

Chromatographic techniques for Quality control of freeze dried Radiopharmaceuticals

Gel filtration / HPLC

January, 2017

Ass. Marija Sterjova, MPharm
Paulina Apostolova, MPharm
Goce Delcev University - Stip

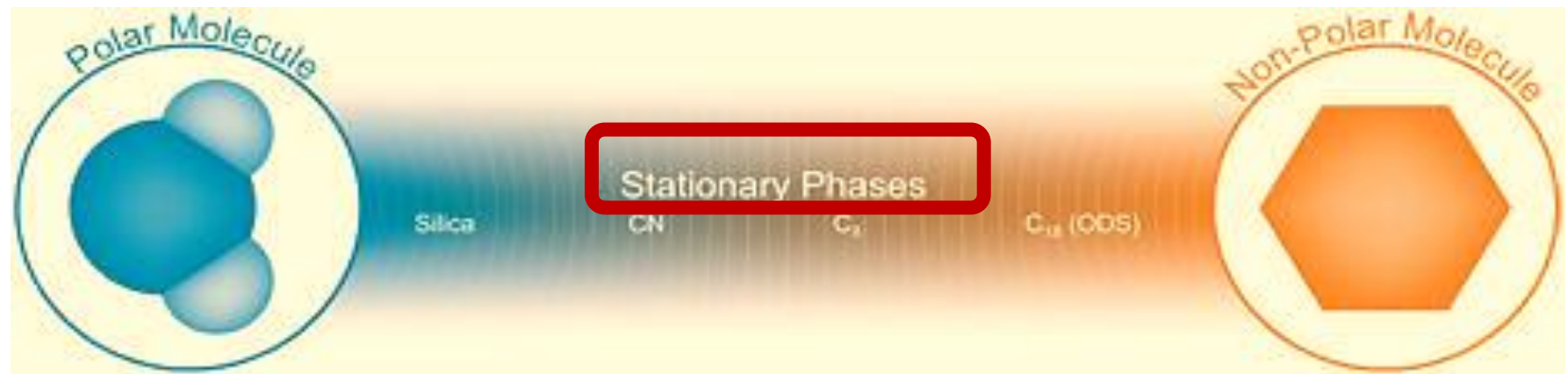
Principles of separation on HPLC

Normal phase liquid chromatography – NPC

- ✓ Non-polar mobile phase
- ✓ Polar stationary phase

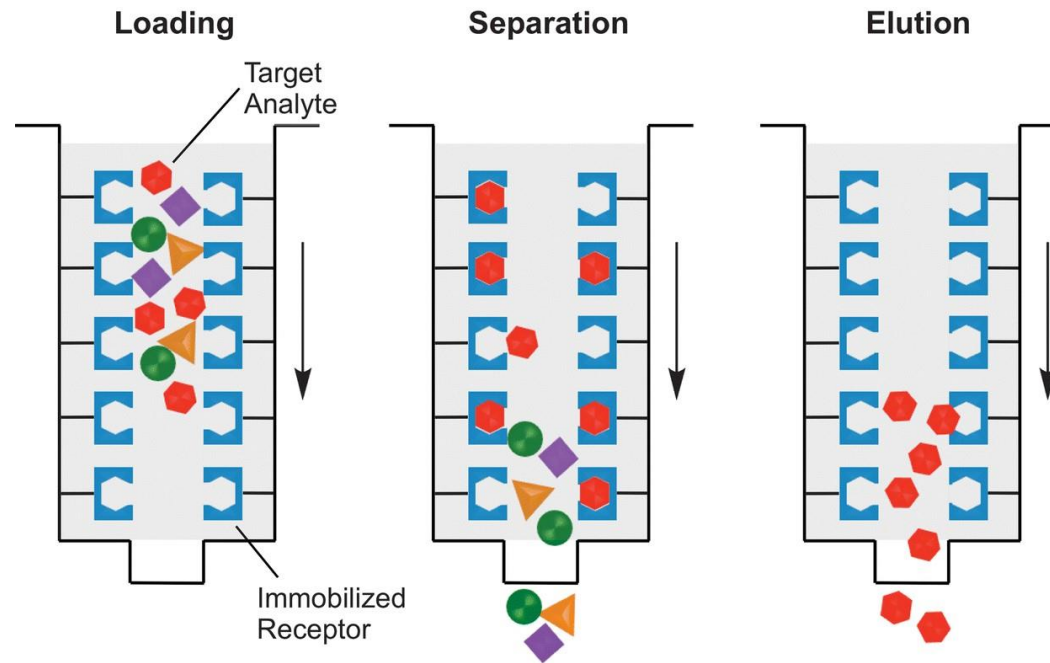
Reversed phase liquid chromatography – RPC

- ✓ Polar mobile phase
- ✓ Non-polar stationary phase

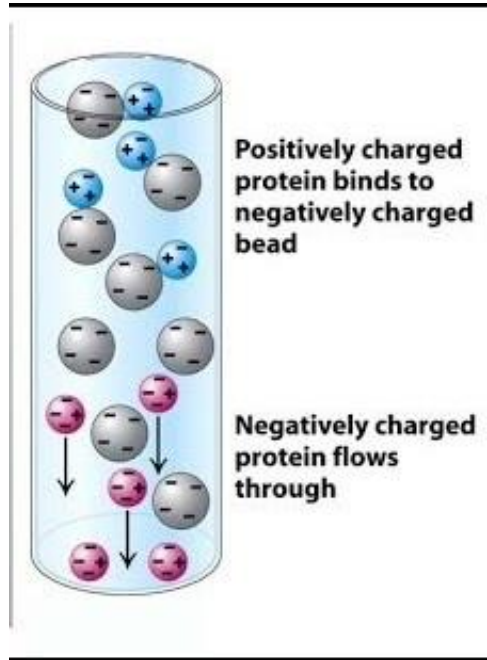


Normal-Phase Liquid Chromatography:	Reversed-Phase Liquid Chromatography:
Stationary phase, Ex. Silica	Stationary phase, Ex. Alkyl and phenyl-modified silica (C8 and C18)
Nonpolar mobile phase, Ex. Hexane	Polar mobile phase, Ex. Water, Methanol

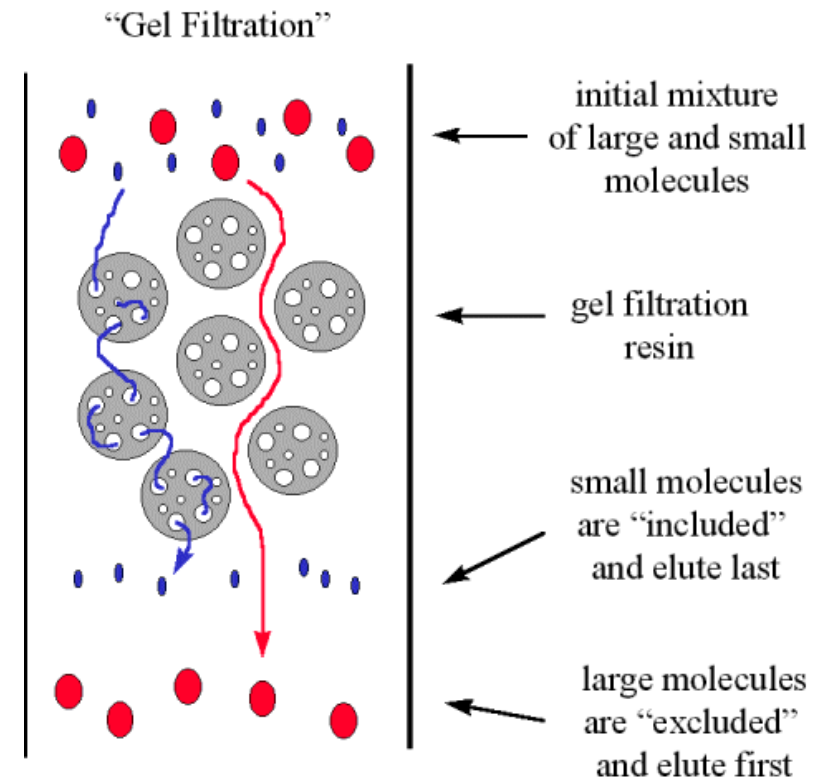
Types of HPLC:	Principle of separation:
Ion-Exchange Chromatography	Charge
Affinity Chromatography	Shape-specific binding
Chiral Chromatography	Enantiomers
Size-exclusion Chromatography	Size



Affinity Chromatography



Ion-Exchange Chromatography



Size-exclusion Chromatography

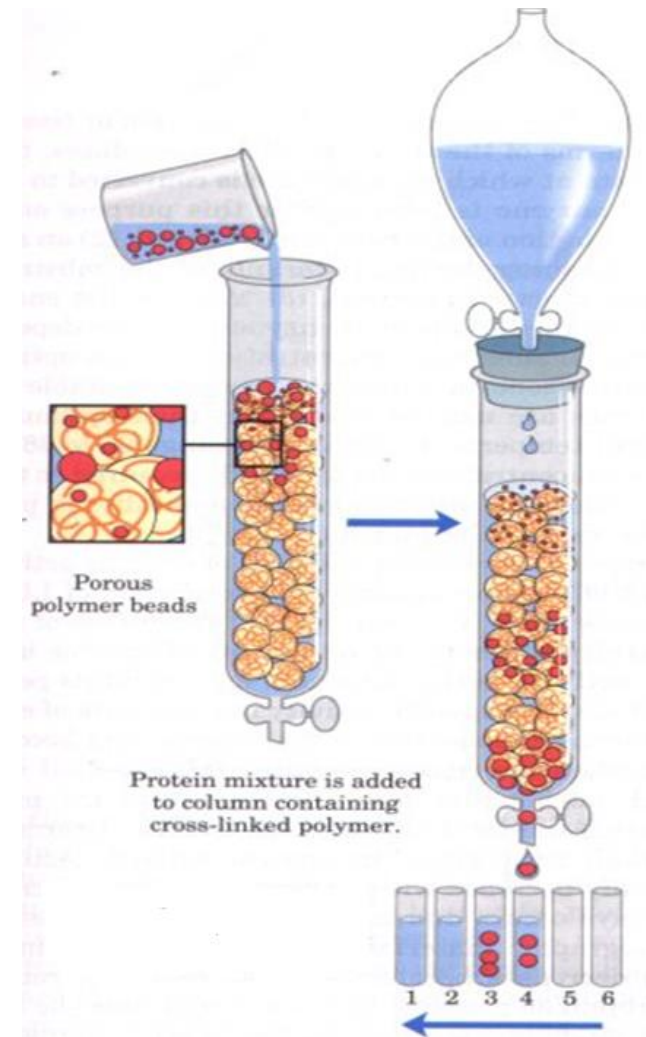
Size-exclusion Chromatography – Gel Filtration

Gel filtration chromatography (referred to as size exclusion chromatography) separates biomolecules based on differences in their molecular size.

The gel media consists of spherical porous particles of carefully controlled pore size through which biomolecules diffuse to different extents based on differences in their molecular sizes. Small molecules diffuse freely into the pores and their movement through the column is retarded, whereas large molecules are unable to enter the pores and are therefore eluted earlier. Hence, molecules are separated in order of decreasing molecular weight, with the largest molecules eluting from the column first.

Types of Gel Media:	Product
Crosslinked dextran	Sephadex G-10 to G-200
Agaroses	Sepharose
Polyacrilamide	BioGel P
Mixtures of these components	

The choice of the Sephadex depends of the size of the pores and the molecular weight of the biomolecules.



Home prepared gel filtration

HPLC – High-performance liquid chromatography



1. Uv-vis detector

2. Solvent bottle tray

3. Front panel display with keypad

4. Syringe access door

5. Column and column heater/cooler

6. Sample compartment access door

7. Solvent delivery tray access door

8. Solvent conditioning tray access door

Fluidic path through Solvent Management system

