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Research Article

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Click-Chemistry Functionalization of Monodisperse Poly(GDMA-co-GDGDA) Microbeads with Propiolic Acid for HILIC Applications

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Abstract

This study aimed to prepare polymeric column packing materials and evaluate their chromatographic performance. Initially, a poly(glycidyl methacrylate) (poly(GMA)) starting latex was prepared by dispersion polymerization. Using this latex as a precursor, monodisperse poly(glycerol dimethacrylate-co-glycerol 1,3-diglycerolate diacrylate) [poly(GDMA-co-GDGDA)] polymeric particles were synthesized via microsuspension polymerization. The morphology and physical characteristics of the obtained polymers were investigated using optical microscopy, scanning electron microscopy (SEM), Brunauer–Emmett–Teller (BET) surface area and pore size analysis, and laser diffraction particle size analysis. The poly(GDMA-co-GDGDA) particles were treated with sodium azide (NaN_3) to introduce azide functionalities onto the particle surface via click chemistry. Subsequently, propiolic acid was coupled to these particles to obtain poly(GDMA-co-GDGDA)-N_3–propiolic acid polymeric particles containing triazole rings. The surface of the particles was functionalized with propiolic acid via copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) using CuBr as a catalyst and PMDETA as a ligand. The success of the functionalization and the high purity achieved through a rigorous washing procedure (DMF, ethanol, and water)

resulted in a robust HILIC stationary phase. The propiolic acid–functionalized poly(GDMA-co-GDGDA) particles were packed into a stainless-steel column (4.6 × 150 mm) and connected to a Shimadzu high-performance liquid chromatography (HPLC) system. Representative analytes, including toluene, acrylamide, thymine, thiourea, cytosine, and inosine, were used to evaluate the chromatographic performance. The analyses were carried out under isocratic conditions using hydrophilic interaction liquid chromatography (HILIC) with UV detection at 254 nm. In the experimental studies, AcN/H_2O mixtures at different compositions were used as the mobile phase. Increasing the AcN content in the mobile phase improved the separation performance of the column while simultaneously reducing the system pressure.

Keywords: Polymeric stationary phase; Click chemistry; HILIC; Monodisperse polymer particles; Surface functionalization; Poly(GDMA-co-GDGDA)

1. Introduction

High-performance liquid chromatography (HPLC) is one of the most widely used analytical techniques for the separation and analysis of complex mixtures. The performance of HPLC systems strongly depends on the physicochemical properties of the column packing materials. In particular, columns packed with monodisperse particles generally exhibit more uniform flow regimes, higher separation efficiency, and lower back-pressure compared with columns containing irregular packing materials. For this reason, the development of monodisperse polymer particles has attracted considerable attention in chromatographic applications.

Chromatographic stationary phases are generally classified into two main categories: silica-based and polymer-based materials. Silica-based stationary phases are widely used due to their high mechanical strength and well-defined pore structures; however, their limited chemical stability under extreme pH conditions restricts their applicability in certain chromatographic systems. In contrast, polymer-based stationary phases offer superior chemical stability, wider pH tolerance, and versatile surface modification possibilities. These advantages make polymeric materials attractive alternatives for the development of novel chromatographic packing materials.

Various multistage polymerization strategies have been developed for the preparation of monodisperse macroporous polymer particles with controlled porosity and surface chemistry. These approaches enable the production of particles with tailored

morphological and physicochemical properties suitable for chromatographic stationary phases [1–14]. Among different polymeric materials, hydroxyl-containing monomers such as glycerol dimethacrylate (GDMA), glycerol-1,3-diglycerolate diacrylate (GDGDA), and their derivatives have been widely used in polymer synthesis due to their high reactivity and versatile functionalization possibilities [15,16].

In recent years, click chemistry has emerged as an efficient and versatile strategy for the surface modification of chromatographic stationary phases. This approach enables the rapid and selective introduction of functional groups under mild reaction conditions. For example, Slater et al. reported the successful surface modification of uniform macroporous polymer particles through the covalent attachment of hydrophobic segments, resulting in improved chromatographic performance [17].

Hydrophilic interaction liquid chromatography (HILIC) is a powerful separation mode particularly suited for polar and highly hydrophilic compounds. In contrast to reversed-phase chromatography, retention in HILIC is governed by the partitioning of analytes between the mobile phase, which is rich in organic solvent, and a thin, water-enriched layer formed on the surface of the stationary phase. The presence of polar functional groups on the stationary phase promotes the formation of this interfacial aqueous layer and enables multiple interaction mechanisms, including hydrogen bonding, dipole–dipole interactions, and, in some cases, electrostatic contributions depending on the ionization state of the analytes and the surface functionalities. Therefore, the rational design of polymeric stationary phases with controllable surface chemistry is essential for achieving enhanced selectivity and improved chromatographic performance in HILIC systems.

The fundamental principles and retention mechanisms of such HILIC systems have been extensively investigated, emphasizing the critical role of surface water layer formation and partitioning [17,18]. Furthermore, achieving high kinetic performance in these polymer-based separation media requires precise control over the morphology and packing homogeneity of the stationary phase [19].

Despite these advances, the development of monodisperse polymer particles with well-defined surface functionalities remains an important challenge. In particular, the controlled surface modification of polymer-based HPLC packing materials has not been extensively explored. Furthermore, the chromatographic performance of poly(GDMA-co-GDGDA)-based stationary phases prepared via such functionalization strategies has not yet been sufficiently investigated, especially in HILIC systems.

In this study, monodisperse polymeric particles based on poly(GDMA-co-GDGDA) were synthesized and subsequently functionalized using a click-based modification strategy to introduce polar surface groups suitable for HILIC applications. Monodisperse poly(glycidyl methacrylate) (poly(GMA)) particles were first prepared by dispersion polymerization and used as seed latex for the synthesis of poly(GDMA-co-GDGDA) particles via microsuspension polymerization. The particle surfaces were then modified using propiolic acid to introduce polar functional groups suitable for HILIC applications. The obtained polymeric particles were packed into an HPLC column, and their chromatographic performance was systematically evaluated. These results provide useful insights into the design of polymer-based stationary phases for HILIC separations.

To date, no studies have reported the preparation of monodisperse macroporous poly(GDMA-co-GDGDA) particles followed by their surface functionalization with propiolic acid via azide–alkyne click chemistry for use as HILIC stationary phases.

2. Experimental

2.1. Materials

Glycidyl methacrylate (GMA, 97%, containing 100 ppm monomethyl ether hydroquinone as inhibitor), glycerol-1,3-diglycerolate diacrylate (GDGDA, technical grade, containing 1000 ppm monomethyl ether hydroquinone), and glycerol dimethacrylate (GDMA, technical grade, 85%, containing 200 ppm monomethyl ether hydroquinone) were obtained from Aldrich Chemical Co. and used as received.

2,2′-Azobis(isobutyronitrile) (AIBN) was supplied by BDH Chemicals Ltd. and purified by recrystallization from methanol prior to use.

Cyclohexanol (99%), dibutyl phthalate (99%), ethanol (HPLC grade), dimethyl sulfoxide (DMSO, ≥99.5%), and isoamyl alcohol (98%) were purchased from Aldrich and used without further purification.

Sodium lauryl sulfate (SLS), poly(vinyl alcohol) (PVA, 87–89% hydrolyzed), and poly(vinyl pyrrolidone) (PVP K-30) were obtained from Sigma Chemical Co.

Benzoyl peroxide (BPO, Merck, Germany) was recrystallized from methanol prior to use. Sodium azide (NaN_3, Si-Al), sodium iodide (NaI, Si-Al), and propiolic acid (Aldrich) were used as received.

Copper(I) bromide (CuBr, 98%, Si-Al, USA) was used as the catalyst for the azide–alkyne cycloaddition reaction without further purification.

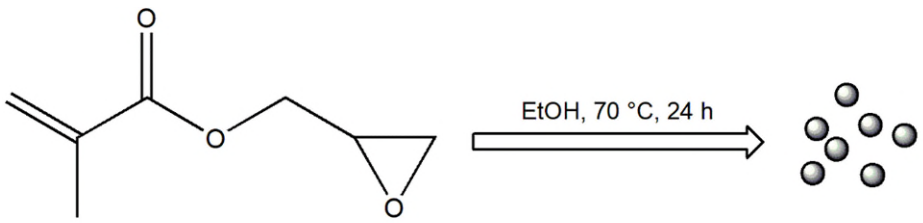
Dimethyl sulfoxide (DMSO, 99.9%, Si-Al) and *N*,*N*,*N*′,*N*″,*N*″-Pentamethyldiethylenetriamine (PMDETA, 99%, Si-Al) were used as the reaction solvent and ligand, respectively, for the click functionalization reactions.

2.2 Preparation of poly(GMA) seed latex

Poly(GMA) seed latex particles were synthesized via dispersion polymerization following previously reported procedures with minor modifications [21-23].

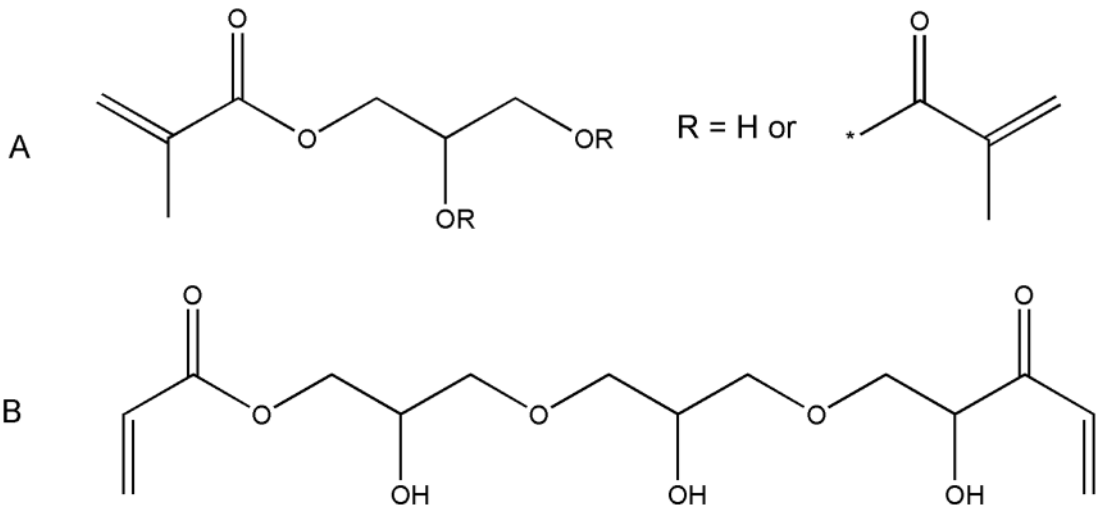
Briefly, glycidyl methacrylate (GMA) and the initiator AIBN were dissolved in absolute ethanol containing poly(vinyl pyrrolidone) (PVP K-30) as a stabilizer. The reaction mixture was transferred into a sealed Pyrex reactor and polymerization was carried out in a temperature-controlled shaking water bath at 70 °C for 24 h.

Upon completion of the reaction, the resulting latex particles were isolated and purified by repeated centrifugation–decantation cycles using ethanol and distilled water. The purified particles were finally redispersed in distilled water to obtain a stable seed latex suspension.



Scheme 1. Preparation of poly(GMA) seed latex by dispersion polymerization.

Monodisperse macroporous particles were prepared by seeded polymerization of a monomer mixture within the poly(GMA) seed particles. The molecular structures of the monomers glycerol dimethacrylate (GDMA) and glycerol-1,3-diglycerolate diacrylate (GDGDA) are presented in Scheme 2.



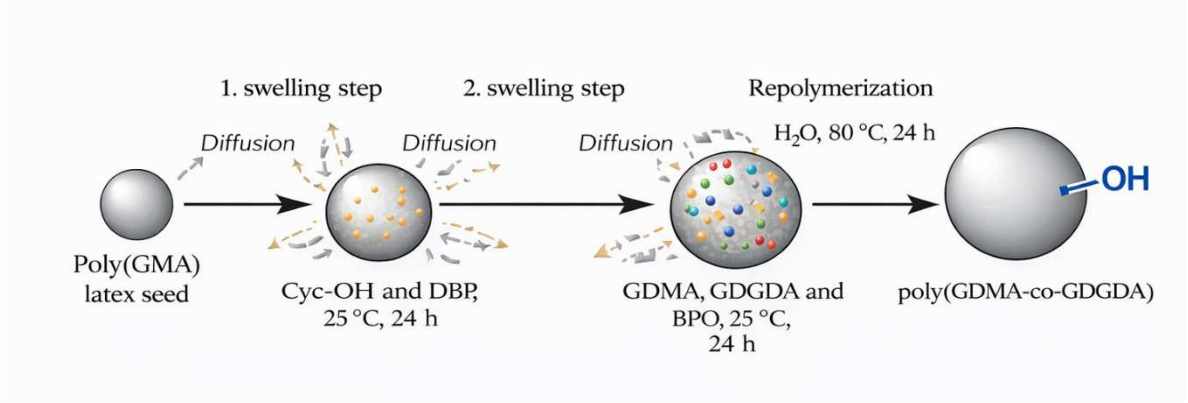
Scheme 2. Chemical structures of the monomers used in the preparation of monodisperse porous P(GDMA-co-GDGDA) particles: (A) glycerol dimethacrylate (GDMA) and (B) glycerol-1,3-diglycerolate diacrylate (GDGDA).

2.3 Synthesis of poly(GDMA-co-GDGDA) particles

Monodisperse macroporous poly(GDMA-co-GDGDA) particles were prepared through a multistage seeded microsuspension polymerization technique using poly(GMA) seed latex as the template.

In the initial stage, the seed particles were swollen with a mixture of cyclohexanol and dibutyl phthalate acting as porogenic solvents. Subsequently, a monomer phase containing GDMA, GDGDA, and benzoyl peroxide (BPO) as the initiator was introduced into the swollen particles, allowing diffusion of the monomers into the polymer matrix.

Following the swelling process, polymerization was conducted in an aqueous medium at 80 °C for 24 h, resulting in the formation of crosslinked macroporous particles. This multistep swelling and repolymerization approach enabled controlled particle growth while preserving the monodispersity of the initial seed latex. A schematic representation of the preparation process is shown in Scheme 3.



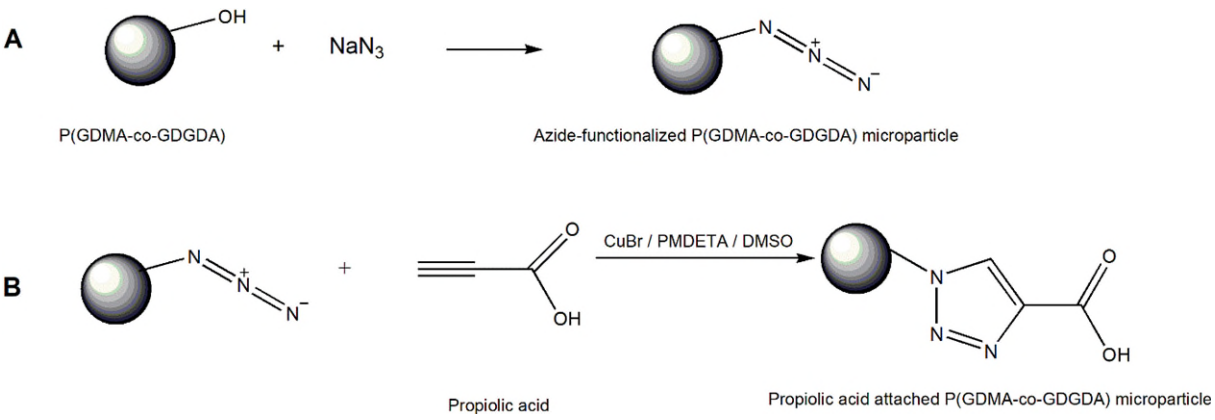
Scheme 3. Preparation of monodisperse poly(GDMA-co-GDGDA) particles by two-step swelling and repolymerization.

2.4 Surface Functionalization of poly(GDMA-co-GDGDA) Particles

Surface modification of poly(GDMA-co-GDGDA) particles was carried out through a two-step functionalization process involving azide introduction followed by copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC).

Initially, the epoxy groups present on the particle surface were reacted with sodium azide (NaN_3), leading to the formation of azide-functionalized particles via nucleophilic ring-opening reactions. This step introduced reactive azide groups onto the particle surface, providing active sites for subsequent modification.

In the second step, the azide-functionalized particles were reacted with propiolic acid in DMSO using CuBr as the catalyst and PMDETA as the ligand. The CuAAC reaction resulted in the formation of triazole linkages, covalently attaching propiolic acid to the particle surface. The corresponding reaction scheme is illustrated in Scheme 4A and 4B.



Scheme 4. Functionalization of P(GDMA-co-GDGDA) microparticles. (A) Formation of azide-functionalized P(GDMA-co-GDGDA) microparticles by reaction with NaN_3. (B) Covalent attachment of propiolic acid via azide–alkyne click chemistry forming a triazole linkage.

After completion of the functionalization, the particles were purified through a sequence of washing steps to remove residual reagents and byproducts. The particles were first treated with DMF, followed by ethanol washing, and finally extensively rinsed with distilled water. This procedure ensured high purity of the final material and promoted the formation of a hydrophilic surface layer suitable for HILIC applications.

The purified poly(GDMA-co-GDGDA)-N_3–propiolic acid particles were subsequently used for column packing.

2.5 Characterization methods

The physicochemical properties of the synthesized polymeric particles were investigated using multiple analytical techniques.

Particle morphology was examined by scanning electron microscopy (SEM), providing detailed information on surface structure and particle uniformity. The porous characteristics, including specific surface area and pore size distribution, were determined using Brunauer–Emmett–Teller (BET) analysis based on nitrogen adsorption–desorption measurements.

Particle size distribution was evaluated by laser diffraction using a Malvern Mastersizer system, allowing accurate determination of the size distribution profile and average particle diameter.

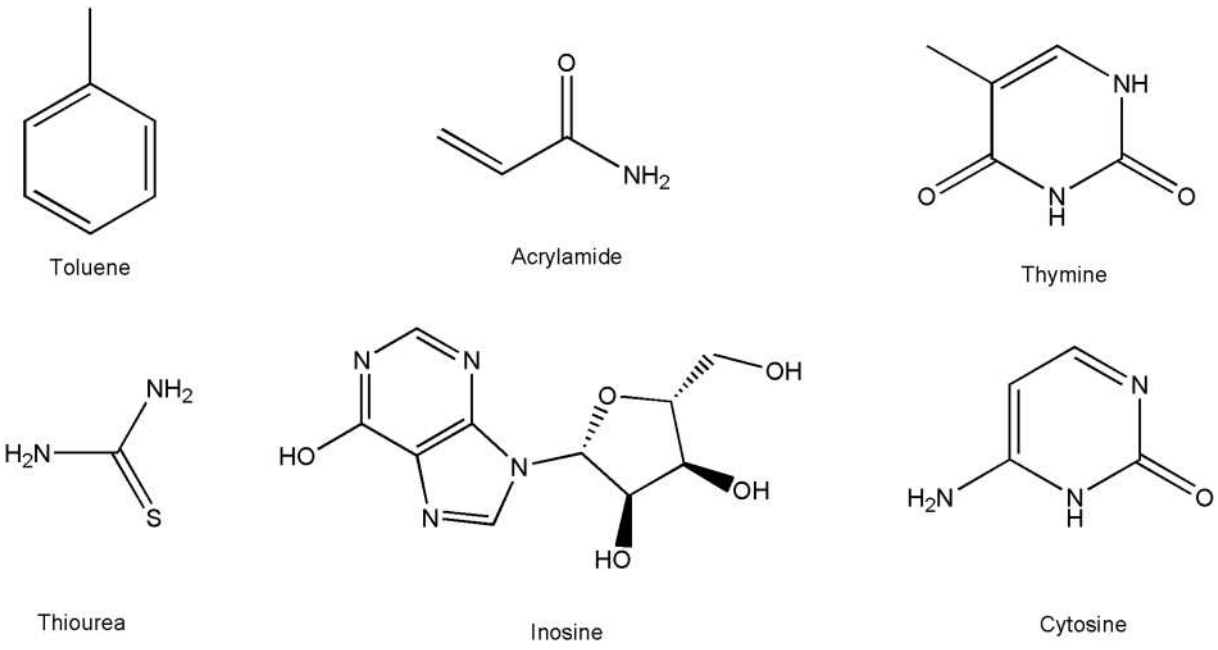
These complementary techniques enabled a comprehensive characterization of the structural and morphological properties of both seed latex and final polymer particles.

2.6 Chromatographic conditions

The chromatographic performance of the prepared polymeric particles was evaluated using high-performance liquid chromatography (HPLC) operated in hydrophilic interaction liquid chromatography (HILIC) mode. The stationary phase was packed into a stainless steel column (4.6 × 150 mm), and the column was operated under isocratic

conditions at ambient temperature (25 °C). UV detection was performed at 254 nm. A mixture of analytes with varying polarity, including toluene, acrylamide, thymine, thiourea, inosine, and cytosine, was used to assess separation performance. Toluene was employed as a non-retained reference compound. The chemical structures of the analytes are shown in Scheme 5.

The mobile phase consisted of acetonitrile/water (ACN/H_2O) mixtures at different volume ratios, enabling evaluation of the effect of solvent composition on chromatographic behavior.



Scheme 5. Chemical structures of the analytes used in the chromatographic experiments.

3. Results and Discussion

3.1 Characterization of poly(GMA) Seed Latex particles

The monodisperse seed latex used for the preparation of poly(GDMA-co-GDGDA) particles was first characterized in terms of particle morphology and size distribution. SEM images of the seed latex particles are presented in Figure 1. As observed in the micrographs, the particles exhibit a uniform spherical morphology with a narrow size distribution. The average particle diameter was approximately 2 μm, indicating that the

seed latex possessed a highly monodisperse structure. The morphology of the particles was confirmed by SEM analysis (Figure 1).

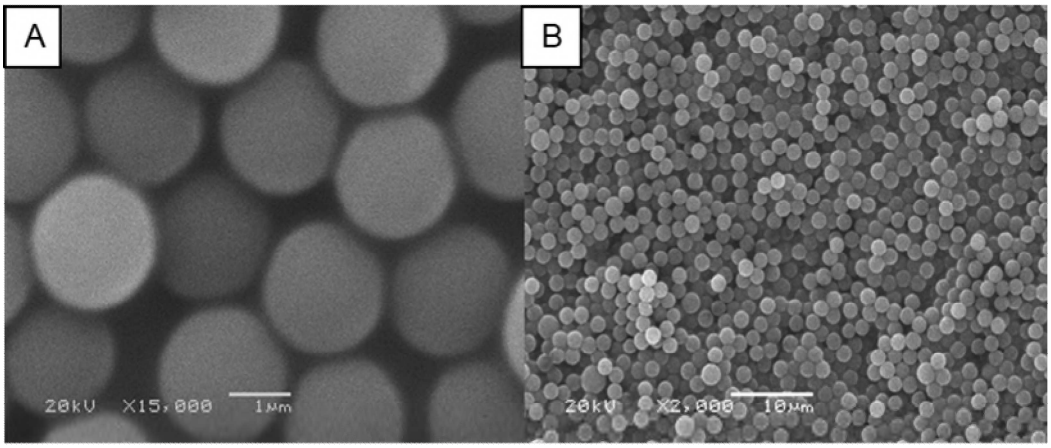


Figure 1. SEM micrographs of monodisperse poly(GMA) seed latex particles at different magnifications: (A) 15,000× and (B) 2,000×.

Particle size distribution analysis was performed using a Malvern Mastersizer Hydro 2000MU particle size analyzer. The resulting distribution profile is presented in Figure 2, while the corresponding numerical data are summarized in Table 1. The distribution exhibits a relatively narrow profile centered at approximately 2 μm, suggesting the formation of highly uniform microspheres with a controlled particle size distribution.

Table 1. Numerical particle size distribution data of the seed latex particles obtained by laser diffraction analysis (Malvern Mastersizer Hydro 2000MU).

Particle size (μm)	Volume (%)
0.700	0
1.096	1.4
1.259	1.87
1.445	5.41
1.660	21.85
1.905	34.48
2.188	25.31
2.512	7.31
2.884	2.37
5.000	0

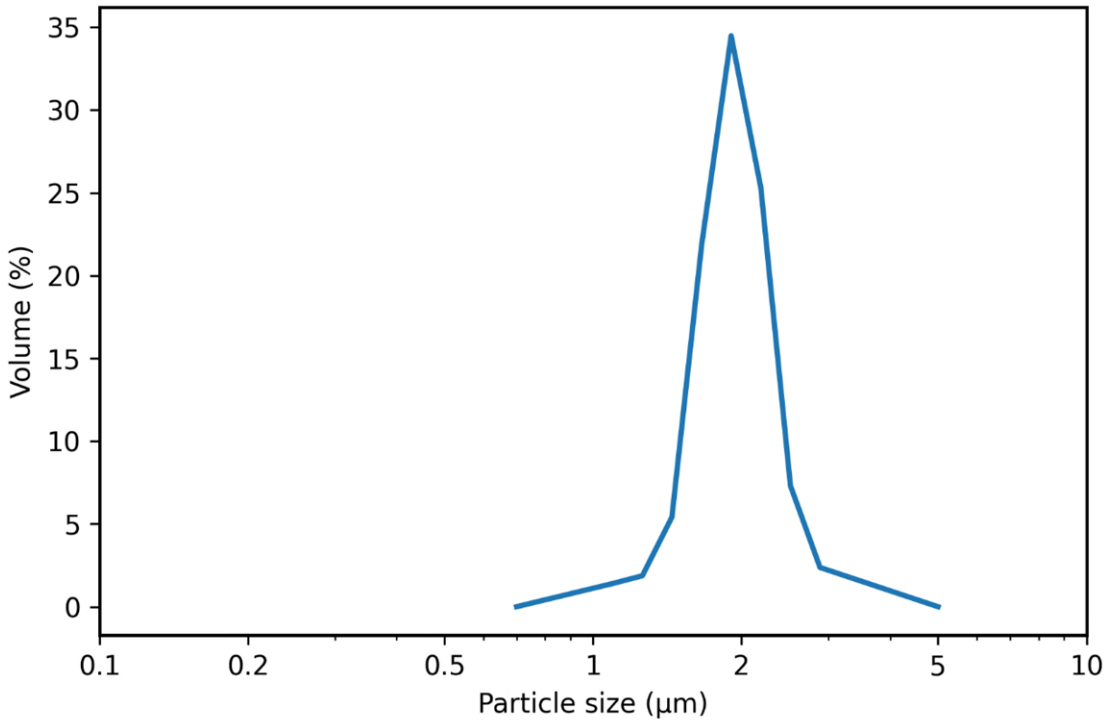


Figure 2. Particle size distribution of the seed latex particles determined by laser diffraction (Malvern Mastersizer Hydro 2000MU).

Using the particle size distribution data and the equations given in the experimental section, the number-average particle diameter (dn) of the seed latex was calculated as 1.94 µm, while the coefficient of variation (CV) was determined as 0.17. These values confirm the narrow particle size distribution and the highly monodisperse nature of the seed latex particles.

The presence of monodisperse seed particles is essential for obtaining monodisperse macroporous particles during the subsequent swelling and repolymerization steps.

3.2 Poly(GDMA-co-GDGDA) Particle Formation

The morphology of the synthesized poly(GDMA-co-GDGDA) particles was investigated by scanning electron microscopy. SEM images of the particles are presented in Figure 3. The particles maintained their spherical morphology after the swelling and repolymerization processes and displayed a relatively uniform particle size distribution.

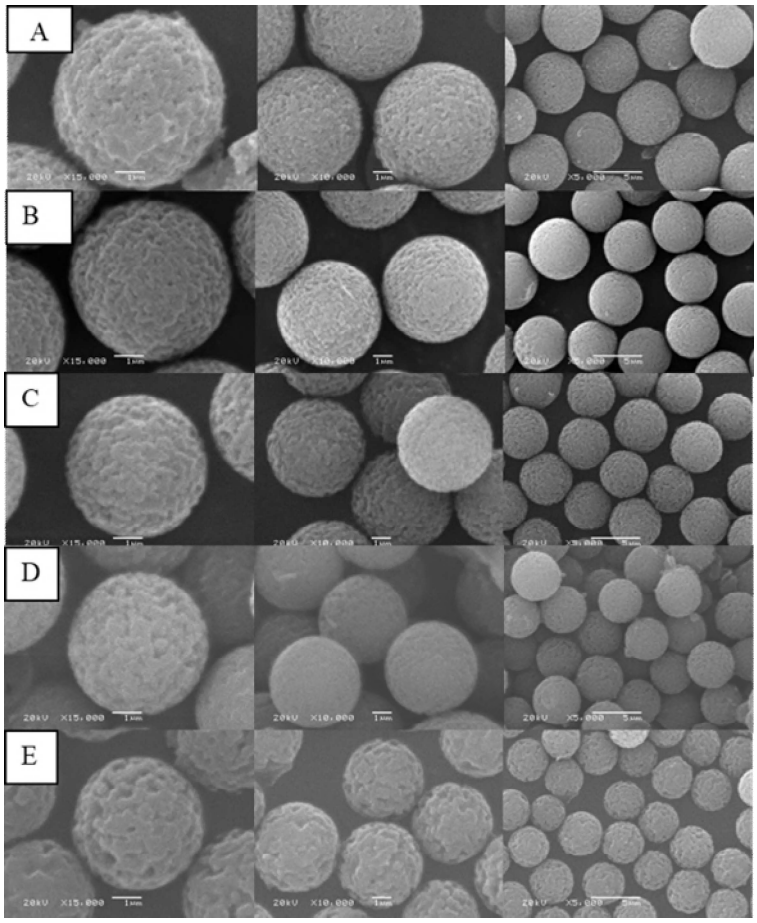


Figure 3. Scanning electron micrographs of poly(GDMA-co-GDGDA) particles prepared with different seed latex-to-monomer ratios (g mL^{-1}): (A) 0.04, (B) 0.08, (C) 0.12, (D) 0.16, and (E) 0.20. Magnifications: 15000×, 10000×, and 5000× (from left to right).

SEM observations revealed that the average particle diameter increased to approximately 6 μm after the polymerization stage. This increase in particle size is attributed to the diffusion of the monomer mixture into the seed latex particles during the swelling step, followed by polymerization inside the particles.

Particle size distribution analysis of the synthesized particles was also performed using the Malvern Mastersizer system. The obtained distribution profile is presented in Figure 4, and the corresponding numerical values are summarized in Table 2. The results indicate that the particles are mainly distributed in the range of 4–8 μm, with a maximum around 6 μm.

Table 2. Numerical particle size distribution data of the synthesized poly(GDMA-co-GDGDA) particles obtained by laser diffraction analysis (Malvern Mastersizer), corresponding to the distribution profile shown in Figure 4.

Particle size (µm)	Volume (%)
2.500	1.8
4.000	3.01
5.012	5.58
5.754	25.56
6.607	32.54
7.586	18.5
8.710	6.14
10.000	3.68
11.482	1.88
13.000	1.31

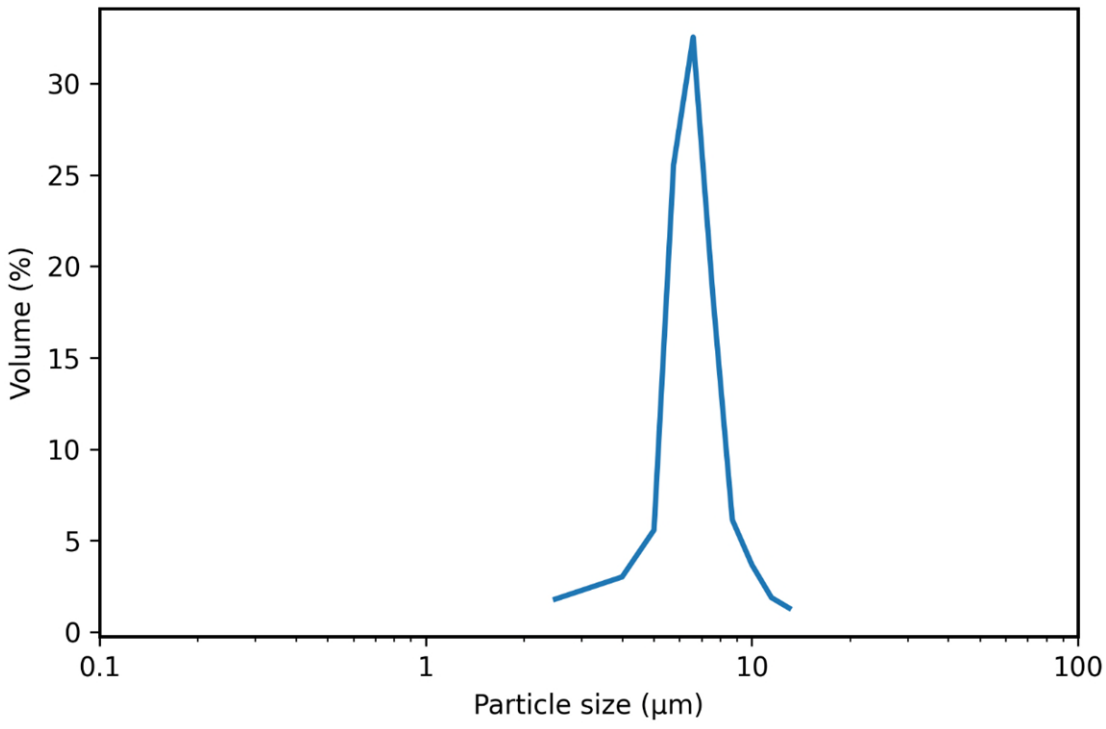


Figure 4. Particle size distribution of the synthesized poly(GDMA-co-GDGDA) particles measured by laser diffraction (Malvern Mastersizer), demonstrating a size distribution predominantly in the range of 4–8 µm with a peak centered at approximately 6 µm.

The calculated **number-average particle diameter** of the final particles was **6.75 µm**, while the **coefficient of variation (CV)** was **0.25**. These results indicate that the particles retained a relatively narrow size distribution even after the swelling and repolymerization process.

3.3 Effect of Seed Latex / Monomer Ratio

The morphology of the poly(GDMA-co-GDGDA) particles prepared at different seed latex-to-monomer ratios was examined by scanning electron microscopy (SEM). The corresponding micrographs are presented in Figure 3. In all cases, the obtained particles exhibit a highly spherical morphology with a relatively narrow particle size distribution, indicating that the seeded microsuspension polymerization method provides good control over particle formation. However, slight variations in the surface morphology can be observed depending on the seed latex-to-monomer ratio. At lower ratios, the particles exhibit relatively smoother surfaces, whereas at higher ratios the particle surfaces become slightly rougher, suggesting the development of a more pronounced porous structure within the polymer matrix.

The average particle size and surface area values of the poly(GDMA-co-GDGDA) particles prepared at different seed latex-to-monomer ratios are summarized in Table 3. As shown in the table, both the average particle diameter (Dp) and the specific surface area (SBET) decrease gradually with increasing seed latex-to-monomer ratio. The average particle size decreases from 6.75 µm to 4.82 µm as the seed latex-to-monomer ratio increases from 0.04 to 0.20 g mL^{-1}. A similar trend is observed for the surface area values, which decrease from 20.82 to 17.92 m^2 g^{-1}.

Table 3. Properties of poly(GDMA-co-GDGDA) particles prepared with different seed latex-to-monomer ratios.

SL/M (g mL^{-1})	Dp (µm)	SBET (m^2 g^{-1})
0.04	6.75	20.82
0.08	6.22	20.05
0.12	5.66	19.26
0.16	5.24	18.47
0.20	4.82	17.92

The results presented in Table 3 clearly demonstrate that the seed latex-to-monomer ratio has a significant influence on both the particle size and the specific surface area of the resulting poly(GDMA-co-GDGDA) particles. As the seed latex-to-monomer ratio increases, the average particle diameter gradually decreases. This behavior can be attributed to the increasing number of seed particles present in the system while the total amount of monomer mixture remains constant. Consequently, the amount of monomer available for each individual seed particle during the swelling stages becomes smaller.

As a result, the degree of swelling of the seed particles and the subsequent particle growth during the repolymerization stage are limited, leading to smaller final particle sizes. A similar tendency is observed for the specific surface area values. The slight decrease in SBET with increasing seed latex-to-monomer ratio can be attributed to the reduced amount of monomer mixture diffusing into each seed particle and the corresponding decrease in pore development within the polymer matrix. These findings demonstrate that the seed latex-to-monomer ratio is an important parameter for controlling both the particle size and the porous structure of the resulting poly(GDMA-co-GDGDA) particles.

Such behavior is consistent with previous studies on seeded polymerization systems, where the monomer-to-seed particle ratio plays a key role in determining particle growth and internal porosity development [24-26].

To evaluate the flow characteristics of the packed columns, chromatographic columns filled with poly(GDMA-co-GDGDA) particles synthesized at different seed latex-to-monomer (SL/M) ratios were first examined by measuring the relationship between the mobile phase flow rate and the corresponding column back pressure.

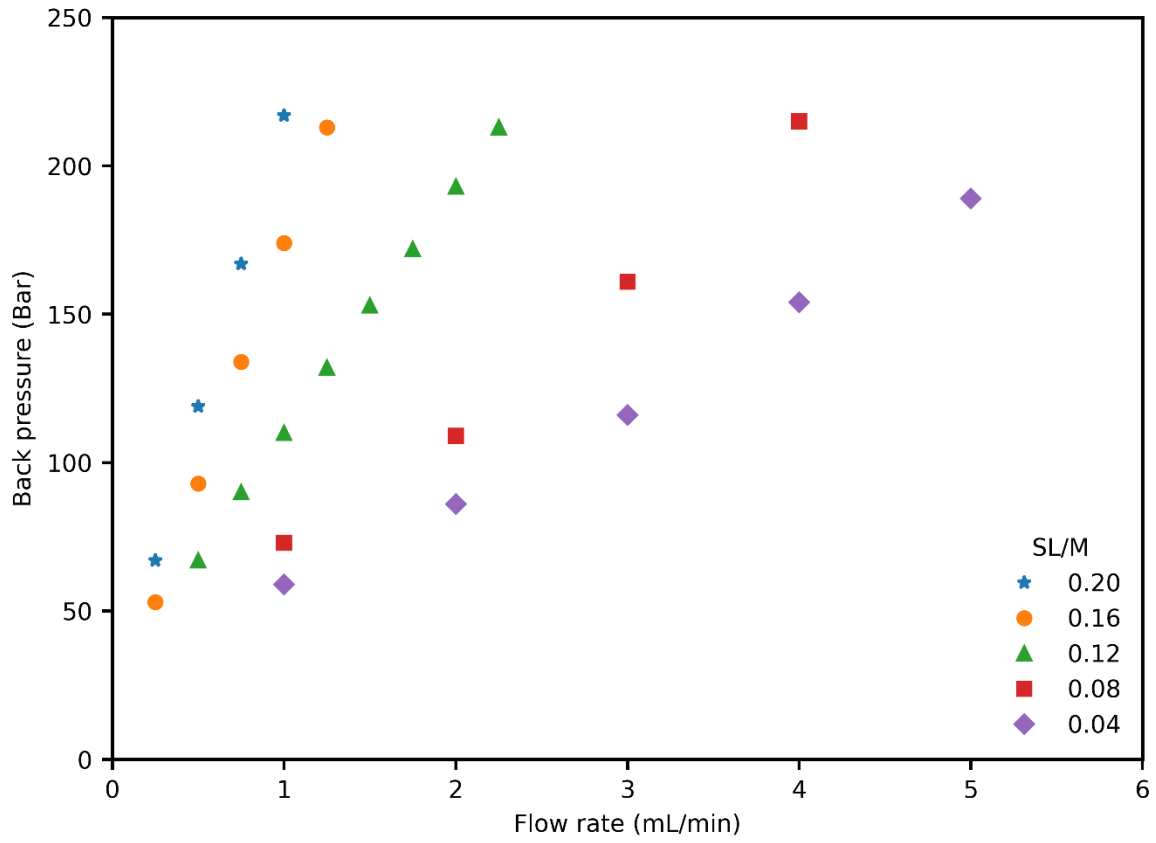


Figure 5. Column back pressure as a function of mobile phase flow rate for columns packed with poly(GDMA-co-GDGDA) microspheres prepared at different seed latex-to-monomer (SL/M) ratios. Column: 4.6 × 150 mm; mobile phase: distilled water.

The relationship between the column back pressure and the flow rate of the mobile phase was investigated to evaluate the permeability and mechanical stability of the packed column. As shown in Figure 5, the column back pressure increased linearly with increasing flow rate. Such linear behavior is typical for well-packed chromatographic columns and indicates low flow resistance of the stationary phase. The linear increase in back pressure with increasing flow rate is consistent with Darcy's law and suggests good permeability and homogeneous packing of the P(GDMA-co-GDGDA) microparticles within the column. In addition, the absence of any abrupt pressure increase indicates that the synthesized microparticles possess sufficient mechanical strength to withstand the applied chromatographic conditions without significant deformation or compression. Therefore, an SL/M ratio of 0.12 was selected as the optimum condition, since it provides a relatively high surface area while keeping the column back pressure within a suitable operational range.

The porous structure of the synthesized particles was evaluated by BET analysis. The specific surface area (SBET) was determined as 15.17 $m^2\ g^{-1}$, while the total pore volume (Vtotal) was found to be 0.124 $cm^3\ g^{-1}$ under optimized conditions corresponding to an SL/M ratio of 0.12. The combination of moderate surface area and appreciable pore volume indicates the formation of a well-developed porous structure, which is expected to facilitate efficient analyte diffusion and contribute positively to chromatographic performance. The pore volume was calculated from nitrogen adsorption data at a relative pressure (P/P_0) close to unity. These results confirm that the synthesized poly(GDMA-co-GDGDA) particles possess a well-developed porous structure that facilitates efficient analyte diffusion and is suitable for chromatographic applications.

3.4 Evaluation of Surface Functionalization

The surface functionalization of poly(GDMA-co-GDGDA) particles was carried out via a two-step modification involving azide formation followed by azide–alkyne cycloaddition with propiolic acid (Scheme 4). This modification introduces polar functional groups, including triazole rings, onto the particle surface. The presence of these functionalities enhances the hydrophilicity of the stationary phase and promotes the formation of a water-enriched layer, which is essential for HILIC-type separation mechanisms. This modification plays a key role in improving the interaction between the stationary phase and analytes.

SEM images of P(GDMA-co-GDGDA), azide-functionalized P(GDMA-co-GDGDA), and propiolic-acid-modified P(GDMA-co-GDGDA) microparticles are presented in Figure 6.

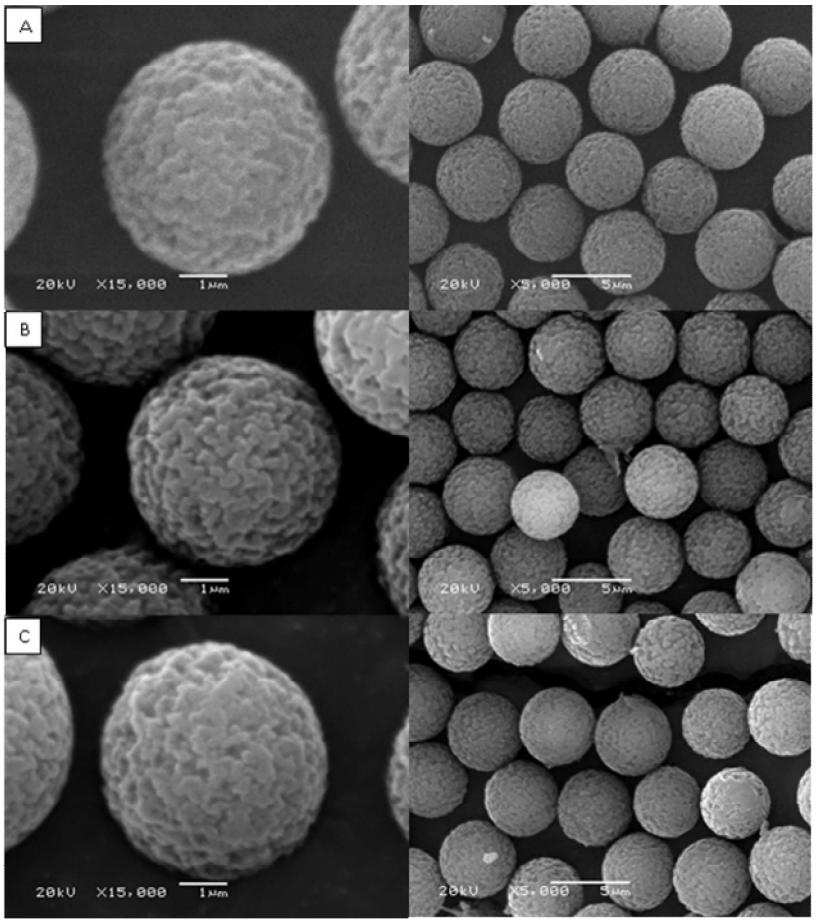


Figure 6. Scanning electron micrographs of the synthesized microparticles: (A) P(GDMA-co-GDGDA); (B) azide-functionalized P(GDMA-co-GDGDA); and (C) propiolic-acid-modified P(GDMA-co-GDGDA).

3.5 Chromatographic Performance of the propiolic-acid-modified P(GDMA-co-GDGDA) particles

The chromatographic behavior was investigated using mobile phases with different acetonitrile/water (ACN/H_2O) ratios to evaluate the effect of mobile phase composition. In these experiments, the flow rate was kept constant at 1 mL min^{-1}. The chromatograms obtained under these conditions are presented in Figure 7.

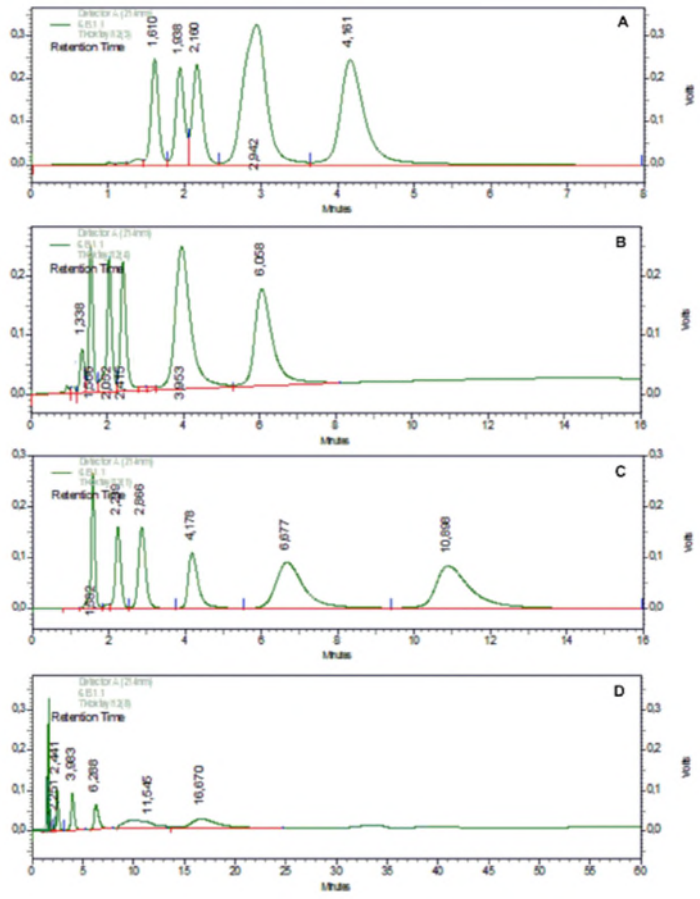


Figure 7. Separation behavior of propiolic-acid-attached poly(GDMA-co-GDGDA) microparticles at different acetonitrile concentrations under isocratic conditions for the separation of analytes with different polarities. Conditions: column size, 4.6 × 150 mm; pH 7; room temperature (25 °C); flow rate, 1 mL min^{-1}; UV detection at 254 nm. Elution order: (1) toluene, (2) acrylamide, (3) thymine, (4) thiourea, (5) inosine, (6) cytosine. Mobile phase (ACN/H_2O, v/v): (A) 80/20; (B) 85/15; (C) 90/10; (D) 95/5.

As the acetonitrile content in the mobile phase increased, the retention of polar analytes increased due to stronger hydrophilic interactions between the analytes and the hydrophilic stationary phase. This behavior is typical for HILIC, where a water-enriched layer forms on the hydrophilic stationary phase surface and polar analytes partition into this layer through hydrogen bonding and dipole–dipole interactions [27-29]. In contrast, the nonpolar compound toluene showed little or no retention and eluted close to the void volume of the column, which is characteristic behavior in HILIC systems. The observed elution order (toluene < acrylamide < thymine < thiourea < inosine < cytosine) reflects the increasing polarity of the analytes and confirms the hydrophilic interaction mechanism of the stationary phase. Among the analytes, cytosine exhibited the strongest retention, which can be attributed to its higher polarity and the presence of multiple hydrogen-bonding functional groups that enhance its interaction with the hydrophilic stationary phase. The good separation performance further supports the presence of an accessible porous structure and effective surface functionalization.

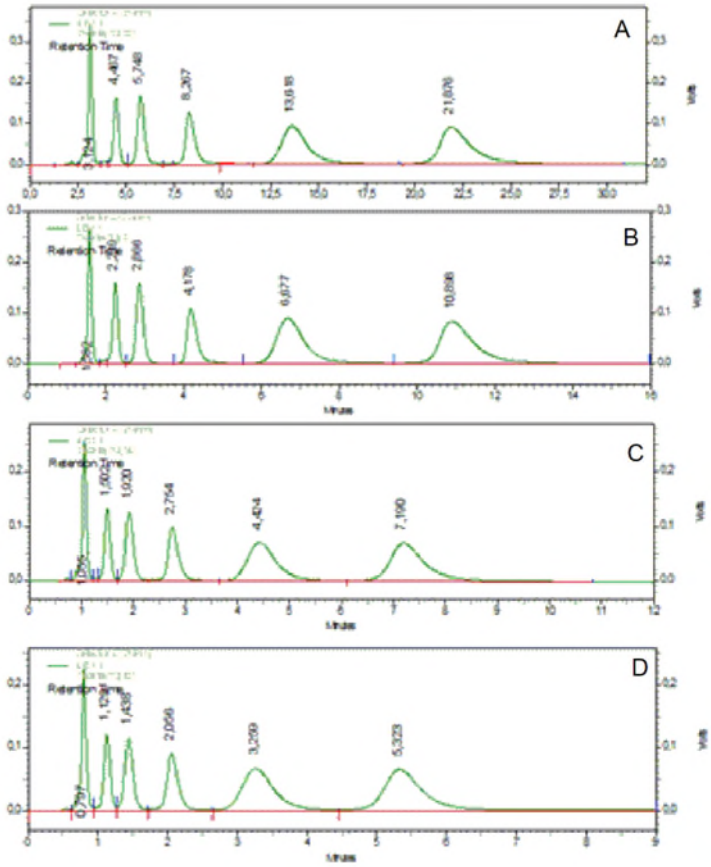


Figure 8. Liquid chromatograms obtained from isocratic separation of polar analytes on a 150 × 4.6 mm i.d. column packed with propiolic-acid-modified poly(GDMA-co-GDGDA) particles. Chromatographic conditions: ACN/H_2O ratio (v/v) 90/10; UV detection at 254 nm; pH 7; T = 25 °C. Order of peaks: 1. Toluene, 2. Acrylamide, 3. Thymine, 4. Thiourea, 5. Inosine, 6. Cytosine. Flow rate (mL min^{-1}): A: 0.5; B: 1.0; C: 1.5; D: 2.0.

The effect of the flow rate on the chromatographic separation of the analytes was investigated, and the resulting chromatograms are presented in Figure 8. As the flow rate increased from 0.5 to 2.0 mL min^{-1}, the retention times of all analytes decreased, which is expected due to the reduced interaction time between the analytes and the stationary phase. Despite the increase in flow rate, the separation of the analytes was maintained, indicating that the prepared stationary phase provides sufficient interaction with the analytes even under relatively high flow conditions. These results demonstrate that the packed column exhibits stable chromatographic performance over a wide range of flow rates.

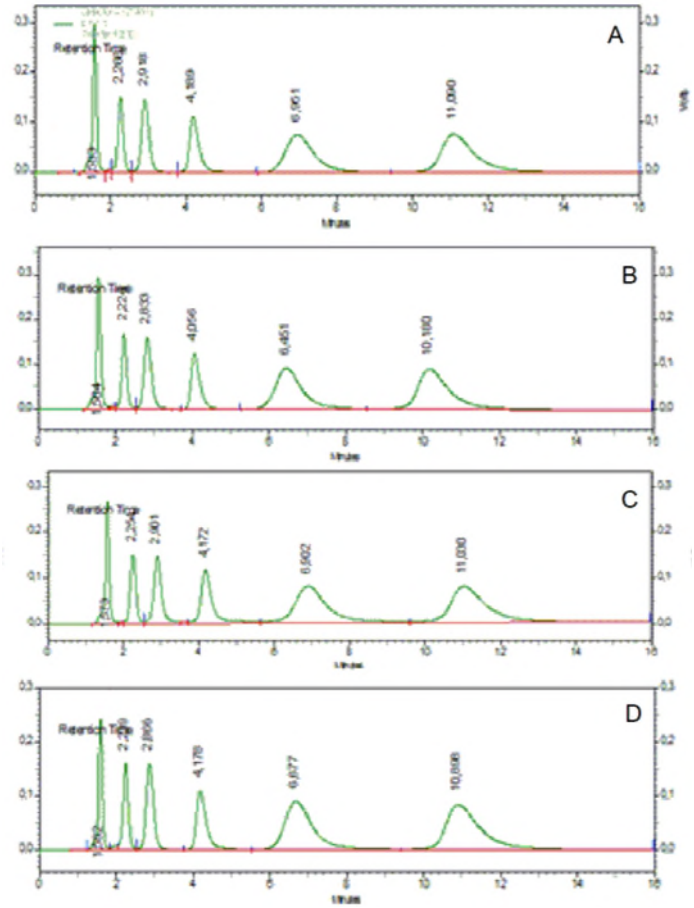


Figure 9. Liquid chromatograms obtained from isocratic separation of polar analytes on a 150 × 4.6 mm i.d. column packed with propiolic-acid-functionalized poly(GDMA-co-GDGDA) particles. Chromatographic conditions: ACN/H_2O ratio (v/v) 90/10; UV detection at 254 nm; flow rate 1 mL min^{-1}; T = 25 °C. Order of peaks: 1. Toluene, 2. Acrylamide, 3. Thymine, 4. Thiourea, 5. Inosine, 6. Cytosine. pH: A: 3; B: 4; C: 5; D: 7.

The effect of mobile phase pH on the chromatographic behavior of the analytes was also investigated, and the corresponding chromatograms are shown in Figure 9. Changes in pH can influence the ionization state of the analytes and the stationary phase, thereby affecting their interactions. As the pH of the mobile phase increased from 3 to 7, slight changes in retention times were observed for the analytes, particularly for the more polar compounds. This behavior can be attributed to variations in hydrogen bonding and polar interactions between the analytes and the hydrophilic stationary phase. Overall, the stationary phase maintained stable separation performance over the investigated pH range.

3.6 Column Efficiency and Van Deemter Analysis

The variation of theoretical plate number with acetonitrile concentration is presented in Figure 10. The results clearly show that the column efficiency reaches a maximum around 90% acetonitrile, indicating that this composition represents the optimum operating condition for the column.

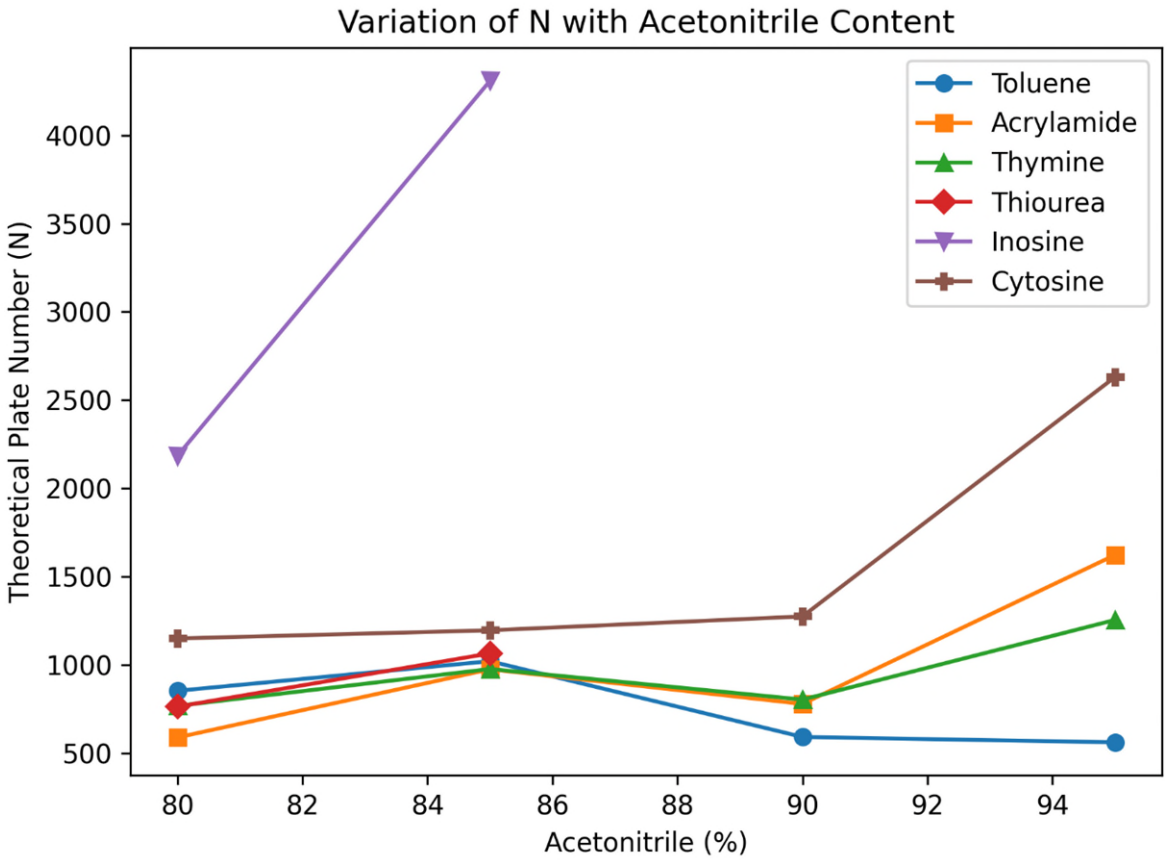


Figure 10. Variation of theoretical plate number with acetonitrile content in the mobile phase for the propiolic-acid-functionalized poly(GDMA-co-GDGDA) column.

The effect of mobile phase flow rate on chromatographic efficiency was investigated by varying the flow rate between 0.5 and 2 mL min^{-1}.

The results show that column efficiency strongly depends on the mobile phase flow rate. As the flow rate increased from 0.5 to 1 mL min^{-1}, the resolution values increased significantly, indicating improved chromatographic efficiency.

The highest resolution values were generally obtained at 1 mL min^{-1}, suggesting that this flow rate represents the optimum operating condition for the column.

At higher flow rates (1.5–2 mL min^{-1}), a decrease in resolution was observed. This behavior can be attributed to insufficient interaction time between the analytes and the stationary phase at higher mobile phase velocities.

The variation of reduced plate height with mobile phase flow rate is presented in Figure 11, indicating that the column exhibits a typical chromatographic efficiency profile.

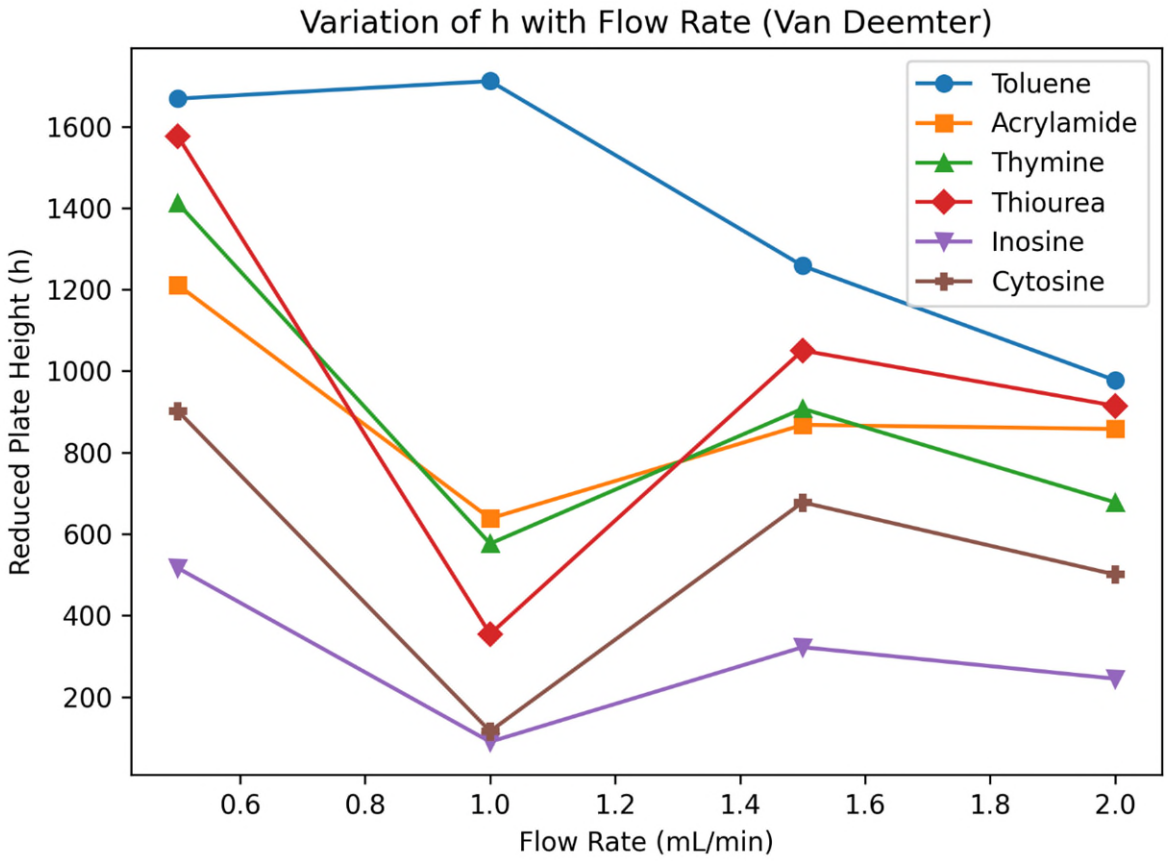


Figure 11. Variation of reduced plate height with mobile phase flow rate (Van Deemter plot) for the poly(GDMA-co-GDGDA)NaN_3-propiolic acid column.

The influence of mobile phase pH on chromatographic performance was also investigated. The results indicate that the separation efficiency is influenced by the pH of the mobile phase. At lower pH values (pH 3–5), relatively lower resolution values were obtained for most analyte pairs.

When the pH was increased to pH 7, a noticeable improvement in resolution was observed. This behavior indicates that the interaction between the analytes and the stationary phase becomes more favorable under near-neutral pH conditions.

The variation of theoretical plate number with pH is presented in Figure 12, showing that the highest column efficiency was obtained at pH 7.

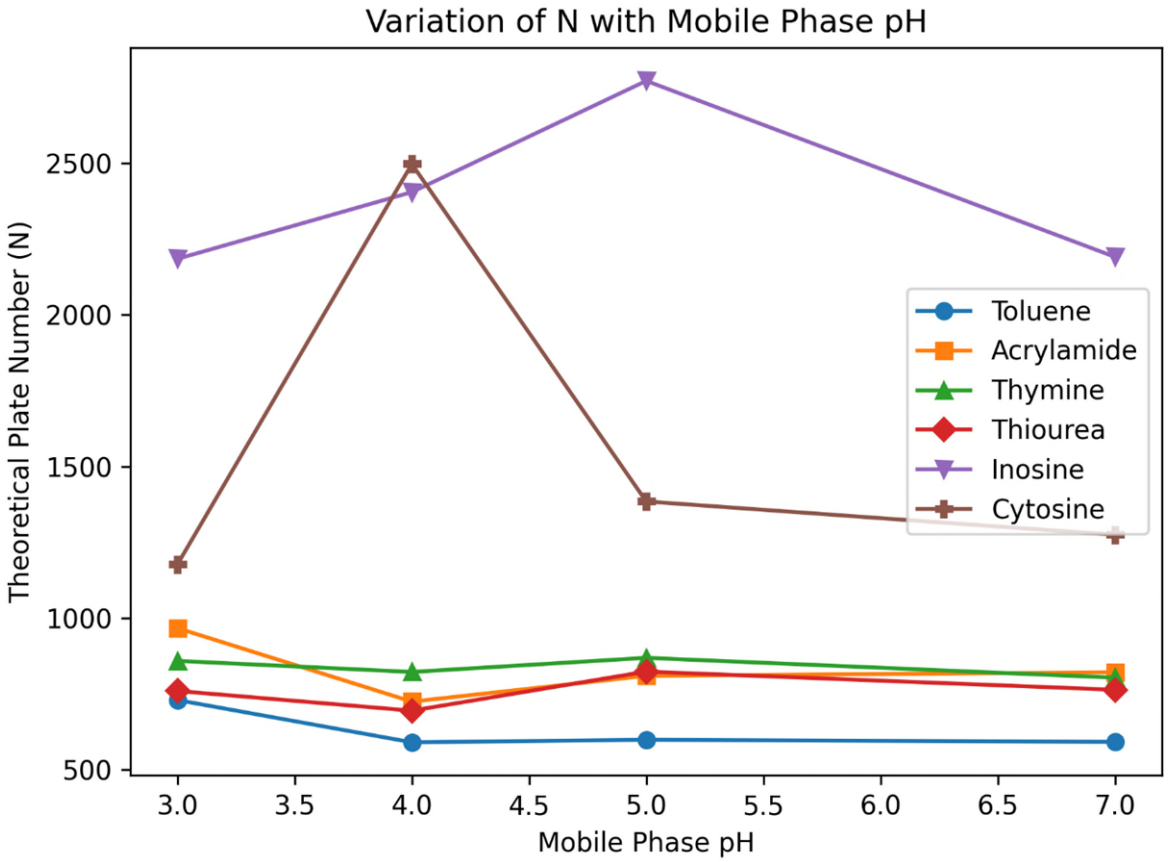


Figure 12. Variation of theoretical plate number with mobile phase pH.

Column efficiency was further evaluated by examining the variation of reduced plate height with the mobile phase flow rate. The relationship between band broadening and linear velocity in chromatographic columns is described by the Van Deemter equation (Eq. 1) [30].

$$H = A + \frac{B}{u} + Cu \qquad (1)$$

where H is the height equivalent to a theoretical plate, u is the linear velocity of the mobile phase, A represents eddy diffusion, B/u represents longitudinal diffusion, and

Cu represents resistance to mass transfer in both stationary and mobile phases. This relationship is widely used to describe efficiency behavior in liquid chromatography columns.

In order to compare column performance independently of particle size, the reduced plate height (h) was calculated using Eq. 2.

$$h = \frac{H}{d_p} \qquad (2)$$

Where H is the plate height and d_p is the particle diameter.

The variation of reduced plate height with mobile phase flow rate for the poly(GDMA-co-GDGDA)NaN_3-propiolic acid column is shown in Figure 13.

The results indicate that column efficiency strongly depends on the mobile phase flow rate. When the flow rate increased from 0.5 to 1 mL min^{-1}, the reduced plate height decreased significantly, indicating improved mass transfer and reduced band broadening.

The minimum reduced plate height was observed at approximately 1 mL min^{-1}, indicating that this flow rate corresponds to the optimum operating condition for the column.

At higher flow rates (1.5–2 mL min^{-1}), the reduced plate height increased again due to increasing mass-transfer resistance in the stationary phase. Under these conditions, analyte molecules do not have sufficient time to equilibrate between the stationary and mobile phases, resulting in peak broadening.

The observed Van Deemter profile therefore shows a typical U-shaped behavior, which is characteristic of efficient chromatographic columns packed with porous polymeric particles. Similar reduced plate height values have been reported for monodisperse polymer-based stationary phases used in HPLC columns [27].

These results confirm that the synthesized particles exhibit favorable packing characteristics and efficient mass-transfer properties.

The relatively low reduced plate height obtained in this study indicates that the prepared monodisperse polymer particles provide efficient chromatographic performance.

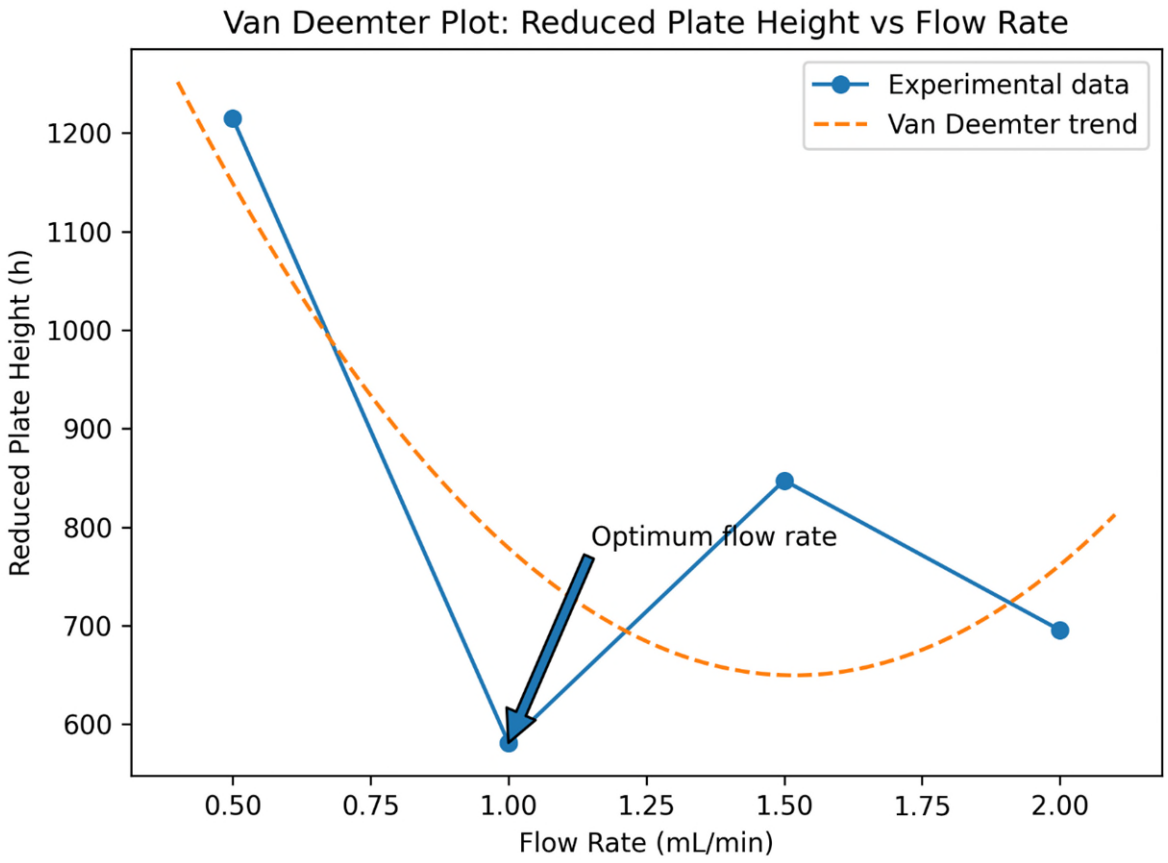


Figure 13. Van Deemter plot showing the variation of reduced plate height (h) with mobile phase flow rate for the poly(GDMA-co-GDGDA)NaN_3-propiolic acid column. The minimum plate height is observed at approximately 1 mL min^{-1}, indicating the optimum operating flow rate.

3.7 Separation Mechanism of the Stationary Phase

The chromatographic behavior observed in this study suggests that multiple interaction mechanisms contribute to the separation process on the poly(GDMA-co-GDGDA)NaN_3-propiolic acid stationary phase.

The stationary phase contains triazole rings formed via azide–alkyne cycloaddition, together with polar oxygen-containing functional groups originating from the glycidyl methacrylate backbone. These functional groups provide several possible interaction sites for analytes and may participate in hydrogen bonding, dipole–dipole interactions, and π-related interactions, depending on the chemical structure of the analytes.

The influence of mobile phase composition provides important insight into the separation mechanism. The observation that the highest column efficiency was obtained at approximately 90% acetonitrile suggests that the stationary phase exhibits behavior similar to hydrophilic interaction chromatography (HILIC) systems. In highly organic mobile phases, a water-enriched layer forms on the surface of polar stationary phases, allowing analytes to partition between this immobilized aqueous layer and the organic mobile phase. Such partition-based retention mechanisms are characteristic of HILIC systems [27,29].

In addition to hydrophilic partitioning, specific interactions between analytes and the stationary phase may also contribute to retention. Aromatic or heterocyclic analytes such as nucleobase derivatives can interact with the triazole moieties through hydrogen bonding and π-related interactions, which may enhance chromatographic selectivity [29].

The influence of mobile phase pH further supports the presence of multiple interaction mechanisms. Changes in pH may modify the ionization state of both analytes and functional groups present on the stationary phase surface, affecting electrostatic interactions as well as hydrogen bonding.

Overall, the separation mechanism of the poly(GDMA-co-GDGDA)NaN_3-propiolic acid stationary phase appears to involve a combination of hydrophilic interaction, hydrogen bonding, and specific polar interactions, which together contribute to the observed chromatographic selectivity and column efficiency.

4. Conclusion

In this study, monodisperse poly(GMA) seed latex particles were successfully synthesized and employed as templates for the preparation of macroporous poly(GDMA-co-GDGDA) microparticles via a multistage swelling and repolymerization approach. The resulting particles exhibited well-defined spherical morphology, controlled particle size distribution, and a developed porous structure suitable for chromatographic applications.

Subsequent surface functionalization through azide introduction followed by copper(I)-catalyzed azide–alkyne cycloaddition enabled the incorporation of propiolic acid onto the particle surface via stable triazole linkages. This modification significantly enhanced the surface polarity and facilitated the formation of a hydrophilic interfacial layer, which is essential for HILIC-type separation.

The chromatographic evaluation demonstrated that the prepared stationary phase provides effective separation of polar analytes, with retention behavior strongly dependent on the mobile phase composition. Increased acetonitrile content led to enhanced retention, consistent with the characteristic mechanism of hydrophilic interaction chromatography. In addition, the column exhibited stable performance over a broad range of flow rates and pH conditions, indicating good mechanical stability and packing homogeneity.

Van Deemter analysis further confirmed that the column operates with high efficiency, showing a typical U-shaped profile and an optimum flow rate around 1 mL min^{-1}. The relatively low reduced plate height values obtained in this study indicate favorable mass transfer characteristics and efficient column packing.

Overall, the results demonstrate that propiolic-acid-functionalized poly(GDMA-co-GDGDA) microparticles represent a promising alternative to conventional stationary phases for HILIC applications. The combination of controlled particle morphology, tunable porosity, and versatile surface functionalization via click chemistry provides a flexible platform for the design of advanced polymer-based chromatographic materials. This study contributes to the development of functional polymeric stationary phases and offers new opportunities for tailoring separation performance in HILIC systems.

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Author Contributions

M. Solener: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision.

A.S. Satilmis: Methodology, Formal analysis, Investigation, Validation, Data curation.

All authors have read and agreed to the published version of the manuscript.

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