High performance (pressure, problematic) liquid chromatography HPLC

- Sixties – massive development of chromatography

  - **Development of:**
  - HPLC – high performance (pressure) chromatography
  - MPLC – medium pressure liquid chromatography

Requirements laid on HPLC:

1. Columns filled with very smooth sorbent particles
2. High flow rates of mobile phases

Solution:

Usage of
- high pressure pumps
- on-line continuous detectors
- complicated injection systems
- novel chromatographic sorbents

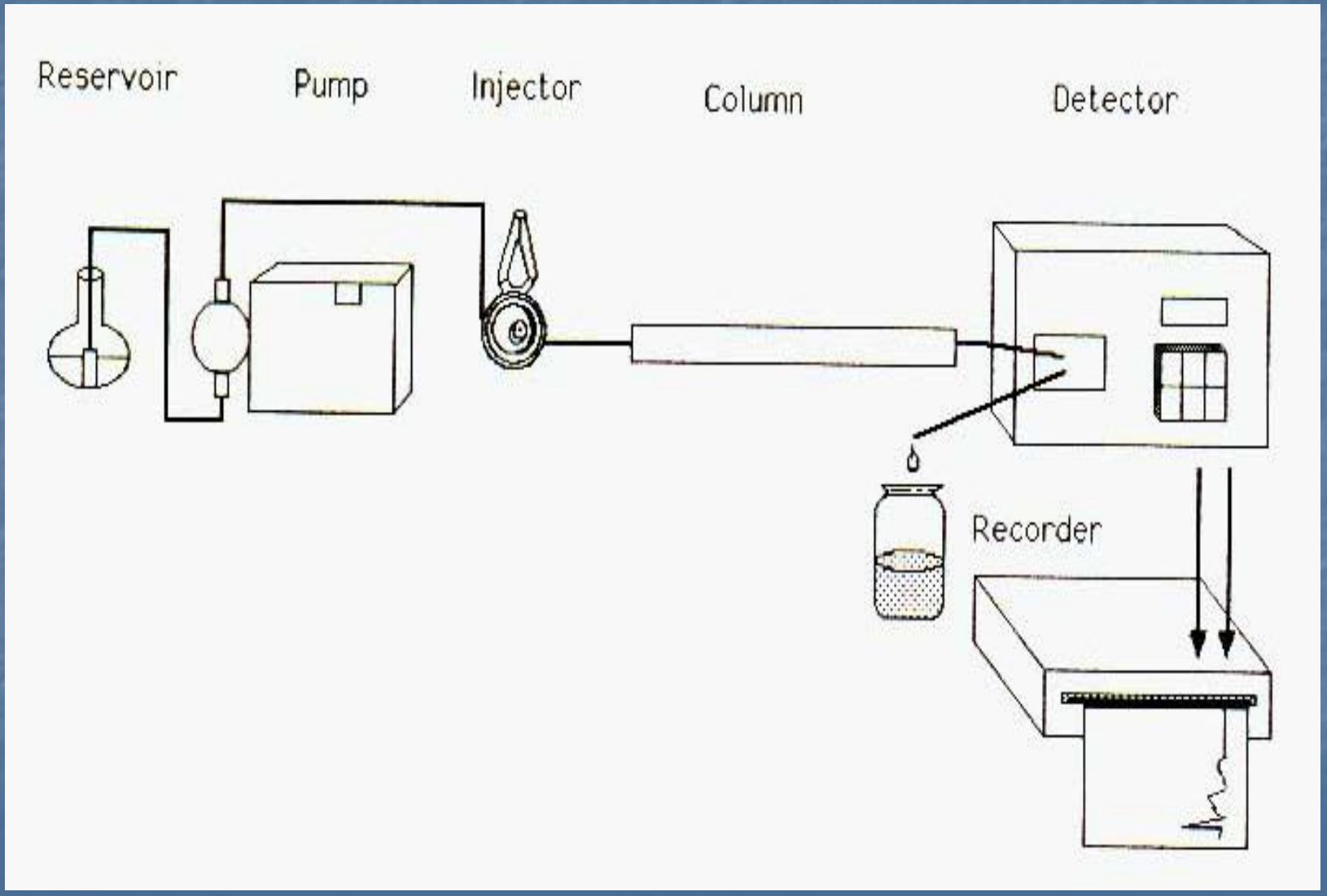
Advantages:

- Rapidity
- Effectiveness
- Automation
- The possibility of easy result description and quantification
- Reproducibility

Equipment for HPLC chromatography contains

- Reservoir of mobile phase
- Degassing equipment
- Pump
- Injector
- Column with chromatographic sorbent
- Fraction collector

HPLC



HPLC pumps

- **Alternating piston pumps with volume 35-400 µL, 2-4 pistons, alternating work in 90-180°, piston volume and velocity of movement can be changed**

- **A reciprocating pump is used where relatively small quantity of liquid is to be handled and where delivery pressure is quite large.**
- **A piston is a component of reciprocating pumps**

- Characteristic sign is pulse flow rate of mobile phase
  - Pumped liquid is pushed out by a piston or a membrane
  - Pump head possess inlet and outlet valve

HPLC pumps

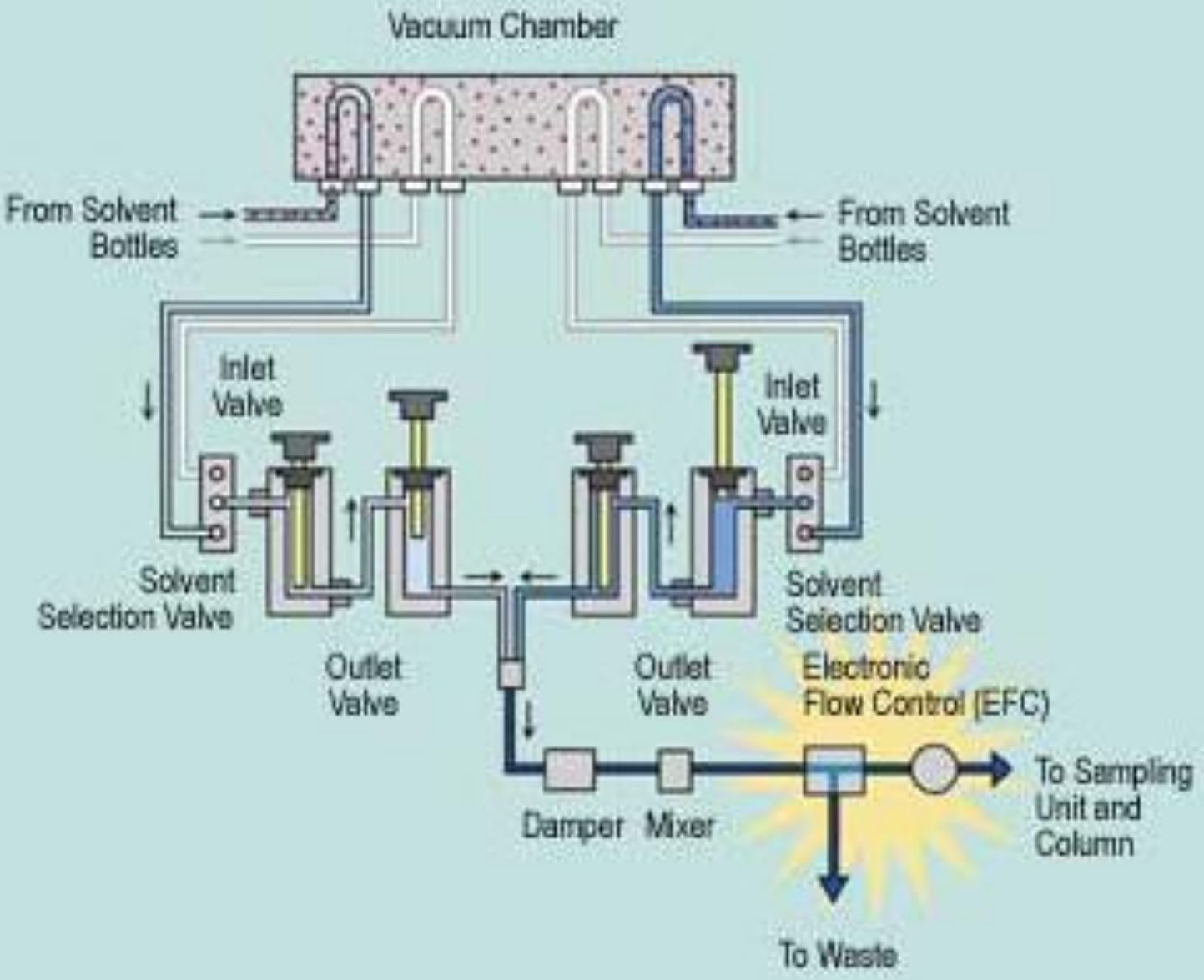
- **Syringe shaped pumps with volume 250-500 mL, piston with constant volume**
  - Pressure on piston is produced by an electric engine
  - Working volume 100 - 500 ml
  - Main advantage is constant flow without pulsing
    - High stability of detector signal
  - Generally high price of pump
    - Very precise manufacturing of single parts
  - Stable flow rate after 15 to 60 min
  - Necessary to degas mobile phase in advance
  - No gradient

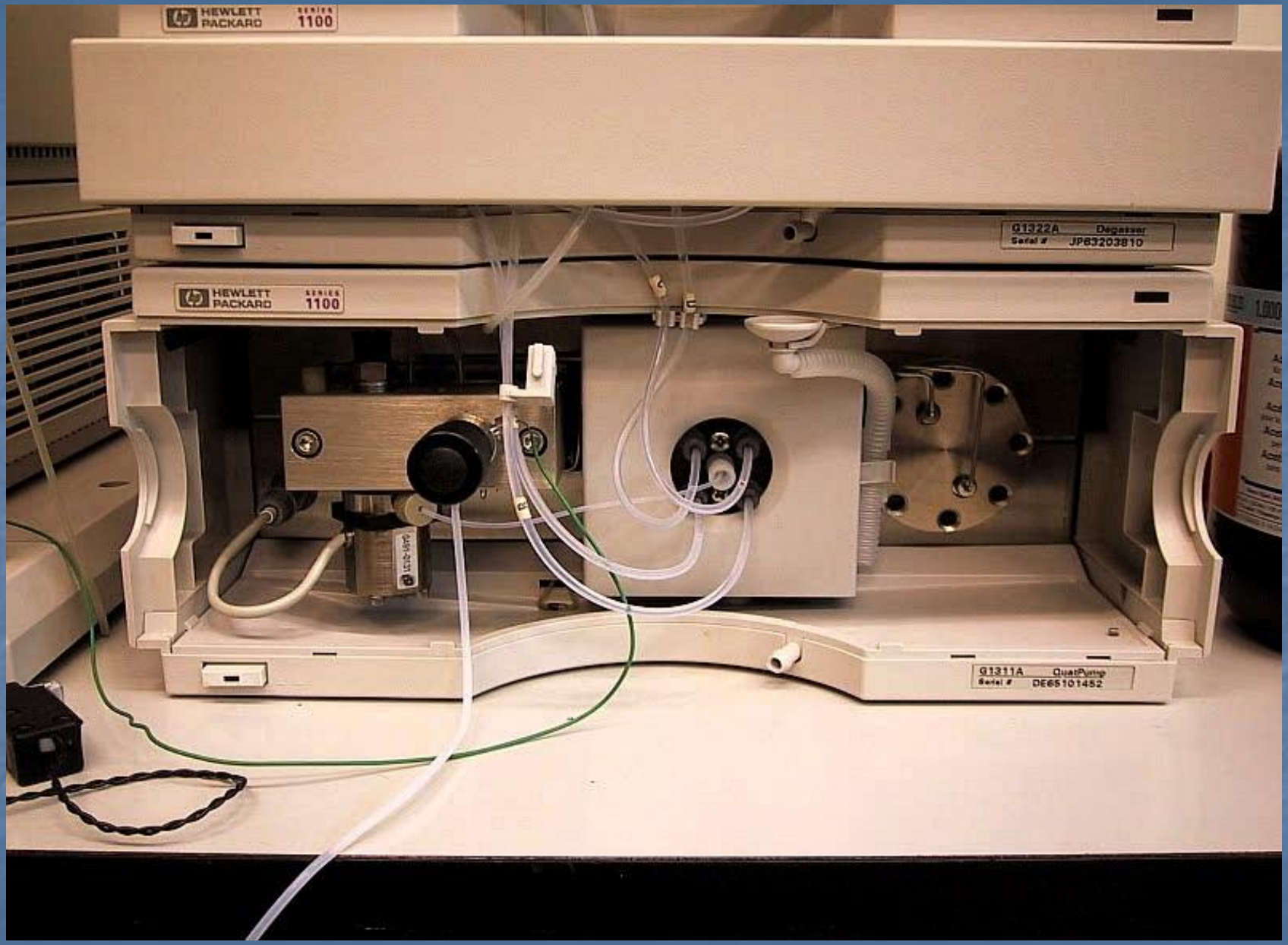
HPLC pumps

- **Pumps produce a constant flow by a stream of gas from the bomb, low gas pressure can produce high pressure in liquid**
  - Pneumatic pumps
  - Source of energy is compressed gas
  - Gas is flowing directly on the liquid level or is compartmentalized by using pistons
    - During the contact of the gas with liquid, the gas is partially dissolved
    - Advantageous separation of gas from liquid
  - Pumps are pulseless, very simple and cheap

Softening of pressure pulses

- Necessary to use multi-piston pumps with low internal volume
- Possibility of using the next „reversed“ phased pump
- Possibility of using more pump heads with coordinated inlets and outlets
- Often programmed movement of pistons
- Usage of dumpers (elastic tubing system)
- Residual pulses are in software filtered and removed


Vacuum Chamber
From Solvent Bottles
From Solvent Bottles
Inlet Valve
Inlet Valve
Solvent Selection Valve
Solvent Selection Valve
Outlet Valve
Outlet Valve
Electronic Flow Control (EFC)
Damper
Mixer
To Sampling Unit and Column
To Waste

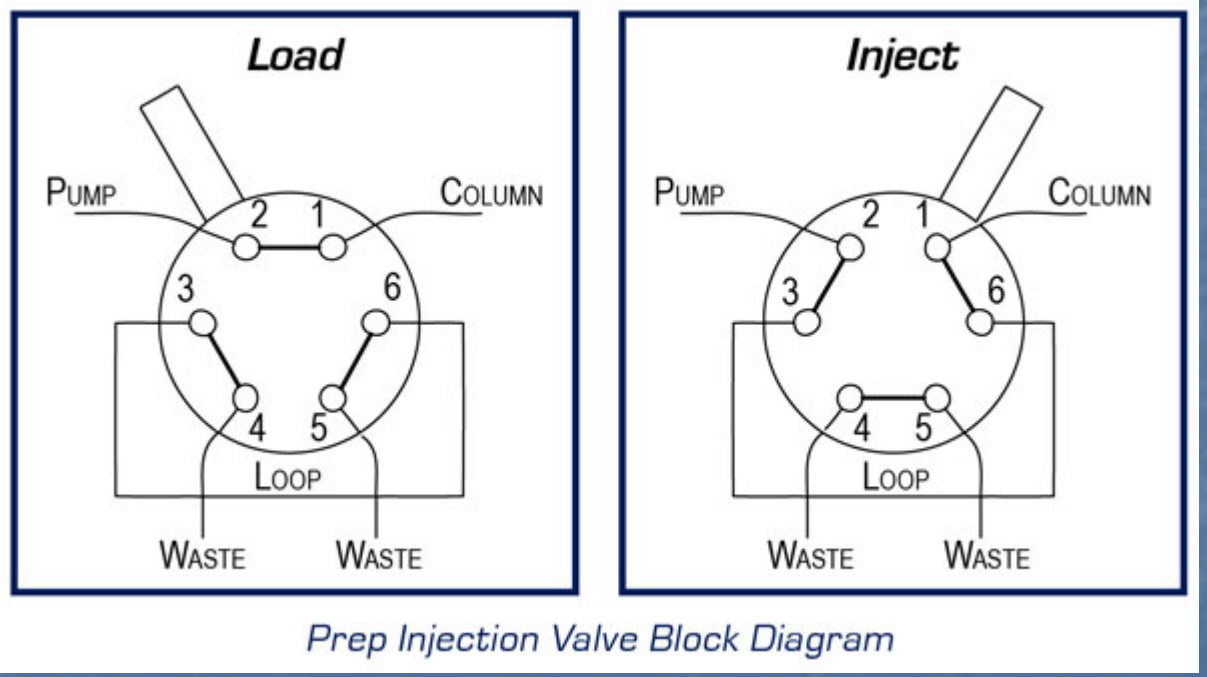

HEWLETT PACKARD
SERIES 1100
G1322A Degasser
Serial # JP83203810
HEWLETT PACKARD
SERIES 1100
G1311A QuatPump
Serial # DE65101452

Mobile phase

- Single component
- Multi component
  - isocratic elution
  - gradient elution
- Gradient elution
  - change of ratio of mobile phase components shorten time of analysis
  - improves separation of complex mixtures
  - iincreases sensitivity of detector
  - step wise or continuous

Injection in HP liquid chromatography

- During injection it is necessary to overcome the high pressure inside the column
- It is not possible to use simple injectors as for gas chromatography
- Most common HPLC injector is 6-path injection valve with exchangeable loop
- Injector used in HPLC possess very high reproducibility of injection volume



Prep Injection Valve Block Diagram

HPLC columns

According to usage:

- Micro columns
- guard
- analytical
- preparative

According to sorbent:

- normal phased
- reversed phased


G1315A DAD
Serial # DE64301787
HEWLETT PACKARD
SERIES 1100
5-8985
HPLC Column
FLOW
G1316A Col Comp
Serial # DE64301902
HEWLETT PACKARD
SERIES 1100

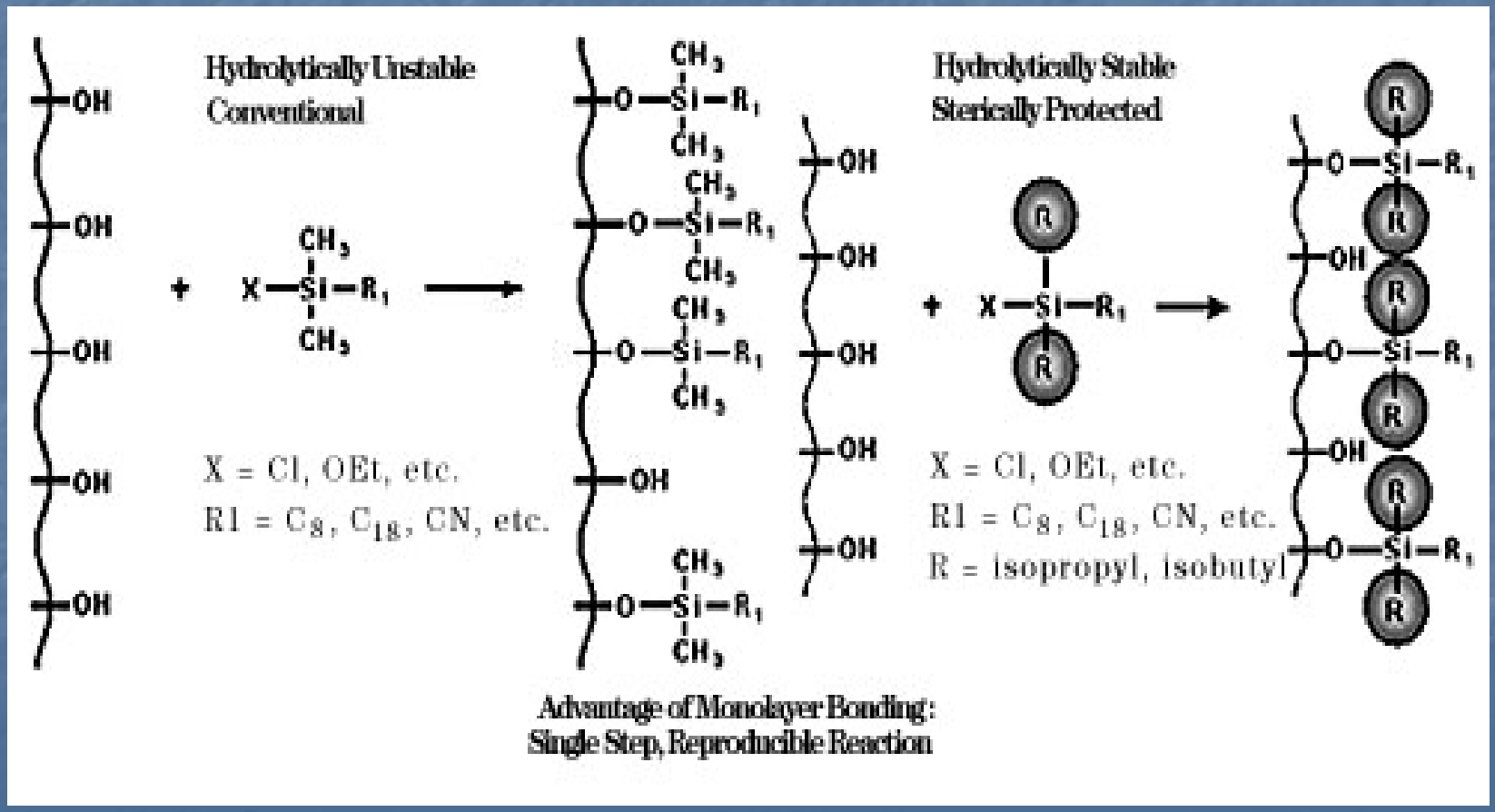
- More smooth particles of sorbent = higher pressure, but better separation abilities:

  1) silica gel or Al_2O_3
  2) silica gel or other carrier bonded phases
  3) gels
  4) other materials with pores of controlled size

5) Ion exchangers

6) others

Preparation of modified silica gel



Choice of column

- chromatographic column is chosen according to the analysis demands and used technique
- Besides analytic columns we have special column for preparative separation (big diameter and length)
- Conditions of analysis are selected as a compromise in relation to the analysis time, demanded resolution, and demanded load with material:
  - **If high resolution is needed**, the time of analysis is prolonged, and the column should not be overloaded with high volume of the sample
  - **If a short time of analysis is needed**, lower resolution will be obtained, and the column should not be overloaded with high volume of the sample
  - **If high amounts of sample should be separated**, the time of analysis will be prolonged, and lower resolution will be obtained

Analytical columns

- Internal diameter less than 4 mm
- Length 30, 100 up to 250 mm
- Stainless steel
- Filled with particles with diameter 2-10 μm, usually inorganic matrix – silica gel, with bonded stationary phase
- So called sectioned columns for separation of very complex mixtures of compounds

Preparative columns

- Bigger diameter
- Bigger length
- Higher amount of loaded and separated sample
- Designated for the separation of higher amounts of compounds (milligrams to tens of milligrams)

- Guard column
  - The tasks of guard column
    - To saturate the mobile phase with sorbent material to prevent the damage of the analytical column
    - Protection of the analytical column during the analysis of biological material
    - Filtration of mobile phase
  - As guard column can be used old or damaged „normal" column