Thin-layer chromatography

Thin-layer chromatography is one of the separation methods that separate substances from a mixture based on the different affinity of the individual components of the mixture to the stationary and mobile phase. In thin-layer chromatography, a glass, aluminum or plastic plate is coated with a thin layer of a stationary phase (silica gel , aluminum oxide, etc.), onto which the analyzed sample is applied. The substance amount of the applied sample should not exceed the absorption capacity of the stationary phase (it is necessary to optimize the amount of the sample and the thickness of the stationary phase used).

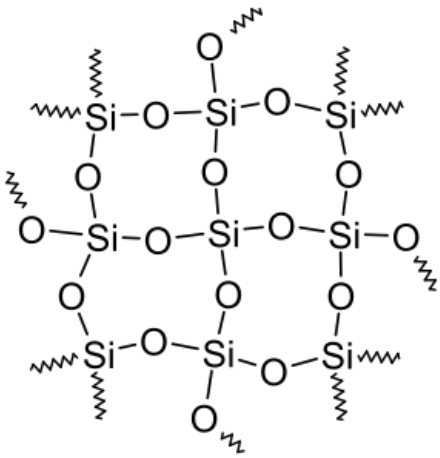
Silica gel

Silica gel is a form of silicon dioxide in which silicon atoms are cross-linked to each other via oxygen molecules. Towards the surface of the wafer, –OH groups are attached to the silicon atoms. The whole structure then looks like this:

Bound –OH groups give the surface of the plate polar properties with the possibility of forming hydrogen bridges and other non-covalent interactions with separated substances.

Mobile phase

The mobile phase is a specifically defined mixture of solvents, which we pour in a small layer (up to 1 cm column of liquid) into the chromatography bath. Then we insert the chromatography plate with the applied sample into the bath so that the sample is above the surface of the solvent and that the TLC plate rests against the wall only on the back side. The mobile phase rises up the plate and carries the analyzed sample with it. The rate at which the sample is carried upward depends on the solubility of the sample in the mobile phase and the degree of interaction with the stationary phase (affinity for the stationary phase). The greater the sample's affinity for the stationary phase, the slower it rises. When the solvent arrives far enough from the start (mostly more than ¾ of the plate), we remove the plate from the chromatographic bath and mark the position on the plate where the solvent has traveled with a line (= front).



Silicon dioxide

Retardation factor

Then we measure the distance of the individual substances from the start and calculate the so-called **retention (retardation) factor Rf** for each substance . The Rf value indicates how far the analyte spot lags behind the solvent front. Rf is characteristic for a given substance in a given system, i.e. if we repeat the experiment, we should get the same Rf in the same arrangement. In our case, for substance A Rf=a/c, for substance B Rf=b/c (see figure).

If they are not colored substances, they must be **visualized** before analysis . There are several methods, from the presence of a fluorescent substance directly in the plate, when individual substances appear as black spots under a UV lamp, to chemical detection, when the developed chromatographic plate is sprayed with a reagent (ninhydrin for amino acids , etc.), which reacts with the given substances and creates such a colorful product.

Method:

In this hands-on exercise, we will use a plastic plate coated with silica gel. The entire procedure should be carried out due to the organic mobile phase in the digester. We do not touch the active stationary phase, we use gloves when handling the plate.

1. **Pour the solvent into the chromatography bath** , close it with a lid and let it equilibrate for a while. Solvent vapors should saturate the internal space above the surface.
2. **Apply the sample** to the TLC plate with a pipette or syringe and let the applied mixture dry.
3. We flip the lid and **insert the plate** inside as quickly as possible. Close the lid immediately.
4. We check **that the front of the solvent does not reach beyond the upper edge of the plate** . In that case, part of the sample would "go out" and it would not be possible to determine the Rf.
5. **We end the analysis** by removing the plate from the chromatography bath and marking the front of the analysis.

Links

Related articles

- Chromatography

Source

Leníček M., Muchová L.: Organika I