크로마토그래피의 원리와 분석법

Thin Layer Chromatography의 원리-1

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- **흡착제를 균일한 박층 (두께 0.1-0.25 mm)으로 만들어 사용**
- TLC plate : glass, metal, plastic sheets
- **흔히 silica gel 혹은** alumina **박층을 사용**
- **분해능이 좋으며 분석소요시간이 짧다**
- **용매, 발색시약에 제한이 없어 여러 종류의 물질 분석에 이용**
- **간단하고 빠르며 경제적인 화합물 확인 방법**
- **혼합물 내의 화합물의 수를 확인**
- **표준물질과 Rf 치를 비교 : 혼합물내의 화합물의 종류를 확인**

❖ Preparation

Developing chamber, Capillary tube, TLC plate, UV lamp
Filter paper, Sample solution, Mobile phase (developing solvents)
etc

❖ Chamber

Lid가 있어 전개용매가 전개조 내에 균일하게 포화되어야 함
필요에 따라 필터를 전개조 내에 넣어 용매를 포화시킨다
전개용매는 바닥에서 **0.5 cm** 내외로 한다**.**
혼합물의 전개를 쉽게 확인할 수 있도록 투명전개조 사용

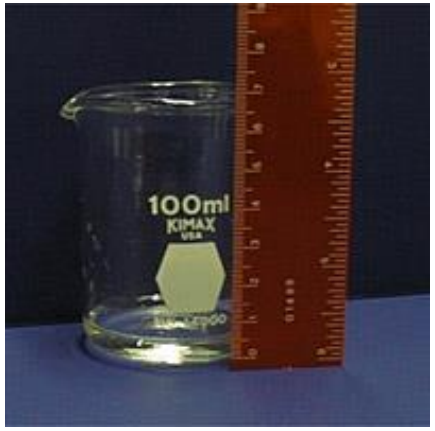
❖ TLC plate

Precoated Merck Kiesel gel 60 F254 plate
0.25, 0.5, 1 mm 두께의 **TLC**가 있다
5 cm or 20 cm x 20 cm sheets.

How to Run Thin Layer Chromatography

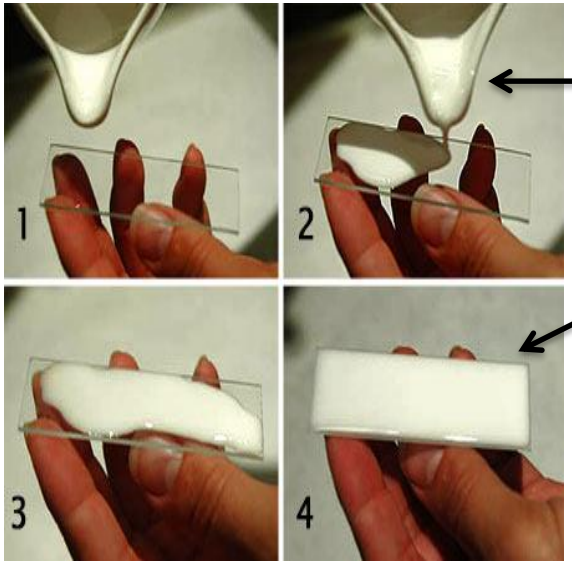
- Step 1: Prepare the developing container
- Step 2: Prepare the TLC plate
- Step 3: Spot the TLC plate
- Step 4: Develop the plate
- Step 5: Visualize the spots

Preparation of a developing chamber



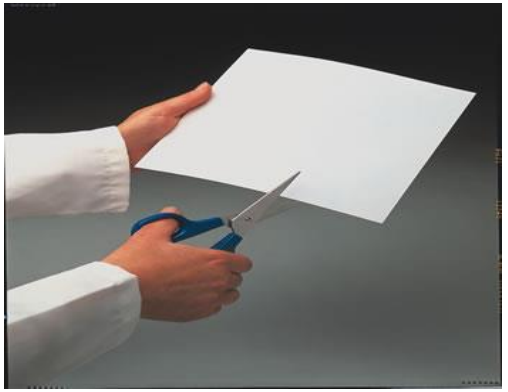
- To aid in the saturation of the TLC chamber with solvent vapors, you can line part of the inside of the beaker with filter paper.

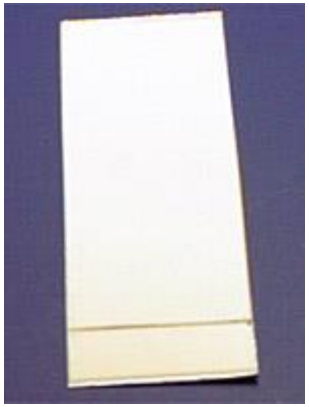
Preparation of a TLC plate

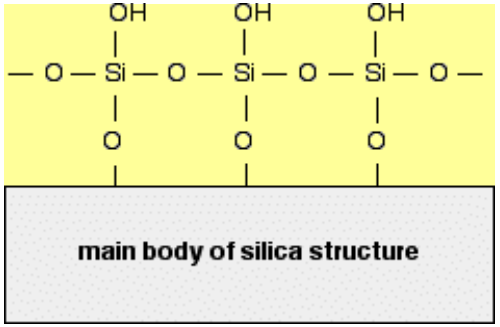


실리카

형광물질이 혼합됨







❖ Preparation of Sample solution

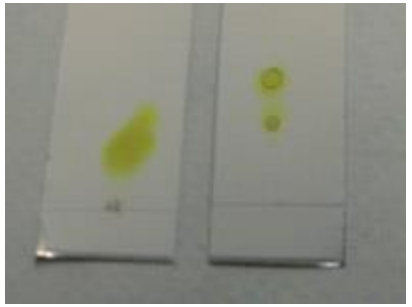
용액의 농도가 약 1% or 1 gram in 100 mL되도록 만든다

1 mg in a few drops : 정확할 필요가 없다

사용하는 용매는 시료가 잘 녹은 것을 선택하되 휘발성인 것을 사용 (Hexanes, ethyl acetate, or methylene chloride)

농도가 너무 진하거나 연하면 물질을 확인할 수 없다

이 경우 시료를 희석하여 다시 TLC한다



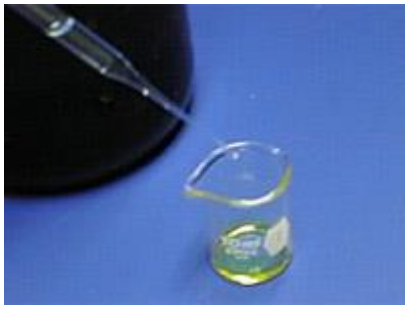
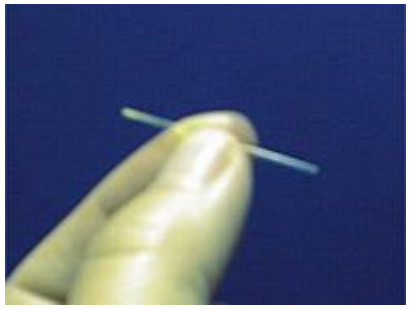
❖ Spotting of Sample solution

모세관 혹은 microtube를 사용

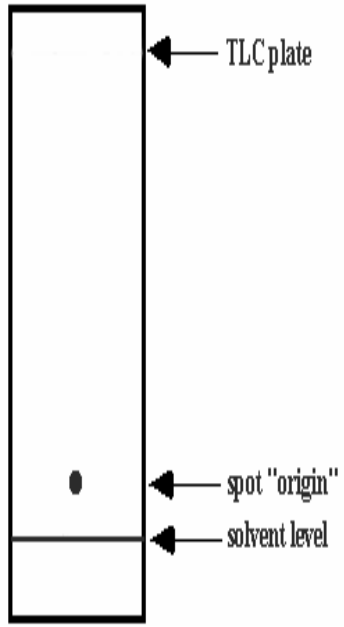
TLC coating이 손상되지 않도록 조심하며 Spot 의 직경이 너무 크지 않게 loading

너무 넓게 loading하면 각 화합물의 spot이 겹치게 되어분리가 효율적이지 않다

Spotting of sample on TLC plate

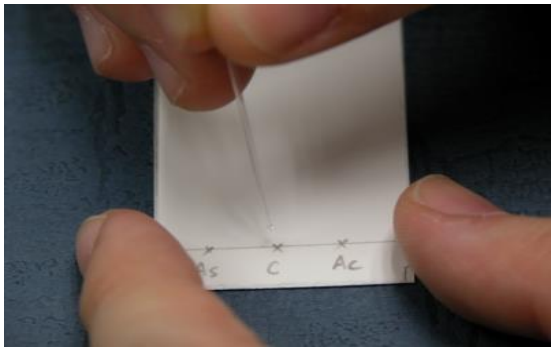



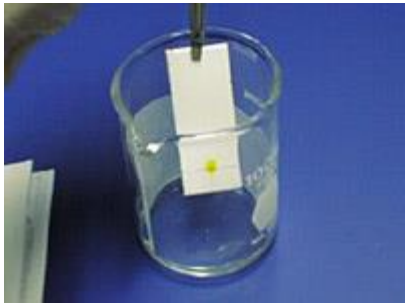
- Using a pencil, draw a line across the plate at the 0.5 cm mark. This is the origin: the line on which you will spot the plate. Take care not to press so hard with the pencil that you disturb the adsorbent.
- Under the line, mark lightly the samples you will spot on the plate, or mark numbers for time points. Leave enough space between the samples so that they do not run together; about 4 samples on a 5 cm wide plate is advised.
- Prepare 1% solution of drug dissolving in volatile solvents like hexanes, ethyl acetate, or methylene chloride.
- Dip the microcap or microcapillary into the solution and then gently touch the end of it onto the proper location on the TLC plate.

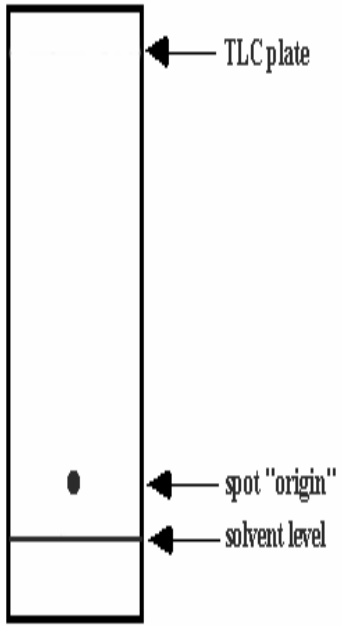


Spotting of sample on TLC plate



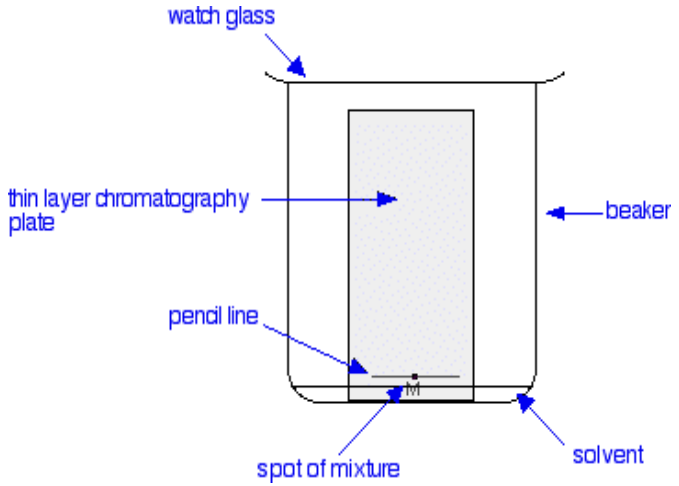


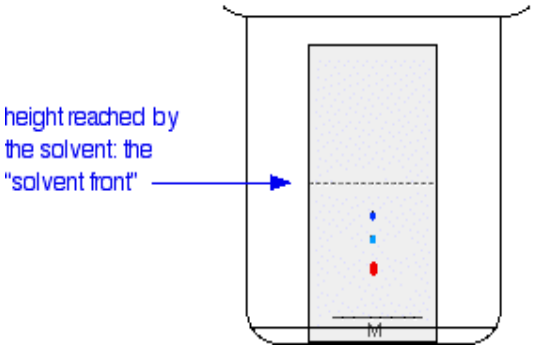


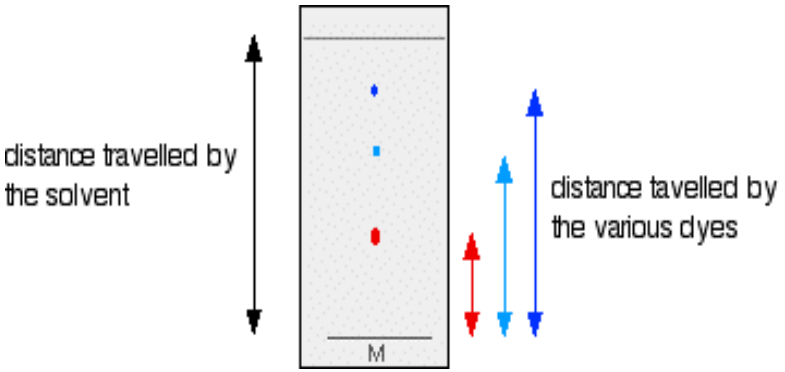



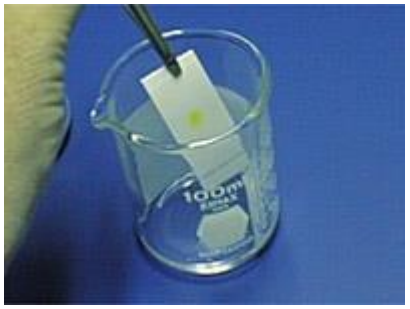
- Place the prepared TLC plate in the developing beaker, cover the beaker with the watch glass, and leave it undisturbed on your bench top.
- A solvent, or mixture of solvents, called the eluent, is allowed to flow up the plate by capillary action.
- Make sure the solvent does not cover the spot.
- Do chamber saturation to avoid "edge effect"

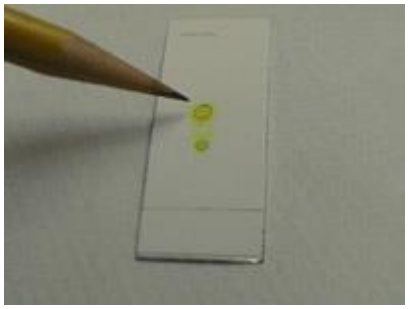
Developing



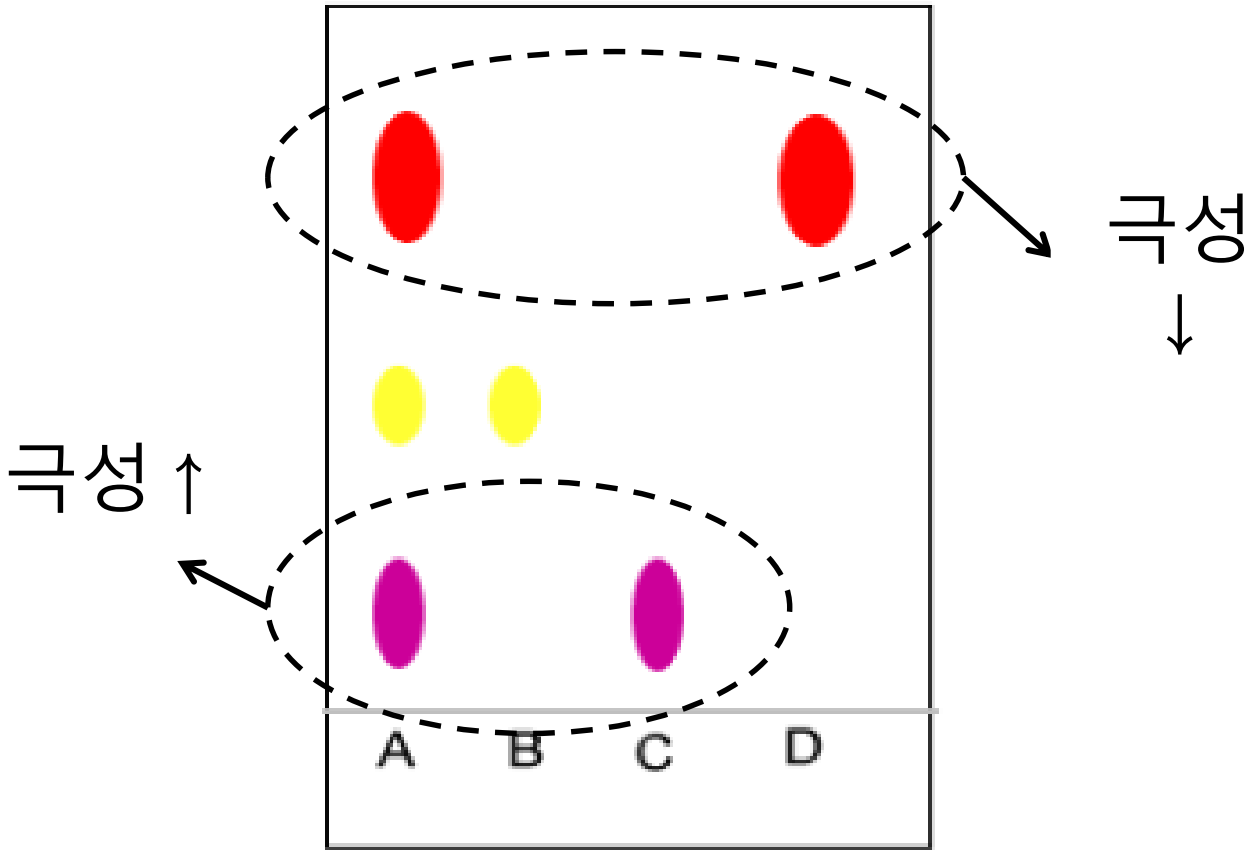








- Allow the plate to develop until the solvent is about half a centimeter below the top of the plate.
- Remove the plate from the beaker and immediately mark the solvent front with a pencil.
- Allow the plate to dry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