SEPARATION TECHNIQUES

Chromatography

How mixtures separate by differential affinity, retention factors, and the main chromatography types, with a 10-question practice set and full solutions.

SUBJECT	LEVEL	INCLUDES
Chemistry	High school and early college	Practice set, answer key, revision sheet

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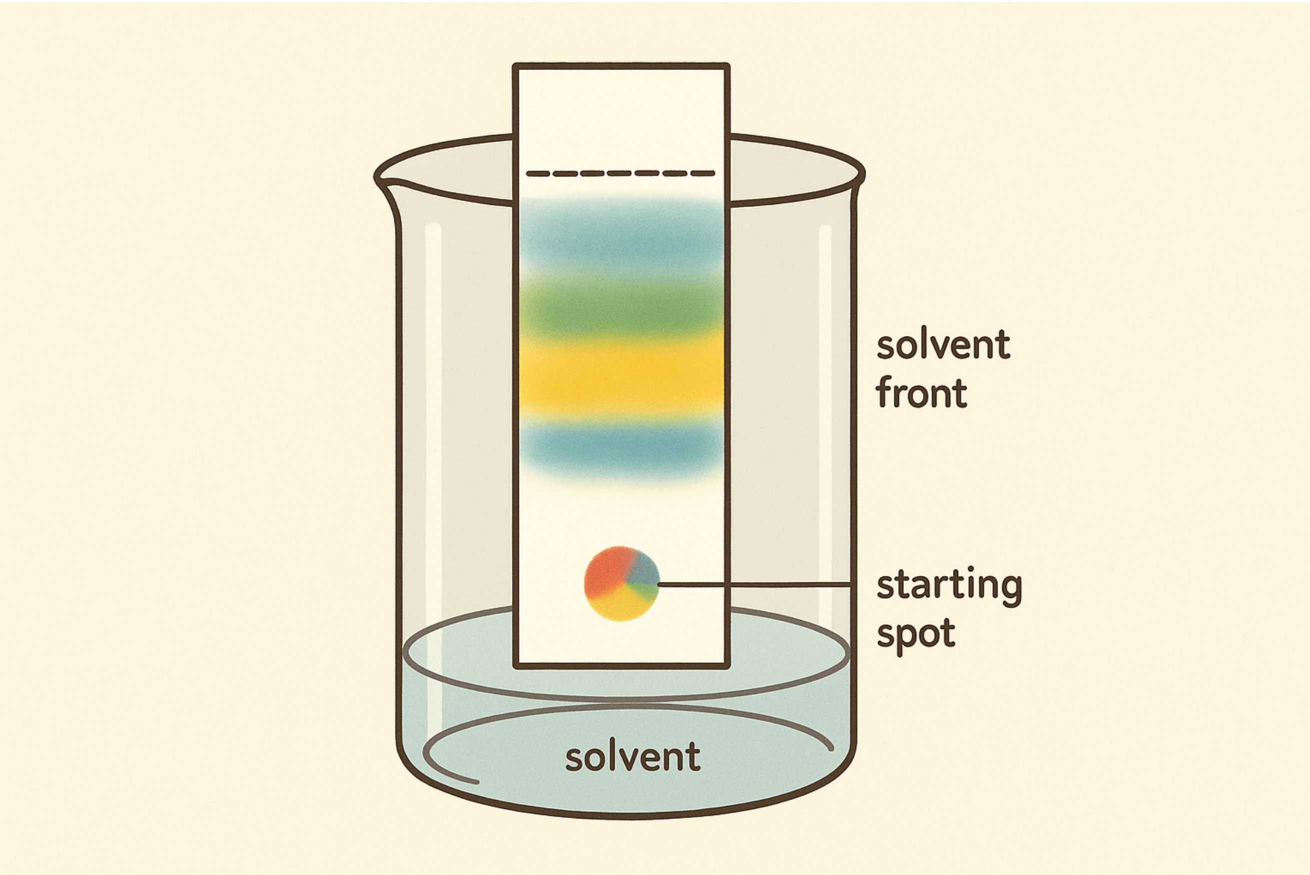
INSIDE

Chromatography is a family of laboratory techniques used to separate the components of a mixture by exploiting the different ways those components interact with two phases: a stationary phase that stays put and a mobile phase that moves through it. Components that bind tightly to the stationary phase move slowly; components that prefer the mobile phase move quickly. The result is a spatial separation that lets chemists identify, quantify, or purify each component.



Paper chromatography, different components in a mixture travel at different speeds, separating into bands.

How Chromatography Works

Every chromatographic technique has two phases:

- **Stationary phase.** A solid or a liquid coated onto a solid support that does not move, paper, silica gel, a packed column, a thin layer.
- **Mobile phase.** A liquid or gas that carries the sample through the stationary phase.

As the mobile phase flows past the stationary phase, each component of the sample partitions between the two phases according to its chemical affinity. Components that interact strongly with the stationary phase are held back; components that prefer the mobile phase travel faster. Given enough distance, the original mixture spreads out into distinct bands or peaks.

Major Types

TECHNIQUE	STATIONARY PHASE	MOBILE PHASE	USED FOR
Paper chromatography	Cellulose (filter paper)	Solvent (water, ethanol)	Pigments, dyes, simple mixtures
Thin-layer chromatography (TLC)	Silica or alumina on glass/plastic	Organic solvent	Quick reaction monitoring, ID
Column chromatography	Silica gel in a vertical tube	Solvent driven by gravity	Purification of organic compounds
Gas chromatography (GC)	Liquid coating in capillary column	Inert gas (helium, nitrogen)	Volatile compounds, forensics
HPLC	Microparticles in pressurized column	Liquid solvent at high pressure	Pharmaceuticals, biomolecules
Ion-exchange chromatography	Charged resin beads	Aqueous buffer	Proteins, amino acids, ions

The Retention Factor

For TLC and paper chromatography, each component is characterized by its retention factor (Rf):

$$R_f = \frac{\text{distance traveled by component}}{\text{distance traveled by solvent front}}$$

R_f is always between 0 and 1. It depends on the compound, the stationary phase, and the mobile phase, so under standardized conditions R_f acts like a fingerprint for identification.

Worked Example: Separating Ink

Place a small dot of black water-soluble ink near the bottom of a filter paper strip. Dip the bottom edge in water (not submerging the ink). As the water travels up the paper by capillary action, it carries the ink components along. Within minutes you see distinct bands, typically yellow, magenta, cyan, and sometimes black, revealing that the 'black' ink is actually a mixture of dyes. Measuring each band's distance gives the R_f for each dye.

Applications

- **Pharmaceutical analysis.** HPLC verifies drug purity and quantifies active ingredients in tablets.
- **Forensic chemistry.** GC-MS identifies trace drugs, explosives, accelerants in fire debris, and ink composition in document analysis.
- **Food and beverage.** Detecting pesticide residues, verifying flavor compounds, checking for adulteration.
- **Environmental monitoring.** Identifying pollutants in water and air samples at parts-per-billion levels.
- **Biochemistry.** Purifying proteins, separating amino acids, sequencing DNA fragments by electrophoretic chromatography.
- **Doping control.** Olympic and professional sports use GC-MS and LC-MS to detect banned substances.

Related study notes: Titration, Distillation, Solubility, Molarity.

Practice Questions

Work each question before reading its solution. The set runs from direct recall and substitution to the applied questions that exams actually use to separate grades.

Q1. What physical principle lets chromatography separate a mixture into its components?

SOLUTION

Components partition differently between a stationary phase and a mobile phase: a molecule that clings to the stationary phase lags behind, one that prefers the mobile phase races ahead. Different affinities mean different travel speeds, and speed differences become spatial separation.

Q2. In paper chromatography, a dye travels 6.4 cm while the solvent front travels 8.0 cm. Compute the Rf value.

SOLUTION

$R_f = \dfrac{\text{distance moved by substance}}{\text{distance moved by solvent}} = \dfrac{6.4}{8.0} = 0.80$. Rf always sits between 0 (never left the origin) and 1 (moved with the solvent), a fingerprint for identifying a substance under fixed conditions.

Q3. Two dyes have Rf 0.3 and 0.75 on the same paper. Which is more soluble in the mobile solvent, and which is more strongly adsorbed to the paper?

SOLUTION

The Rf 0.75 dye travels closer to the solvent front: more soluble in the mobile phase, weaker attraction to the stationary paper. The Rf 0.3 dye clings to the paper and lags. Rf ranks affinity directly: higher means mobile-phase-loving.

Q4. Why must the solvent level in TLC or paper chromatography start below the sample spot?

SOLUTION

If the spot sits underwater, the sample dissolves straight into the bulk solvent instead of migrating by capillary action through the stationary phase, and the whole separation is ruined. The rule ”spot above the solvent line” protects the entire experiment.

Q5. Distinguish adsorption chromatography from partition chromatography, with 1 stationary/mobile phase pair for each.

SOLUTION

Adsorption: separation by surface binding strength to a solid stationary phase, as in silica-gel TLC with an organic solvent. Partition: separation by differential solubility between 2 liquid phases, as in paper chromatography, where the stationary phase is water held in the paper's cellulose fibers and the mobile phase is the running solvent.

Q6. In gas chromatography, why does a compound with a lower boiling point generally elute first?

SOLUTION

Lower-boiling compounds are more volatile: they spend more time as vapor in the mobile carrier gas and less time condensed in the stationary liquid film, so they move through the column faster. Elution order in GC largely tracks the volatility ladder, modified by how strongly each compound interacts chemically with the stationary phase.

Q7. HPLC forces solvent through a column under high pressure instead of relying on gravity or capillary action. What does this buy?

SOLUTION

A tightly packed column with far smaller particles resolves closely related compounds that gravity-fed methods blur together, and the process finishes in minutes instead of hours. The tradeoff is more expensive instrumentation. HPLC is the workhorse of pharmaceutical purity testing for exactly this resolving power.

Q8. A crime lab needs to prove an ink sample matches a suspect pen. How does chromatography build that evidence?

SOLUTION

Run the questioned ink and a known reference from the pen side by side on the same TLC plate under identical conditions. Matching Rf values and matching color-band patterns support a common origin; any mismatched spot rules it out. The method is comparative, not absolute: it excludes non-matches with confidence and supports (never proves outright) a match.

Q9. Column chromatography can purify a natural pigment like chlorophyll. What does the column separate it from, and how is progress tracked visually?

SOLUTION

It separates chlorophyll a, chlorophyll b, and the carotenoid pigments (carotenes, xanthophylls) that co-exist in plant extract, each traveling at its own rate due to differing polarity. As solvent flows through, distinctly colored bands, green, yellow-green, orange, become visible and are collected separately as they exit: chromatography's original 1900s demonstration, which is also where the technique's name (color-writing) comes from.

Q10. Why does changing the mobile-phase solvent change every Rf value in the experiment, and what must you record alongside a reported Rf?

SOLUTION

Rf reflects a specific balance of solubility in that particular solvent versus adsorption to that particular stationary phase; a different solvent shifts every compound's balance, often unevenly. A useful Rf report always specifies the solvent system and stationary phase used, since the number alone is meaningless without them.

Answer Key

QUICK ANSWERS

Q1 differential affinity for 2 phases **Q2** 0.80 **Q3** 0.75 dye is more soluble; 0.3 dye adsorbs more strongly **Q4** the spot would dissolve into the reservoir instead of migrating **Q5** surface binding (silica) vs solubility partition (paper)

Q6 higher volatility means faster passage through the column **Q7** much finer resolution and speed, at higher equipment cost **Q8** compare Rf and band patterns of questioned vs known ink **Q9** chlorophylls from carotenoids; tracked by colored bands **Q10** Rf is solvent-specific; always report the solvent system

Revision Sheet

The principle

Mixture components partition differently between a stationary and a mobile phase, so they travel at different speeds.

GC logic

More volatile compounds spend more time in the gas phase and elute sooner.

Rf value

$R_f = \frac{\text{distance by substance}}{\text{distance by solvent}}$, between 0 and 1; solvent-and-phase specific.

HPLC

High pressure through fine packing: sharper resolution, faster runs, costlier equipment.

2 mechanisms

Adsorption (surface binding, e.g. silica TLC) vs partition (solubility split, e.g. paper).

Applications

Ink/forensic comparison, pigment separation, drug purity testing, amino acid identification.

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