काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

AN INSTITUTION OF NATIONAL IMPORTANCE ESTABLISHED BY AN ACT OF PARLIAMENT

Semester VI (CHB-601)

Separation of molecules by chromatographic techniques

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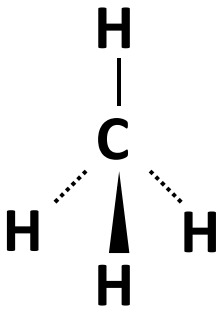
Polarity of Substances

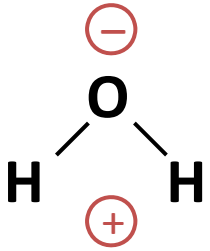
- Polarity
  - Property of a substance whereby the positions of the electrons give rise to positive and negative poles
  - Water: Polar

    Methane: Nonpolar

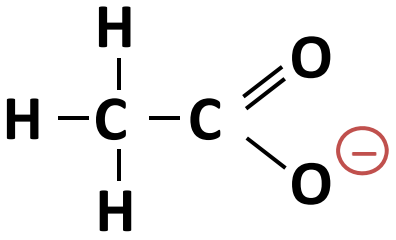
- Miscibility of solvents
  - Solvents of similar polarities can be easily dissolved together.
  - Polar and nonpolar molecules have a similar relationship to that of water and oil.



Methane



Water



Acetic acid

Nonpolar (Hydrophobic) Functional Groups and Polar (Hydrophilic) Functional Groups

- Nonpolar Functional Groups
  - $-(CH_2)_nCH_3$
    - Alkyl groups
  - $-C_6H_5$
    - Phenyl groups
- Polar Functional Groups
  - -COOH
    - Carboxyl groups
  - $-NH_2$
    - Amino groups
  - -OH
    - Hydroxyl groups

Partition Chromatography

- A liquid (or a substance regarded as a liquid) is used as the stationary phase, and the solute is separated according to whether it dissolves more readily in the stationary or mobile phase.
- Liquid-liquid chromatography

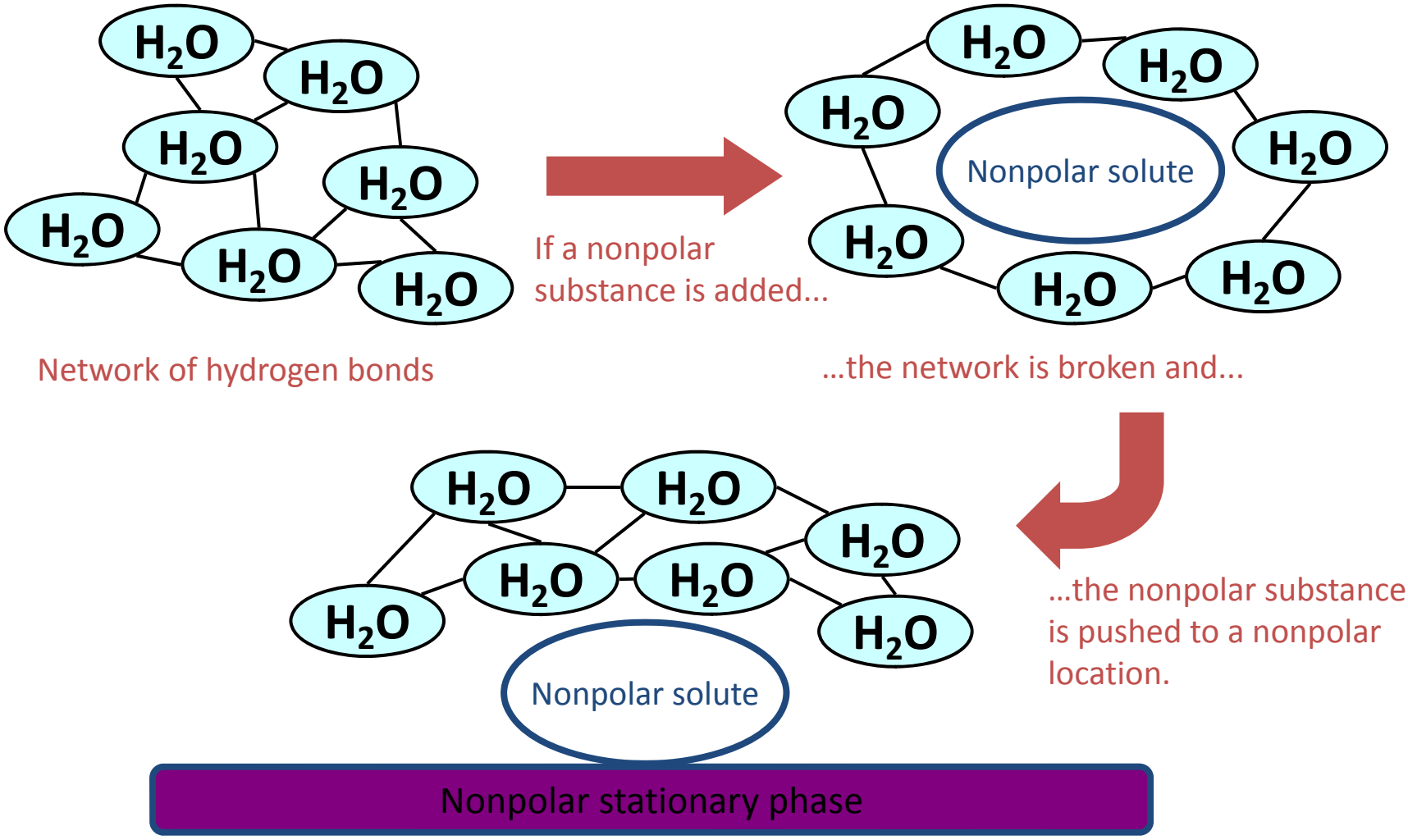
Normal Phase / Reversed Phase

	Stationary phase	Mobile phase
Normal phase	High polarity (hydrophilic)	Low polarity (hydrophobic)
Reversed phase	Low polarity (hydrophobic)	High polarity (hydrophilic)

Reversed Phase Chromatography

- Stationary phase: Low polarity
  - Octadecyl group-bonded silical gel (ODS)
- Mobile phase: High polarity
  - Water, methanol, acetonitrile
  - Salt is sometimes added.

Hydrophobic Interaction



Stationary Phases

Silica

- Silica, silica gel and silicic acid- terms commonly applied to materials produced by acidification of silicate solutions followed by washing and drying.
- enormous surface area ~500 m^2/g.
- surface consists of –Si-OH groups spaced at intervals of ~5Å

Alumina

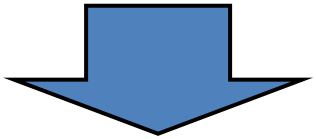
- Highly active alumina is produced by overnight air drying at 400^oC.
- surface area 150 m^2/g.
- a monolayer of water amounts 3.5 x 10^{-4} mL/ m^2

Cellulose

- It holds microscopic pools of water

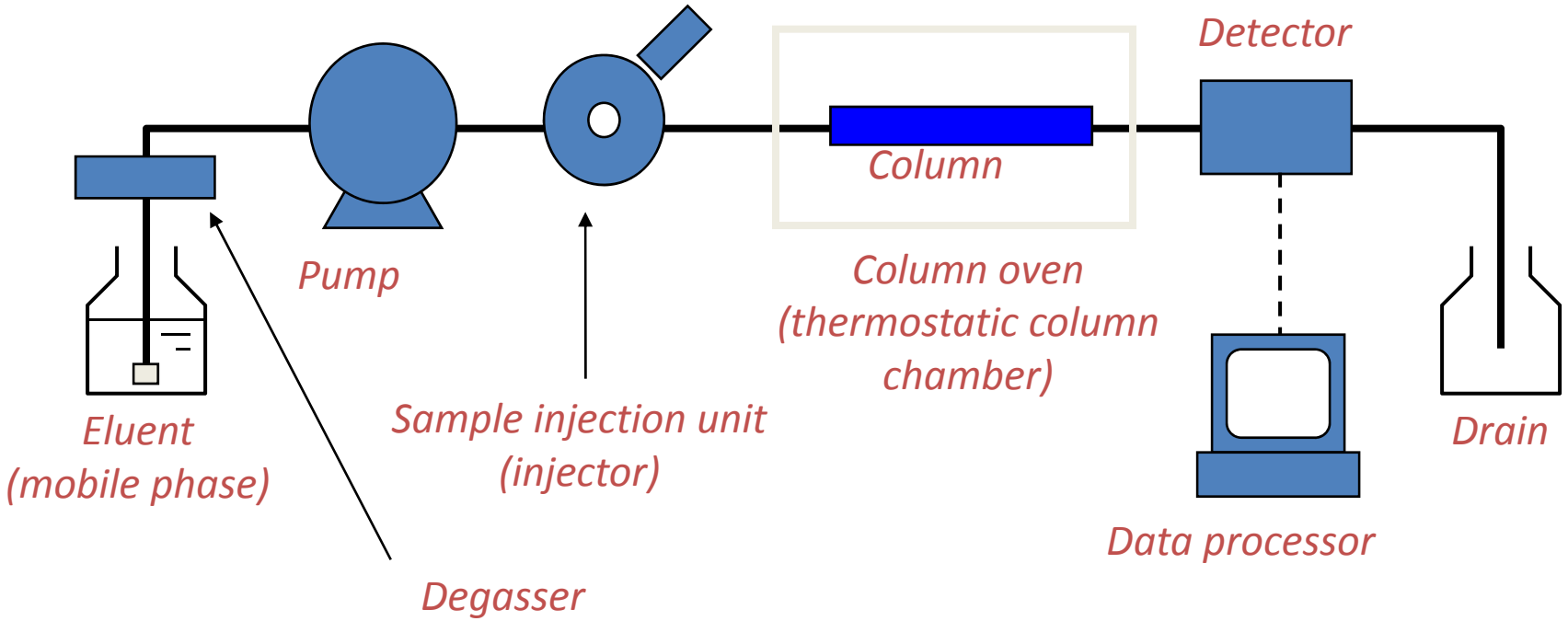
From Liquid Chromatography to High Performance Liquid Chromatography

- Higher degree of separation!
  → Refinement of packing material (3 to 10 µm)
- Reduction of analysis time!
  → Delivery of eluent by pump
  → Demand for special equipment that can withstand high pressures



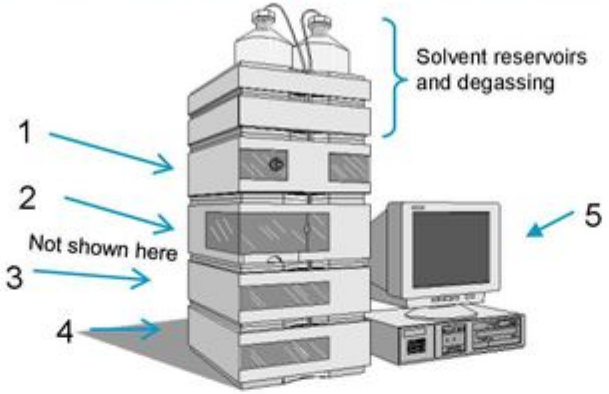
The arrival of high performance liquid chromatography!

Flow Channel Diagram for High Performance Liquid Chromatograph



What does a high pressure LC look like?

(1) Describing the 5 major HPLC components and their functions …



1. Pump:

- The role of the pump is to force a liquid (called the mobile phase) through the liquid chromatograph at a specific flow rate, expressed in milliliters per min (mL/min).
  - Normal flow rates in HPLC are in the 1- to 2-mL/min range.
  - Typical pumps can reach pressures in the range of 6000-9000 psi (400- to 600-bar).
- During the chromatographic experiment, a pump can deliver a constant mobile phase composition (isocratic) or an increasing mobile phase composition (gradient).

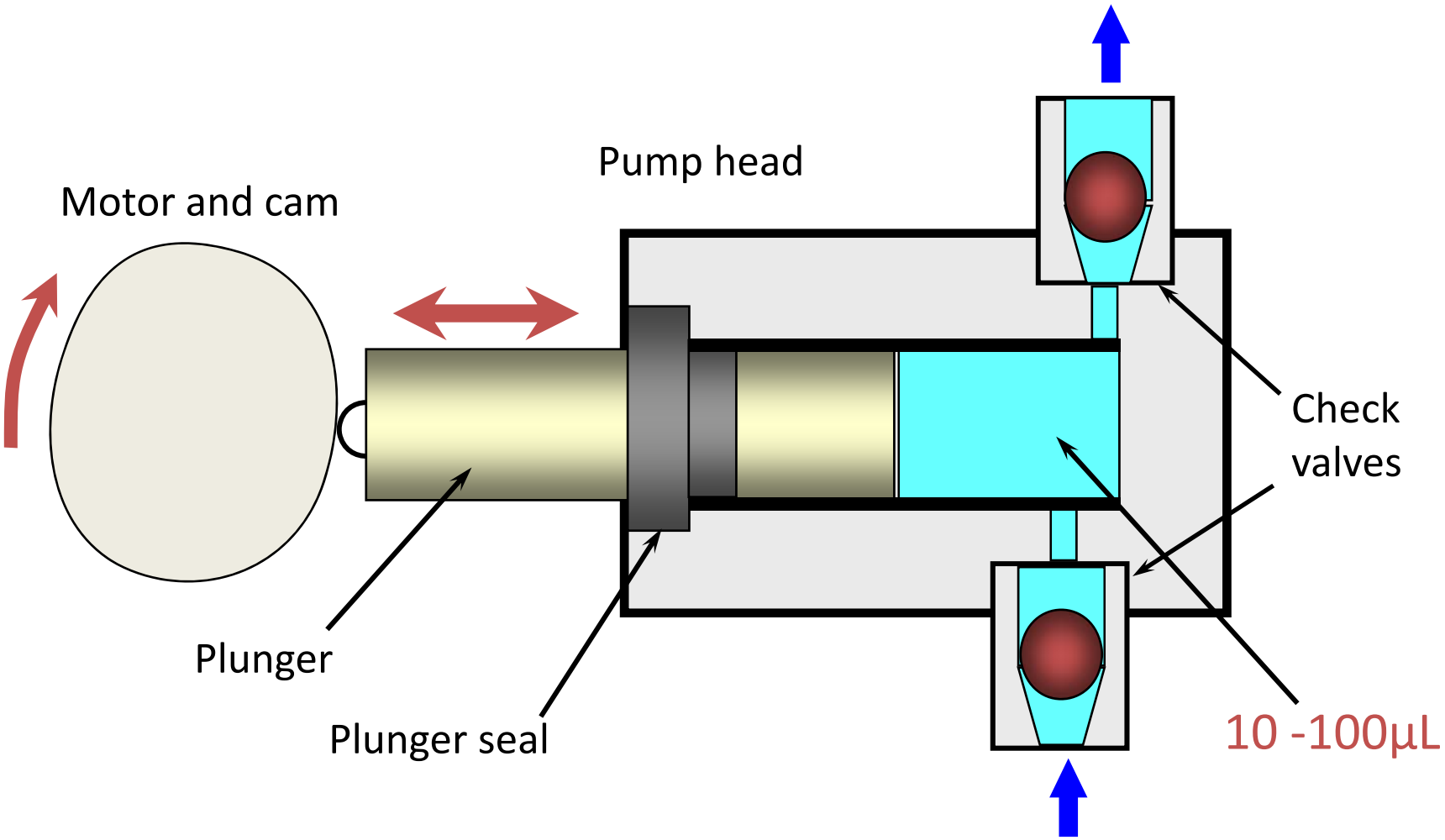
2. Injector:

- The injector serves to introduce the liquid sample into the flow stream of the mobile phase.
  - Typical sample volumes are 5- to 20-microliters (µL).
  - The injector must also be able to withstand the high pressures of the liquid system.
- An autosampler is the automatic version for when the user has many samples to analyze or when manual injection is not practical.

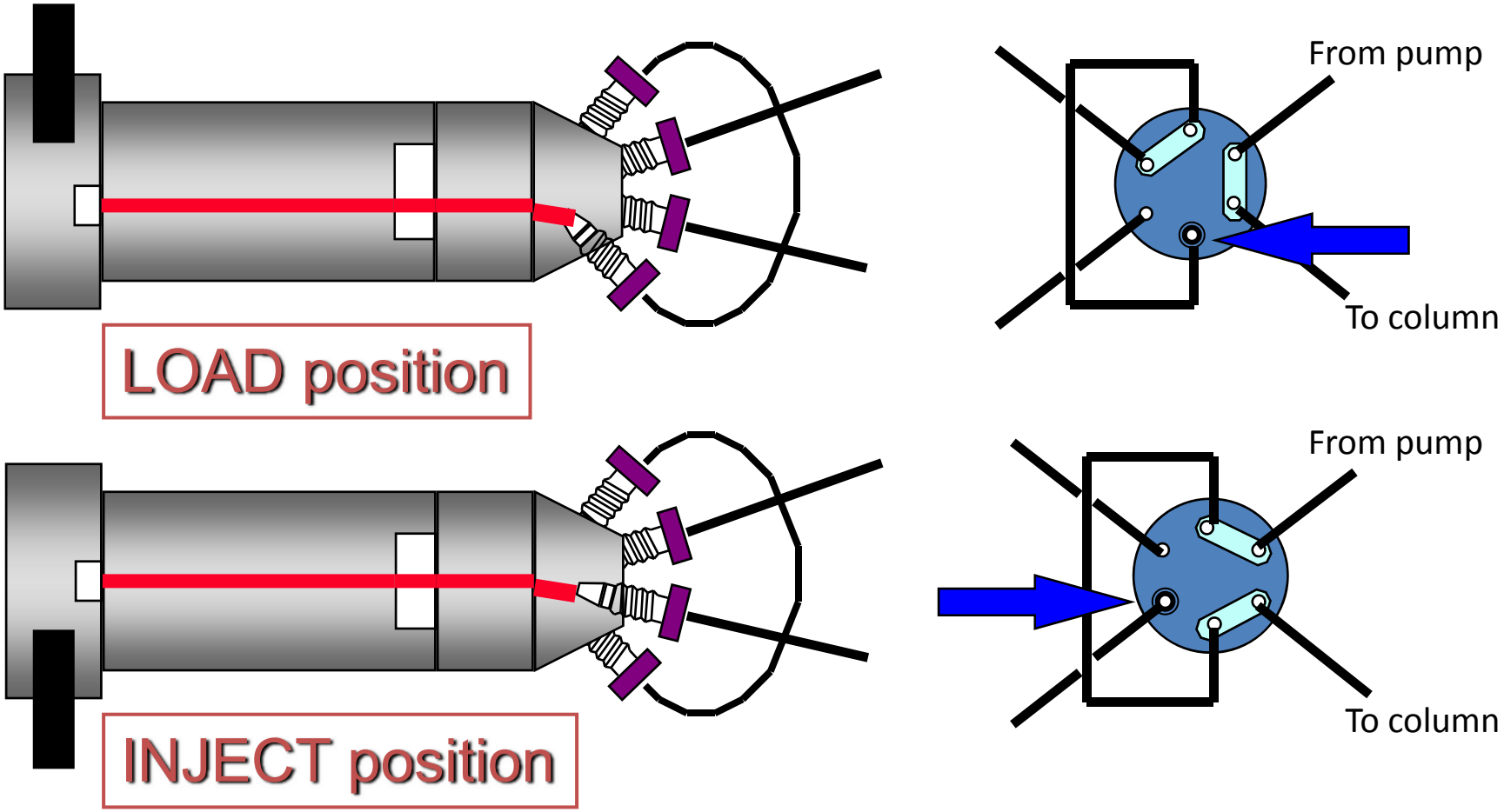
Solvent Delivery Pump: Representative Pumping Methods

- Syringe pump
- Plunger pump
- Diaphragm pump

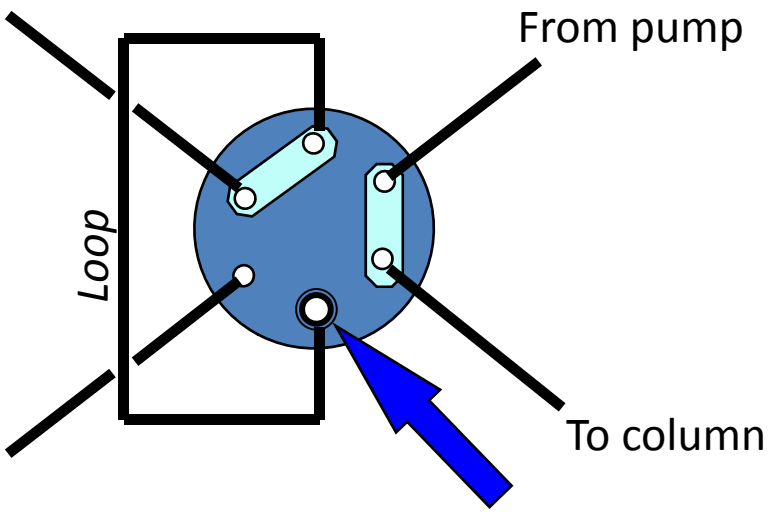
Solvent Delivery Pump: Schematic Diagram of Plunger Pump



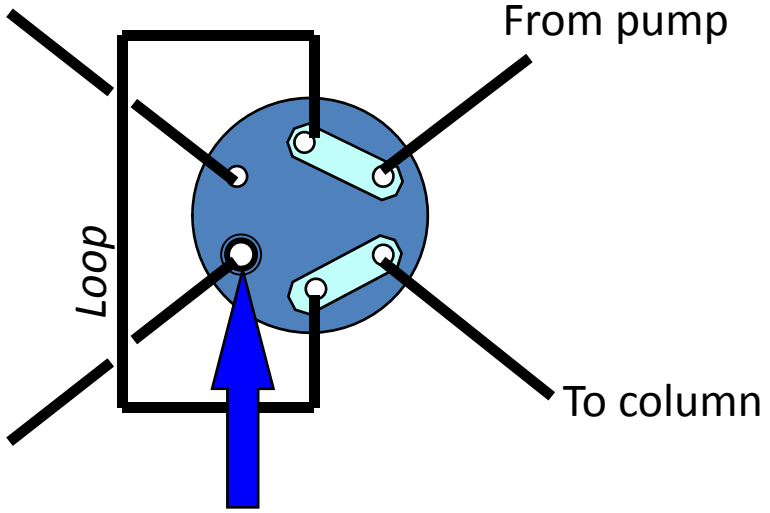
Manual Injector



Manual Injector:
Operating Principle of Sample Injection



LOAD



INJECT

Manual Injector: Injection Method

- Syringe measurement method
  - It is desirable that no more than half the loop volume is injected.
- Loop measurement method
  - It is desirable that at least 3 times the loop volume is injected.