3 色層分析法 Chromatography

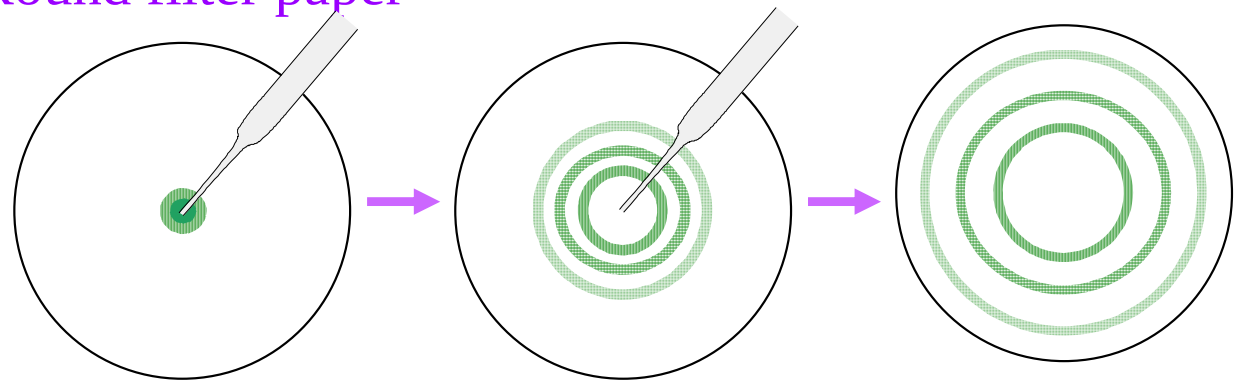
- 3.1 色層分析原理 Basic principles
  極性不同的分子在兩相中有不同分佈比例
- 3.2 膠体過濾法 Gel filtration
  依樣本分子量的不同來做分離純化
- 3.3 離子交換法 Ion exchange
  利用樣本分子的表面帶電性質不同來進行分離
- 3.4 親和層析法 Affinity chromatography
  利用分子間的親和性大小不同來進行分離
- 3.5 HPLC 及 FPLC
  改善介質的材質及吸附容量可增加速度及解析力

色析法演進過程 Historical review

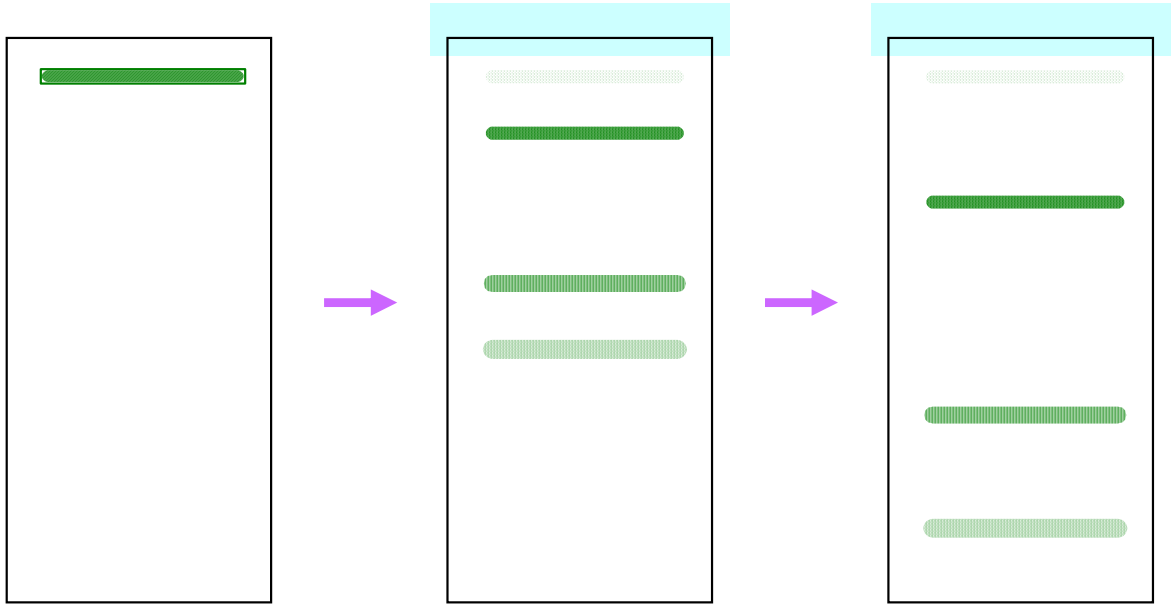
Martin, Synge (1952)

Paper partition chromatography (PPC)

Round filter paper

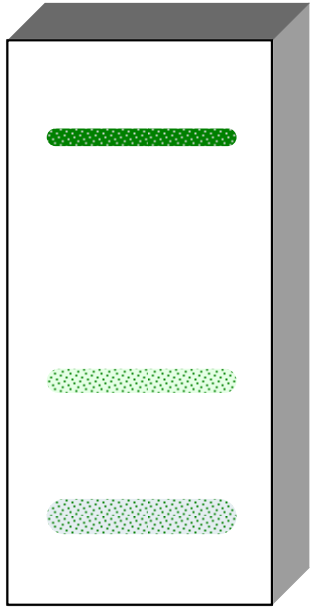


Development



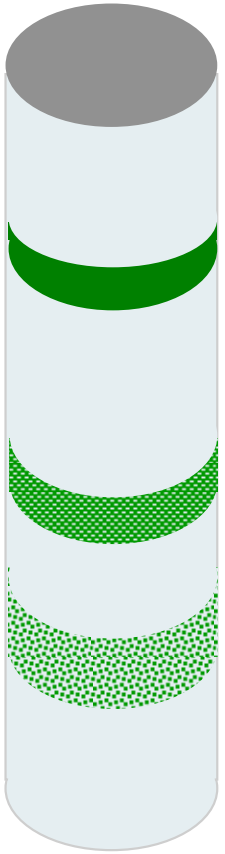
Long rectangle paper

Thin layer (TLC)



Sample capacity increased

Column



Larger capacity

色層分析法的基本機制 Essential mechanism

Like
Dissolves
Like

- 極性相似的兩分子間，其親和力較強。

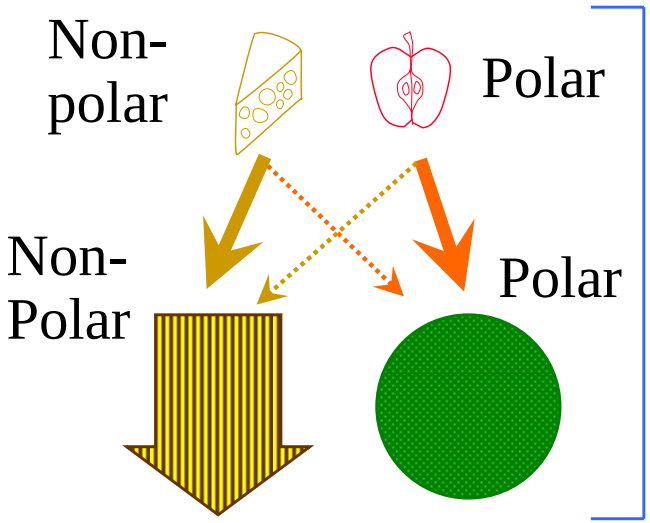
Polar → Polar

Non-polar → Non-polar

色層分析法原理 Two-phase separation system

A

Two-phase



Mobile phase 流動相

Stationary phase 固定相

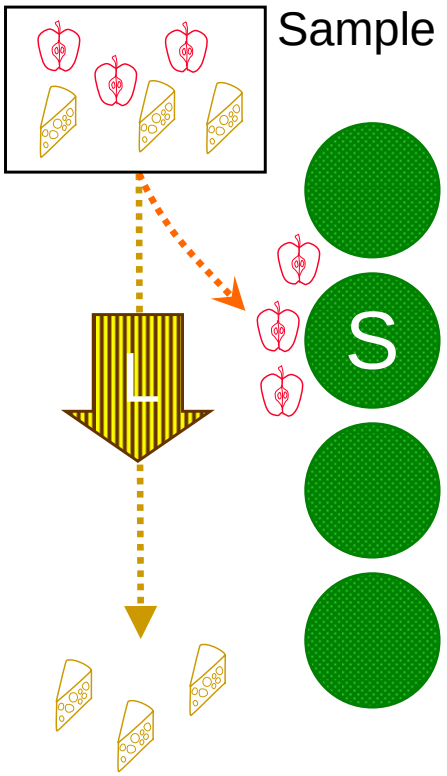
樣本分子依其分子極性
選擇親和兩相之一

每次分離樣本
都要做一次選擇

One Plate of Separation

Theoretical plate number

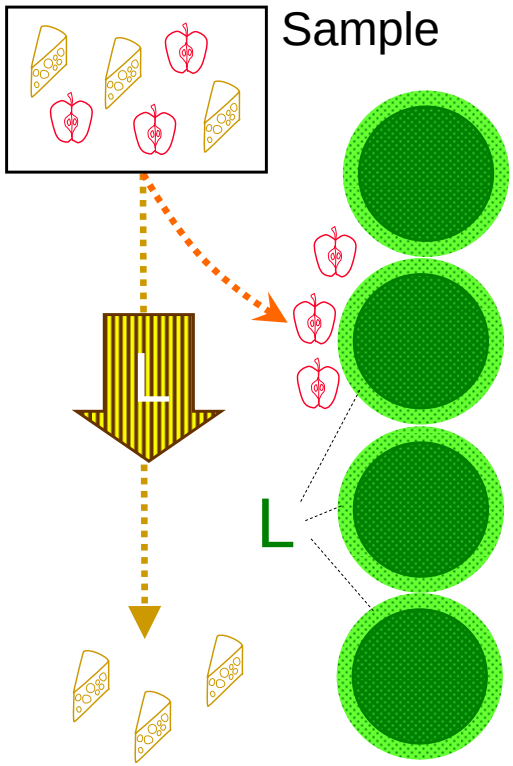
B



ADSORPTION

Liquid - Solid

C

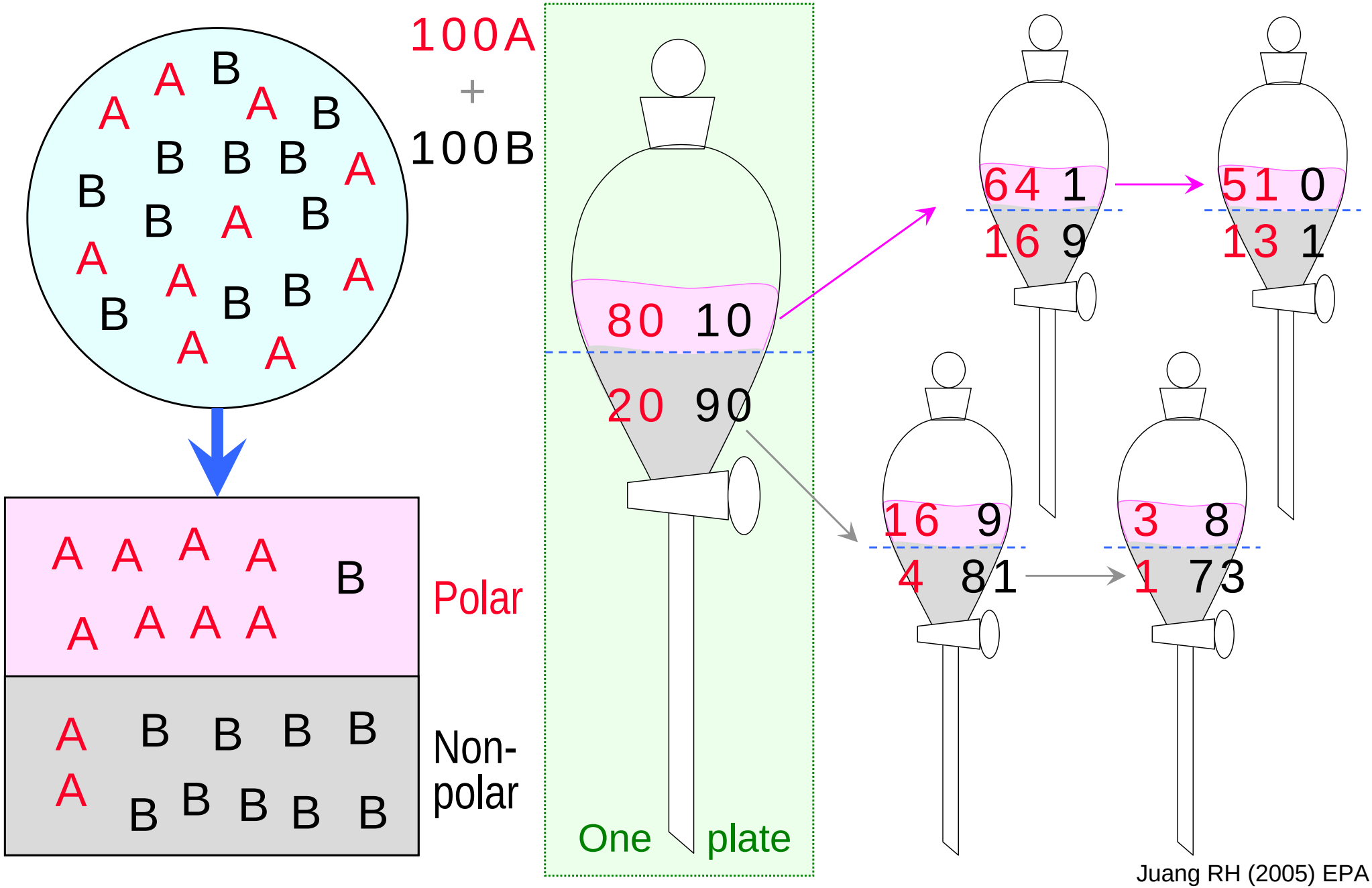


PARTITION

Liquid - Liquid

Juang RH (2005) EPA

層析法的板數概念 One separation, one plate



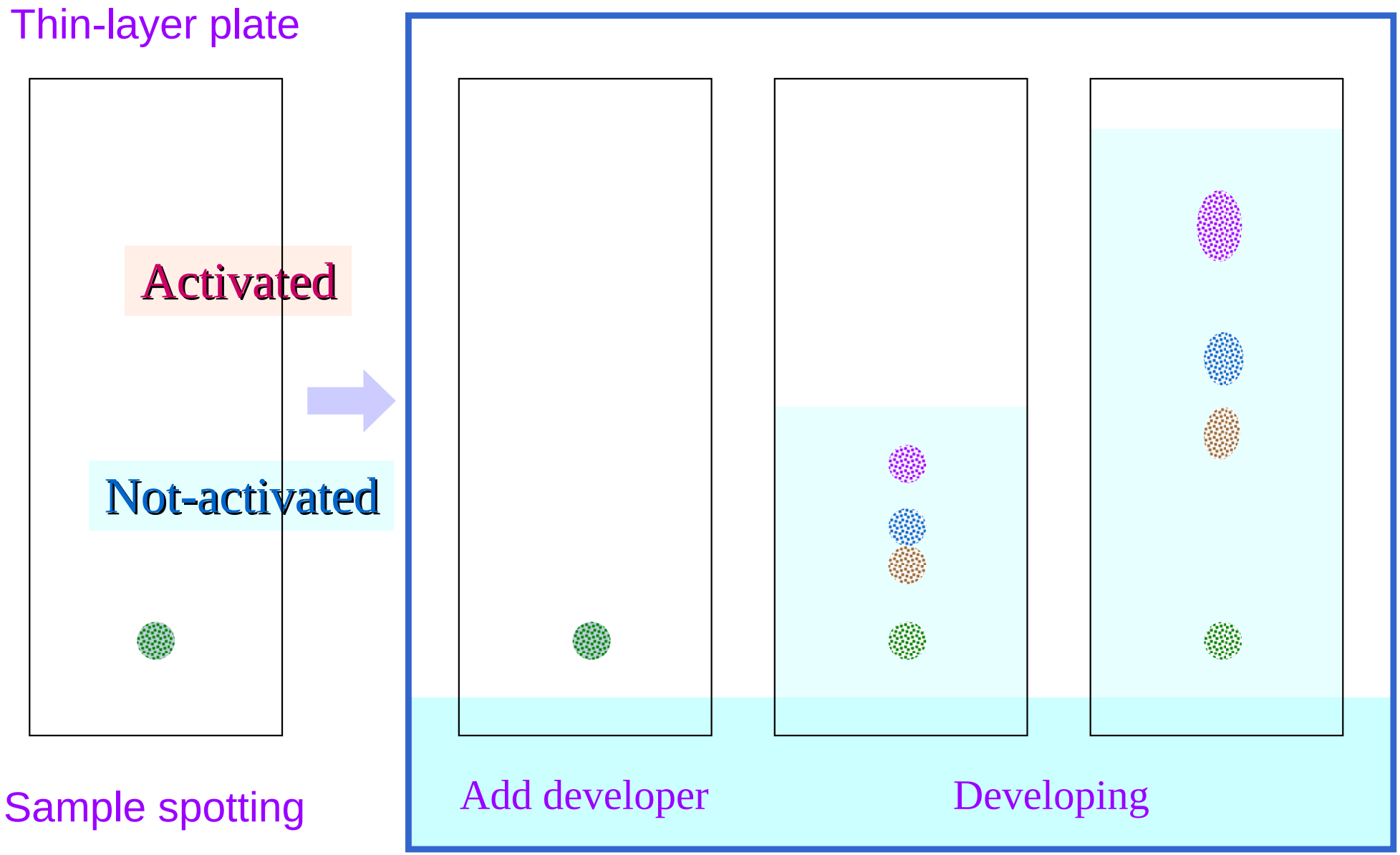
Juang RH (2005) EPA

常用層析法 Common chromatographic methods

	Partition	Adsorption
Large molecules	Gel filtration Reverse phase chromatography	Ion exchange Affinity chromatography Hydrophobic interaction Hydroxyapatite
Small molecules	PPC, TLC, GC	TLC, GC

Juang RH (2005) EPA

薄層層析法操作 Thin-layer chromatography

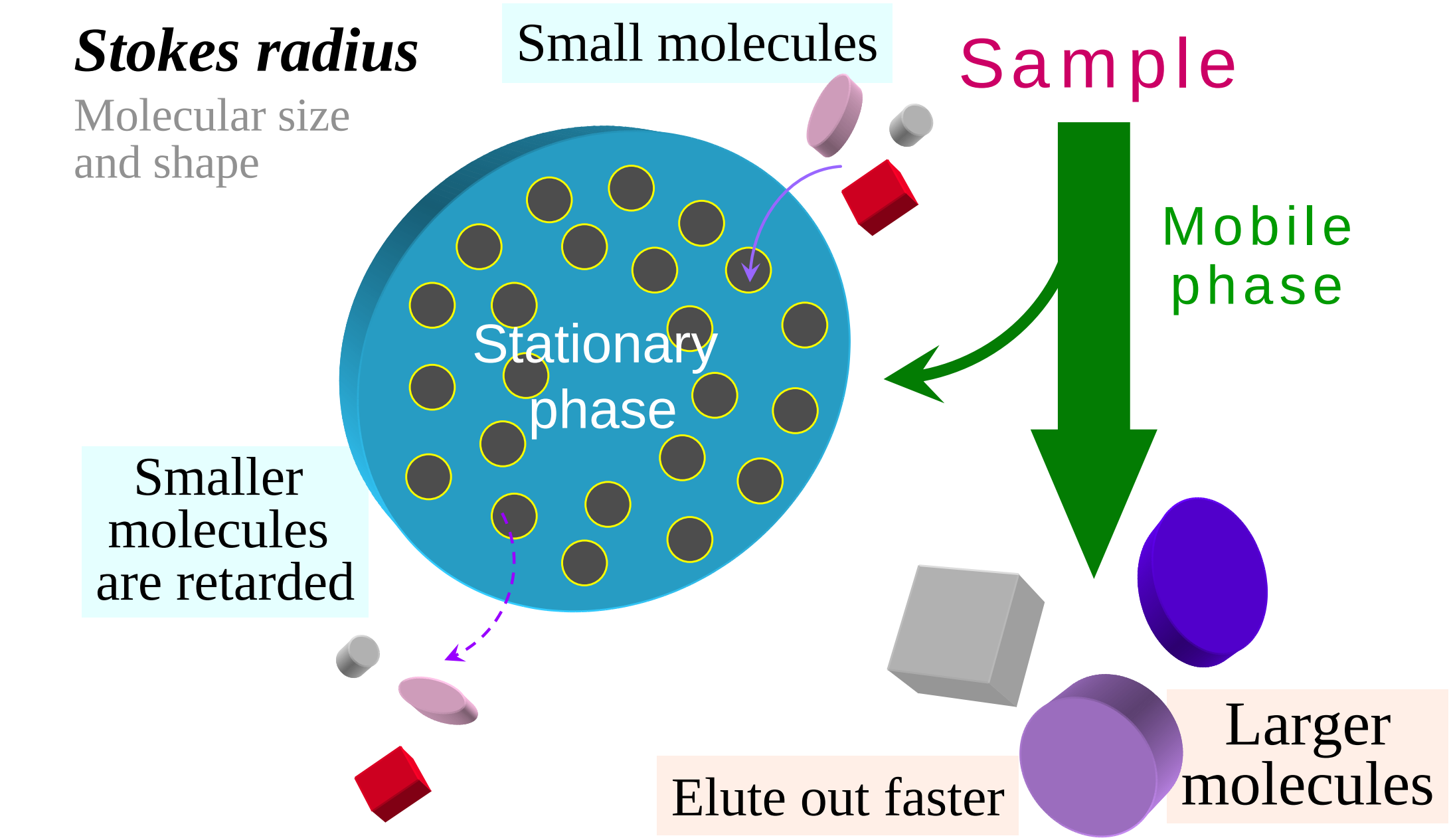


Juang RH (2005) EPA

3.2 膠体過濾法 Gel filtration

- 3.2.1 原理概述 Basic principles
  是一種 partition 層析法
- 3.2.2 膠体介質 Gel materials
  是一種長鏈的大分子聚合物
- 3.2.3 膠体管柱 Gel and column
  管柱性質、影響因素及管柱系統
- 3.2.4 管柱操作 Column operation
  裝填並操作一支膠体過濾管柱
- 3.2.5 問題及解決 Problem and solution
  常見的錯誤要先避免之

膠体過濾法是一種 Partition 層析法



Gel filtration is a partition type chromatography

Juang RH (2005) EPA

各種色析膠體

Pharmacia

Sephadex	glucose (dextrose)
Sepharose	agarose
Sephacryl	glucose + acrylamide
Sephacel	cellulose

FPLC

Superose, Superdex

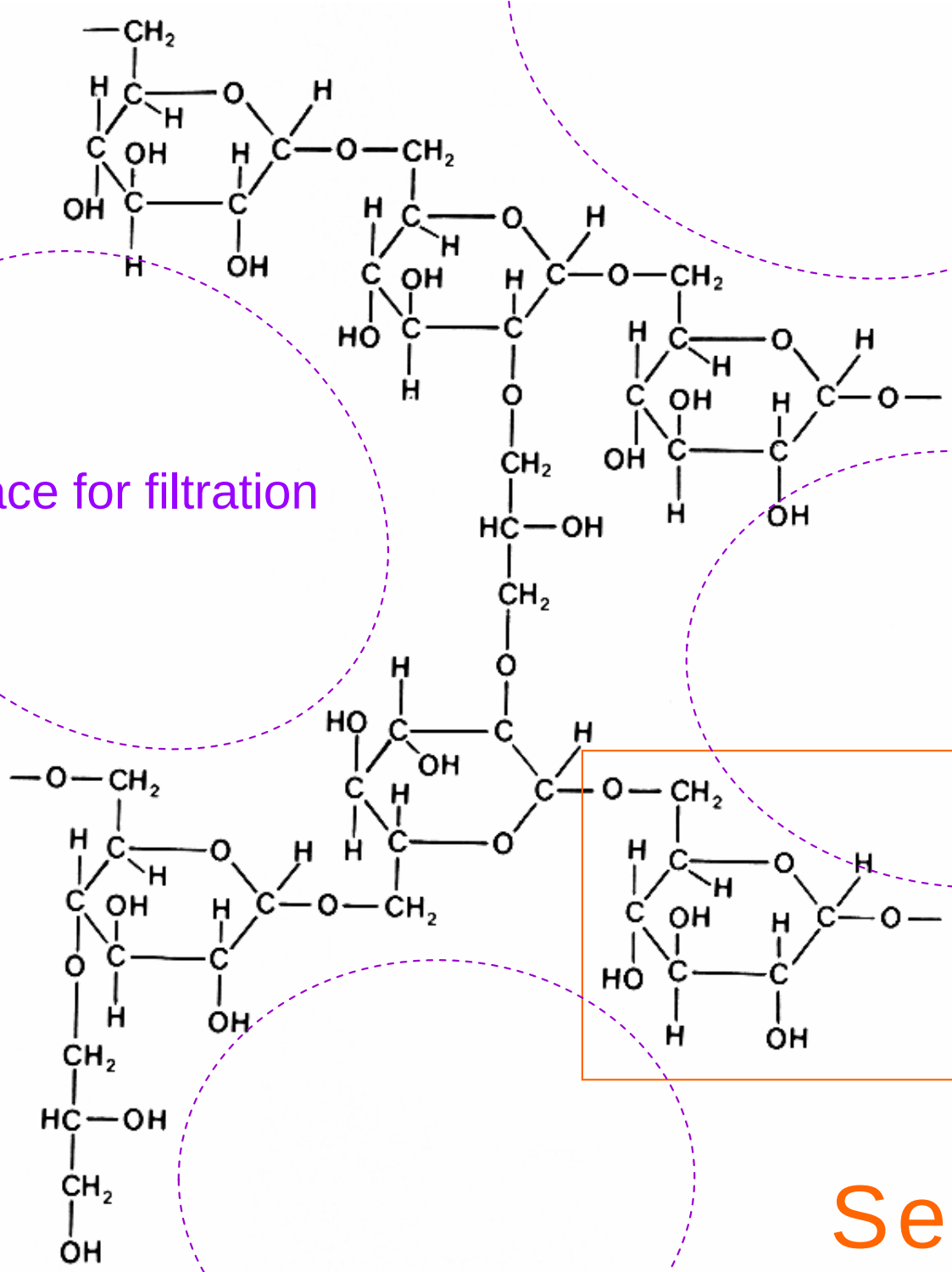
Mono Q, Mono S

Bio-Rad

BioGel P	acrylamide
BioGel A	agarose

Gel material is a polymer of carbohydrate or acrylamide

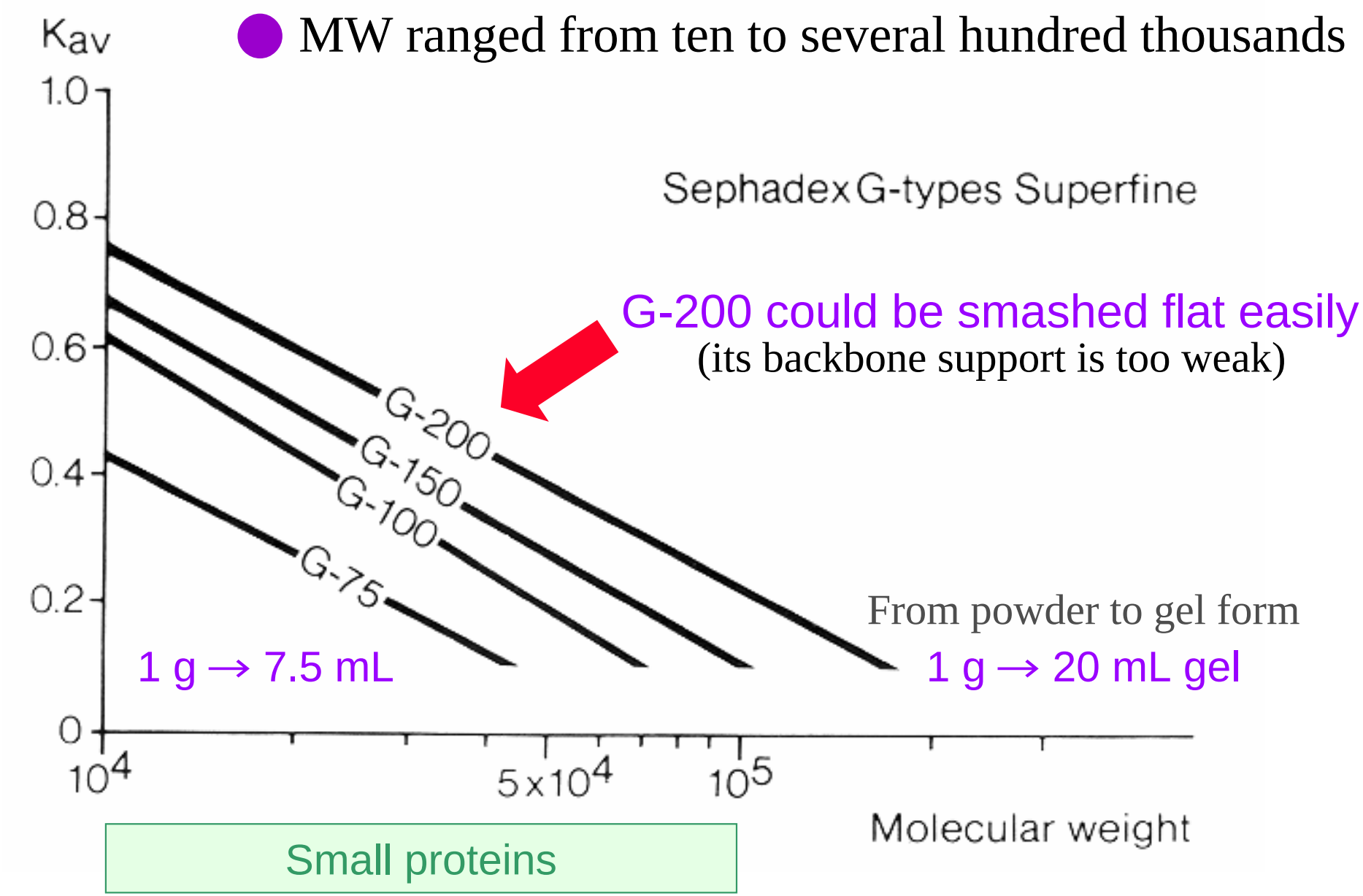
膠體的構成



Sephadex

Sephadex is the polymer of glucose with chemical cross-linking

膠體的使用範圍 Sephadex G



- MW ranged from ten to several hundred thousands

Sephadex G-types Superfine

G-200 could be smashed flat easily
(its backbone support is too weak)

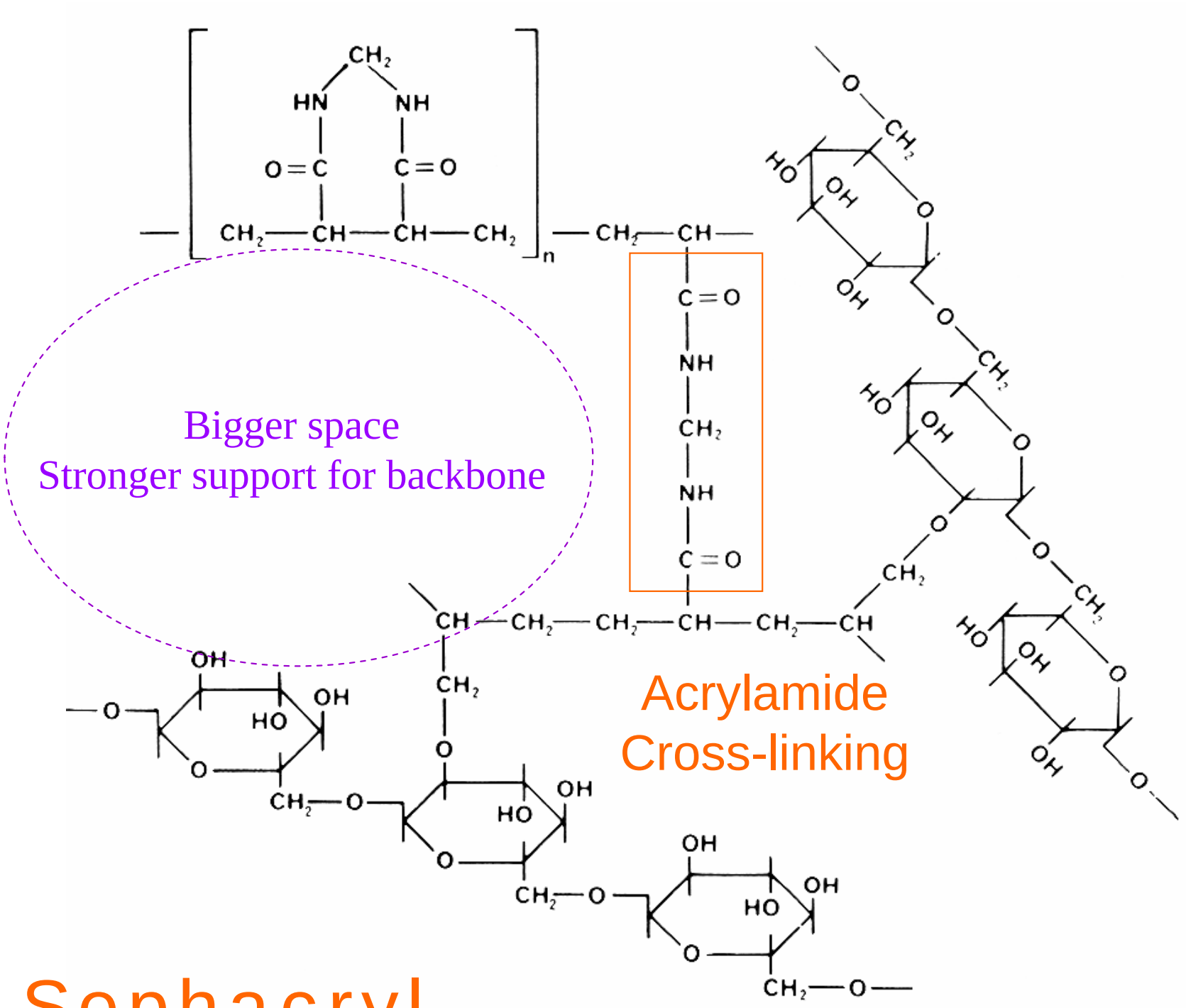
From powder to gel form
1 g → 20 mL gel

1 g → 7.5 mL

Small proteins

Pharmacia (1991) Gil Filtration – Principles and Methods p.37

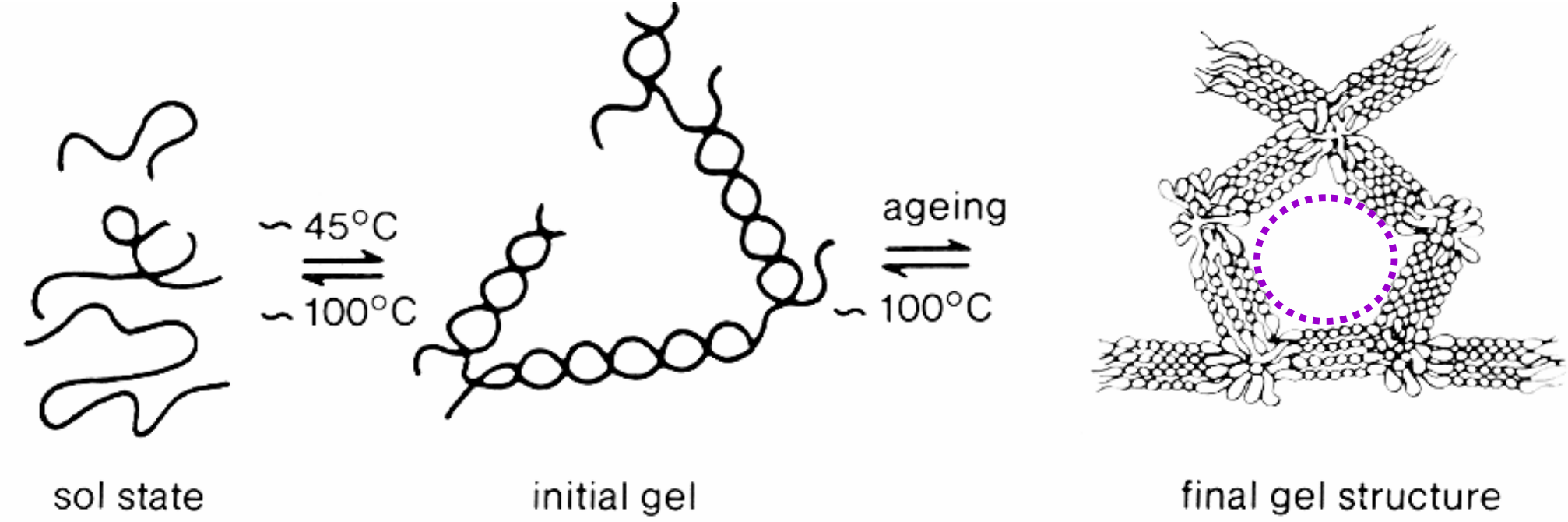
膠體的構成



Sephacryl

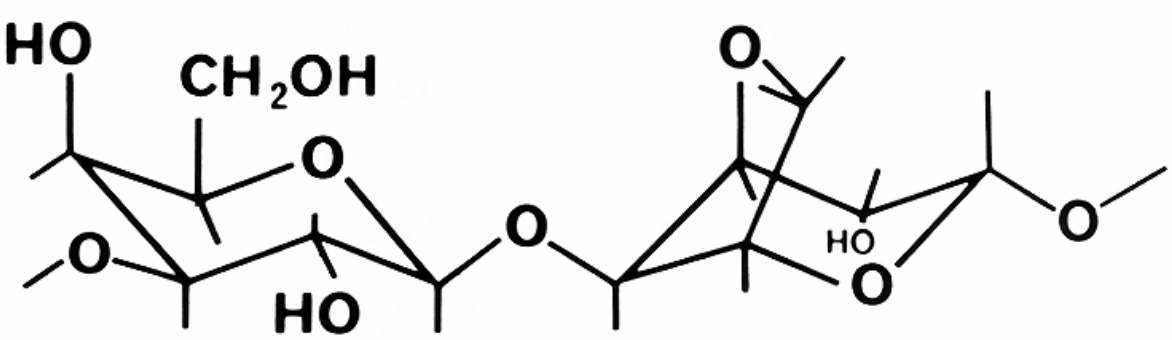
Pharmacia (1991) Gil Filtration – Principles and Methods p.22

洋菜膠體的成膠反應 Agar gel formation



Even stronger backbone

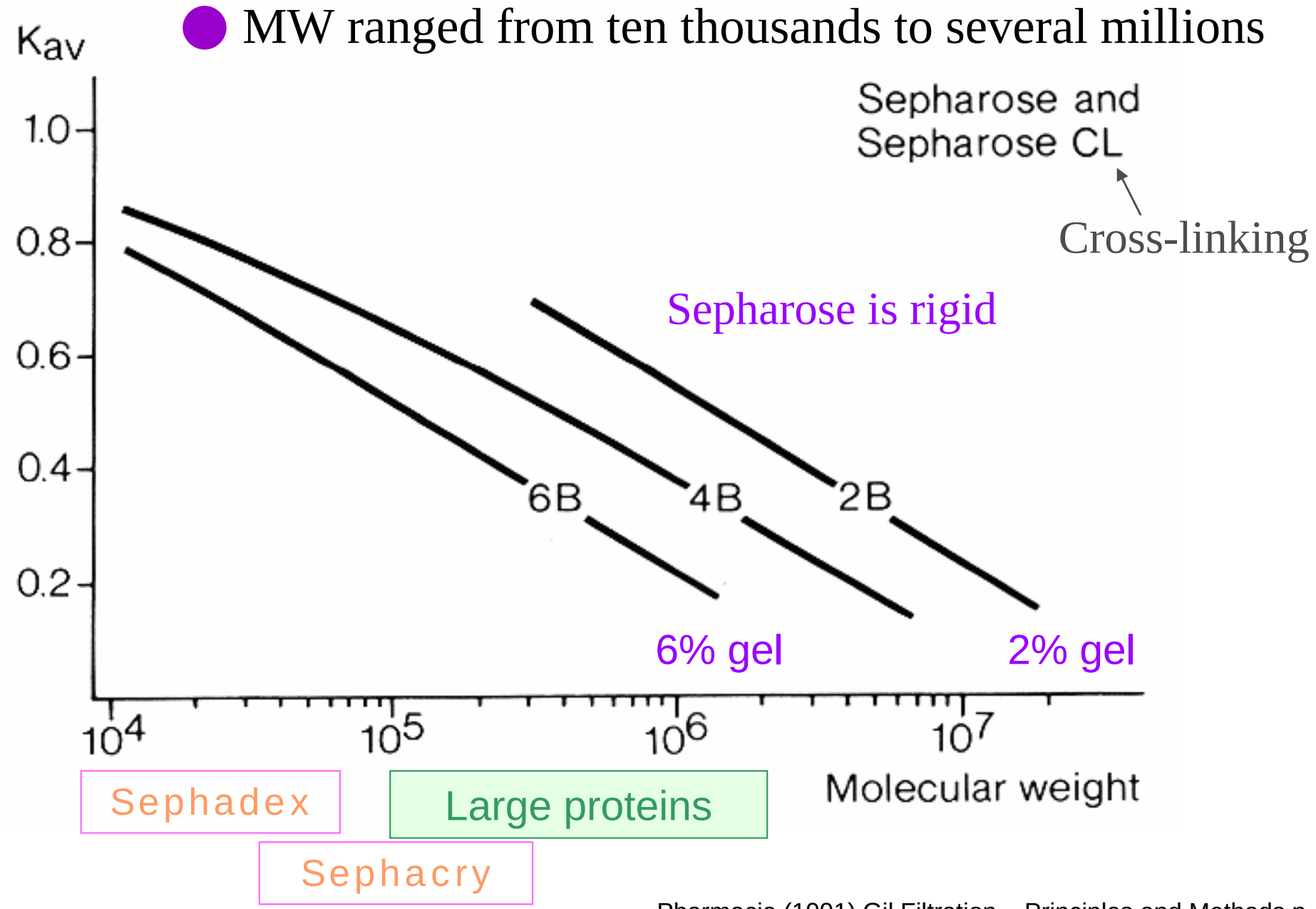
Much bigger space



Pharmacia (1991) Gil Filtration – Principles and Methods p.38, 39

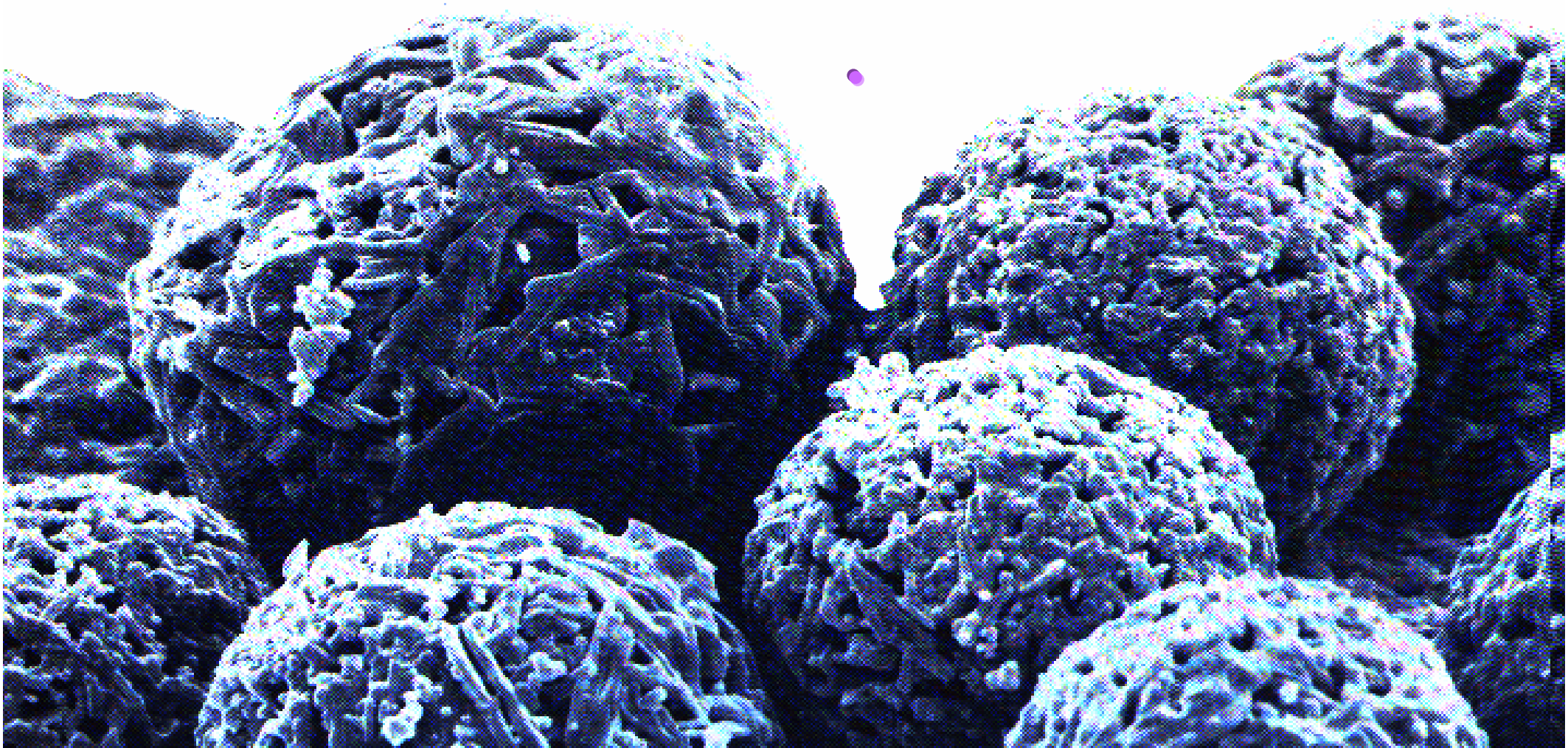
膠體的使用範圍 Sepharose

- MW ranged from ten thousands to several millions

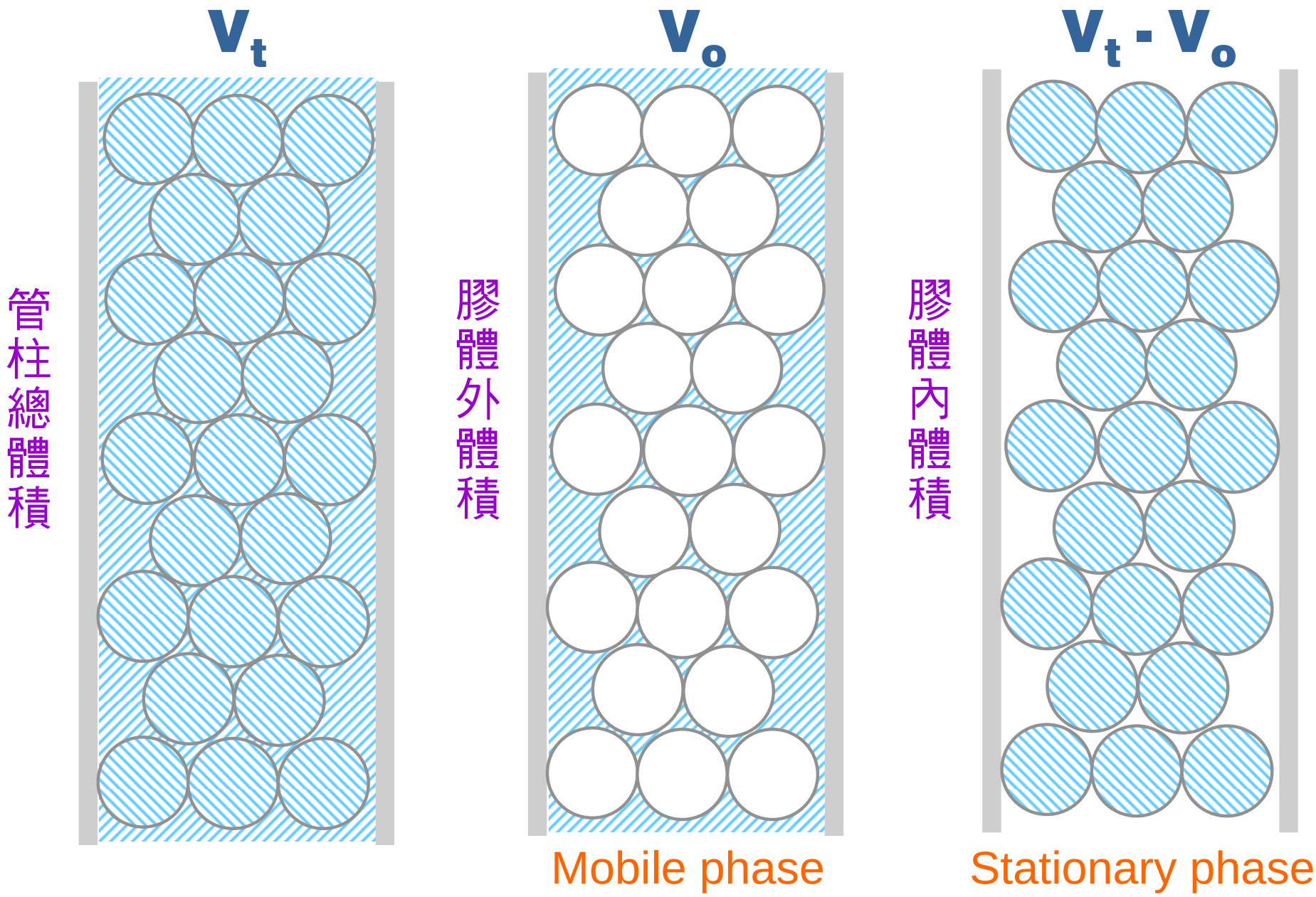


Pharmacia (1991) Gil Filtration – Principles and Methods p.40

膠体過濾法的膠球 Gel filtration beads

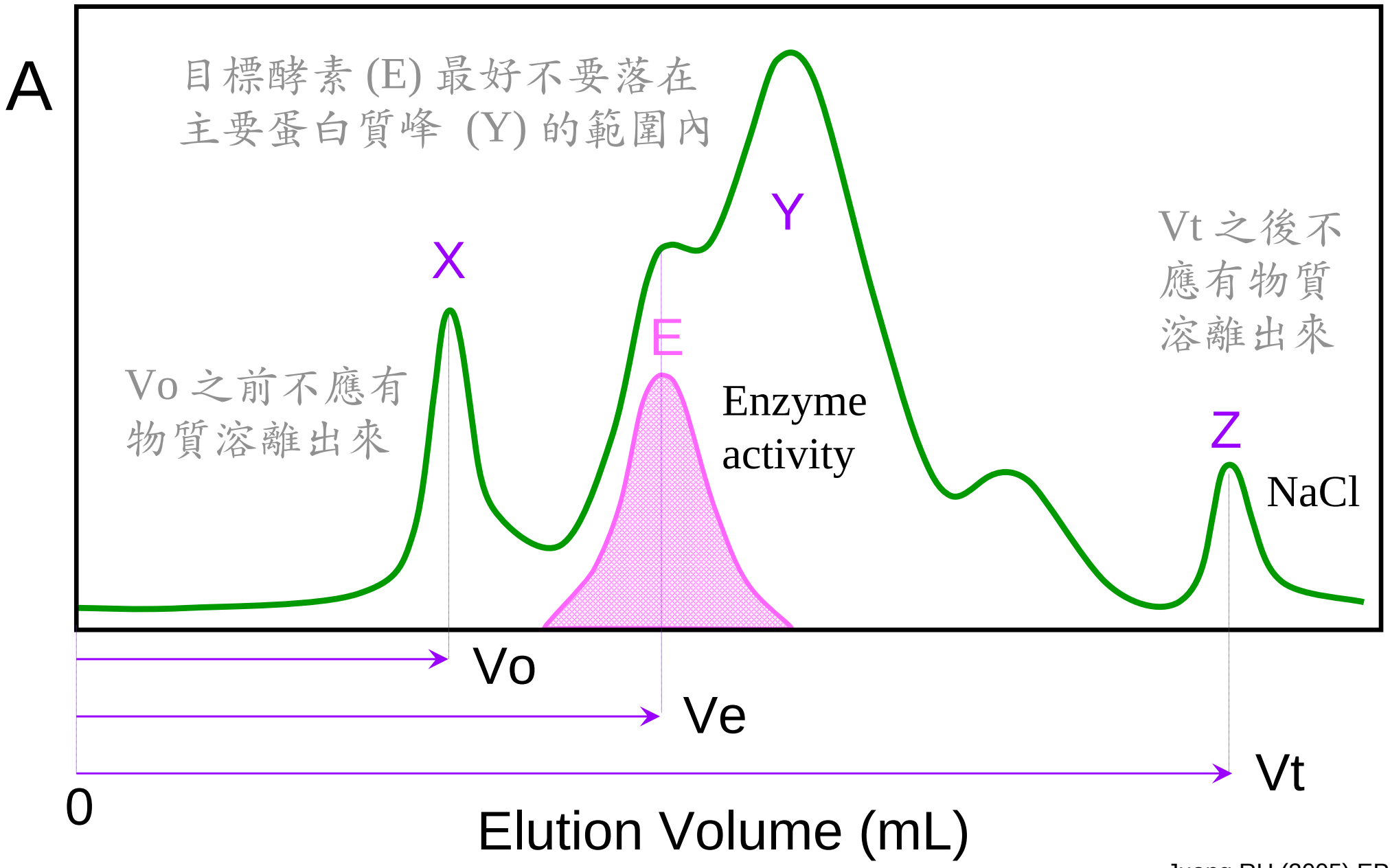


管柱內膠體的組成區隔 Spaces in a column



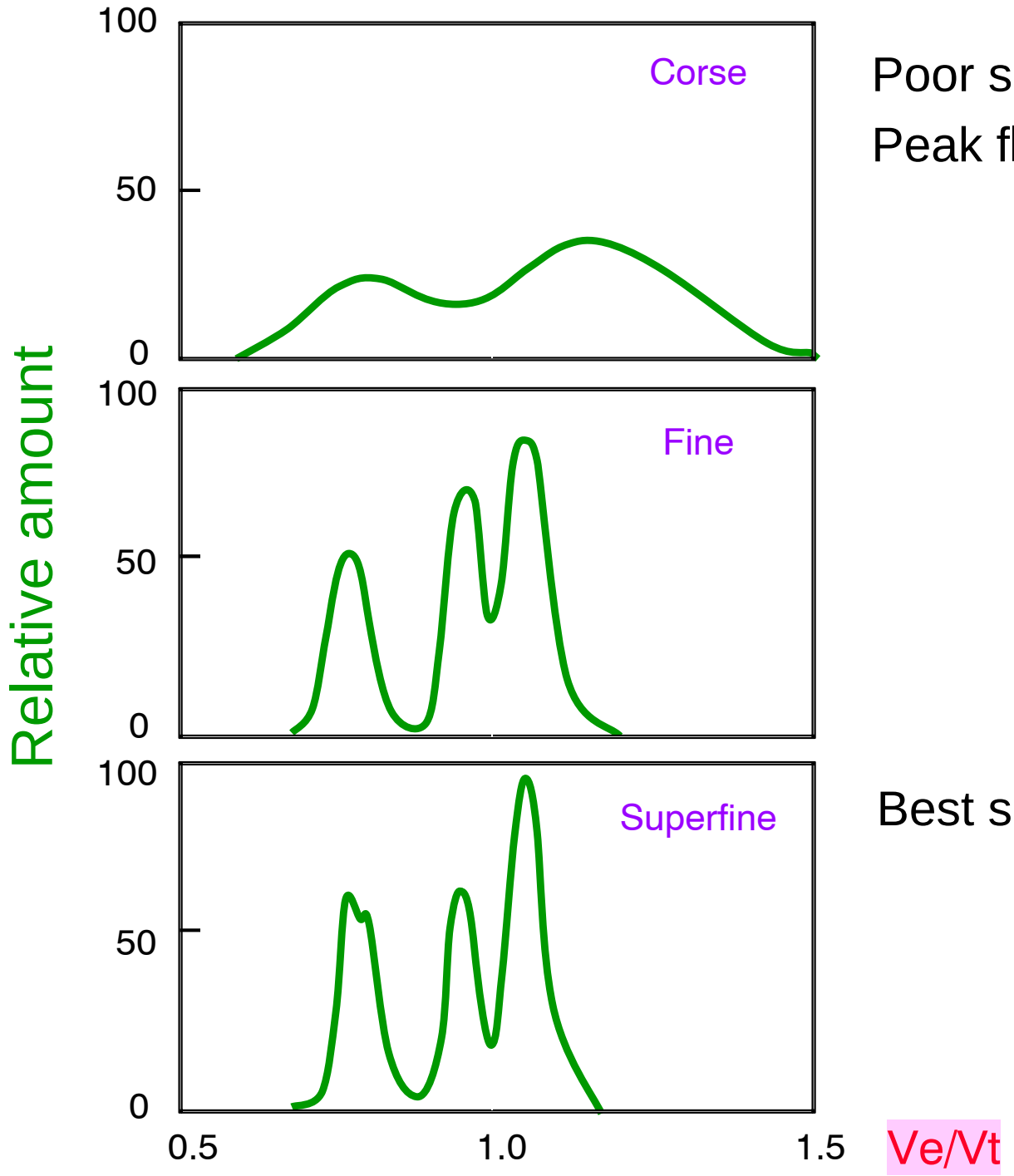
Adapted from Pharmacia (1991) Gil Filtration – Principles and Methods p.11

膠体過濾的溶離圖譜 A typical chromatogram



Juang RH (2005) EPA

介質的粗細影響解析力



Poor separation

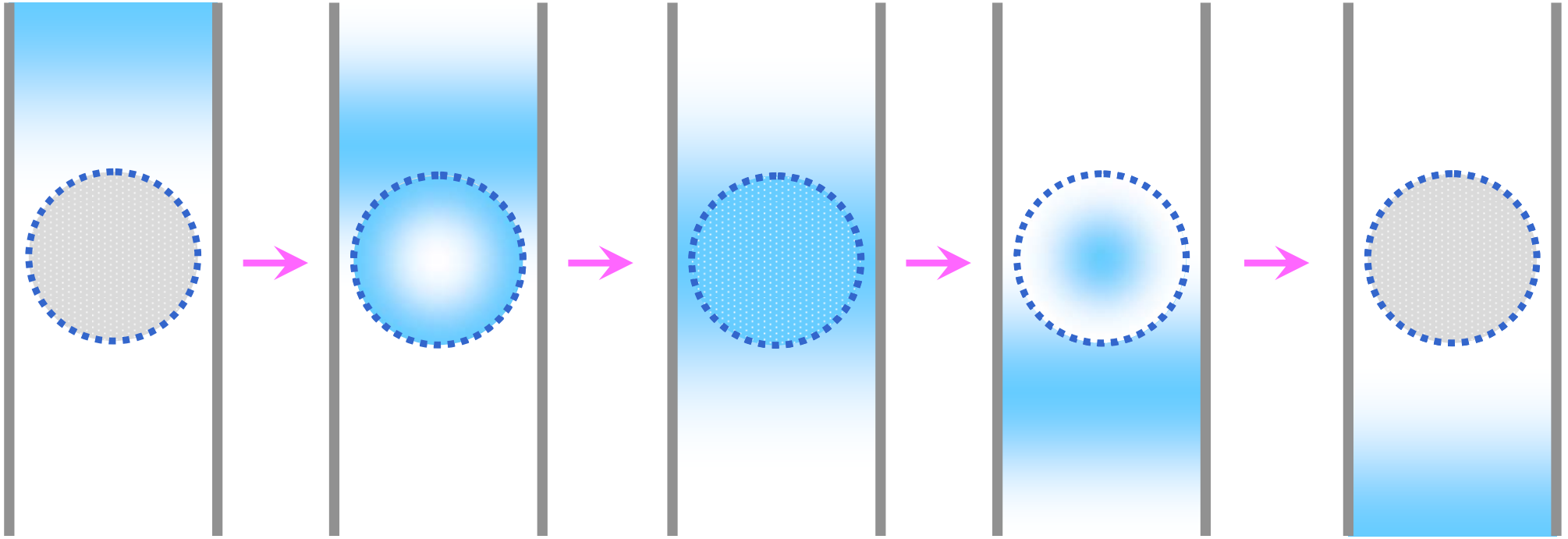
Peak flattened

Best separation

Bead size is critical to the resolution of gel chromatography

Adapted from Pharmacia: Gil Filtration – Principles and Methods

溶離液擴散進出膠球 Diffuse in and out bead

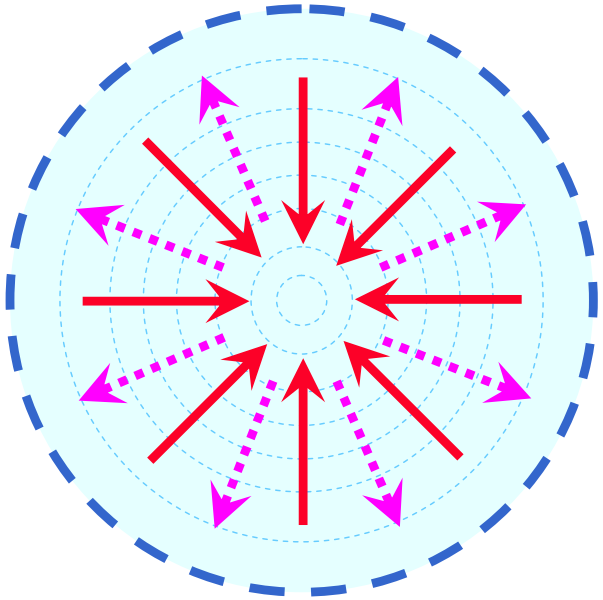


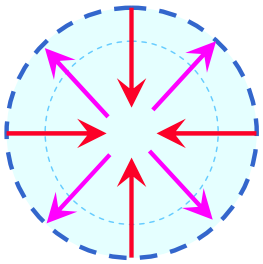
溶離液或樣本分子，由膠體粒子外圍均勻向內或外擴散。

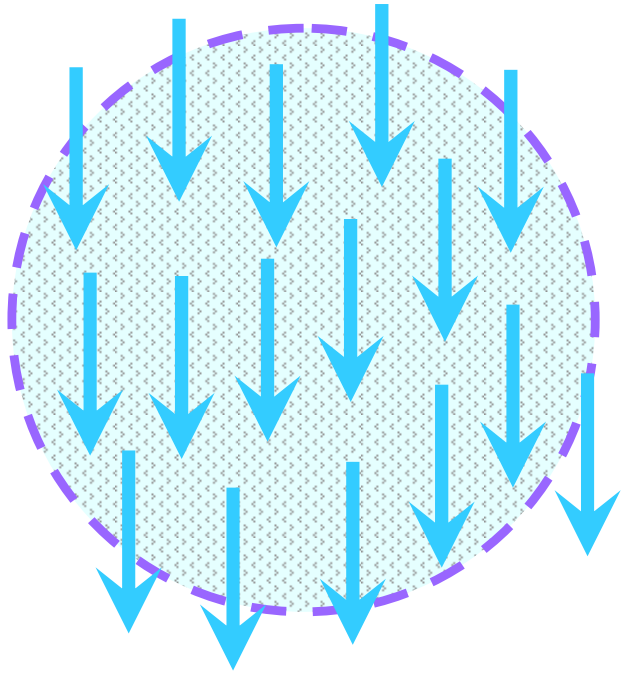
Sample or buffer is diffusing in and then out of the gel particle.

Diffusion → Dispersion

溶離液對膠球的擴散或瀰散 Two types of gel







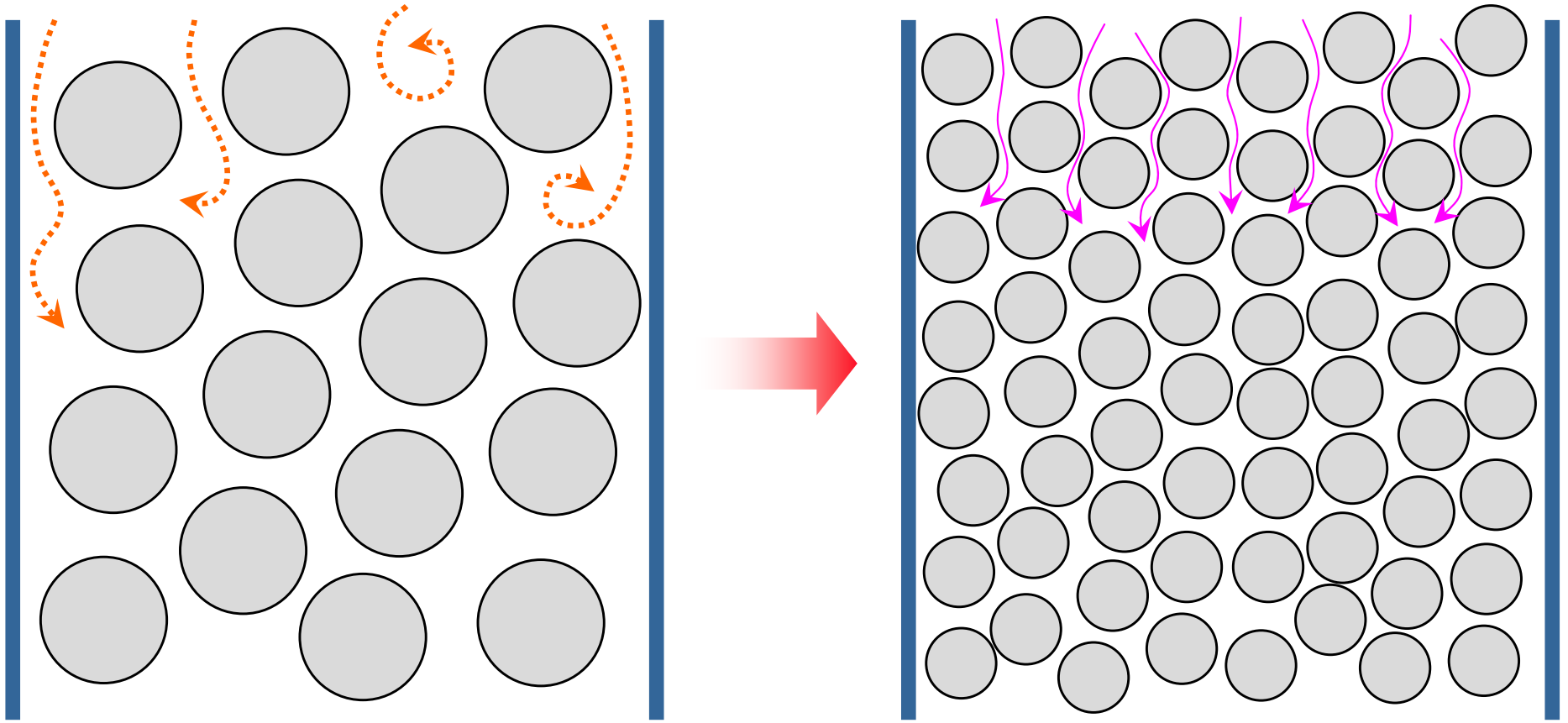
若膠體粒子太大，則由外圍擴散到內部的距離長，通過粒子所需要的時間拉長，降低分離效果。

Small particle size reduces the diffusion time and increases the resolution of the gel

Dispersion (瀰散) 方式的膠體因通透性佳，溶離液可直接流入膠體，不再靠擴散作用。

Dispersion type gels let the sample molecules flow directly through the gel body, and have better resolution

粒子粗細影響溶離液流動 Bead size vs flow rate

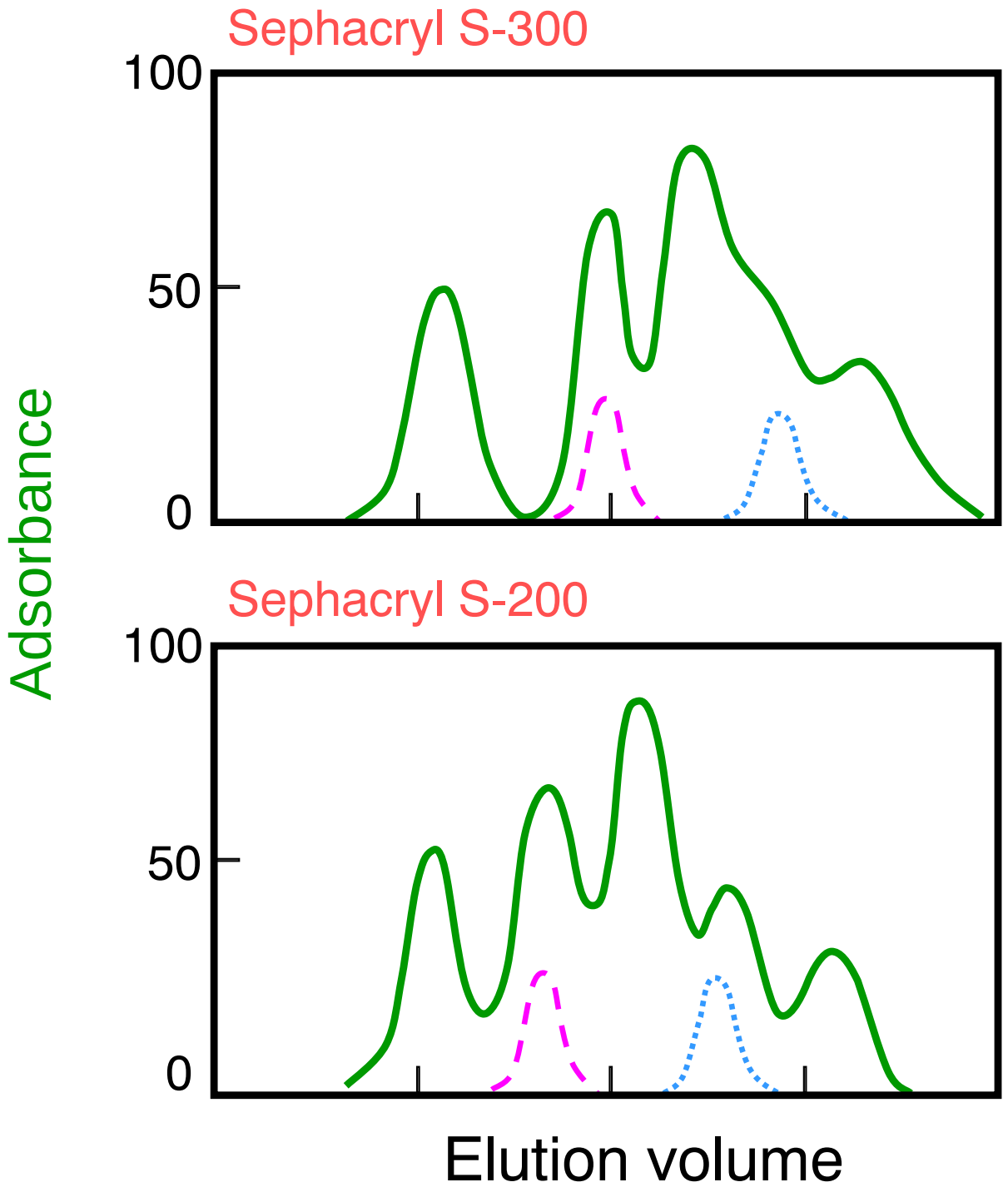


膠體的顆粒越小 其解析力越大 但流速變慢

Smaller particles also reduce the space between the beads, and prevent the turbulence as the buffer flows, but the flow rate might be decreased

Adapted from Scope RK (1987) Protein Purification – Principles and Practice p.192

不同介質的運用選擇



高分子量處
分離較好
色帶都較晚
溶離出來

低分子量處
分離較好
色帶都較快
溶離出來

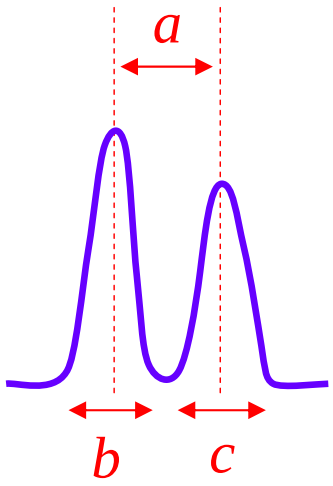
Choose the gel which brings your target protein out of the column earlier

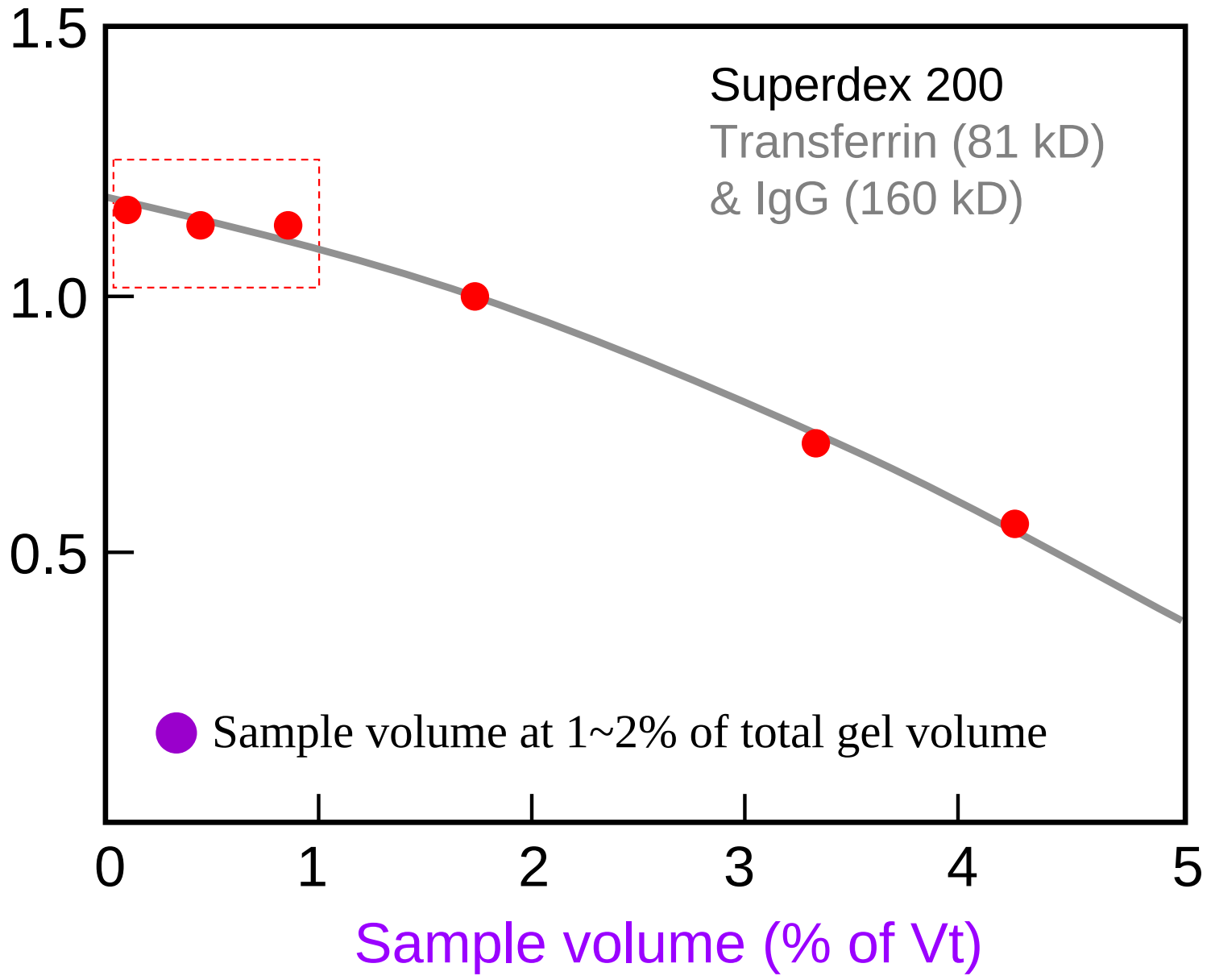
Adapted from Pharmacia: Gil Filtration – Principles and Methods

樣本體積的影響 Sample volume at 1% of Vt

Resolution

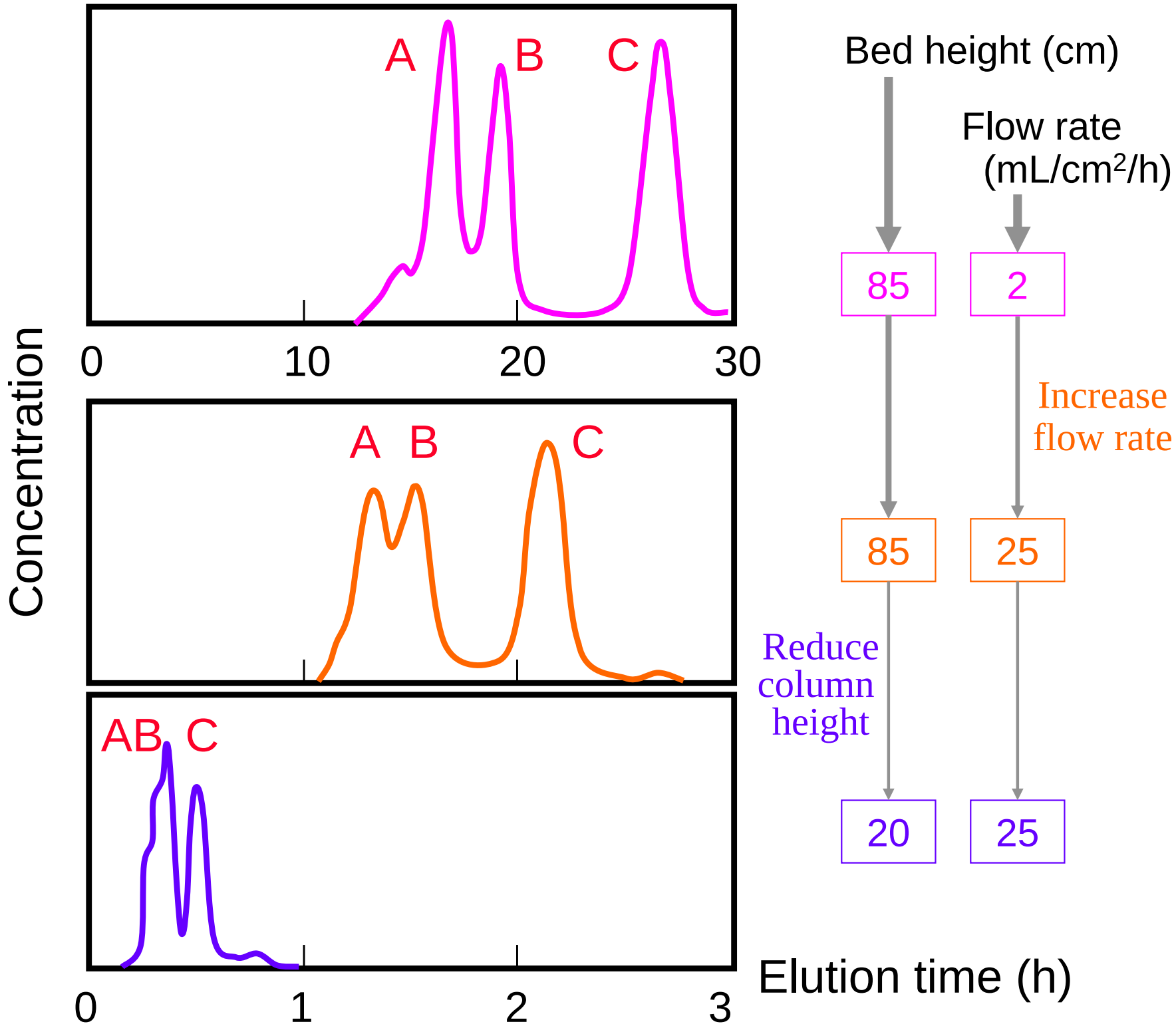
$$R = \frac{a}{(b+c)/2}$$





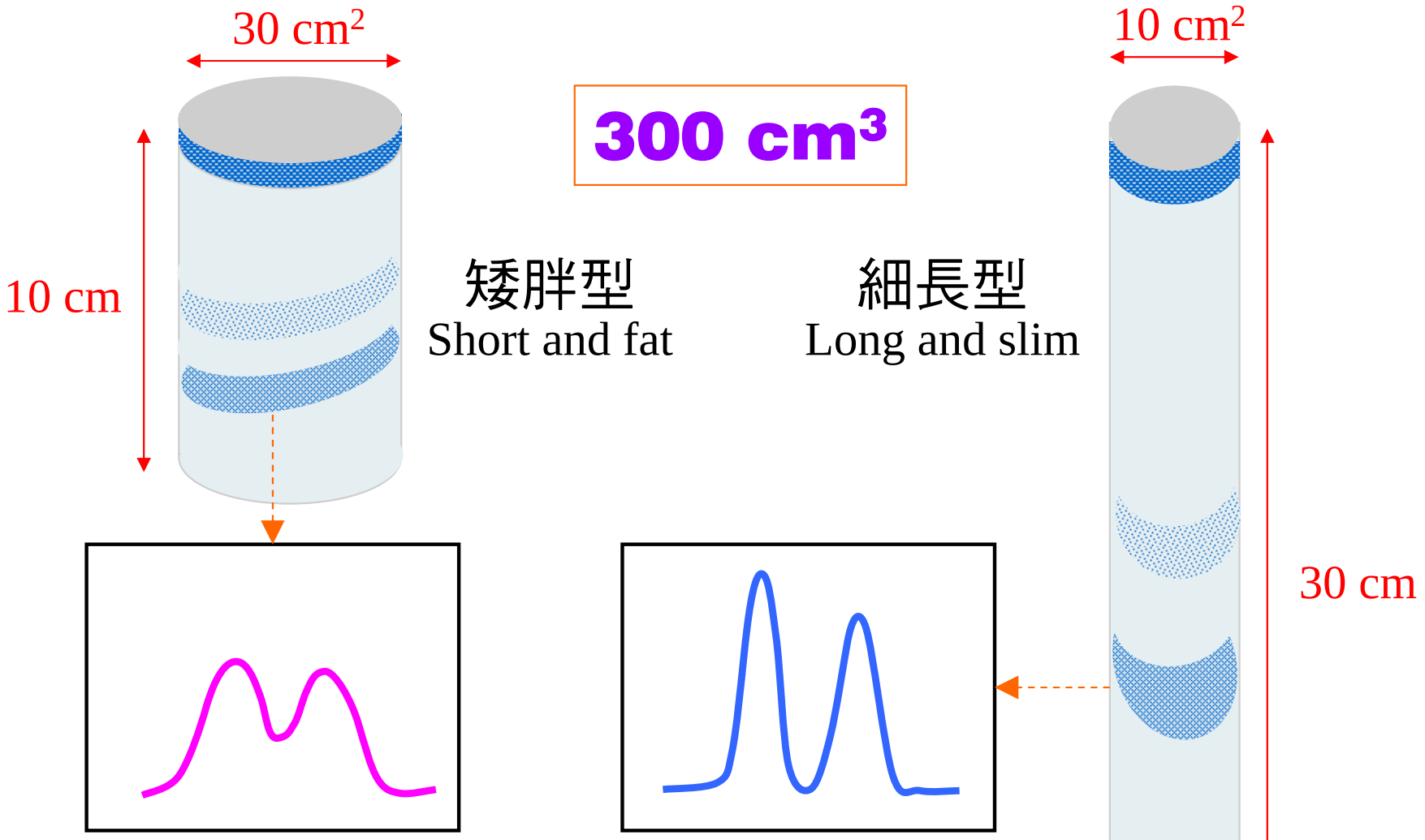
Adapted from Pharmacia (1991) Gil Filtration – Principles and Methods p.46

膠體管柱的最佳化調整



Adapted from Pharmacia (1991) Gil Filtration – Principles and Methods p.59

色析管柱的形狀

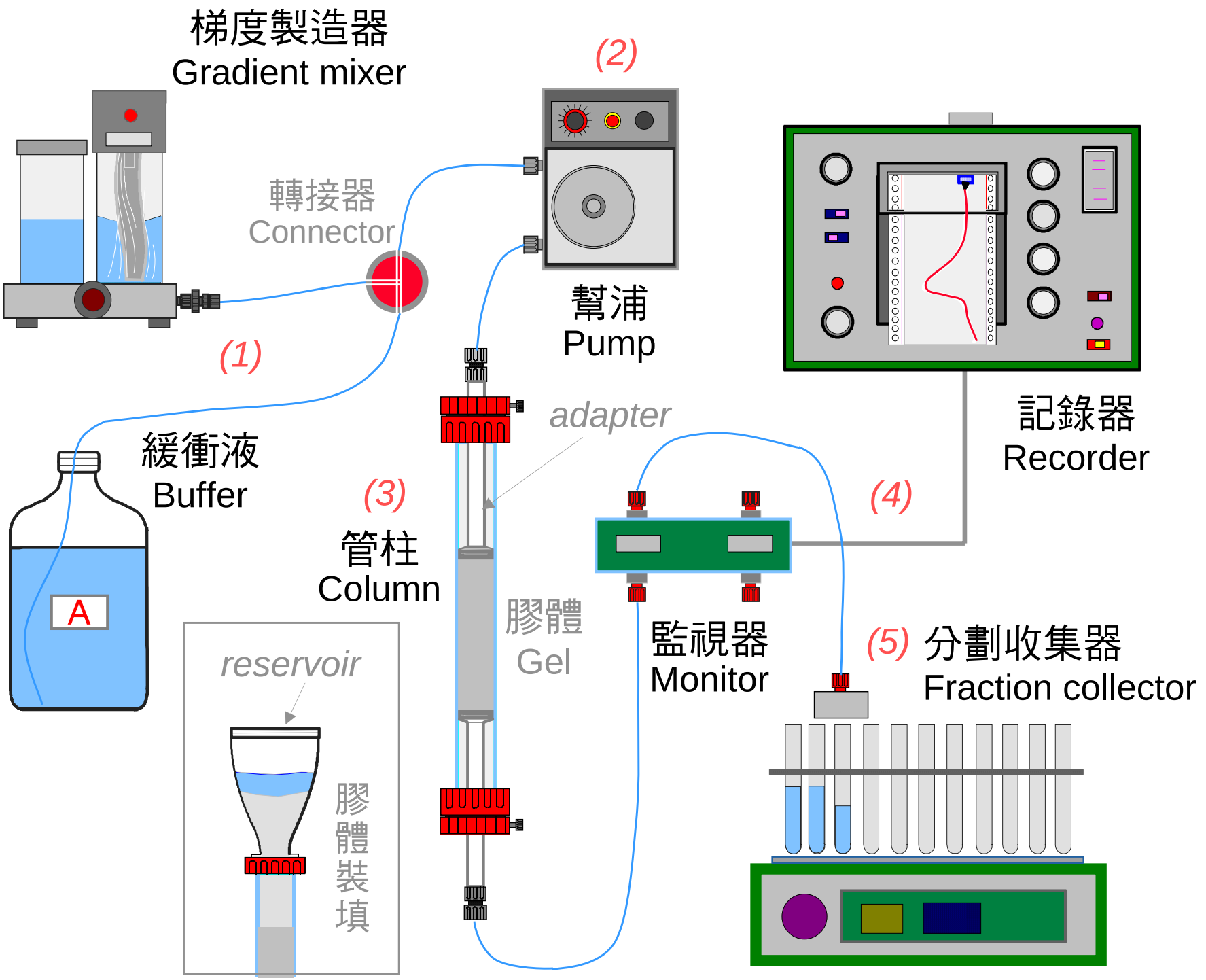


- 矮胖型管柱不能容忍分離不佳
  Fat column cannot tolerate poor separation

但其流速及容量均較大
But it has better flow rate and higher capacity

Adapted from Scope RK (1987) Protein Purification – Principles and Practice p.195

液相層析管柱系統



The whole family of liquid chromatography apparatus

Juang RH (2005) EPA

膠柱裝填方法 Packing column step by step

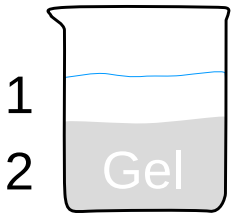
清洗膠體
Wash gel well

預估體積
Estimate
gel volume

緩衝液
平衡
Equilibrated
in buffer

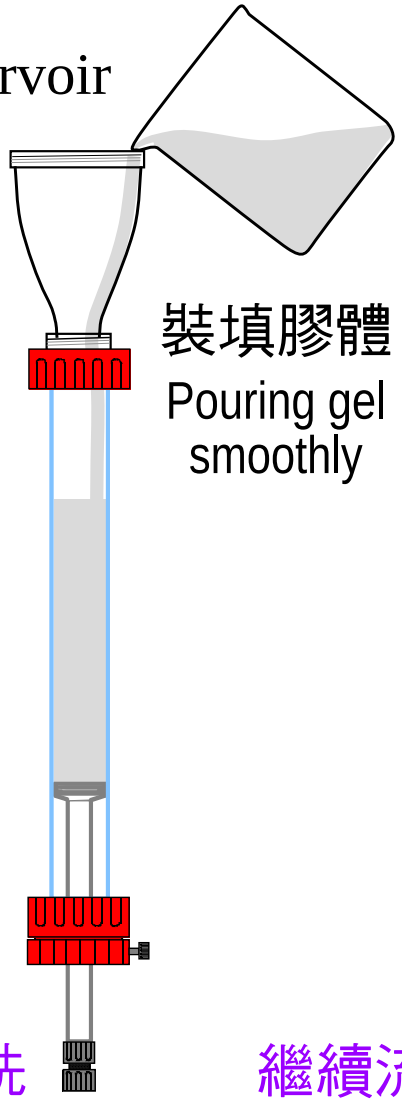
靜置膠體
Gel stands o/n

溫度平衡
Temperature
equilibrated



檢查管柱
是否暢通
Check flow
rate of empty
column

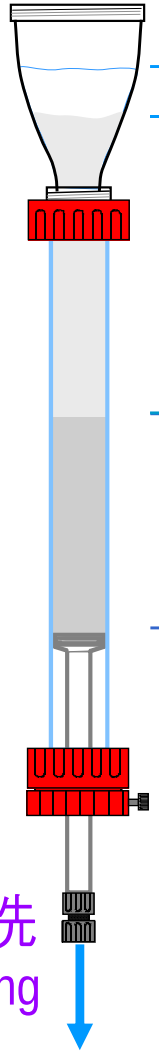
Put on reservoir



裝填膠體
Pouring gel
smoothly

暫停流洗
Stop elution

X



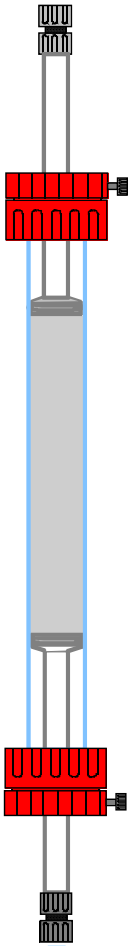
上清
Supernatant

沈積中
In progressing

已堆積
Sediment

繼續流洗
Keep eluting

Put on adaptor



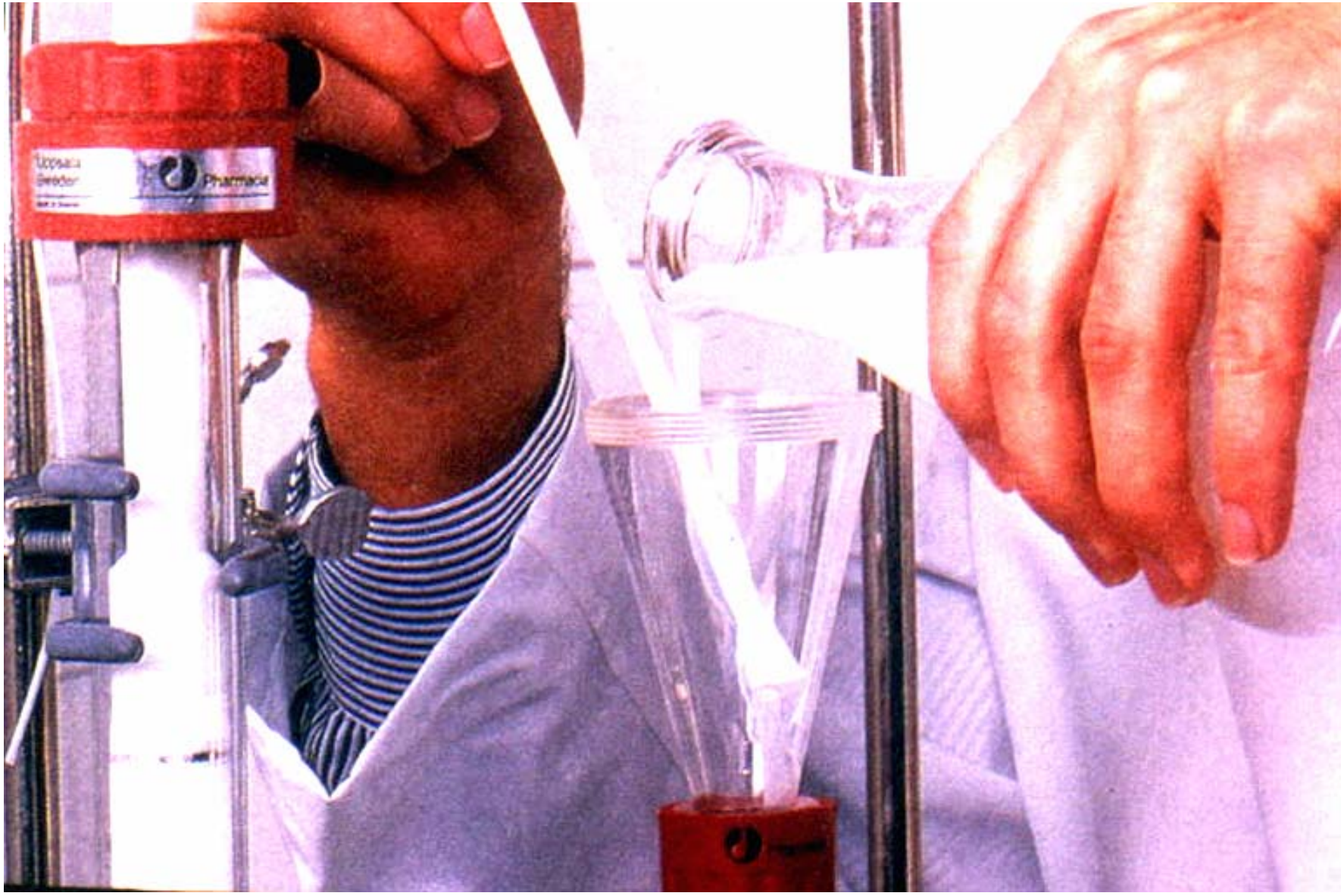
緊密堆積

加壓流洗
Elute under
High pressure

Gel should be packed tightly

Juang RH (2005) EPA

色析膠體的裝填 Packing column



一口氣倒入膠體，勿陷入氣泡。

Pour gel slurry smoothly (non-stop), avoid trapping any bubble

Pharmacia (1991) Gil Filtration – Principles and Methods

General principle for column chromatography

Gel selection	Make target protein elute out column earlier
Bead size	Finer bead has better resolution, slower flow rate
Column size	Use larger column size but consider practical need
Column shape	Slim column for gel filtration, fat column for others
Pack tightly	Pack the gel tightly for better resolution
Flow rate	Fast flow reduces resolution, slow
Sample volume	Apply 1% of total gel volume for sample