GEL FILTRATION (SIZE EXCLUSION CHROMATOGRAPHY)

BACKGROUND

Chromatography is a commonly used method in chemistry, biochemistry and biotechnology to separate and purify different substances. The separation can be based on the size, polarity, charge or solubility of the chemical substances. In chromatography, there is a mobile phase (such as liquid or gas) and a stationary phase, which separates the substances carried by the mobile phase. There are various chromatographic methods such as paper-, thin layer-, ion-, reverse phase-, affinity- and size exclusion chromatography.

Gel filtration (size exclusion chromatography) is used to separate chemical substances based on their molecular size. In the stationary phase, there is a porous material and the substances can move through the pores. The smallest substances can move inside the pores, which makes them move slower than the bigger molecules.

Gel filtration is often conducted in separation columns and the substances are washed out of the column by mobile phase.

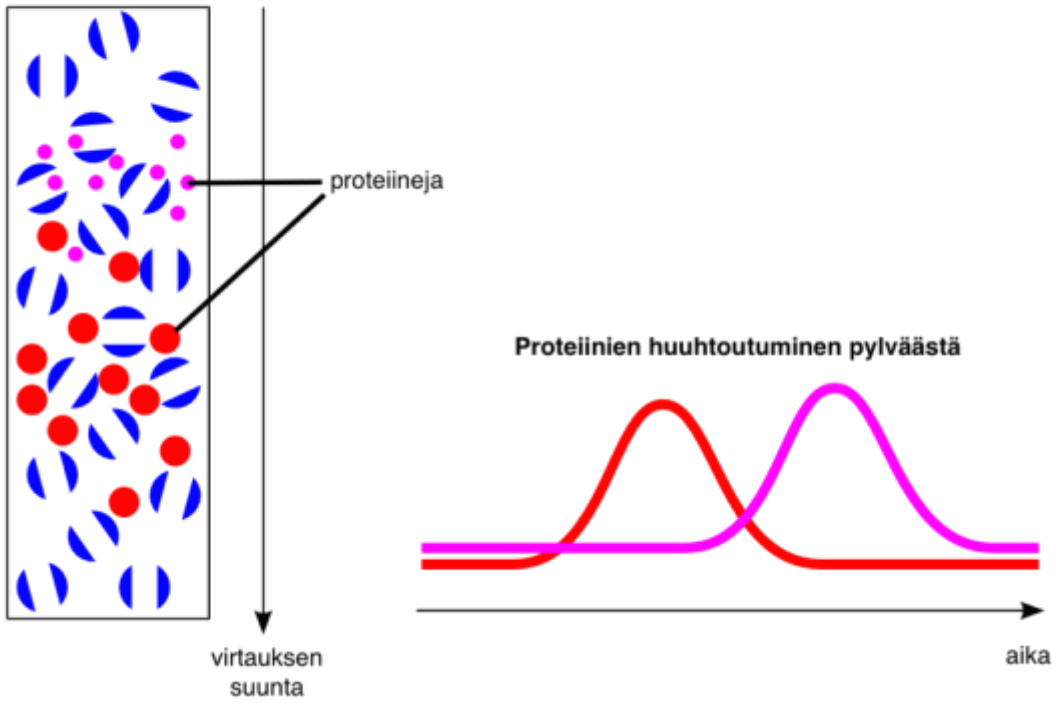

Image 1. The principle of gel filtration. (Takometer / Wikimedia Commons)

In this task, there are two colourful substances, which makes possible to follow the separation in the column. You should separate hemoglobin (brownish red) and vitamin B12 (pink) based on their molecular size. In gel filtration, you can separate the substances and collect them in different test tubes.

Hemoglobin contains a heme (with a iron ion) and a protein part (globin). The protein complex binds to oxygen and carries it from lungs to other tissues. The Fe^{2+} causes the brownish red colour of the substance.

Vitamin B12 (cobalamin) is a common cofactor and it is important for many methyl transferase enzymes. Vitamin B12 is needed e.g. for cell division, and deficiency symptoms include anemia, muscle weakness and nervous malfunction. The colour of vitamin B12 is pink.

LAB SAFETY

- Wear a protective coat and gloves during the task.

QUESTIONS BEFORE THE TASK

- What is the biological function of hemoglobin and vitamin B12?
- Which one moves faster in gel filtration: protein with molar mass of 10 000 M or 100 000 M?
- Where could you use gel filtration?

EQUIPMENT

- Bio-Radin kit: Biotechnology Explorer™: Size Exclusion Chromatography, with
  - protein mixture
  - gel filtration columns
  - column buffer (0,1 M NaCl)
  - the cap of the column (yellow)
- Pasteur pipettes
- Micropipettes and tips
- Test tubes and racks

INSTRUCTIONS

The instructions are based on"Biotechnology Explorer™: Size Exclusion Chromatography".

1. Take 17 test tubes. Mark a tube as "buffer", a tube as "waste" and number the rest from 1 to 15.
2. Move 4,5 ml column buffer to the "buffer" tube.
3. Break the tip of the column and/or remove the caps. Collect the flow-through (drain) to the "waste" tube.
4. When the flow of the buffer has finished, move the column to the tube 1.
5. Add 15 µl of protein mixture to the column. The drop should be loaded just above the top of the grainy part of the column. Do not touch the grainy part.
6. Add 250 µl of column buffer to the column. Let the buffer run down the side of the column.
7. When the buffer has drained through the column, add 250 µl of column buffer (like the previous step). Let the buffer run to the tube 1.
8. When the buffer has drained through the column, move it to the tube 2.
9. Add 3,5 ml column buffer to the column.
10. Collect 5 drops of flow-through to the tubes 2–15.
11. When you're ready, cap the column (both caps) and store it in the refrigerator.

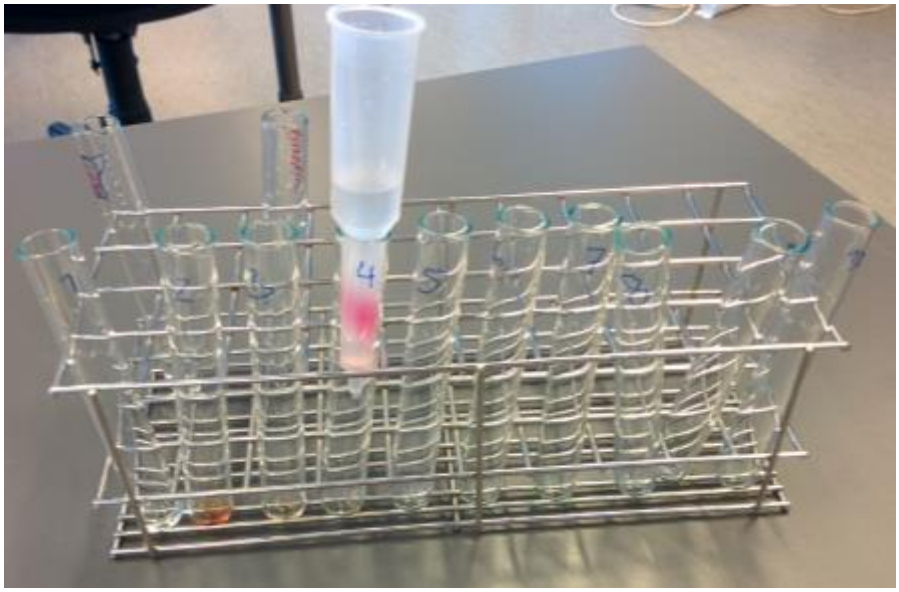

Image 2. Collecting the flow-through to the test tubes.

QUESTIONS AFTER THE TASK

- Which one of the substances was the bigger one? How did you end up in this conclusion?
- Do you know any other chromatographic methods?
- Why you did not add all the buffer in the column at once?