크로마토그래피의 원리와 분석법

Thin Layer Chromatography의 원리-2

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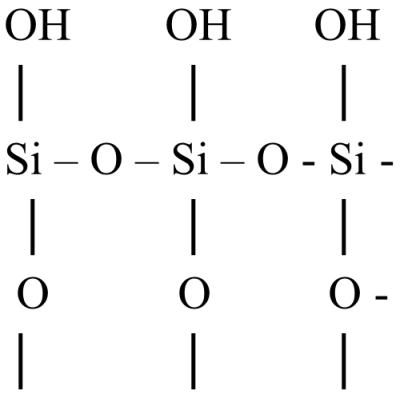
Silica As a Stationary Phase



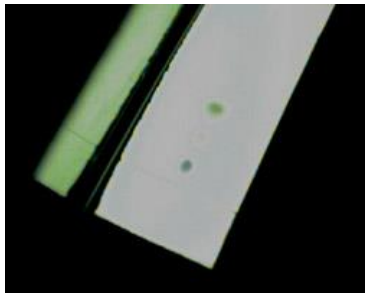
- Silica (SiO_2) is a solid with an extended structure of tetrahedral silica atoms bridged together by bent oxygen atoms.
- On the surface of the silica particles, the solid terminates in very polar silanol (Si-O-H) groups.
- The silica is the stationary phase because it remains adhered to the glass plate and does not move during the chromatography process.

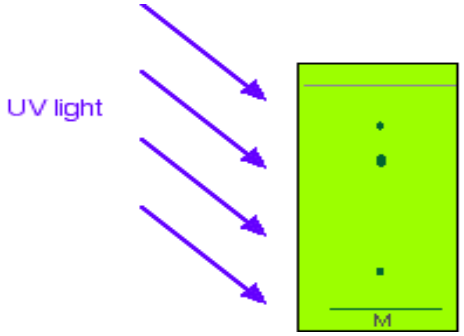
Adsorbability of organic compounds

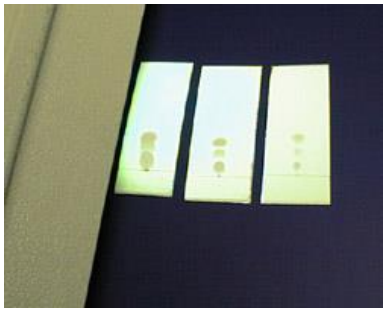
- Absorbability of organic compounds by functional group

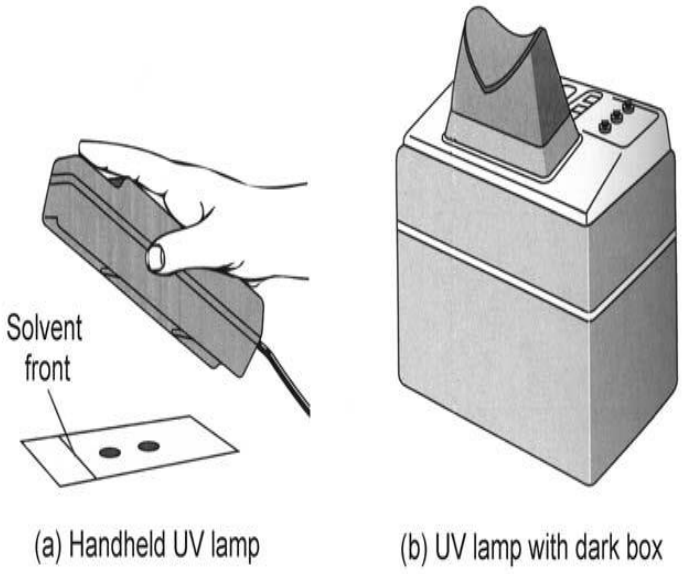
- **Least Strongly Adsorbed :**
  - Saturated hydrocarbons; alkyl halides
  - Unsaturated hydrocarbons; alkenyl halides
  - Aromatic hydrocarbons; aryl halides
  - Polyhalogenated hydrocarbons
  - Ethers
  - Esters
  - Aldehydes and ketones
  - Alcohols
- **Most Strongly Adsorbed :**
  - Acids and bases (amines)

Visualization of sample on TLC plate









(a) Handheld UV lamp

(b) UV lamp with dark box

- If there are any colored spots, circle them lightly with a pencil.
- Most samples are not colored and need to be visualized with a UV lamp.
- Hold a UV lamp over the plate and circle any spots you see.
- Make sure you are wearing your goggles and do not look directly into the lamp. Protect your skin by wearing gloves.

❖ UV detector

시료에 색이 있을 경우 : 탈색되기 전에 연필로 표시

시료에 색이 없을 경우 : UV lamp를 사용하여 육안으로 확인할 수 없었던 spot에 연필로 표시 (볼펜, 샤프사용 X)

보통 장파장 (365 nm)과 단파장 (254 nm)으로 구성

장, 단파장에서 detect되지 않는 시료인 경우 정색반응 시행

❖ Mobile phase, developing solvent

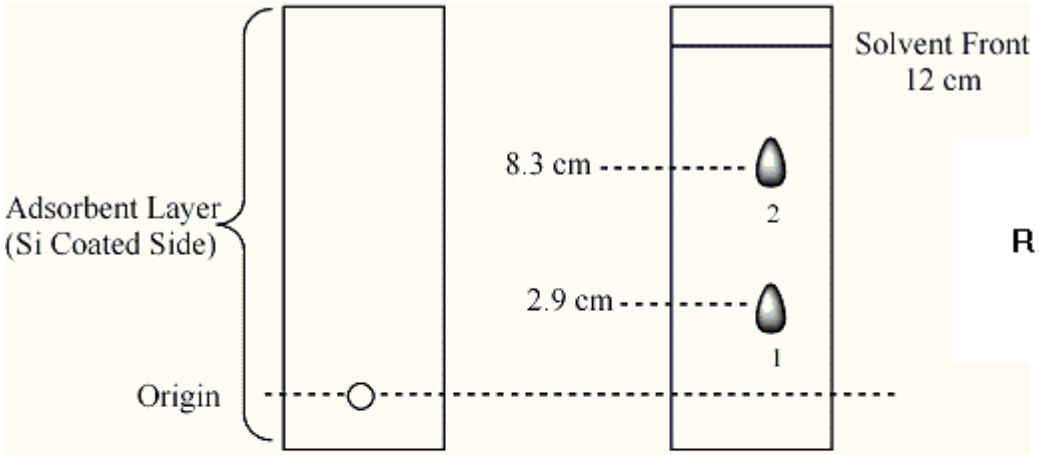
Rf value : 0.2 ~ 0.7, 둥근 spot

분리 혹은 정제시 다양한 전개용매를 사용할 필요가 있다.

구조내 OH (hydroxyl) group이 많은 화합물 : tailing현상

산을 첨가하면 바람직한 TLC analysis 가능

❖ R_f (retardation factor) value



R_f = Distance Traveled by Spot / Distance Traveled by Solvent

$R_f(1) = 2.9\ cm / 12\ cm = 0.24$

$R_f(2) = 8.3\ cm / 12\ cm = 0.69$

Note: R_f Values Are Always Less Than 1

$$R_f = \frac{\text{Distance from start to center of substance spot}}{\text{Distance from start to solvent front}}$$

❖ Rf depends on the following parameters:

solvent system

absorbent (grain size, water content, thickness)

amount of material spotted

temperature

What if the substances you are interested in are colourless?

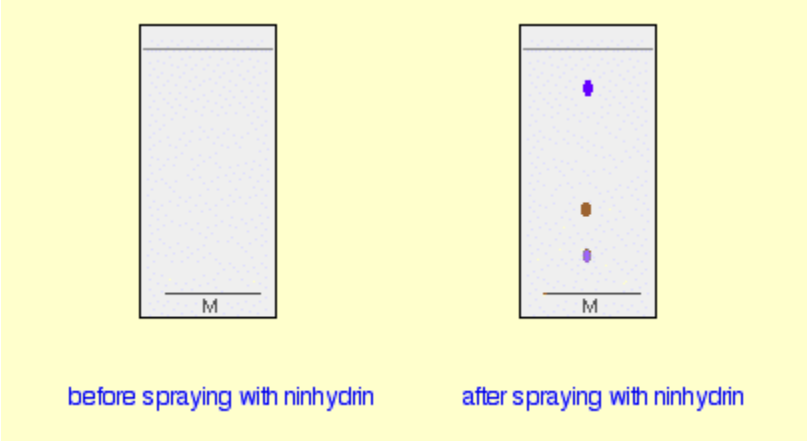
There are two simple ways of getting around this problem.

1) Using fluorescence

2) Showing the spots up chemically

(1) Ninhydrin solution

The chromatogram is allowed to dry and is then sprayed with a solution of ***ninhydrin***. Ninhydrin reacts with amino acids to give coloured compounds, mainly brown or purple.

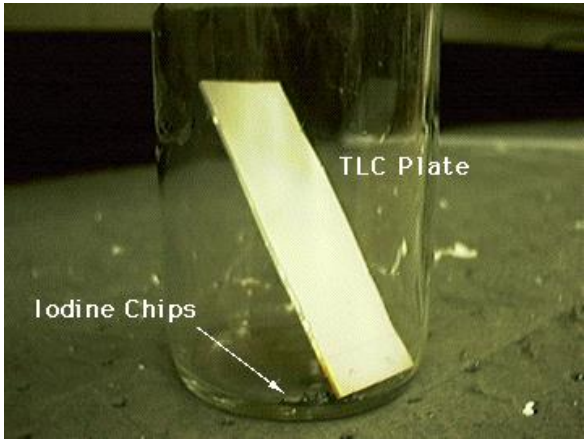


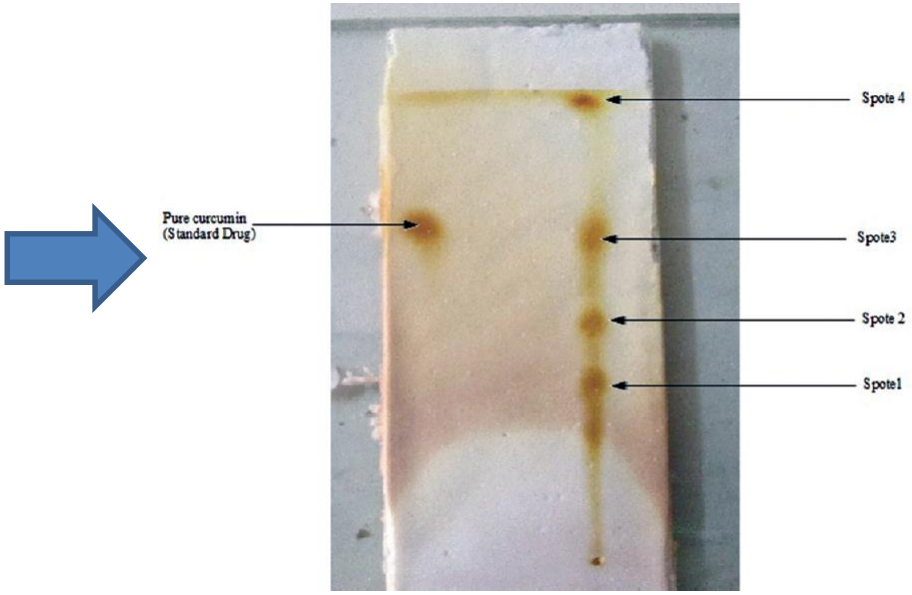
2) Showing the spots up chemically

(2) Iodine crystals

The chromatogram is again allowed to dry and then placed in an enclosed container (such as another beaker covered with a watch glass) along with a few ***iodine crystals***.

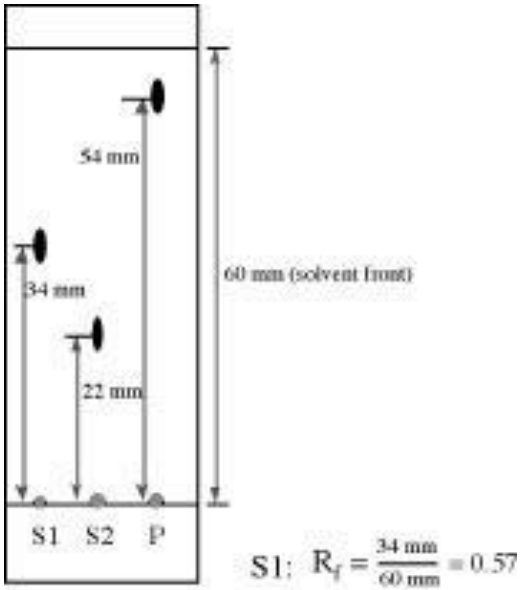
The iodine vapour in the container may either react with the spots on the chromatogram, or simply stick more to the spots than to the rest of the plate. Either way, the substances you are interested in may show up as brownish spots.





❖ 표준물질과 **Rf**치 비교 : 미지물질을 확인

Rf stands for "ratio of fronts" and is characteristic for any given compound on the same stationary phase using the same mobile phase for development of the plates. Hence, known Rf values can be compared to those of unknown substances to aid in their identifications.

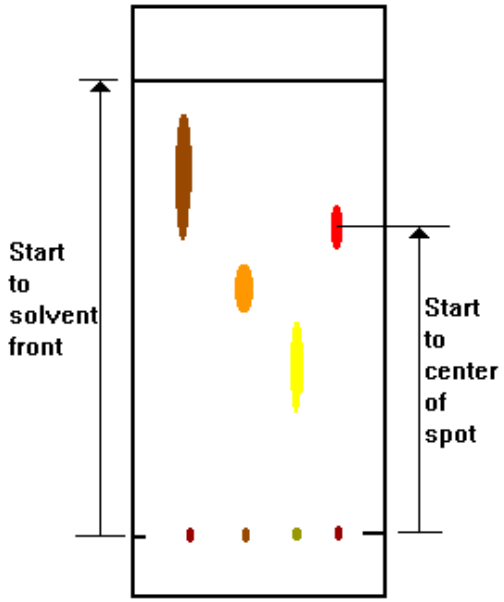


calculate R_f's

S1: $R_f = \frac{34\text{ mm}}{60\text{ mm}} = 0.57$

S2: $R_f = \frac{22\text{ mm}}{60\text{ mm}} = 0.37$

P: $R_f = \frac{54\text{ mm}}{60\text{ mm}} = 0.90$



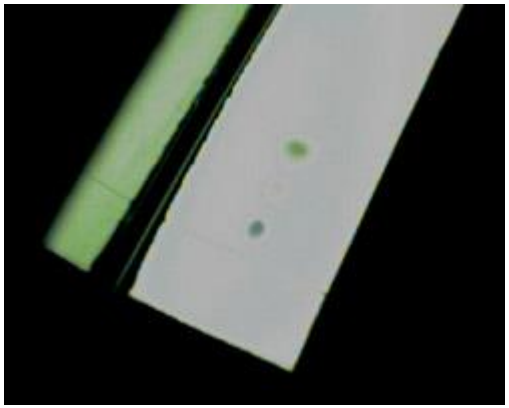
ADVANTAGES OF TLC

- simple mtd. & cost of the equipment is low
- rapid technique & not time consuming like C.C
- separation of µg of the substances can be achieved
- any type of compound can be analyzed
- corrosive spray reagents can be used without damaging the plate & needs less solvent

APPLICATIONS OF TLC

- Purity of sample
- Examination of reaction
- Identification of compounds
- Biochemical analysis
- In pharmaceutical industry
- Separation of multicomponent pharmaceutical formulations
- In food and cosmetic industry

Identification of sample on TLC plate



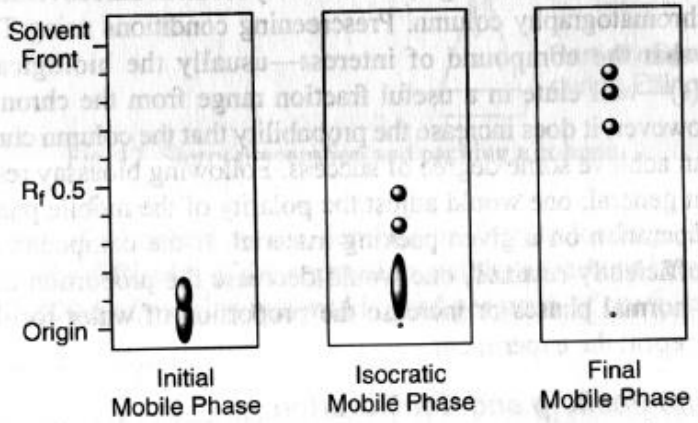


Fig. 10. Thin-layer chromatography plates sprayed with a general purpose detection reagent.

repeatition three times

- The two most common classes of TLC are:
  - Normal phase
  - Reversed phase

The End.