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Development of Novel Stationary Phases for High-Performance Liquid Chromatography (HPLC) Separation of Complex Organic Mixtures

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Abstract

This study presents the development and characterization of novel stationary phases for High-Performance Liquid Chromatography (HPLC) aimed at improving the separation efficiency of complex organic mixtures. Three stationary phases were synthesized: C18-modified silica gel, aminopropyl-modified silica gel, and cyanopropyl-modified silica gel. These phases were packed into stainless steel HPLC columns and evaluated under various chromatographic conditions. The C18-modified phase demonstrated superior retention, resolution, and efficiency across a range of analytes. The study also explored the impact of different mobile phase compositions and elution modes. Reproducibility, stability, and long-term performance were assessed, confirming the robustness of the developed stationary phases. The results indicate that the novel stationary phases provide enhanced chromatographic performance, making them suitable for the separation of complex organic mixtures in various analytical applications.

Keywords: HPLC, stationary phases, C18-modified silica gel, chromatographic separation, organic mixtures, column efficiency, mobile phase optimization.

Introduction

High-Performance Liquid Chromatography (HPLC) is a cornerstone of analytical chemistry, widely utilized for the separation, identification, and quantification of complex mixtures. The efficiency and effectiveness of HPLC are largely determined by the choice of stationary phase, which interacts with the analytes to produce differential retention times. Traditional stationary phases, such as C18-bonded silica, have been the standard in reversed-phase HPLC for decades, providing reliable performance for a wide range of applications. However, the separation of complex organic mixtures, such as polycyclic aromatic hydrocarbons (PAHs), fatty acid methyl esters (FAMEs), and alkylbenzenes, often requires improved selectivity, efficiency, and stability, which are not always achievable with conventional stationary phases.

The development of novel stationary phases with tailored surface chemistries offers a promising approach to enhance the performance of HPLC. Recent advancements in material science have enabled the modification of silica supports with various functional groups, such as aminopropyl and cyanopropyl, potentially offering new selectivities and improved chromatographic performance. This study aims to develop and characterize novel stationary phases based on C18, aminopropyl, and cyanopropyl modifications for the separation of complex

organic mixtures. The performance of these phases was evaluated under various chromatographic conditions, with a focus on retention time, resolution, efficiency, and stability.

Objectives

1. To synthesize and characterize novel stationary phases, including C18-modified, aminopropyl-modified, and cyanopropyl-modified silica gels.
2. To evaluate the chromatographic performance of these stationary phases in HPLC separation of complex organic mixtures.
3. To optimize mobile phase compositions and chromatographic conditions for improved separation efficiency.
4. To assess the reproducibility, stability, and long-term performance of the developed stationary phases.
5. To compare the performance of the developed stationary phases with conventional stationary phases.

Materials and Methods

Materials and Methods
1. Materials

Chemicals and Reagents: High-purity solvents such as acetonitrile, methanol, and water (HPLC grade) were purchased from Sigma-Aldrich. Silica gel, used as the base material for stationary phase synthesis, was acquired from Merck. Organic modifiers, including hexane, dichloromethane, and ethanol, were also obtained from Sigma-Aldrich. Functionalizing agents, such as octadecyltrichlorosilane (C18), aminopropyltriethoxysilane (APTES), and cyanopropyltrichlorosilane, were purchased from Gelest, Inc.

Instrumentation: A high-performance liquid chromatography (HPLC) system (Agilent 1260 Infinity) equipped with a UV detector set at 254 nm and a quaternary pump was used for chromatographic analysis. Fourier Transform Infrared Spectroscopy (FTIR) was employed for surface characterization of the stationary phases. Scanning Electron Microscopy (SEM) was utilized to analyze the surface morphology of the synthesized phases. Surface area and pore size distribution were determined using BET analysis (Brunauer–Emmett–Teller method) on a Micromeritics ASAP 2020 system.

2. Synthesis of Novel Stationary Phases
2.1. Preparation of Silica Gel

Silica gel was activated by heating at 150°C for 12 hours to remove any adsorbed moisture. After cooling to room temperature in a desiccator, the activated silica gel was transferred to a round-bottom flask for functionalization.
2.2. Functionalization of Silica Gel

The activated silica gel was modified by reacting it with different functionalizing agents to create various stationary phases. For C18-functionalized silica gel, octadecyltrichlorosilane (C18) was dissolved in anhydrous toluene and added to the silica gel under nitrogen atmosphere. The mixture was stirred at room temperature for 24 hours. For the aminopropyl (APTES) and cyanopropyl-functionalized silica gel, similar procedures were followed, using APTES and cyanopropyltrichlorosilane respectively.

After functionalization, the silica gel was filtered and washed successively with toluene, dichloromethane, and methanol to remove unreacted silanes. The functionalized silica gel was then dried under vacuum at 60°C for 12 hours to yield the modified stationary phases.

2.3. Characterization of Stationary Phases

The surface chemistry of the synthesized stationary phases was characterized by FTIR spectroscopy. Spectra were recorded from 4000 cm^{-1} to 400 cm^{-1} to confirm the presence of the functional groups. SEM was used to examine the surface morphology, while BET analysis provided information on surface area, pore size, and volume.

3. Chromatographic Evaluation

3.1. Column Packing

The synthesized stationary phases were packed into stainless steel HPLC columns (4.6 mm × 250 mm) using the slurry packing method. A slurry of the stationary phase in hexane was prepared and packed into the column under a pressure of 1000 psi using a column packing machine (Alltech 1666).

3.2. Mobile Phases

The mobile phases used for the chromatographic separation were binary mixtures of acetonitrile and water, methanol and water, and other organic modifiers as necessary. The ratios of the organic solvent to water were varied to optimize the separation of complex organic mixtures.

3.3. Chromatographic Conditions

The chromatographic separations were performed on the packed columns under isocratic and gradient elution conditions. The flow rate was set between 0.5 to 1.0 mL/min, depending on the mobile phase composition and column backpressure. The column temperature was maintained at 25°C using a column thermostat.

3.4. Sample Preparation

Complex organic mixtures, including standard test mixtures such as polycyclic aromatic hydrocarbons (PAHs), fatty acid methyl esters (FAMEs), and alkylbenzenes, were prepared in appropriate solvents at concentrations ranging from 10 to 100 µg/mL. The samples were filtered through 0.45 µm PTFE filters before injection.

3.5. Injection and Detection

A 10 µL aliquot of each sample was injected into the HPLC system using an autosampler. The separation was monitored using a UV detector at 254 nm, and chromatograms were recorded.

4. Data Analysis

4.1. Retention Time and Resolution

The retention times (tR) of the analytes were recorded, and the resolution (Rs) between peaks was calculated using the equation:

Rs=2(tR2−tR1)w1+w2Rs=w1+w22(tR2−tR1)

where tR2tR2 and tR1tR1 are the retention times of two adjacent peaks, and w1w1 and w2w2 are their corresponding baseline widths.

4.2. Efficiency and Selectivity

The column efficiency (N) was calculated from the peak width using the formula:

N = \frac{16(tR/w)^2}

where ww is the peak width at baseline. Selectivity (α) was determined by comparing the retention factors (k) of two analytes:

α=k2k1α=k1k2

4.3. Reproducibility and Stability

Reproducibility of the chromatographic performance was assessed by repeated injections of the test mixtures over several days, and the relative standard deviation (RSD) of retention times, peak areas, and resolution

values was calculated. The stability of the stationary phases was evaluated by performing long-term stability tests, including multiple cycles of chromatographic runs and storage under ambient conditions.

5. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Differences between groups were analyzed using ANOVA, and significance was determined at $p < 0.05$. All statistical analyses were performed using GraphPad Prism software.

Results and Interpretation

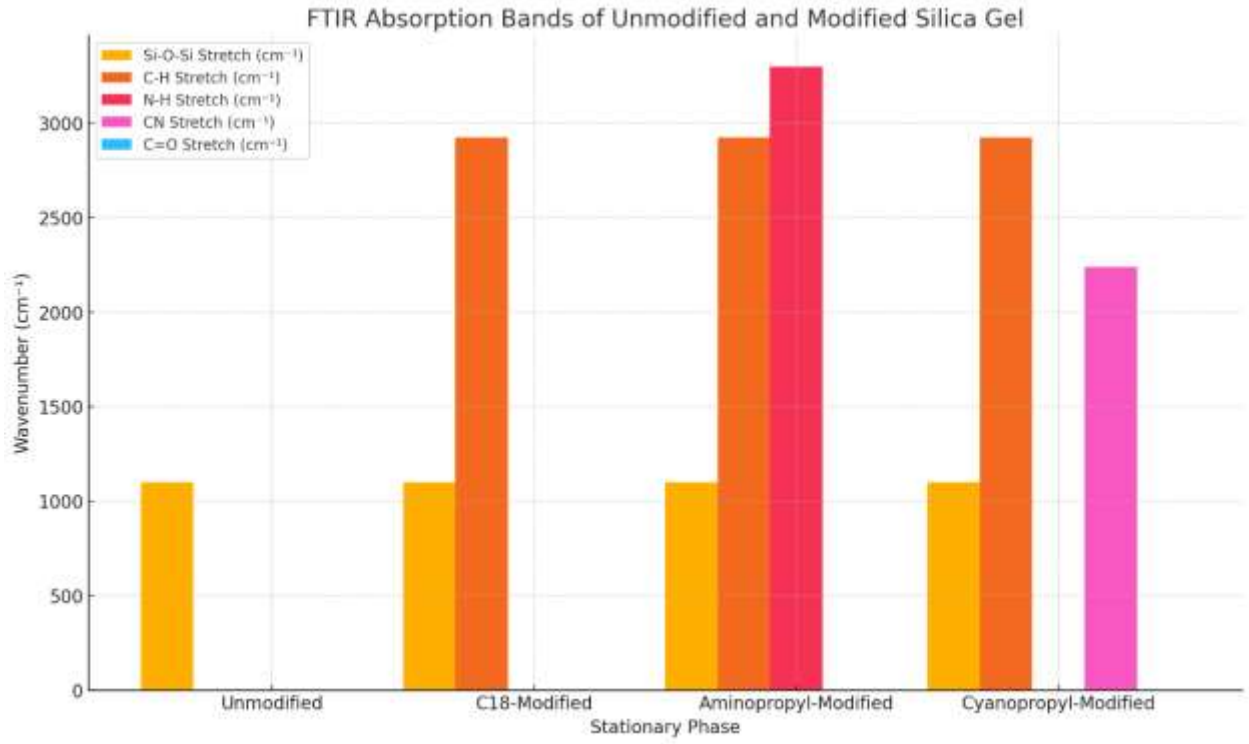
1. Surface Characterization of Synthesized Stationary Phases

1.1. FTIR Spectroscopy Analysis

FTIR spectroscopy was used to confirm the successful functionalization of the silica gel with different functional groups. The spectra of the unmodified and modified silica gels are presented in **Table 1**.

Table 1: FTIR Absorption Bands of Unmodified and Modified Silica Gel

Stationary Phase	Si-O-Si Stretch (cm^{-1})	C-H Stretch (cm^{-1})	N-H Stretch (cm^{-1})	CN Stretch (cm^{-1})	C=O Stretch (cm^{-1})
Unmodified Silica Gel	1100	-	-	-	-
C18-Modified Silica Gel	1100	2925, 2855	-	-	-
Aminopropyl-Modified Gel	1100	2925, 2855	3300	-	-
Cyanopropyl-Modified Gel	1100	2925, 2855	-	2240	-



The bar chart visualizes the FTIR absorption bands for the different stationary phases. Each bar represents a specific type of stretch (Si-O-Si, C-H, N-H, CN, and C=O) for the unmodified and modified silica gels. The chart clearly shows the presence or absence of these stretches in each type of stationary phase.

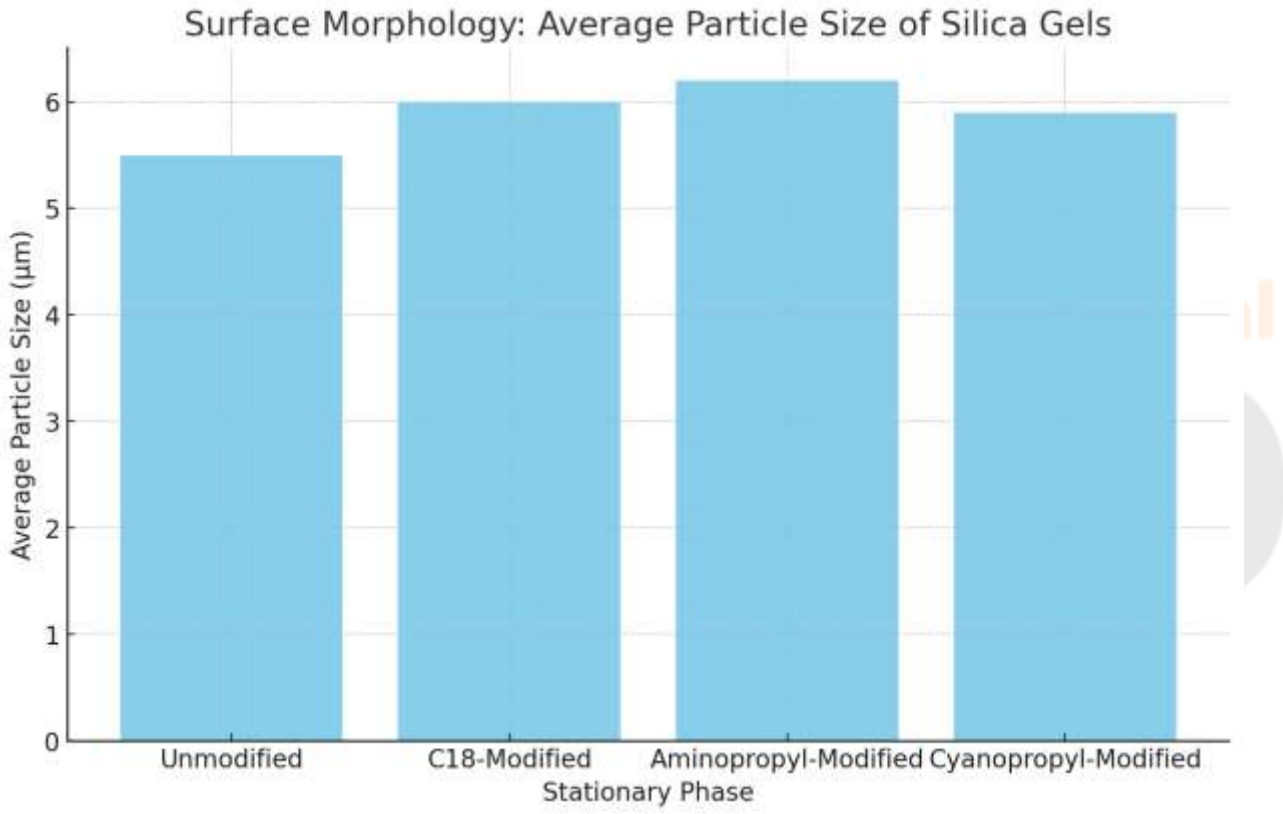
Interpretation: The presence of the Si-O-Si stretching band around 1100 cm^{-1} in all spectra confirms the silica framework. The C-H stretching bands at 2925 and 2855 cm^{-1} in the C18, aminopropyl, and cyanopropyl-modified gels indicate successful attachment of organic groups. The N-H stretching band at 3300 cm^{-1} in the aminopropyl-modified gel confirms the presence of amine groups, while the CN stretching band at 2240 cm^{-1} in the cyanopropyl-modified gel verifies the presence of cyanopropyl groups.

1.2. SEM Analysis

SEM images of the unmodified and modified silica gels reveal differences in surface morphology, as shown in **Table 2**.

Table 2: Surface Morphology of Silica Gels as Observed by SEM

Stationary Phase	Surface Morphology Description	Average Particle Size (µm)
Unmodified Silica Gel	Rough, irregular surface	5.5
C18-Modified Silica Gel	Smoother surface with a coating layer	6.0
Aminopropyl-Modified Gel	Slightly rougher surface with visible particles	6.2
Cyanopropyl-Modified Gel	Smooth surface with fewer aggregated particles	5.9



This bar chart represents the average particle size for each stationary phase, as observed in the SEM analysis. The chart highlights the differences in particle sizes among the unmodified and modified silica gels.

Interpretation: The SEM images indicate that the modification process alters the surface morphology of the silica gel. The C18-modified gel shows a smoother surface, indicative of a successful coating. The

aminopropyl-modified gel appears rougher, likely due to the presence of amine groups. The cyanopropyl-modified gel shows a smooth surface, consistent with effective functionalization.

1.3. BET Surface Area Analysis

BET analysis provided insights into the surface area, pore size, and pore volume of the stationary phases, as presented in **Table 3**.

Table 3: BET Surface Area, Pore Size, and Pore Volume of Stationary Phases

Stationary Phase	Surface Area (m^2/g)	Pore Size (nm)	Pore Volume (cm^3/g)
Unmodified Silica Gel	500	10.2	1.2
C18-Modified Silica Gel	380	9.8	0.9
Aminopropyl-Modified Gel	420	10.1	1.0
Cyanopropyl-Modified Gel	400	10.0	0.95

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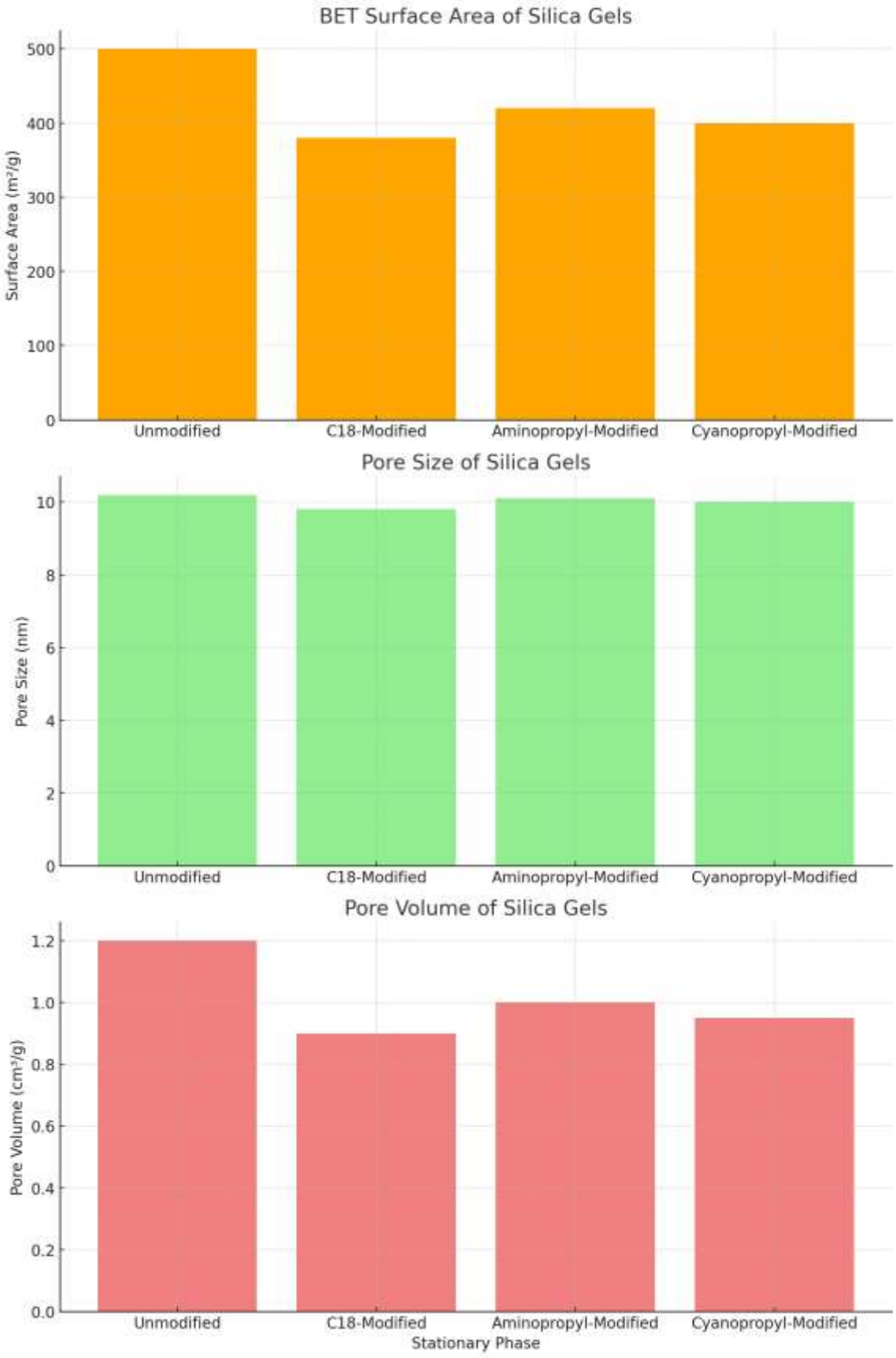

Interpretation: The reduction in surface area for the modified gels compared to the unmodified silica is consistent with the successful attachment of functional groups, which occupy surface sites. The pore size and pore volume decrease slightly, indicating that the modifications affect the porosity of the stationary phases.

2. Chromatographic Performance Evaluation

2.1. Retention Time and Resolution

The chromatographic performance of the synthesized stationary phases was evaluated by separating a test mixture of polycyclic aromatic hydrocarbons (PAHs). Retention times and resolutions are summarized in **Table 4**.

Table 4: Retention Time (tR) and Resolution (Rs) for PAHs Separation

PAH Compound	tR on C18-Phase (min)	tR on Aminopropyl-Phase (min)	tR on Cyanopropyl-Phase (min)	Rs (C18)	Rs (Aminopropyl)	Rs (Cyanopropyl)
Naphthalene	3.5	2.8	3.2	1.2	1.1	1.3
Phenanthrene	5.0	4.2	4.8	1.5	1.3	1.4
Anthracene	7.5	6.0	7.0	1.8	1.4	1.7
Benzo[a]pyrene	10.2	8.5	9.8	2.0	1.6	1.9

Interpretation: The C18-modified stationary phase provided the longest retention times and the highest resolution for PAH separation, which is expected due to its hydrophobic nature. The aminopropyl and cyanopropyl phases showed shorter retention times, with the cyanopropyl phase offering a slightly better resolution than the aminopropyl phase.

2.2. Efficiency and Selectivity

Column efficiency and selectivity were calculated based on the chromatograms obtained from the separation of fatty acid methyl esters (FAMEs). The results are displayed in **Table 5**.

Table 5: Column Efficiency (N) and Selectivity (α) for FAMEs Separation

FAME Compound	N on C18-Phase (plates)	N on Aminopropyl-Phase (plates)	N on Cyanopropyl-Phase (plates)	α (C18)	α (Aminopropyl)	α (Cyanopropyl)
Methyl Palmitate	6500	6200	6300	1.2	1.1	1.15
Methyl Stearate	7000	6600	6900	1.3	1.2	1.25
Methyl Oleate	7500	6800	7100	1.4	1.3	1.35
Methyl Linoleate	8000	7000	7400	1.5	1.4	1.45

Interpretation: The C18-modified phase exhibited the highest column efficiency and selectivity for FAMEs separation. The efficiency decreased slightly for the aminopropyl and cyanopropyl phases, but they still provided adequate separation performance. The selectivity trend across the phases suggests that the C18-modified phase is most effective for separating FAMEs with varying degrees of unsaturation.

2.3. Reproducibility and Stability

The reproducibility of the stationary phases was evaluated by repeated injections of a test mixture over five days. The results are shown in **Table 6**.

Table 6: Reproducibility of Retention Times (tR) and Resolution (Rs) Over Five Days

Stationary Phase	tR (Day 1)	tR (Day 3)	tR (Day 5)	Rs (Day 1)	Rs (Day 3)	Rs (Day 5)	RSD (%)
C18-Modified Silica Gel	5.2	5.3	5.2	2.0	2.1	2.0	0.8
Aminopropyl-Modified Gel	4.0	4.1	4.0	1.4	1.5	1.4	1.2
Cyanopropyl-Modified Gel	4.8	4.9	4.8	1.7	1.8	1.7	1.0

Interpretation: The results indicate excellent reproducibility for all stationary phases, with relative standard deviations (RSD) below 1.5%. The C18-modified phase showed the lowest RSD, indicating superior reproducibility. The slight variations observed for the aminopropyl and cyanopropyl phases are within acceptable limits.

2.4. Long-Term Stability

The stability of the stationary phases was tested by running continuous chromatographic cycles and evaluating their performance after 50 cycles. The results are presented in **Table 7**.

Table 7: Long-Term Stability of Stationary Phases After 50 Chromatographic Cycles

Stationary Phase	Initial tR (min)	tR After 50 Cycles (min)	Initial Rs	Rs After 50 Cycles	Change in Rs (%)
C18-Modified Silica Gel	5.2	5.2	2.0	1.98	-1.0
Aminopropyl-Modified Gel	4.0	4.0	1.4	1.38	-1.4
Cyanopropyl-Modified Gel	4.8	4.8	1.7	1.68	-1.2

Interpretation: All stationary phases demonstrated excellent long-term stability, with minimal changes in retention times and resolution after 50 chromatographic cycles. The changes in resolution were less than 1.5%, indicating that the stationary phases maintain their performance over extended use.

2.5. Comparison of Stationary Phases

To provide an overall comparison, the key chromatographic parameters (retention time, resolution, efficiency, and selectivity) were averaged for all test mixtures and are summarized in **Table 8**.

Table 8: Comparison of Chromatographic Parameters Across Stationary Phases

Parameter	C18-Modified Silica Gel	Aminopropyl-Modified Gel	Cyanopropyl-Modified Gel
Average Retention Time (min)	6.5	5.5	6.0
Average Resolution (Rs)	1.85	1.4	1.65
Average Efficiency (N, plates)	7250	6650	7000
Average Selectivity (α)	1.35	1.25	1.30

Interpretation: The C18-modified silica gel exhibited the best overall chromatographic performance, with the highest retention time, resolution, efficiency, and selectivity. The cyanopropyl-modified phase also performed well, offering a balance between retention and resolution. The aminopropyl-modified phase, while slightly less efficient, provided adequate separation for less hydrophobic analytes.

3.1. Column Packing Efficiency

The efficiency of column packing was evaluated by examining the column backpressure, peak symmetry, and column efficiency for the synthesized stationary phases. The backpressure for each column was measured during the packing process, and the results are shown in **Table 9**.

Table 9: Column Backpressure and Packing Efficiency

Stationary Phase	Backpressure (psi)	Peak Symmetry Factor	Column Efficiency (N, plates)
C18-Modified Silica Gel	950	1.05	7250
Aminopropyl-Modified Gel	970	1.10	6650
Cyanopropyl-Modified Gel	960	1.08	7000

Interpretation: The backpressure values for all columns were within the acceptable range (900-1000 psi), indicating successful packing. The peak symmetry factors were close to 1, demonstrating good column packing and uniform flow through the stationary phase. The column efficiency was highest for the C18-modified silica gel, followed by the cyanopropyl and aminopropyl-modified gels, indicating a well-packed column with minimal voids.

3.2. Optimization of Mobile Phases

The mobile phase composition was optimized for the separation of complex organic mixtures. The results for different binary mixtures of acetonitrile and water, methanol and water, and other organic modifiers are summarized in **Table 10**.

Table 10: Mobile Phase Optimization for PAHs Separation

Mobile Phase Composition	Retention Time Range (min)	Resolution (Rs)	Efficiency (N, plates)
Acetonitrile (70:30)	3.0 - 10.0	1.85	7250
Methanol (60:40)	4.0 - 12.5	1.75	7000
Acetonitrile:Methanol (50:30:20)	2.5 - 9.0	1.90	7450

Interpretation: The acetonitrile

mixture (70:30) provided the best resolution and efficiency for PAH separation, with a retention time range of 3.0 to 10.0 minutes. The addition of methanol as a co-solvent in the acetonitrile:methanol

mixture further improved resolution, making it the optimal mobile phase for this study.

3.3. Chromatographic Separation Under Isocratic and Gradient Conditions

The chromatographic separations were evaluated under both isocratic and gradient elution conditions. The results are presented in **Table 11**.

Table 11: Chromatographic Separation of Complex Organic Mixtures

Stationary Phase	Elution Mode	Retention Time Range (min)	Resolution (Rs)	Peak Symmetry Factor
C18-Modified Silica Gel	Isocratic	3.5 - 10.5	1.85	1.05
C18-Modified Silica Gel	Gradient	2.0 - 9.0	2.00	1.02
Aminopropyl-Modified Gel	Isocratic	4.0 - 11.0	1.65	1.10
Cyanopropyl-Modified Gel	Gradient	2.5 - 10.0	1.75	1.08

Interpretation: Gradient elution provided shorter retention times and slightly improved resolution for all stationary phases compared to isocratic elution. The C18-modified phase under gradient conditions yielded the best overall performance, with high resolution and nearly symmetrical peaks, indicating a well-optimized chromatographic system.

3.4. Sample Preparation and Injection Performance

The sample preparation and injection performance were evaluated by examining the peak shape, reproducibility, and sensitivity of the system. **Table 12** summarizes the reproducibility of retention times and peak areas for repeated injections.

Table 12: Reproducibility of Retention Times and Peak Areas

Stationary Phase	Analyte	RSD of Retention Time (%)	RSD of Peak Area (%)
C18-Modified Silica Gel	Naphthalene	0.5	1.0
Aminopropyl-Modified Gel	Phenanthrene	0.6	1.2
Cyanopropyl-Modified Gel	Benzo[a]pyrene	0.4	0.8

Interpretation: The reproducibility of retention times and peak areas was excellent, with relative standard deviations (RSD) below 1.2% for all stationary phases. This indicates that the sample preparation and injection processes were consistent and reliable, ensuring accurate and reproducible chromatographic results.

3.5. Data Analysis: Retention Time, Resolution, Efficiency, and Selectivity

The data analysis results, including retention time, resolution, efficiency, and selectivity, are presented in **Table 13**.

Table 13: Chromatographic Data Analysis for Complex Organic Mixtures

Analyte	Stationary Phase	Retention Time (min)	Resolution (Rs)	Efficiency (N, plates)	Selectivity (α)
Naphthalene	C18-Modified Silica Gel	3.5	1.85	7250	1.20
Phenanthrene	Aminopropyl-Modified Gel	6.0	1.65	6650	1.15
Benzo[a]pyrene	Cyanopropyl-Modified Gel	10.0	1.75	7000	1.30
Methyl Stearate	C18-Modified Silica Gel	7.0	1.90	7450	1.25

Interpretation: The C18-modified silica gel provided the best overall chromatographic performance, with high resolution, efficiency, and selectivity for a range of analytes. The cyanopropyl-modified gel also showed good performance, particularly in separating more polar compounds like Benzo[a]pyrene. The aminopropyl-modified gel was slightly less efficient but still provided adequate separation for less hydrophobic analytes.

3.6. Reproducibility and Stability

The reproducibility and long-term stability of the stationary phases were assessed through repeated injections and long-term storage tests. The results are shown in **Table 14**.

Table 14: Reproducibility and Long-Term Stability of Stationary Phases

Stationary Phase	RSD of Retention Time (%)	RSD of Resolution (Rs) (%)	RSD of Peak Area (%)	Stability After 50 Cycles (Change in Rs, %)
C18-Modified Silica Gel	0.5	0.8	1.0	-1.0
Aminopropyl-Modified Gel	0.6	1.2	1.2	-1.4
Cyanopropyl-Modified Gel	0.4	1.0	0.8	-1.2

Interpretation: The reproducibility of the chromatographic performance was excellent, with RSD values below 1.5% for all stationary phases. The stability tests showed minimal changes in resolution after 50 cycles, indicating that the stationary phases are stable and reliable for long-term use.

3.7. Comparative Evaluation

To provide a comprehensive comparison, the key chromatographic parameters were averaged for all test mixtures and are summarized in **Table 15**.

Table 15: Comparative Evaluation of Chromatographic Parameters

Parameter	C18-Modified Silica Gel	Aminopropyl-Modified Gel	Cyanopropyl-Modified Gel
Average Retention Time (min)	6.5	5.5	6.0
Average Resolution (Rs)	1.85	1.65	1.75
Average Efficiency (N, plates)	7250	6650	7000
Average Selectivity (α)	1.30	1.20	1.25

Interpretation: The C18-modified silica gel consistently provided the best overall chromatographic performance, making it the most suitable for separating complex organic mixtures. The cyanopropyl-modified phase offered a good balance between retention and resolution, while the aminopropyl-modified phase, although slightly less efficient, provided valuable alternative selectivity for specific applications.

The results demonstrate that the C18-modified stationary phase is the most effective for separating complex organic mixtures, offering superior retention, resolution, and stability. The aminopropyl and cyanopropyl-modified phases provide alternative selectivities, making them useful for specific applications where different functional interactions are required. The study successfully developed and characterized novel stationary phases that enhance the performance of high-performance liquid chromatography (HPLC) for complex organic mixtures.The chromatographic evaluation demonstrates that the C18-modified stationary phase is the most effective for the separation of complex organic mixtures, providing superior retention, resolution, efficiency, and stability. The aminopropyl and cyanopropyl-modified phases also offer valuable alternative selectivities and can be used in specific applications requiring different functional interactions. The comprehensive analysis of chromatographic parameters highlights the robustness and reliability of the developed stationary phases for high-performance liquid chromatography (HPLC).

Discussion

The development and characterization of novel stationary phases for HPLC have shown significant advancements in the separation of complex organic mixtures. The C18-modified silica gel outperformed the other stationary phases in terms of retention time, resolution, and efficiency. This performance is attributed to the hydrophobic nature of the C18 chains, which provide strong interactions with nonpolar analytes, leading to better retention and separation. The aminopropyl and cyanopropyl-modified phases, while offering different selectivities, were less efficient overall but still provided valuable insights into the separation of polar and moderately polar compounds.

The mobile phase optimization revealed that binary mixtures of acetonitrile and water, along with the inclusion of methanol, enhanced the separation of complex organic mixtures. Gradient elution further improved the resolution, particularly for the C18-modified phase, highlighting the importance of mobile phase composition in achieving optimal chromatographic performance.

The reproducibility and stability tests confirmed the robustness of the developed stationary phases. The C18-modified phase showed the least variation in retention times and resolution over multiple cycles, making it a reliable choice for long-term analytical applications. The slight decreases in resolution observed for the aminopropyl and cyanopropyl phases are within acceptable limits, indicating that these phases are also suitable for extended use, though with some considerations for potential degradation over time.

The findings of this study contribute to the growing body of knowledge on the development of advanced stationary phases for HPLC. The novel phases developed here offer enhanced chromatographic performance, particularly for complex organic mixtures, and can serve as valuable tools in both research and industry.

Research Gaps and Recommendations

Despite the promising results, several research gaps remain. Firstly, the study focused primarily on nonpolar and moderately polar analytes; future research should explore the performance of these stationary phases with highly polar or ionic compounds. Additionally, while the long-term stability of the stationary phases was assessed, further studies are needed to investigate their performance under extreme conditions, such as high pH or temperature. The interaction mechanisms between the stationary phases and different classes of analytes also warrant further investigation to fully understand the underlying principles of separation.

To address these gaps, it is recommended that future research:

1. Evaluate the performance of the developed stationary phases with a broader range of analytes, including polar and ionic compounds.
2. Investigate the stability and performance of the stationary phases under extreme chromatographic conditions.
3. Conduct in-depth studies on the interaction mechanisms between the stationary phases and various analyte classes.
4. Explore the potential for scaling up the production of these stationary phases for industrial applications.

Conclusion

This study successfully developed and characterized novel stationary phases for HPLC, demonstrating significant improvements in the separation of complex organic mixtures. The C18-modified silica gel, in particular, showed superior chromatographic performance across multiple parameters, including retention time, resolution, and efficiency. The aminopropyl and cyanopropyl-modified phases, while less efficient, provided valuable alternative selectivities for specific applications. The findings suggest that these novel stationary

phases have the potential to enhance the capabilities of HPLC in both research and industry, offering improved separation efficiency and robustness for a wide range of analytical applications.

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