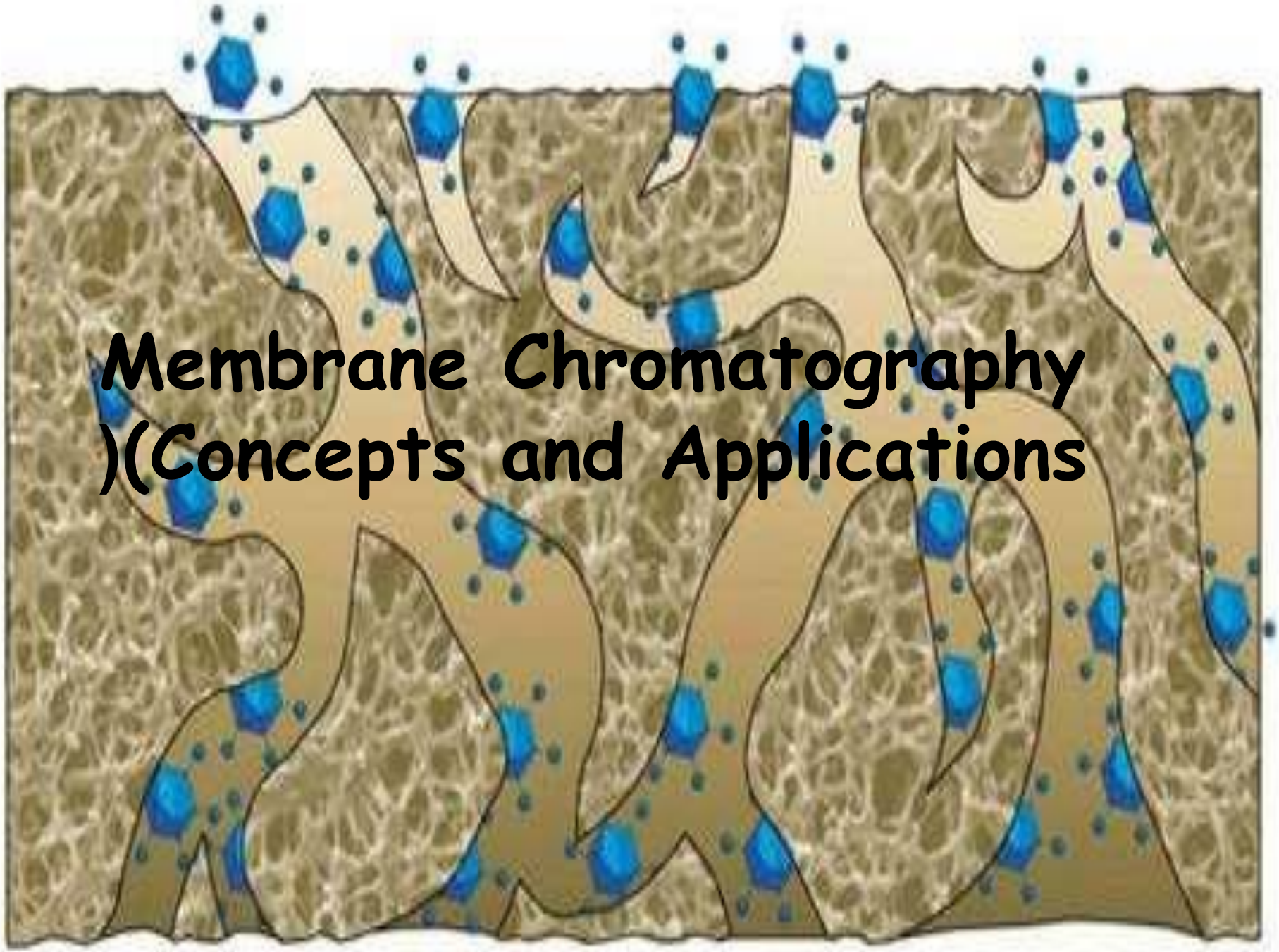
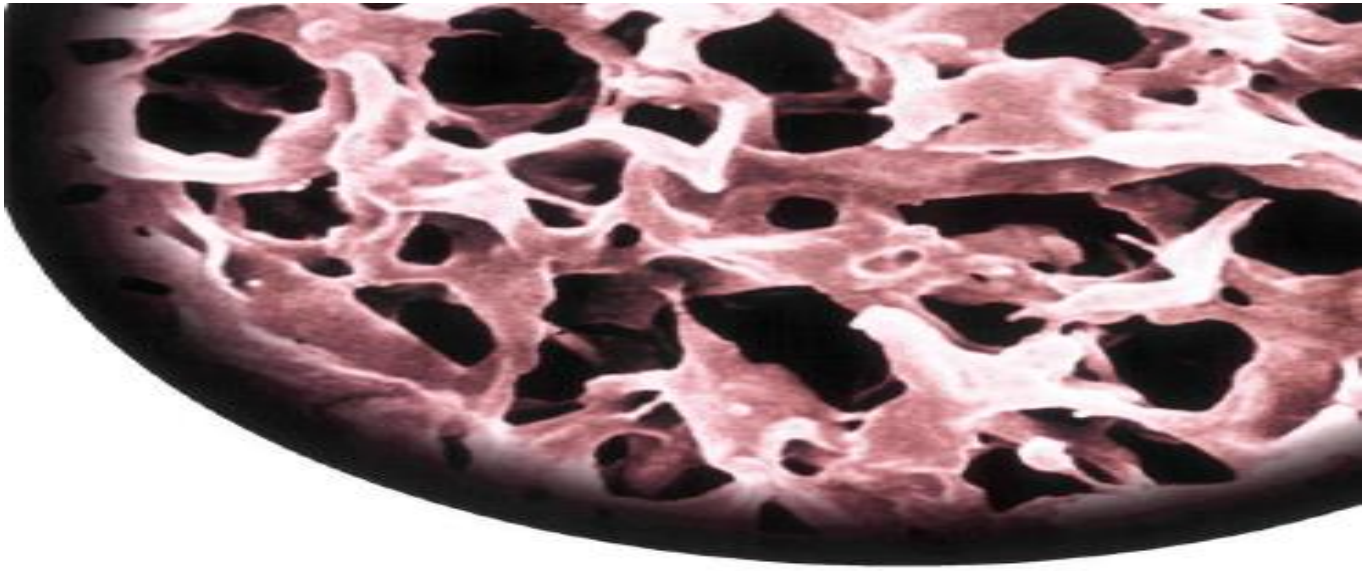

Membrane inlet
To MS ion source
Four-port valve
Mass flow controller
Sample vessel
Oven
Synthetic air

Table 4. Analytical Results of Two Authentic Soil Samples Measured by PAM-MS and HSGC

	sample/content, mg/kg					
	sample 1			sample 2		
compound	PAM-MS	HSGC lab 1	portable HSGC	PAM-MS	HSGC lab 1	portable HSGC
benzene	6.3	5.0	17		0.1	1.0
toluene	140	110	>140	6.8	7.0	19
xylenes	680[a]	610	>350	23[a]	43	70
ethylbenzene		130	110		5.8	16
tetramethylbenzene	100	27[b]		9.2	6.1[b]	
trimethylbenzene	1000	280[c]		52	33[c]	
aliphatics	400			26		
MTBE	18	32	37			
TVOC[f]	2400	2900[d]	>490[e]	120	240[d]	300[e]

[a] The sum of xylenes and ethylbenzene. [b] Only 1,2,3,5-tetramethylbenzene. [c] Only 1,2,4-trimethylbenzene. [d] Estimated with toluene. [e] Estimated with xylene. [f] TVOC (total volatile organic compounds) is the sum of identified compounds and unknown compounds. The amount of unknown compounds was estimated using toluene (lab 1) or xylene (lab 2).


Membrane Chromatography
)(Concepts and Applications



PRINCIPLES OF BIOSEPARATIONS ENGINEERING

RAJA GHOSH

2006

Comparision with packed bed and membrane chromatography:

Adsorption and chromatographic separations are traditionally carried out using packed beds.

Some of the major limitations or disadvantages of using packed beds are:

1. High pressure drop across packed beds

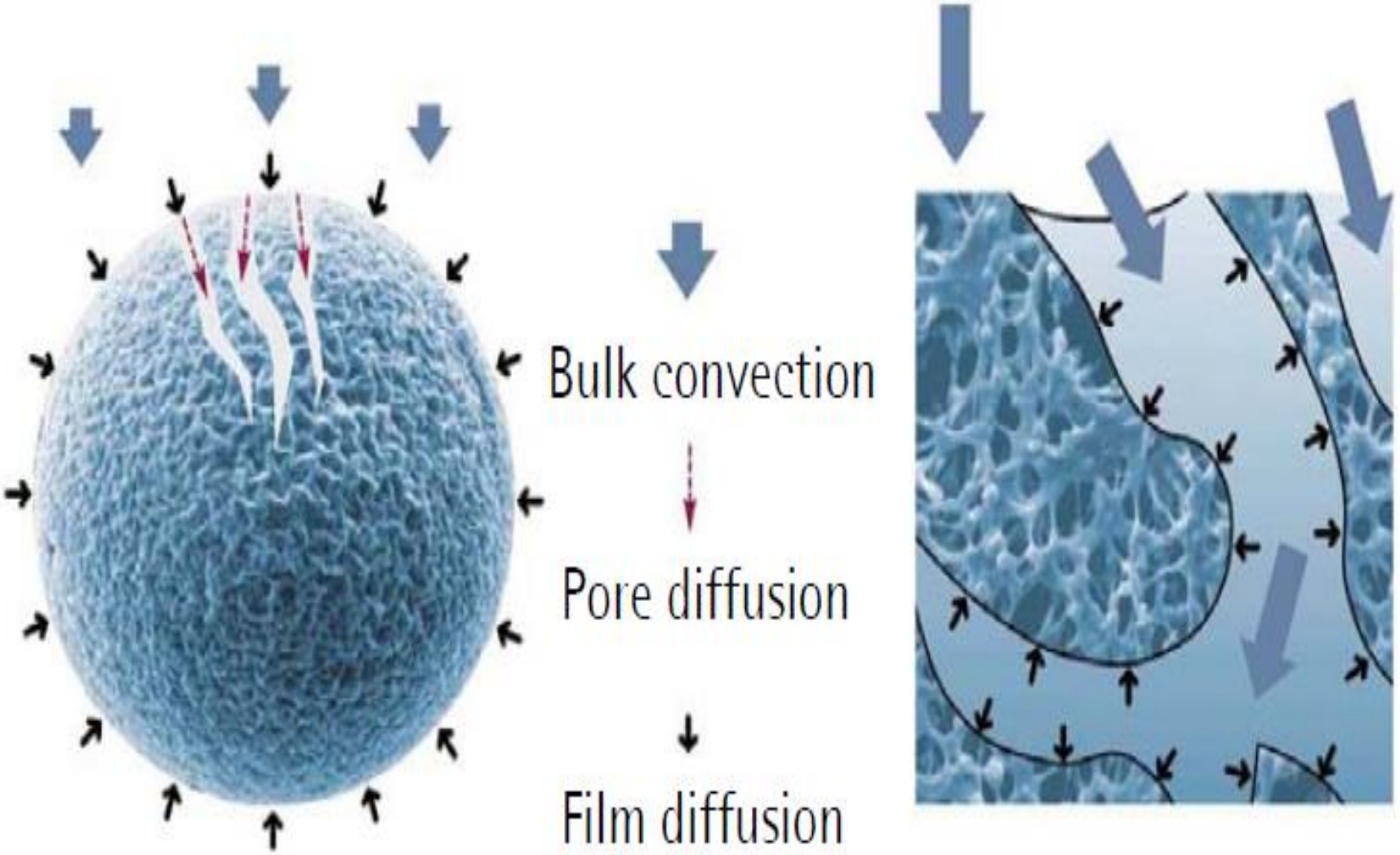
2. Increase in pressure drop during operation

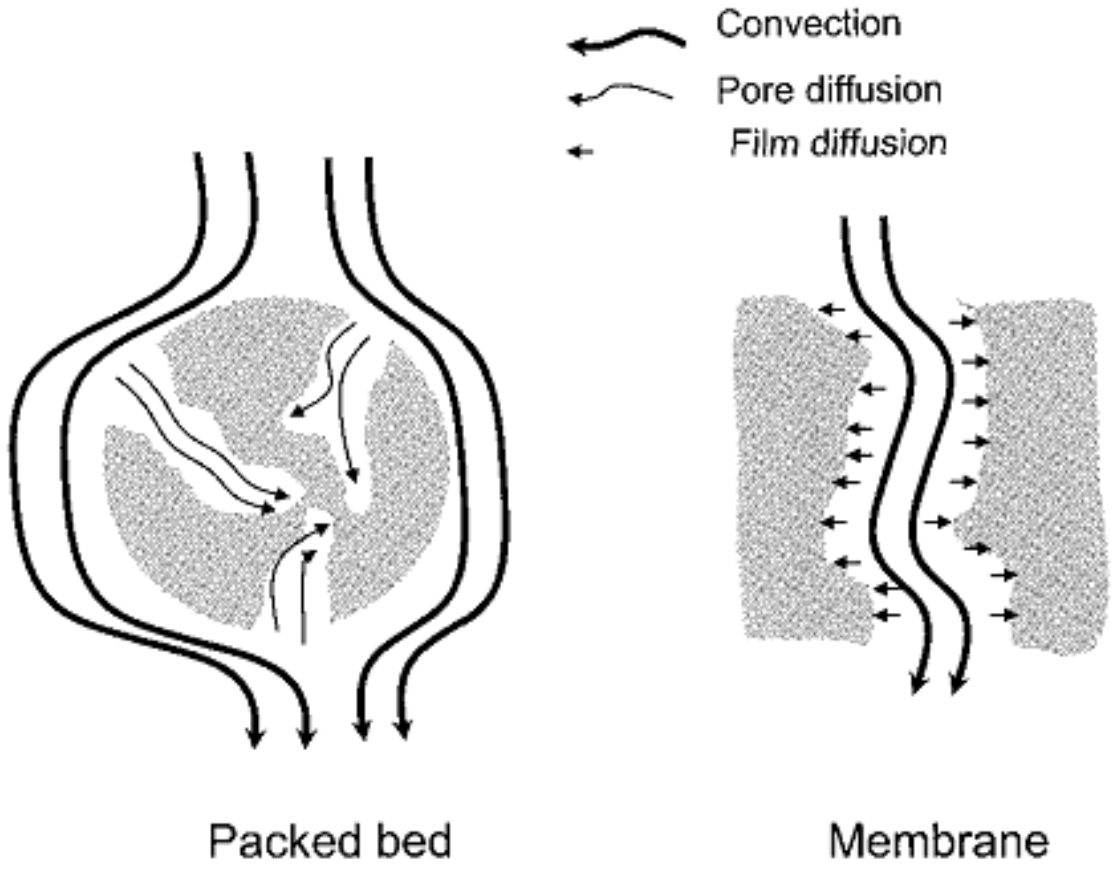
3. Packed bed blinding by biological macromolecules

4. Dependence on intra-particle (or pore) diffusion for solute transport

5. Difficulty in scaling-up

Mass transfer effects: conventional bead vs. membrane adsorber



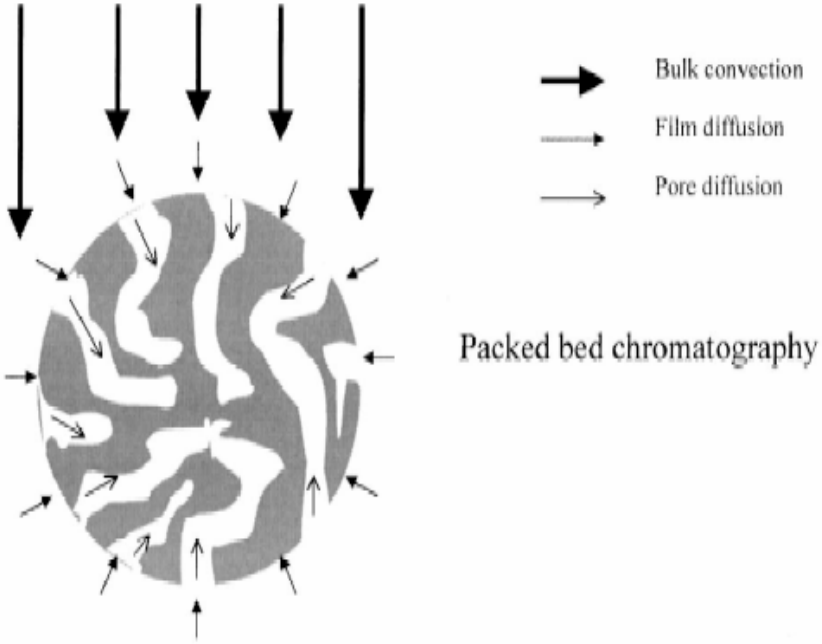

Convection
Pore diffusion
Film diffusion
Packed bed
Membrane

Mechanisms of separation in membrane chromatography

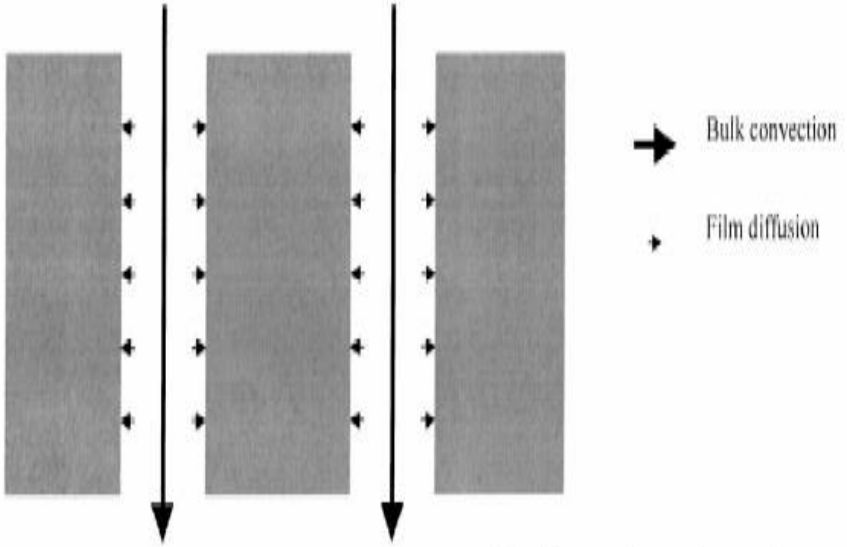
1. Affinity binding

2. Ion-exchange interaction

* Hydrophobic interaction Size exclusion based separation using membrane beds has not yet been feasible.



Packed bed chromatography



Membrane chromatography

Advantages:

1. Low process time

2. Low process liquid requirement

3. Possibility of using very high flow rates

4. Lower pressure drop(very low bed height to diameter ratio)

5. Less column blinding

6. Ease of scale-up

7. Fewer problems associated with re-validation (if a disposable membrane device is used)

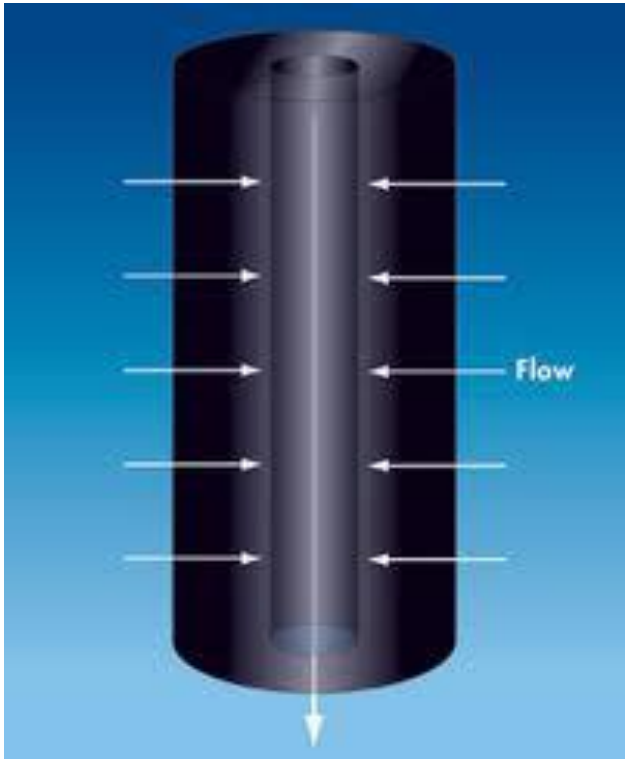
Based on the membrane geometry, three types of membrane absorbers are used:

flat sheet

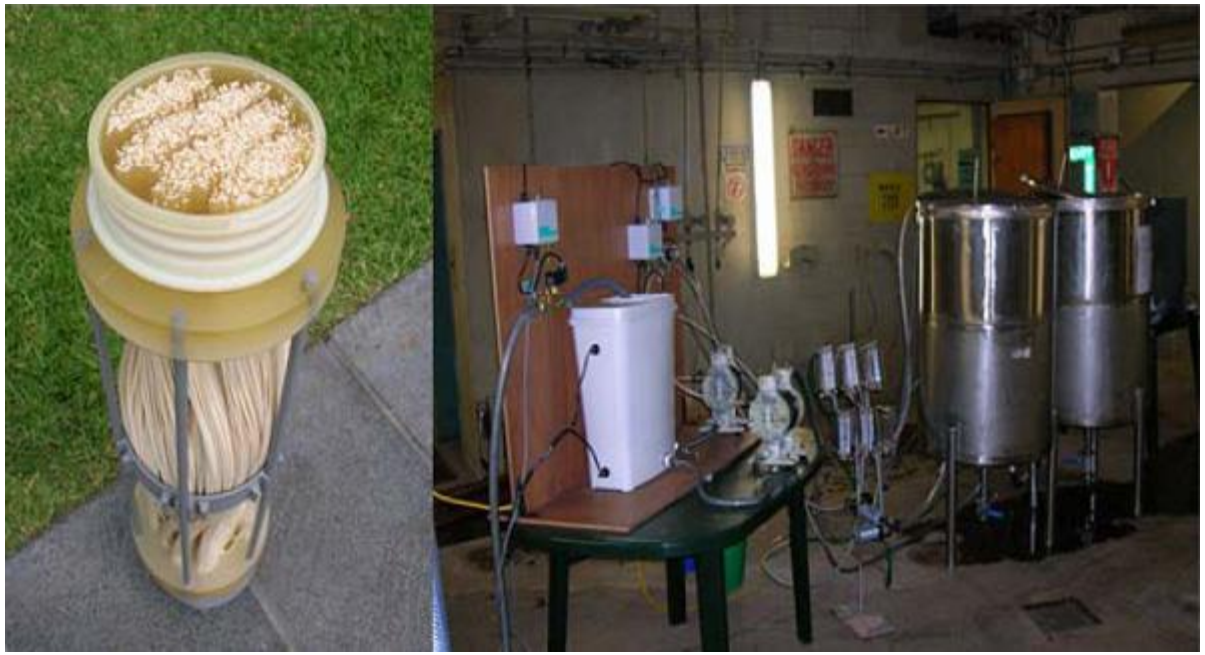
(A)

(B)

radial flow



hollow fiber



Applications:

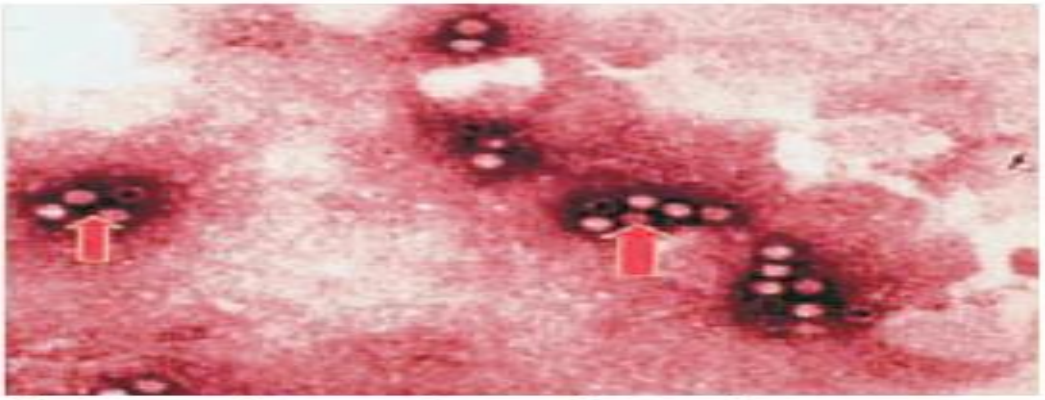
The high dynamic binding capacity of membrane chromatography makes it useful in various potential applications, including DNA, virus, and host-cell protein removal as well as purification of gene therapy vectors.

Membrane proteins

1. Removal of unbroken cells from the cell lysate by low speed centrifugation (20 min at 10,000 g).
2. Isolation of the membrane particles from the supernatant by ultracentrifugation (60 min at >100 000 g).
3. Washing of the membrane particle to remove all soluble proteins.
4. Solubilization of protein from the membrane particles by a mild detergent. (detergent: protein ratio = 1:10)
5. Phosphate buffers(0.1M-0.5M), 5-50% glycerol helps.

(http://www.ls.huji.ac.il/~purification)

Biotech Trends



New membrane chromatography technology has been used for numerous biopharmaceutical applications, including the removal of small (~20 nm) viruses such as this parvovirus. A quaternary amine (Q) anion-exchange membrane has successfully removed greater than 7 log of parvovirus from a protein solution at a high flow rate of 10 CV/min. (Photo provided by Pall Corp.)

Capturing Large Biomolecules With Membrane Chromatography

As demand for protein-based and genetic therapies increases worldwide, ion-exchange membrane chromatography provides a fast, efficient purification technology that can help streamline production and lower overall manufacturing costs.

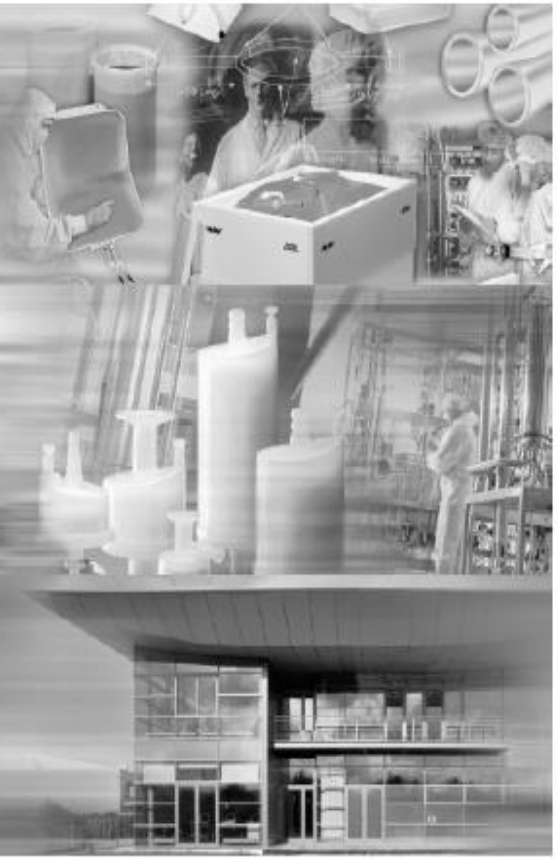
By Ian Sellick at Pall Life Sciences

Ian Sellick graduated from Bristol University (UK) after studying zoology, cell biology, medical microbiology and pharmacology. Postgraduate research included studies on the production of fish mucus and environmental effects on fish growth. After working for several years in the laboratories of CSL, he moved to the field of technical separations – most recently at Pall Corporation, where he has been involved in the marketing of purification products for over 11 years. Dr Sellick is the author of numerous publications, including many on purification in the biotechnology industry. He is a senior member of the Society for Technical Communication and a member of the American Chemical Society.

October 2006

Keep it clean: Sartobind® - The high speed Membrane Chromatography for Contaminant Removal

Dr. Stefan Fischer-Frühholz
Product Manager

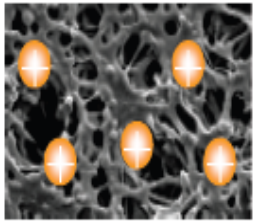


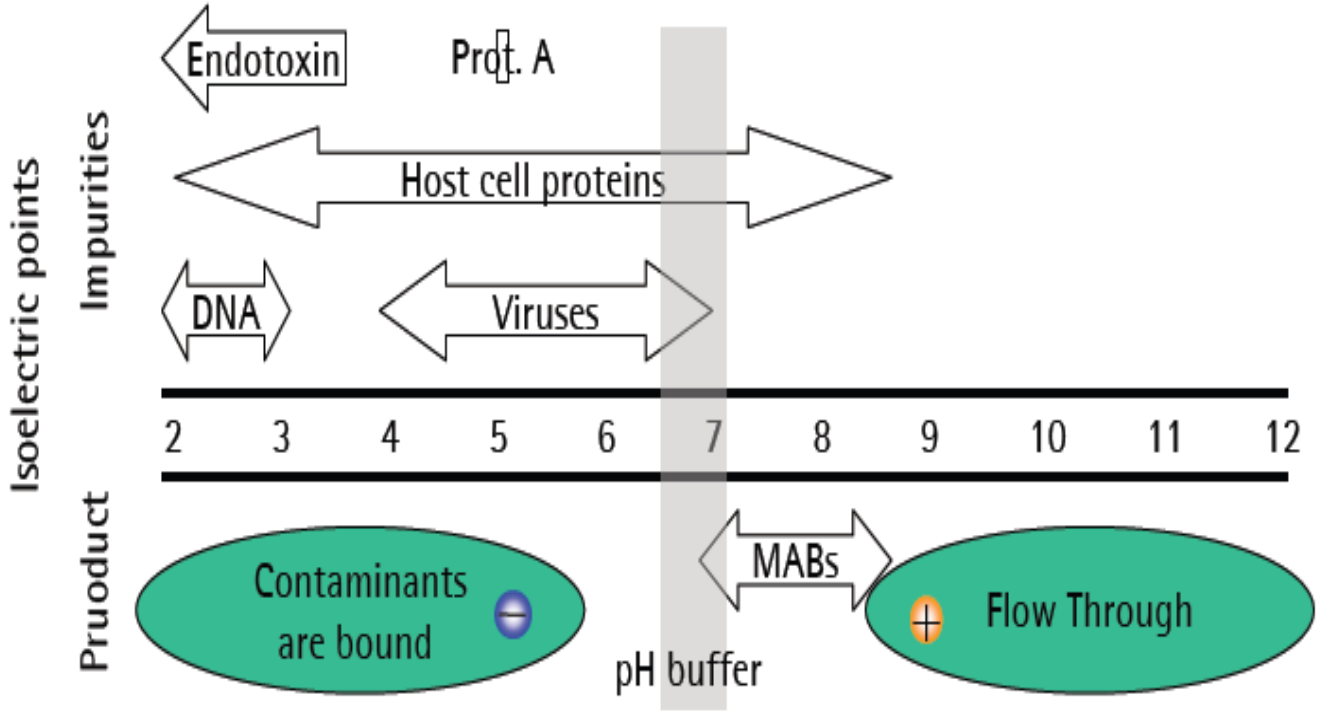
sartorius

Removal of contaminants during monoclonal antibody production

: Sartobind Q

: Membrane charge:





sartorius

SEPARATION OF PROTEINS BY ION EXCHANGE AND MEMBRANE CHROMATOGRAPHY: BUFFER COMPOSITION, INTERFERING IMPURITIES AND FOULING CONSIDERATIONS

A Thesis

by

TAHMINA IMAM

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Chemical Engineering

Protein Separation with Ion-exchange Membrane Chromatography

by

Liming Cao

A thesis

Submitted to the Faculty

Of the

Worcester Polytechnic Institute

in partial fulfillment to the requirements for the

Degree of Master of Science

in Chemical Engineering

by

Liming Cao

Journal of Membrane Science 390–391 (2012) 263–269

Contents lists available at SciVerse ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Simultaneous removal of leached protein-A and aggregates from monoclonal antibody using hydrophobic interaction membrane chromatography

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ARTICLE INFO

Article history:
Received 1 November 2011
Received in revised form
26 November 2011
Accepted 28 November 2011
Available online 7 December 2011

Keywords:
Hydrophobic interaction
Membrane chromatography
Monoclonal antibody
Aggregates
Protein-A
Polishing

ABSTRACT

Protein-A affinity chromatography is the standard capture technique used for purification of monoclonal antibodies (mAbs). A major problem with this technique is its inability to remove monoclonal antibody aggregates from the monomeric form of the antibody. Moreover, the elution of mAbs from a protein-A column is carried out using acidic conditions which in turn could accelerate the formation of antibody aggregates and causes leaching of protein-A from its supporting media. These impurities have to be removed using appropriate separation methods before the mAb can be used for therapeutic applications. There is currently a limited repertoire of polishing techniques which simultaneously remove both mAb aggregates and protein-A. In this paper, we describe a polishing method based on hydrophobic interaction membrane chromatography for simultaneously removing these impurities. The flow-through mode was used whereby monomeric mAb flowed through a membrane stack while impurities were retained by reversible adsorption. These impurities were subsequently eluted by lowering the salt concentration and the membrane stack was thus regenerated. The operating conditions for this method were systematically optimized using pure mAb, aggregates, protein-A, and mAb/protein-A complexes. The mechanisms by which aggregates and leached protein-A were simultaneously removed are hypothesized.

Journal of Chromatography A, 1242 (2012) 67–74

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Comprehensive two-dimensional HepG2/cell membrane chromatography/monolithic column/time-of-flight mass spectrometry system for screening anti-tumor components from herbal medicines

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ABSTRACT

Cell membrane chromatography (CMC) is a biological affinity chromatographic method using specific cell membrane as stationary phase. It has been proved to be a practical tool for investigating binding interactions between drugs and membrane receptors. In this study, a novel comprehensive two-dimensional (2D) chromatography approach was established for screening anti-tumor components from herbal medicines (HMs). HepG2/CMC model was first developed and applied as the first dimensional column. Using an automatic ten-port switching valve equipped with two sample loops, the fractions of the first-dimension were introduced in the second-dimension consists of a monolithic column and a time-of-flight mass spectrometry (TOFMS) with high resolving ability. Based on the stability, selectivity and suitability assays of the HepG2/CMC/monolithic column/TOFMS system, berberine (BBR) and tetrahydropalmatine (THP) from Cortex *phellodendri amurensis*, oxymatrine and matrine from Radix *sophorae flavescentis* were screened and identified as potential active components. The competitive displacement assay suggested that the four components could act on epidermal growth factor receptor region on the HepG2 cell membrane in similar manner of gefitinib. Furthermore, their inhibiting effects on cell proliferation *in vitro* were also confirmed and, BBR and THP showed concentration dependently inhibitory ability on HepG2 cell proliferation ($p < 0.05$). The result demonstrated that the proposed comprehensive 2D HepG2/CMC/monolithic column/TOFMS system has the advantages of strong recognition and rapid analysis abilities for the total screening procedure, which will be selectable and practical in drug discovery from complex HM samples and can also be applied to other biochromatography models.

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Screening active compounds acting on the epidermal growth factor receptor from *Radix scutellariae* via cell membrane chromatography online coupled with HPLC/MS

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ARTICLE INFO

Article history:
Received 3 October 2011
Received in revised form
16 December 2011
Accepted 20 December 2011
Available online 30 December 2011

Keywords:
Radix scutellariae
Epidermal growth factor receptor
Cell membrane chromatography
High performance liquid chromatography/mass spectrometry
Wogonin

ABSTRACT

Radix scutellariae is a traditional Chinese medicine (TCM) and has many pharmacological effects, including antiviral, antibacterial, antifungal, antipyretic, hypotensive, anti-inflammatory and anti-anaphylaxis effects. However, few studies have screened the active compounds in this complex product for tumor therapy. In this study, a two-dimensional online method was developed to screen the active compounds from *Radix scutellariae* acting on the epidermal growth factor receptor. The screening results showed that wogonin from *Radix scutellariae* was the targeted component which acted on epidermal growth factor receptor specificity. The in vitro inhibitory activity of wogonin on the viability of cells with high epidermal growth factor receptor expression was tested using the MTT assay. In the dosage range of 0.40–50.0 μM, inhibition of HEK293/EGFR by wogonin was 8.94 ± 0.2, 20.64 ± 5.10, 34.16 ± 5.90 and 69.03 ± 7.80 at the concentrations of 0.4×10^{-6}, 2×10^{-6}, 10×10^{-6} and 50×10^{-6} mol L^{-1}, respectively. These results showed that wogonin inhibited the growth of cells with high epidermal growth factor receptor expression in a dose-dependent manner.

Questions and Comments?

It doesn't matter how many resources you have

if you don't know how to use them, they will never be enough