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Molecular travel: Fascinating World of Paper Chromatography

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A B S T R A C T :

Paper chromatography is a simple yet useful analytical technique for studying the migration and relative mobility of organic biomolecules. In this study, the migration behavior and retention factor (R_f) values of individual amino acids—methionine, glycine, and glutamic acid—were examined and compared with those obtained from their binary mixtures. The results demonstrate that paper chromatography can provide a quick and effective means of separating and identifying individual components in a mixed sample. The observed differences in R_f values between individual amino acids and their mixtures further suggest that interactions between components within a mixture may influence their migration during chromatographic separation.

Keywords: Whatman No. 1, stationary phase, mobile phase, ninhydrin, retention factor (R_f), partition coefficient (K), amino acids

Introduction

Chromatography is a powerful and versatile separation technique that plays an indispensable role in modern analytical chemistry. Originally developed in the early 20th century by Mikhail Tswett for separating plant pigments by colour, but its name itself is derived from the Greek words “chroma” (colour) and “graphein” (to write), referencing the early separations of plant pigments — resulting in vivid coloured bands.

Although its origins were tied to visible colour separation, chromatography today refers broadly to a family of techniques that separate components in a mixture based on their differential distribution or differing affinities between a stationary phase and a mobile phase.

At its core, chromatography is a laboratory technique used for separating, identifying, and purifying the components of a complex mixture. By exploiting differences in how individual substances interact with the two phases, it enables scientists to isolate even trace amounts of compounds from highly complex mixed samples. This principle underpins a wide range of chromatographic methods, from simple educational demonstrations to sophisticated instrumental systems used in research, forensics, pharmaceuticals, and environmental analysis.

Historical Background of Chromatography

The journey of paper chromatography is deeply intertwined with the broader evolution of chromatographic techniques. Although paper chromatography itself emerged as a simple and practical tool in the mid-20th century, its conceptual and scientific foundations were laid decades earlier by pioneering scientists who transformed a botanical curiosity into one of the most powerful analytical methods in science.

The Birth of Chromatography: Mikhail Semyonovich Tswett (1901–1906)

The story begins with Mikhail Semyonovich Tswett (also spelled Tsvet, Tswett, Zwet, or Cvet), a Russian-Italian botanist born on 14 May 1872 in Asti, Italy. While working at the University of Warsaw, Tswett sought a reliable method to separate the complex mixture of pigments in green leaves, particularly chlorophylls, carotenoids, and xanthophylls. [1]

Between 1901 and 1906, Tswett developed the first true chromatographic technique, which we now recognize as adsorption column chromatography. He packed a glass column with finely divided calcium carbonate (the stationary phase) and poured a petroleum ether extract of crushed leaves through it. As different solvents (mobile phases) such as petroleum ether and alcohol mixtures were added, the pigments separated into distinct, vividly coloured horizontal bands along the column. Lighter yellow xanthophylls moved faster, followed by green chlorophylls and orange-red carotenoids at the bottom.



Fig.1:Mikhail Semyonovich Tswett (14 May 1872 – 26 June 1919)

Fascinated by this visual “colour writing,” Tswett coined the named of this technique “chromatography”. In 1906, he presented his findings to the Warsaw Society of Natural Sciences and later published them in German botanical journals. Despite the elegance and reproducibility of his method, Tswett’s work was largely ignored or dismissed by the chemical community of the time. Many chemists considered it a mere physical phenomenon rather than a genuine analytical tool. Tswett died in 1919 in relative obscurity, never

witnessing the widespread acceptance of his invention.

Rediscovery and Expansion: 1930s – Richard Kuhn and Edgar Lederer

It was not until the 1930s that chromatography began to regain attention. German biochemist Richard Kuhn and his collaborator Edgar Lederer successfully applied Tswett's column adsorption technique to separate and identify fat-soluble vitamins and carotenoids.





Fig.2: a) Richard Johann Kuhn (3 December 1900 – August 1, 1967) b) Edgar Lederer (June 5, 1908- October 19, 1988)

Their work demonstrated that chromatography was not limited to plant pigments but could be used for biologically important molecules.
Kuhn's contributions to carotenoid and vitamin research using chromatographic separation earned him the Nobel Prize in Chemistry in 1938.
This period marked the first significant revival of Tswett's forgotten method and expanded its application beyond botany into biochemistry.

The Revolution: Partition Chromatography by Martin and Synge (1941)

The true transformation of chromatography from an empirical separation tool into a rigorous, quantitative analytical science occurred in 1941 when British biochemists Archer John Porter Martin and Richard Laurence Millington Synge published their groundbreaking paper on partition



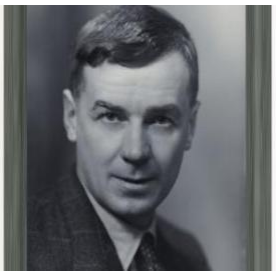


Fig. 3: a) Archer John Porter Martin (1 March 1910 – 28 July 2002) b) Richard Laurence Millington Synge (28 October 1914 – 18

chromatography. [2]

While working at the Wool Industries Research Association in Leeds, Martin and Synge were trying to separate acetylated amino acids from protein hydrolysates, a task that existing methods could not accomplish effectively. They introduced a revolutionary concept: using two immiscible liquid phases instead of a solid adsorbent. In their setup, water (held by silica gel) served as the stationary phase, and an organic solvent like chloroform or butanol acted as the mobile phase. The separation now depended on the partition (distribution) coefficient of each amino acid between the two liquid phases, rather than simple adsorption.

Their apparatus was initially a glass column, but they soon developed a paper-based version to simplify the process and reduce solvent use. They spotted the amino acid mixture at one end of a strip of filter paper, suspended it in a closed chamber saturated with water vapour, and allowed the organic solvent to travel along the paper by capillary action. This was the birth of paper partition chromatography; the direct ancestor of the modern paper chromatography used in classrooms and laboratories worldwide.

The beauty of Martin and Synge's paper method lay in its simplicity, low cost, and remarkable resolving power. They introduced the concept of R_f value (retention factor), defined as the distance travelled by the compound divided by the distance travelled by the solvent front, a parameter that remains central to paper and thin-layer chromatography today.

For their invention of partition chromatography, Archer J.P. Martin and Richard L.M. Synge were jointly awarded the Nobel Prize in Chemistry in 1952. Their 1941 paper is widely regarded as one of the most influential in 20th-century analytical chemistry.

From Paper to Gas: Martin and James (1952)

Even before receiving the Nobel Prize, Martin continued pushing chromatographic boundaries. In 1952, collaborating with Anthony Trafford James, introduced gas-liquid chromatography (GLC), replacing the liquid mobile phase with an inert gas (usually nitrogen or helium) and using a liquid-coated solid as the stationary phase.

Fig. 4:Anthony Trafford James
(6 March 1922 — 7 December 2006)



This marked the beginning of instrumental chromatography but the theoretical foundation remained the same partition principle developed for paper chromatography. Gas chromatography rapidly became indispensable for analyzing volatile organic compounds and further elevated the status of chromatography in analytical science.

The Rise of Paper Chromatography as an Accessible Technique (1940s–1950s)

Following Martin and Synge's 1944 detailed publication on paper chromatography, the technique spread rapidly across laboratories. Its advantages were immediately clear:

- Required only filter paper, a solvent, a glass jar or tank, and simple detection reagents.
- Could separate and identify amino acids, sugars, nucleotides, antibiotics, and dyes from mixed sample.
- Ideal for qualitative and semi-quantitative analysis.
- Served as an excellent teaching tool for demonstrating chromatographic principles.

By the late 1940s, paper chromatography had become a standard method in biochemistry, pharmacology, and clinical chemistry. Consden, Gordon, and Martin (1944) used two-dimensional paper chromatography to create the first "fingerprint" maps of amino acids in proteins, a precursor to modern peptide mapping. [3]

In 1948, the British pharmacologist R.B. Fisher introduced the use of circular (radial) paper chromatography, further simplifying the procedure. Meanwhile, ascending, descending, and horizontal paper chromatography setups were adopted worldwide for educational purpose, especially for separating plant pigments, food dyes, and ink components.

The Golden Age of Chromatographic Diversification (1950s–1970s)

The success of paper and partition chromatography inspired an explosion of new techniques:

- 1950s – Development of thin-layer chromatography (TLC) on glass plates coated with silica or alumina, offering faster separations and better resolution than paper.
- 1960s – Csaba Horváth and others developed high-performance liquid chromatography (HPLC), using high pressure and fine particles to dramatically improve speed and efficiency for separation.



Fig. 5:Csaba Horváth

(25 January 1930 – 13 April 2004)

- 1970s–1980s – Emergence of ion-exchange, affinity, and size-exclusion chromatography, each tailored to specific classes of molecules.

Despite these advances, paper chromatography retained its niche due to its unmatched simplicity and low cost, especially in education, field studies, and developing countries.

Paper Chromatography in the Modern Era

Today, while sophisticated instrumental methods like LC-MS/MS and GC-MS dominate research and industry, paper chromatography remains widely used for:

- Teaching fundamental separation principles in schools and universities.
- Rapid screening of drugs of abuse and pharmaceuticals in resource-limited settings.
- Qualitative analysis of natural products, food colours, and plant extracts.
- Forensic examination of inks and dyes.

Recent innovations include paper-based microfluidic devices ("lab-on-paper") that integrate paper chromatography with colorimetric or electrochemical detection for point-of-care diagnostics, environmental monitoring, and food safety testing in remote areas.

Legacy

From Tswett's coloured bands in a calcium carbonate column in 1903 to Martin and Synge's water-soaked filter paper in 1941, the development of paper chromatography reflects a remarkable journey: a technique born from botanical curiosity, ignored for decades, then reborn through biochemical necessity, and finally democratized into one of the most accessible and enduring analytical methods in science. Its theoretical foundation underpins virtually all modern chromatographic techniques, yet its simplest form, a piece of paper and a drop of solvent, continues to inspire and educate new generations of scientists.

Exploring paper chromatography

1.1. Basis of paper chromatography

Among the various types of chromatography, paper chromatography stands out as one of the simplest, most cost-effective, and widely accessible techniques. It belongs to the category of planar chromatography, where separation occurs on a flat surface. In paper chromatography, a specialized porous filter paper typically Whatman paper, serves as the stationary phase. The paper holds a thin film of water adsorbed on the cellulose fibres, which acts as the actual stationary phase. A suitable solvent or mixture of solvents, known as the mobile phase, travels up (or sometimes across) the paper by capillary action, carrying the components of the sample with it.

Paper chromatography is particularly valued for qualitative analysis of soluble substances such as plant pigments, amino acids, sugars, dyes, inks, food colourings, plant extracts, and certain drugs. Its major advantages include minimal equipment requirements—just paper, a solvent, and a container—making it ideal for educational settings, preliminary investigations, and resource-limited laboratories

In summary, paper chromatography remains a foundational technique in chemical analysis. Despite the advent of advanced methods, it continues to offer valuable insights into mixture composition and serves as an accessible entry point to understanding more complex chromatographic procedures.

Paper chromatography falls under:

Category	**Type**
Based on Physical Setup	Planar chromatography
Based on Stationary Phase	Liquid–solid chromatography
Based on Separation Mechanism	Partition chromatography

1.2. Principle of paper chromatography

Paper chromatography is a simple yet highly effective analytical technique used to separate and identify components of a mixture. Its foundation lies in the universal chromatographic principles of flow, affinity, differential solubility, and selective partitioning. First introduced by Consden, Gordon, and Martin in 1941, this technique demonstrated that filter paper—made of hydrophilic cellulose—can serve as an excellent support medium for separating amino acids, sugars, peptides, and other small biomolecules.

At the core of chromatography lies the concept of differential migration of substances between two distinct phases. In paper chromatography, these are the stationary phase and the mobile phase. The stationary phase consists of water molecules tightly adsorbed or trapped within the cellulose matrix of a special filter paper (usually Whatman No. 1 or No. 3). Cellulose is highly hydrophilic and holds a thin film of water in its pores and on its surface even when the paper appears dry. This bound water acts as the true stationary phase. The mobile phase is a liquid solvent or a mixture of solvents (e.g., n-butanol: acetic acid: water in the ratio 12:3:5, or other combinations like ethanol–water, phenol–water, etc.) that moves through the paper by capillary action. Separation depends on how each component distributes itself between these two phases. [4]

Two essential phases operate in paper chromatography:

- *Stationary Phase:*
    - Water trapped within the pores of cellulose.
    - Hydrophilic in nature, forming a thin, immobilized layer.
    - Provides a medium where polar and hydrogen-bonding interactions occur.
- *Mobile Phase:*
    - Organic solvent or mixture (e.g., butanol–acetic acid–water).
    - Moves through the paper by capillary action.
    - Dissolves and transports sample components upward or across the paper.

When a small drop of the sample mixture (containing substances such as amino acids, sugars, peptides, plant pigments, or inks) is spotted near one end of the paper strip or sheet and allowed to dry, the paper is then placed in a closed chamber containing the mobile phase. Care is taken to ensure that the spot remains above the solvent level. As the solvent rises through the paper by capillary action, it carries the dissolved components of the mixture along with it. Each component continuously partitions itself between the moving organic solvent (mobile phase) and the stationary water layer held by the cellulose fibres.

Each substance distributes itself between the stationary water phase and the flowing solvent based on its:

- Polarity
- Hydrogen-bonding ability
- Solubility in the mobile phase

- Affinity for the stationary phase
- Molecular size and structure

These properties determine how fast or slow each component migrates.

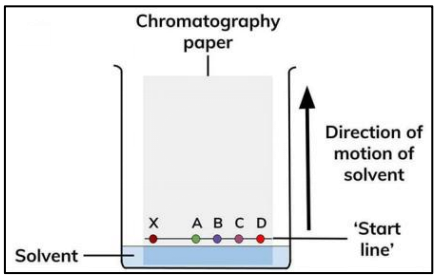


Fig. 6:Paper Chromatography

Basic principle of paper chromatography is as follows:

- Components more soluble in the mobile phase travel quickly.
- Components with greater affinity for the stationary phase travel slowly.
- Constant partitioning occurs as the solvent moves.
- Over time, separation appears as distinct spots or bands across the paper.

This process reflects the principle of partition chromatography, where substances distribute themselves between two phases following separation. The stationary phase attracts more polar compounds, while the mobile organic solvent transports less polar or more soluble compounds farther.
Paper chromatography uses cellulose fibres as the medium, which makes the technique highly effective because:

- Cellulose binds water strongly, forming a stable stationary phase.
- Its fine pore structure allows uniform capillary movement.
- Its hydrophilic property provides strong retention forces for polar solutes.

After the solvent front has travelled a sufficient distance (usually 75–90 % of the paper length), the paper (now called a chromatogram) is removed, the solvent front is immediately marked, and the paper is dried. The separated components may be visible directly if they are coloured (e.g., plant pigments, dyes), or they can be visualized by using suitable locating agents such as:

- Ninhydrin spray for amino acids (purple spots)
- Aniline-diphenylamine reagent for sugars
- Iodine vapour for many organic compounds
- UV light (254 or 365 nm) for fluorescent or UV-absorbing compounds
- Sulphuric acid charring for general organic compounds

The position of each spot is characterized by its Retention Factor (R_f) value, which is calculated as:

$$R_F = \frac{Distance\ travelled\ by\ the\ solute\ from\ the\ origin\ (mid-point\ of\ each\ bands)}{Distance\ travelled\ by\ the\ solvent\ front\ from\ the\ origin}$$

$R_F = R_f$ is usually between 0 and 1 and are characteristic of a particular compound under fixed conditions of temperature, solvent system, paper type, and saturation of the chamber. Identical compounds show the same R_f value under the same experimental conditions, making R_f values useful for

identification and comparison with standards run simultaneously. [5-8]
Paper chromatography is therefore a classic example of liquid–liquid partition chromatography, where separation is governed by the partition coefficient (K) of each solute:
K = (Concentration of solute in stationary phase) / (Concentration of solute in mobile phase)
Substances with higher K values are retained more by the stationary phase and migrate slowly, while those with lower K values move rapidly with the mobile phase.

In summary, the core principle of paper chromatography is differential partitioning of mixture components between a stationary aqueous phase adsorbed on cellulose fibers and a moving organic solvent phase. The continuous distribution and redistribution of each component between these two phases as the solvent ascends results in separation according to individual partition coefficients, solubility, polarity, and molecular interactions. This simple yet powerful mechanism has made paper chromatography an indispensable tool in biochemical, pharmaceutical, forensic, and educational laboratories for qualitative analysis, purity checking, and preliminary identification of complex mixtures.

Materials and methods for paper chromatography

1.3. Materials

3.1.1 Chemicals Used

Chemical	**Purpose**
Sample solution (e.g., dye, amino acid)	Substance to be separated and analyzed
Solvent system (e.g., Butanol: Acetic Acid: Water)	Mobile phase that carries sample along the paper
Ninhydrin solution	Visualizing colourless compounds like amino acids
Distilled water	Used in solvent preparation or for cleaning
Ethanol / Acetone	Solvent for dissolving reagents like ninhydrin

3.1.2 Glassware Used in Paper Chromatography

Glassware	**Purpose**
Beaker	Development chamber where solvent moves through the paper
Measuring cylinder	For accurate measurement of solvents and reagents
Micropipette	Applying precise sample spots at the baseline
Centrifuge Tubes	For preparation of samples

3.1.3 Instruments &Accessories

Item	**Purpose**
Chromatography paper (e.g., Whatman No. 1)	Stationary phase where separation takes place
Pencil & ruler	For marking the baseline and solvent front
Forceps	To handle the paper without contamination
Incubator	For quick drying of the paper after spraying or development
Spray bottle	To apply ninhydrin evenly

1.4. Preparation of Chemicals

3.2.1 Amino Acid Solutions (Sample Preparation)

1. In a Beaker, 90µL of HCl was added to in 9.91 mL of Distilled Water to prepare 35% HCl.
2. Then, in a separate beaker, 1 mL of 10% Isopropanol was added to 9 mL of 35% HCl. Thus, total amount of this solution was 10 ml.
3. Then, 5mg of measure the amino acids (such as Glycine, Methionine, Glutamic Acid) were measured in a Centrifuge tube.
4. Then 2.5ml of above mentioned solution (Isopropanol + HCl) was added, mixed up well and kept in room temperature.

3.2.2 Solvent mixture

1. In a Beaker, n-butanol, glacial acetic acid and distilled water were added in a ratio 200:50:25 i.e. 200 mL of n-butanol, 50 mL Glacial Acetic Acid and 25 mL of Distilled Water.
2. Solution was mixed up well and stored in capped container.

3.2.3 Ninhydrin Solution (Stain Solution)

1. In a spray bottle, 200 mg of Ninhydrin Powder, 0.5 mL of Glacial Acetic Acid, 100 mL of n-butanol and 4.5 mL of Distilled Water were added.
2. Solution was mixed properly and the container was covered with aluminium foils.

1.5. Methodology

3.3.1 Sample Spotting: "Mark the Molecules"

1. Strip of chromatography paper was cut according to the length and breadth of the beaker to be used for this experiment.
2. A line was drawn 2 cm above the bottom (baseline).
3. A small drop of each sample mixture was spotted at the baseline by micropipette.
4. Small spots were allowed to dry.
5. Loading of the samples maybe repeated, if necessary, to increase the concentration of the sample at the respective spots.

3.3.2 Chamber Setup: "Soak and Seal"

1. 10–15 mL of the prepared solvent was poured into a clean beaker, to be used as chamber for this experiment.
2. Solvent containing beaker was covered with another bigger size beaker and was allowed to saturate for a few minutes.

3.3.3 Development: "Let It Rise"

1. Paper containing the sample spot was placed in a beaker, ensuring that the spot remained above the solvent level.
2. Chamber was tightly covered with the larger beaker.
3. Solvent was allowed to travel up the paper until it had covered approximately three-fourths of the paper.

3.3.4 Drying & Visualization: "Revealing the Invisible"

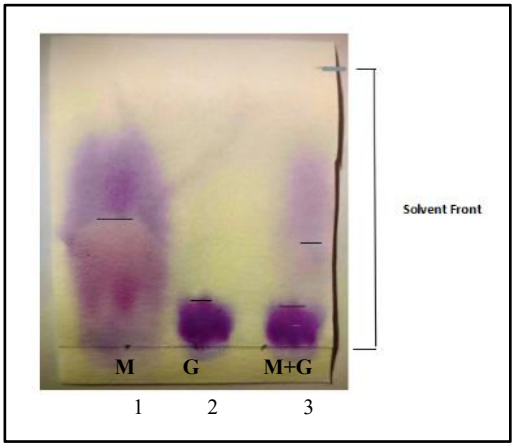
1. When the solvent covered three-fourths of the paper (approx..) the strip of paper was removed and solvent front was marked immediately.
2. As the samples were colourless, Ninhydrin Solution was sprayed all over the paper.
3. Then the paper was dried incubating at 45ºC.
4. The process revealed the distribution of amino acids as purple patches.[9-11]

3.3.5 Analysis: "R_f – The Identity Tag"

1. Distances travelled by the compound and the solvent front was measured from the baseline.[12]

2. Finally, Retention Factor was calculated.

Result and Discussion



1.6. Experiment-1

Experiment:

Paper Chromatography was conducted using 3 different samples of Amino Acid – Methionine, Glycine and a Mixed Sample of Methionine and Glycine. The first spot is of Methionine; second spot of Glycine and the third spot is of the Mixed Sample.

Observations:

These samples are colourless and hence needed a spray of Ninhydrin Solution to visualize the purple bands of Methionine, Glycine and mixed sample of amino acids. After ninhydrin spray, purple bands of respective amino acids were visualized. Accordingly, distance covered by different amino acids were measured for analysis.

Analysis:

According to the paper chromatogram retention factor of Methionine and Glycine (both individual and mixed sample) were calculated.

The Distance travelled by the solvent front from the Baseline – 6.4cm

The Distance travelled by the different Samples are –

Fig. 7: Paper Chromatogram showing separated amino acid spots after staining and drying; M= Methionine, G=Glycine, M+G= mixed sample of Methionine and Glycine

- Methionine – 3.7cm
- Glycine – 0.6cm
- In the Mixed Sample, the distance travelled by each component were –
    - Methionine – 3.1cm
    - Glycine – 0.6cm

The formula of Retention Factor (R_f) is -

$$R_F = \frac{Distance\ travelled\ by\ the\ solute\ from\ the\ origin\ (mid-point\ of\ each\ bands)}{Distance\ travelled\ by\ the\ solvent\ front\ from\ the\ origin}$$

Therefore,

- The R_f of Methionine = $3.7\ cm/6.4\ cm$ = 0.57
- The R_f of Glycine = $0.6\ cm/6.4\ cm$ = 0.09
- The R_f individually of each component of the Mixed Sample

- Methionine = $^{3.1\ cm}/_{6.4\ cm}$ = 0.48
- Glycine = $^{0.6\ cm}/_{6.4\ cm}$ = 0.09

AMINO ACID	DISTANCE TRAVELLED BY AMINO ACIDS FROM ORIGIN	DISTANCE TRAVELLED BY SOLVENT FROM ORIGIN	R_f value
GLYCINE	0.6 *cm*	6.4 *cm*	0.09
METHIONINE	3.7 *cm*	6.4 *cm*	0.57
GLYCINE IN MIXTURE	0.6 *cm*	6.4 *cm*	0.09
METHIONINE IN MIXTURE	3.1 *cm*	6.4 *cm*	0.48

Comment:

Based on the R_f values obtained for the different samples, the R_f values of Methionine and Glycine were 0.57 and 0.09, respectively, in the individual samples, whereas their corresponding R_f values in the mixed sample were 0.48 and 0.09, respectively.

A comparison of the R_f values showed that the R_f of Methionine decreased from 0.57 in the individual sample to 0.48 in the mixed sample, while the R_f of Glycine remained unchanged at 0.09. This observation suggests that the presence of Glycine in the mixed sample may have slightly influenced the migration of Methionine, resulting in a lower R_f value compared with that obtained from the individual sample.

1.7. Experiment-2

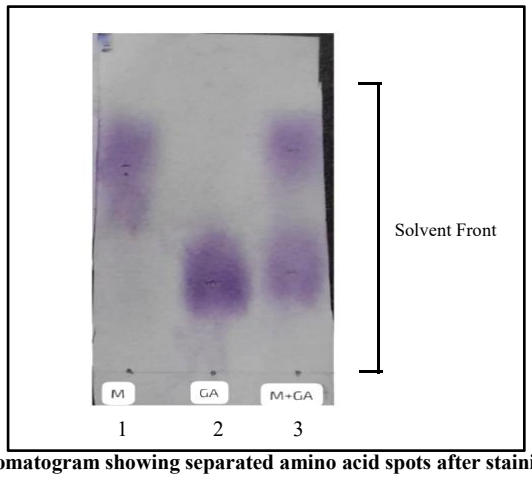


Fig. 8: Paper Chromatogram showing separated amino acid spots after staining and drying; M= Methionine, GA=Glutamic Acid, M+GA= mixed sample of Methionine and Glutamic Acid.

Experiment:

Paper Chromatography was conducted using 3 different samples of Amino Acid – Methionine, Glutamic Acid and a mixed sample of Methionine and Glutamic Acid. The first spot is of Methionine; second spot of Glutamic acid and the third spot is of the mixed sample.

Observations:

These samples are colourless and hence a spray of Ninhydrin Solution was needed to visualize the purple bands of Methionine, Glutamic Acid and a mixed sample of amino acids. After ninhydrin spray, purple bands of respective amino acids were visualized. Accordingly, distance covered by different amino acids were measured for analysis.

Analysis:

According to the paper chromatogram retention factor of Methionine and Glutamic Acid (both individual and mixed sample) were calculated.
The Distance travelled by the solvent front from the Baseline – 6.6cm
The Distance travelled by the different samples are –

- Methionine – 4.2cm
- Glutamic Acid – 1.8cm
- In the Mixed Sample, the distance travelled by each component are –
    - Methionine – 4.6cm

- Glutamic Acid – 2.0cm

The formula of Retention Factor (R_f) is -

$$R_F = \frac{Distance\ travelled\ by\ the\ solute\ from\ the\ origin\ (mid-point\ of\ each\ bands)}{Distance\ travelled\ by\ the\ solvent\ front\ from\ the\ origin}$$

Therefore,

- The R_f of Methionine = $4.2\ cm/7.5\ cm$ = 0.56
- The R_f of Glutamic Acid = $1.8\ cm/7.5\ cm$ = 0.24
- The R_f individually of each component of the Mixed Sample
  - Methionine = $4.6\ cm/7.5\ cm$ = 0.61
  - Glutamic Acid = $2.0\ cm/7.5\ cm$ = 0.27

AMINO ACID	DISTANCE TRAVELLED BY AMINO ACIDS FROM ORIGIN	DISTANCE TRAVELLED BY SOLVENT FROM ORIGIN	Rf value
GLUTAMIC ACID	1.8 *cm*	7.5 *cm*	0.24
METHIONINE	4.2 *cm*	7.5 *cm*	0.56
GLUTAMIC ACID IN MIXTURE	2.0 *cm*	7.5 *cm*	0.27
METHIONINE IN MIXTURE	4.6 *cm*	7.5 *cm*	0.61

Comment:

According to the data, the R_f values of Methionine and Glutamic Acid were 0.56 and 0.24, respectively, in the individual samples, whereas the corresponding R_f values in the mixed sample were 0.61 and 0.27, respectively.

Thus, the R_f value of Methionine increased from 0.56 in the individual sample to 0.61 in the mixed sample, while that of Glutamic Acid increased from 0.24 to 0.27. These observations indicate that the presence of Glutamic Acid and Methionine in the mixed sample may have slightly influenced the migration of each other, as reflected by the differences in their R_f values compared with those obtained from the individual samples.

Significance

1.8. Applications

Paper chromatography remains highly relevant due to its simplicity, low cost, and effectiveness with small sample volumes. Its major applications include: [13]

- *Biochemistry & Molecular Biology* – Separation and identification of amino acids, peptides, sugars, and nucleotides – Analysis of plant pigments (chlorophyll a & b, carotenoids, xanthophylls) – Classic tool for protein hydrolysis studies and metabolic disorder screening
- *Pharmaceutical Industry* – Drug purity testing and detection of impurities/degradation products – Monitoring reaction progress during synthesis – Quality control in resource-limited settings
- *Food Industry* – Detection of artificial colours, preservatives, sweeteners, and adulterants – Identification of natural extracts, spices, and pesticide residues
- *Forensic Science* – Ink analysis in questioned documents – Identification of drugs, poisons, explosives residues, and biological stains – non-destructive; chromatograms can be preserved as evidence
- *Environmental Monitoring*– Screening of pesticides, herbicides, and pollutants in water, soil, and air – Rapid field testing before confirmatory advanced analysis
- *Clinical Diagnostics* – Detection of aminoacidurias, sugar disorders (e.g., PKU, galactosemia) in urine/blood
- *Education & Research* – Hands-on introduction to separation science in schools and colleges – Demonstrates principles of polarity, solubility, and partition coefficient visually

Even with advanced techniques available, paper chromatography continues to be the method of choice for preliminary screening, teaching, field testing, and situations demanding quick, inexpensive, and reliable results.

1.9. Future perspectives

Despite being considered a "classical" technique, paper chromatography is experiencing a remarkable renaissance, driven by the global need for low-cost, sustainable, and field-deployable analytical tools. Its future lies not in competing with high-end instruments like LC-MS, but in complementing them through innovative, accessible formats.

Key emerging and future applications include:

- ***Super-Cheap Medical Testing at Home and Villages***

Paper chromatography will power simple "dip-and-read" strips or foldable cards to test blood, urine, or saliva for diabetes, kidney problems, infections, malaria, and even COVID-like viruses. Cost will be less than ₹10–20 per test, no electricity or lab needed — perfect for rural clinics and home use.

- ***Water and Food Safety in Everyone's Hand***

Small paper kits will let farmers, shopkeepers, and school children quickly check drinking water for poison (lead, arsenic, pesticides) or food for fake colours, melamine, and harmful chemicals using just a smartphone camera to read results.

- ***Eco-Friendly and Disposable Devices***

Future paper devices will be 100 % biodegradable and made from recycled paper or plant waste. They will replace plastic test kits and reduce laboratory waste while remaining accurate.

- ***Wearable Health Monitors***

Tiny paper patches stuck on skin (like bandages) will continuously check sweat for sugar, salt, or stress levels and change of colour will give early warnings for diseases.

- ***Space and Extreme Adventures***

Astronauts and mountaineers will carry lightweight paper strips to test air, water, and body fluids in space stations or high mountains where big machines cannot go.

In short, paper chromatography will not disappear; it will become smarter, greener, and reach every corner of the world to make science simple, fast, and low-cost testing available to all.

1.10. Advantages and disadvantages

Paper chromatography is one of the simplest and most accessible separation techniques, making it ideal for educational and preliminary analytical work. However, like every method, it has its own strengths and limitations. The following table summarizes the major advantages and disadvantages observed during the experiment:

Advantages of Paper Chromatography	**Disadvantages of Paper Chromatography**
1. Cost effective – requires only filter paper, a jar, and solvent	1. Qualitative or semi-quantitative only – not suitable for accurate quantitative analysis
2. Simple and easy to perform – needs minimal training and no sophisticated instruments	2. Low resolution – cannot separate complex mixtures with many similar compounds
3. Requires very small sample volume (2–10 µL is sufficient)	3. Slow process – development often takes 2–8 hours or more
4. Highly suitable for teaching and demonstrating basic principles of separation, polarity, and R_f values	4. Not easily automated – results depend heavily on manual technique and environmental conditions
5. Non-destructive and visual – separated spots can be cut out and further analyzed; coloured compounds are directly visible	5. Limited to relatively polar, water-soluble or solvent-soluble compounds; not effective for volatile or non-polar substances

Conclusion

Therefore, paper chromatography is far more than just coloured spots on a strip of filter paper—it is an elegant demonstration of how the invisible world of molecules can be made visible through patience, precision, and scientific curiosity.

Here in this hands-on experience, components of the mixture of amino acids (methionine, glycine, and glutamic acid) were successfully separated and their characteristic R_f values were calculated. The clear, distinct spots that appeared after ninhydrin spraying confirmed the core principle: compounds migrate differently because of their unique balance of solubility in the mobile phase and affinity for the stationary aqueous layer held by cellulose. Glycine, being small and highly polar, showed the lowest R_f, while methionine with its non-polar thioether side chain travelled farthest—beautiful proof of structure–property relationships in action. [14]

Most importantly, this simple technique opened a window into the molecular world and showed that how fundamental principles of partition, polarity, and differential migration underlie even the most sophisticated separation methods used today. It has strengthened the foundation in analytical biochemistry and practical biochemistry, sharpened laboratory skills of investigator, and deepened the appreciation for the beauty hidden in everyday scientific processes.

Thus, paper chromatography has not only equipped the investigator with a valuable analytical technique but has also instilled a lasting appreciation for the power of observation and the stories that molecules quietly tell when we learn to interpret them.

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