

Preparation of Silica-Phenyl Modified Methacrylate Polymer as Column Particles for Separation of Paracetamol and Caffeine

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Abstract

Silica-based column particles have been widely developed, but their particle size remains very small and heterogeneous, typically less than 3 μm . This small and inconsistent size poses challenges for interacting with the mobile phase during the separation of specific components due to the instability of the column particles. To address this issue, a method was developed using a methacrylate-based polymer core coated with silica and phenyl groups. The goal was to test column particles with a polymer core as the base, expecting improved performance compared to silica cores, with a target particle size $>3 \mu\text{m}$. This study evaluated the new method for separating paracetamol and caffeine, achieving excellent separation results (Rs 3.86). The method was optimized using a mobile phase consisting of methanol and water (60:40), a flow rate of 1.0 mL/min, and a wavelength of 274 nm. A single injection resulted in peak 1 for paracetamol and peak 2 for caffeine. The success of this method is evidenced by the production of sphere, homogeneous, with an average size of 3.17 μm . This analytical method has been effectively applied to the separation of paracetamol and caffeine, demonstrating its suitability for continuous use in drug separation validation.

Keywords: *particle column, liquid chromatography, mma-co-dvb, phenyl modification*

Abstrak

Partikel kolom berbasis silika telah banyak dikembangkan, tetapi ukuran partikelnya masih sangat kecil dan heterogen, biasanya kurang dari 3 μm . Ukuran yang kecil dan tidak konsisten ini menimbulkan tantangan untuk berinteraksi dengan fase gerak selama pemisahan komponen tertentu karena ketidakstabilan partikel kolom. Untuk mengatasi masalah ini, sebuah metode dikembangkan dengan menggunakan inti polimer berbasis metakrilat yang dilapisi dengan silika dan gugus fenil. Tujuannya adalah untuk menguji partikel kolom dengan inti polimer sebagai dasar, dengan harapan peningkatan kinerja dibandingkan dengan inti silika, dengan target ukuran partikel $>3 \mu\text{m}$. Penelitian ini mengevaluasi metode baru untuk memisahkan parasetamol dan kafein, dengan hasil pemisahan yang sangat baik (Rs 3,86). Metode ini dioptimalkan dengan menggunakan fase gerak yang terdiri dari metanol dan air (60:40), laju alir 1,0 mL/menit, dan panjang gelombang 274 nm. Injeksi tunggal menghasilkan puncak 1 untuk parasetamol dan puncak 2 untuk kafein. Keberhasilan metode ini dibuktikan dengan dihasilkannya sphere yang homogen dengan ukuran rata-rata 3,17 μm . Metode analisis ini telah diterapkan secara efektif pada pemisahan parasetamol dan kafein, yang menunjukkan kesesuaiannya untuk digunakan secara terus menerus dalam validasi pemisahan obat.

Kata kunci: *kolom partikel, kromatografi cair, mma-co-dvb, modifikasi fenil*

1. Introduction

The development of silica-based stationary phases has advanced significantly, with various modifications to the polymer. However, creating silica particles smaller than 3 μm remains challenging. In contrast, the development of polymer cores, particularly those coated with silica, is still limited. The compatibility of particles packed as stationary phases greatly affects the performance of liquid chromatography columns. Polymer cores have covalent bonds between monomers, and crosslinking is strengthened by free radicals during the polymerization process. It is possible to manufacture these polymer cores to larger particle sizes, potentially exceeding 3 μm .

Exploring the potential of polymer cores to achieve larger and more stable particle sizes, which can then be coated with silica and interact with other components, is promising. Polymer synthesis using solvothermal methods, which are widely used for nanomaterials and composite materials with controlled structures, involves high temperatures and pressures in certain organic solvents[1].

The preparation of polymer synthesis is crucial in the development of macroporous materials for liquid chromatography. Macroporous systems are well-designed with interconnected pores to facilitate the separation process. Poly(MMA-co-DVB) was synthesized through free radical polymerization using the solvothermal method. This synthesis process involves radical suspension polymerization with an appropriate composition of monomers and porogenic organic solvents to produce macroporous polymers in the form of 3D spherical spheres [2]. The Bourgeat-Lami method prepared silica-poly(styrene) core-shell microspheres (SiO_2 -PS). Asher's method successfully synthesized monodisperse SiO_2 -polymer core-shell spheres at submicrometer sizes through dispersion polymerization, though this method is still limited to the nanoscale. In research conducted by [3] monodisperse porous polymer particles were synthesized by mixing glycidyl methacrylate (GMA) with ethylene dimethacrylate (EDMA) crosslinks and adding diphenylethylene (DPE) to polystyrene seeds to control the shape and size of the resulting polymer particles, optimizing them for efficient chromatography. carried out microsphere polymerization using P(MMA-AA-DVB) as an initial attempt to form a spherical core, which can then be modified with other materials [4].

The modified stationary phase aims to achieve high charge density and increased column efficiency [5]. Column modification produces ordered pores that affect the diffusion or flow of the mobile phase. Introducing functional groups into the nonpolar copolymer structure can significantly alter selectivity. Any chemical modification affects the porous structure of the sorbent, so understanding the sorbent's structure is crucial [6]. Silica synthesis is time-consuming due to its amorphous nature and heterogeneous surface, making it challenging to achieve the desired properties [7]. Therefore, polymer synthesis is a more practical alternative to silica cores. It offers a simpler and more efficient process, allowing for the production of larger particle sizes. In this approach, the silica component is used as a coating on the polymer core and binds directly to other groups during modification.

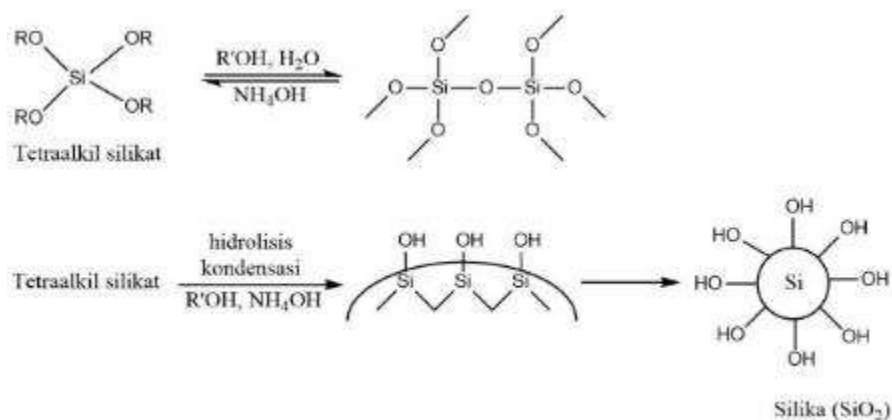


Fig.1: Silica Polymer Structure through the Stober Process [8].

In this study, we developed a polymerization-based synthesis method to create copolymer microsphere cores with mono-crosslinked polymer structures and narrow dispersions/pores for good porosity. The microspheres were then coated with silica and modified to enhance binding interactions with phenyl groups. The resulting materials were characterized using diffuse reflectance spectroscopy (DRS-FTIR) and scanning electron microscopy (SEM). It is anticipated that this method will produce microspheres in the micrometer range, comparable to commercial columns. The synthesized material was packed into a column and used for the separation of paracetamol and caffeine via high-performance liquid chromatography (HPLC). The method was optimized, and the separation was assessed by identifying the single components in peaks 1 and 2.

2. Material and Methods

The materials used in this study include: mineral-free water, tetraethyl orthosilicate (TEOS, Merck), phenyltrimethoxysilane (PTES, Merck), methanol (Merck), acetonitrile (p.a., Merck), toluene (Merck), ammonia (Merck), methyl methacrylate (MMA, Sigma Aldrich), divinylbenzene (DVB, Sigma Aldrich), benzoyl peroxide (BPO, Merck), and standard paracetamol and caffeine (10–100 ppm).

Instrumentations used in this research are scanning electron microscopy (SEM, SU3500), Fourier Transform Infrared (FTIR) Spectrophotometer (Shimadzu IR Prestige 21), Diffuse Reflectance Spectroscopy (DRS) attachment, a column with dimensions 150 mm × 4.6 mm, high-performance liquid

chromatography (HPLC, Agilent 1260 Infinity II), a centrifuge (Centurion Scientific model Pro-Analytical C2006), and a column packing tool (GALAK GLK2000).

Methacrylate Polymer Synthesis

The method begins with the polymerization stage. First, 18.8×10^{-3} mol MMA and 16.8×10^{-3} mol DVB prepare a solution in a 250 mL duran bottle containing 1 wt % (to monomer) or 37 mg of BPO in 200 mL of acetonitrile (p.a.). Magnetic stirrer added and stir the mixture on a hotplate, heating it at 70°C for 24 hours. After this, continue stirring for an additional 20 hours, centrifuged and dried in a vacuum oven at 50°C for 3-4 hours before characterization.

Silica Modification

The solution obtained from the polymer synthesis process was then modified by adding 8 mL of TEOS. This mixture was stirred at room temperature for 8 hours, after which 10 mL of ammonia and 20 mL of water were added. Stirring continued for 20 hours at room temperature. The modified product was then centrifuged for 10 minutes at 3500 rpm, washed with methanol three times for 3 minutes each, and the precipitate was dried in a vacuum oven at 50°C for approximately 2 hours.

Phenyl Modification

For the phenyl modification, 2 grams of solids from the silica-modified polymer synthesis were placed in a 50 mL bottle containing 4 mL of PTES in 40 mL of toluene. A magnetic stirrer was added, and the mixture was stirred at room temperature for 20 hours. The product was then centrifuged for 10 minutes at 3500 rpm, washed with methanol three times for 3 minutes each, and the MPSM precipitate was dried in a vacuum oven at 50°C for approximately 2 hours..

FTIR Characterization

Characterization of all components, including elemental analysis and modification procedures, was performed by drying the samples at 100°C for 2-3 hours. Following this, the samples were characterized using a Diffuse Reflectance Attachment (DRS) FTIR spectrophotometer, with measurements taken in the wavelength range of 400-4000 cm^{-1} and a resolution of 4 cm^{-1} . The FTIR spectra of the varied silica are provided in the electronic supplementary material.

SEM Characterization

Scanning Electron Microscopy (SEM, SU3500, Hitachi, Japan) was used to analyze and identify the morphology and structure of the microspheres obtained from synthesis and modification, in order to evaluate the pore yield. For SEM analysis, a small amount of the sample was placed on carbon tape and coated with gold using an ion sputtering instrument. The gold disk was turned into plasma and used to coat the sample. The prepared sample was then inserted into the SEM chamber and vacuumed. The SEM results provided a visual representation of the modified microspheres, displaying the morphology and micro-sized details of the results. The analyzed images were saved in TIF, PNG, and PDF formats..

Column Packing

Column particle packing was prepared by multipacking: 3.5 g of solids were dissolved in 25 mL of methanol, sonicated for 10 minutes, and degassed for 5 minutes. The particles were then loaded into a homogeneous tank in the column packing device, with a pressure set to 40 MPa. During the column loading process, methanol was continuously flowed through the column until 200 mL of solvent was discarded as waste. This ensured that the solids were fully packed into the column, as indicated by the increasingly slow flow of solvent droplets. The column loading was performed using stainless steel chromatography columns (150 mm \times 4.6 mm, with 2 μm diameter particles). Once packed with microsphere solids, the column was sealed with a special filter, metal cover, and plastic caps at both ends, and then stored.

Performance Testing of Modified Columns

Columns containing modified solids that had undergone synthesis and modification were then installed into a chromatographic device. Preparatory conditions included washing the columns with 100% methanol for 15-20 minutes at a flow rate of 1.0 mL/min and heating the detector. Performance testing of the modified silica was conducted for the separation of caffeine and paracetamol at concentrations ranging from 10 to 100 ppm, as well as for mixtures of caffeine and paracetamol. Testing was performed in reverse phase gradient elution mode, with variations in the mobile phase composition (MeOH:H₂O ratios of 100:0, 90:10,

80:20, 70:30, 60:40, and 50:50), flow rate (0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 mL/min), and UV wavelength (202, 246, 250, 274, and 280 nm).

$$\text{Resolution (Rs)} = \frac{2 \times (t_{R2} - t_{R1})}{(W_2 + W_1)}$$

t_{R1} : Retention time of peak 1

t_{R2} : Retention time of peak 2

w_1 : Peak Width (area) of peak 1

w_2 : Peak Width (area) of peak 2

3. Results and Discussion

Methacrylate Polymer Synthesis

In testing the performance of modified columns, optimal conditions were achieved using MMA and DVB as monomers and crosslinkers. The optimal crosslinker was found to be 3,6-DVB, producing particles with a size equivalent to commercial columns, approximately 3 μm . Composition C also resulted in precipitation because DVB, as a crosslinking agent, can form a 3D network within the methacrylate. This network formation results in a polymer that precipitates and does not dissolve in the reaction medium or solvent used, further supported by high temperatures that enhance the success of the process. Therefore, the researcher chose polymer spheres from composition MMA-DVB it produced polymer spherical with a large and more homogeneous size range (1.98-2.02 μm

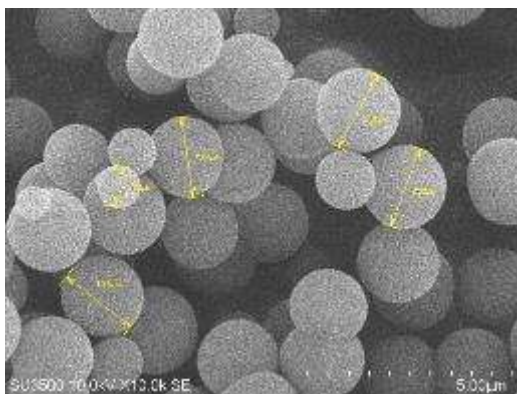


Fig. 2: SEM Characterization of MMA-DVB

Silica-Phenyl Modification

Silica was used to coat the core of the synthesized polymer, this coating was aimed at increasing the particle size and ensuring uniformity. Silica does not directly interact with the polymer but rather coats it. The coated polymer then interact with other components to enhance the stationary phase's performance during the separation process. The results of the coating are shown in **Figure 3** [9,10].

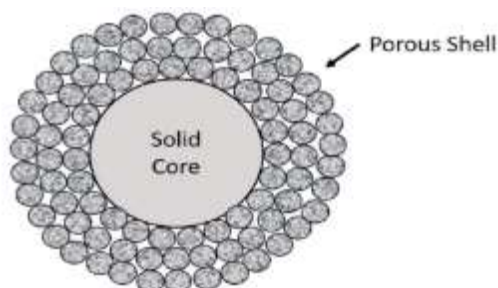


Fig. 3: Coating Results on Polymer Core

The stationary phase performance improves when the silica-coated polymer core is combined with phenyl groups. This combination lengthens the alkyl chain, leading to better interactions between the analyte and the mobile phase. As the result, peak width is reduced, and resolution during separation is enhanced [11]. Monomers that balance lipophilic and hydrophilic properties also improve the retention of polar compounds. Polar groups in hydrophilic monomers increase wettability, which enhances interaction with the functional groups of the analyte [12].

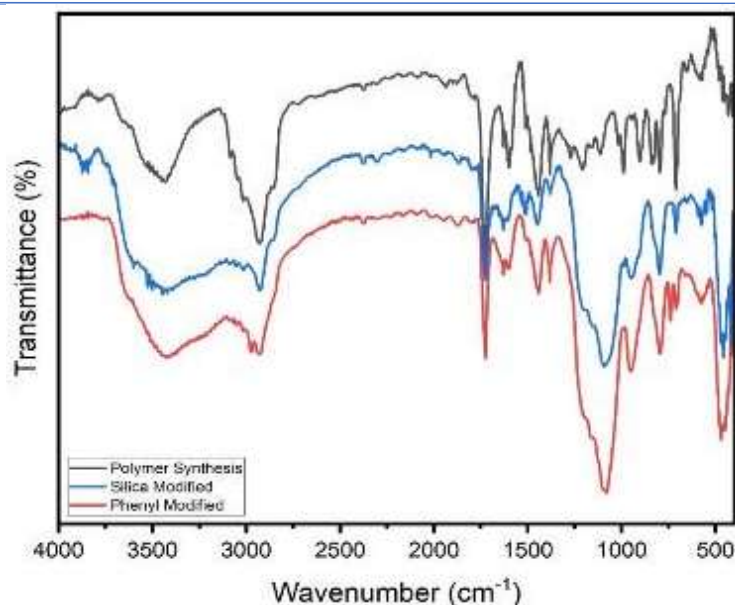


Fig. 4: FTIR of spectrum Polymer Synthesis, Silica modified, dan Phenyl modified

In the methacrylate polymer synthesis process, characteristic peaks were observed: a sharp peak at 1720 cm^{-1} corresponding to the C=O ester carbonyl group, peaks at 1638 and 816 cm^{-1} for conjugated C=C bonds, and peaks at 1198 and 1158 cm^{-1} associated with C-O-C stretching. CH_2/CH_3 groups were detected at 1452 , 1325 , 1300 , and 832 cm^{-1} . C-H bond vibrations were indicated by a strong peak at 2855 cm^{-1} , while hydroxyl (OH) group stretching was observed in the band between $3100\text{--}3700\text{ cm}^{-1}$ [4,13,14].

In the modification process with silica, a peak at 1081 cm^{-1} was observed, representing the alkoxide group of TEOS hydrolyzed from siloxane bonds, forming symmetric Si-O-C and asymmetric C-O bonds. The band at 816 cm^{-1} corresponds to Si-C stretching. Peaks at 1000 , 800 , and 500 cm^{-1} are attributed to silicon dioxide (SiO_2). The TEOS structure is represented by bands at 1169 , 1075 , 960 , and 700 cm^{-1} , which correspond to CH_3 groups [15]. Si-O-Si groups are indicated by peaks at 1081 and 447 cm^{-1} [16]. The success of the phenyl modification is evidenced by the appearance of new peaks at 722 and 817 cm^{-1} , which indicate Si-C bonds, and peaks around 3000 cm^{-1} , specifically at 2965 and 2855 cm^{-1} , indicating C-H bonds associated with the stretching of the benzene ring on phenyl [16]. These results confirm that this method effectively incorporates phenyl and silica modifications into the pre-synthesized polymer components. MMA dan DVB compositions were further crosslinked to evaluate the effect of achieving an optimal particle size for use in column particles.

Table 1. Variation of Crosslinker

Composition	Monomer ($\times 10^{-3}$ mol)	Crosslinker ($\times 10^{-3}$ mol)	Particle size
C1	18.8 MMA	16.8 DVB	1,98-2,02 μm
C2	18.8 MMA	8.4 DVB	1,24-1,35 μm
C3	18.8 MMA	25.2 DVB	2,88-3,03 μm

Increasing the amount of divinylbenzene (expressed in moles) and applying high temperatures will enhance the density of crosslinking. This is because a higher amount of divinylbenzene leads to greater polymerization incorporation, which in turn tends to reduce the polymer's solubility. For effective degradation and copolymerization with the monomer, the crosslinker must be present in a greater amount than the monomer. Therefore, as the number of moles of monomer increases, the number of moles of crosslinker must also increase to ensure that all monomers are effectively polymerized, while maintaining thermal stability at a certain temperature. The ratio of crosslinker to monomer affects the bond density due to crosslinking, which correlates with the amount of methacrylate present and subsequently influences the size of the particles produced. The optimal results were achieved using a composition of 18.8×10^{-3} mol MMA + 25.2×10^{-3} mol DVB. This composition was used for the column particles (after being modified with silica-phenyl), which were then applied to analyte separation applications.

Particle size characterized by SEM on polymer synthesis obtained results 2.64 to $3.03\text{ }\mu\text{m}$. After silica modification, the particle size of 2.95 to $3.10\text{ }\mu\text{m}$. This increase is attributed to the silica coating on the polymer core. Further modification with phenyl, following immersion in toluene, resulted in a particle

size of 3.06 to 3.17 μm . This indicates improved homogeneity in particle size due to the conjugated bonds formed between silica and phenyl, leading to more uniform particle sizes.

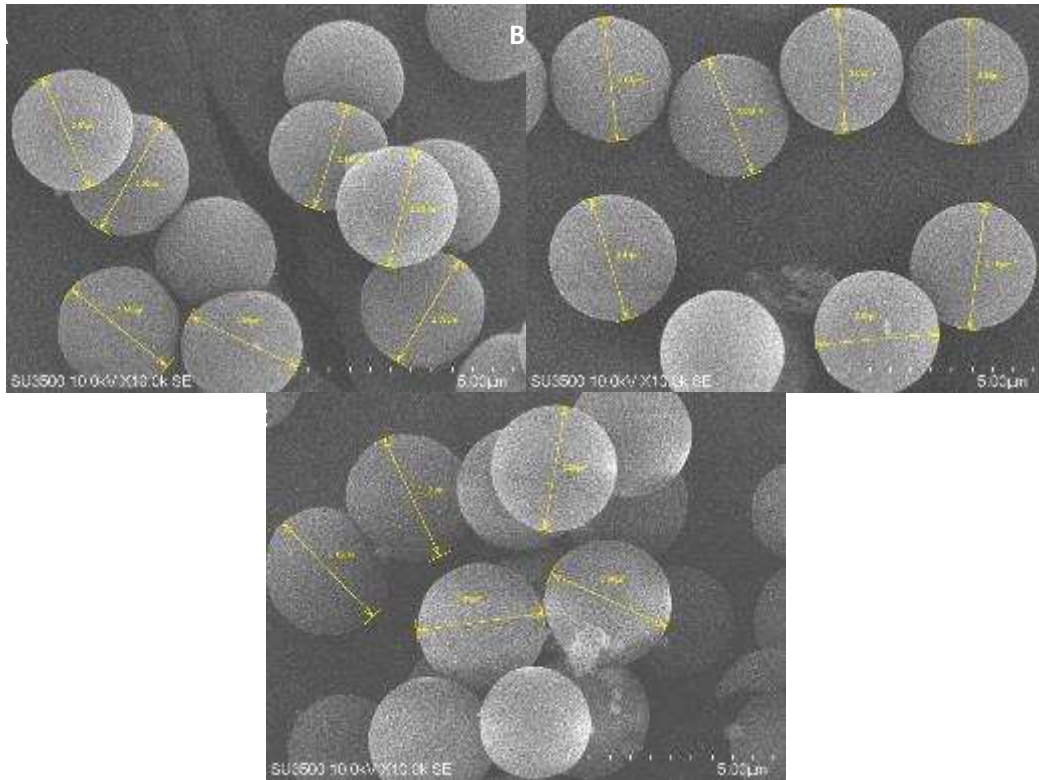
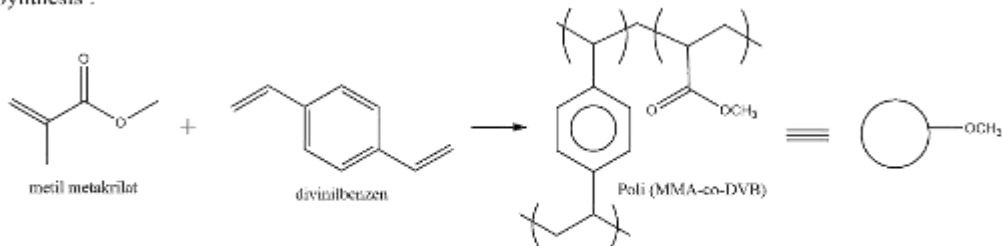


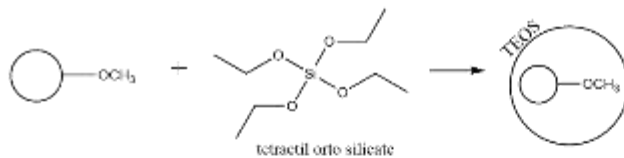
Fig. 5: SEM Characterization: A) Polymer Synthesis, B) Silica Modified, dan C) Phenyl Modified.

The interactions that occurred during the polymer synthesis process, silica modification, and phenyl modification are illustrated in Figure 6. In the silica modification process depicted in Figure 6, silica doesn't interact directly with the polymer; instead, it serves to coat the polymer core. Following this, phenyl modification allows for interaction between the silica and phenyl groups.

Synthesis :



Silica Modification :



Phenyl Modification :

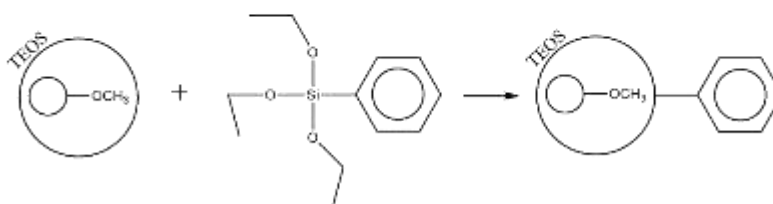


Fig. 6: Reaction of MMA-co-DVB/Silika/Phenyl

The polymerization of MMA and DVB starts with the formation of free radicals from the BPO initiator, which decomposes at a specific temperature to generate free radicals. These radicals then react with MMA monomers, breaking the double bonds in MMA to create active MMA radicals. These active radicals initiate the formation of new polymer chains. The radicals subsequently attack DVB in the blend, and the two vinyl groups on the DVB radicals form a cross-linked network with the MMA polymer. This cross-linking enhances the mechanical strength and thermal stability of the polymer. The bonding between MMA and DVB involves covalent bonds during the polymerization process, resulting in a cross-linked polymer. The incorporation of DVB into the MMA polymer creates a three-dimensional network with a more complex structure. As shown in Figure 4, the carbon double bond (C=C) in MMA is the center of reactivity in radical polymerization. This C=C bond is broken and attacked by free radicals, leading to the formation of polymer chains. Additionally, MMA radicals attack the double bonds in DVB, resulting in MMA-co-DVB covalent bonds and a three-dimensional network structure. This polymer interaction results in the formation of macroporous structures.

Phenyl modification involves bonding an aromatic group to the polymer chain through covalent bonds, either via substitution reactions or the addition of vinyl groups. This bonding occurs between the carbon atom on the phenyl group and the carbon atom on the polymer chain, which is coated with silica. Additionally, π - π interactions occur between the phenyl aromatic ring and the polymer structure, influencing the mechanical and optical properties of the polymer. The hydrophobic nature of the phenyl ring can also affect the physical properties of the polymer, particularly through hydrophobic interactions with other components in the polymer matrix.

Optimization of HPLC

In general, porous materials characterized by a bimodal or large pore size distribution are known as macropores or through-pores, while smaller-sized bimodal pore structures are referred to as mesopores. In macropores, the interconnected pores form channels that facilitate the convective flow of the mobile phase. The size and meandering nature of these pores significantly impact the column's permeability. Very small pore sizes can hinder effective liquid transportation through the column and may not be suitable for chromatography due to difficulties in separating certain components [9]. Columns containing solids that have undergone synthesis and modification were packed and tested for the separation of paracetamol and caffeine. Based on Figure 7, the single injection test of each component, under the conditions of a mobile phase MeOH: H₂O 70:30, a flow rate of 1.0 mL/min, and wavelength of 274 nm, showed that both components were well-identified with different retention times. The single peak of paracetamol was detected at a faster retention time than a single peak of caffeine, though the dead time was still associated with the peak of paracetamol. This observation indicates an opportunity to separate the two components more effectively by optimizing variations in the mobile phase, flow rate, and wavelength parameters using HPLC, aiming for optimal separation results.

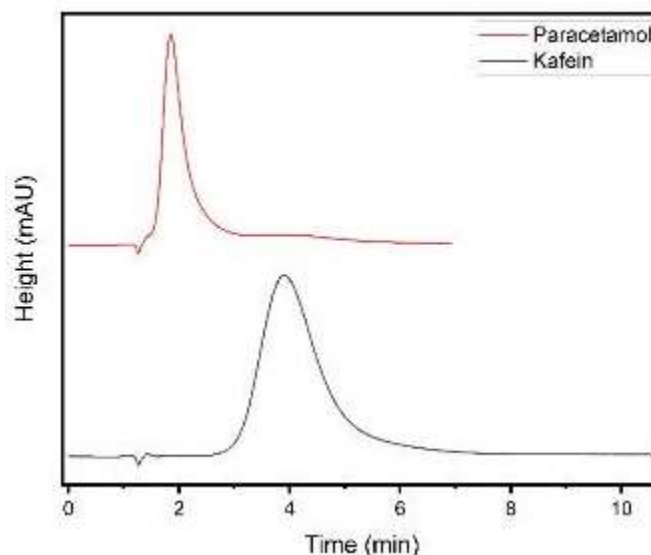


Fig. 7: Single injection for each peak

Optimization involved varying the methanol:water (MeOH:H₂O) mobile phase from 100% MeOH to a 50:50 ratio. The results showed that the resolution value (R_s) of the two peaks increased as the

concentration of the organic solvent (methanol) decreased. A lower concentration of the organic solvent also resulted in longer retention times for the two peaks. The optimal composition was found to be MeOH:H₂O 60:40, which provided an Rs value of 2.86 and a dead time of 1.47 minutes. Peaks 1 and 2 were observed at retention times of 2.46 and 5.51 minutes, respectively, as shown **Fig. 8**.

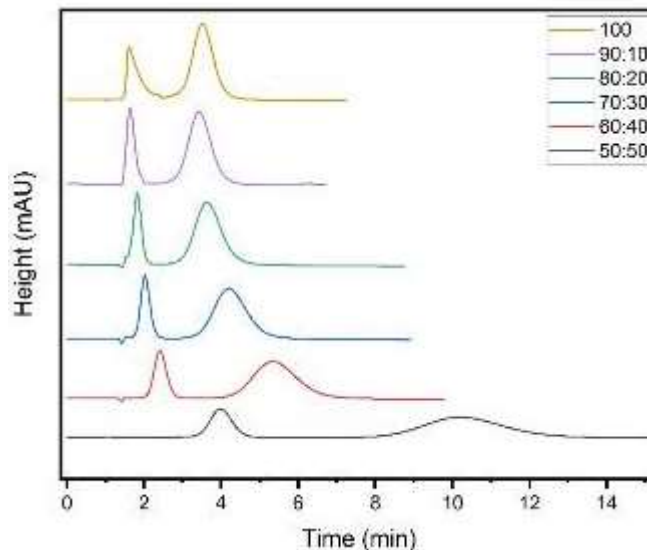


Fig. 8: Optimization of mobile phase

Table 2. Mobile phase variation

MeOH : water	Solvent retention time (minutes)	Retention time (minutes)		Width		Rs
		Peak 1	Peak 2	Peak 1	Peak 2	
100:0	-	1.62	3.52	0.3	0.63	4.09
90:10	-	1.63	3.41	0.23	0.66	2.76
80:10	1.56	1.82	3.64	0.23	0.75	3.71
70:30	1.51	2.05	4.29	0.29	0.99	3.50
60:40	1.47	2.46	5.51	0.36	1.22	3.86
50:50	1.44	3.98	10.17	0.65	2.54	3.88

Flow rate optimization was performed using a MeOH:water as mobile phase at an optimal ratio of 60:40, with flow rates varied between 0.5 and 1.0 mL/min. An increase in flow rate generally affects retention time, resulting in more efficient and effective separation. This is characterized by a decreasing Rs value as the flow rate increases, meaning the peaks move closer together. However, at a flow rate of 1.0 mL/min, the peaks remained well-separated. The optimum results were achieved at a flow rate of 1.0 mL/min, which yielded an Rs value of 3.86.

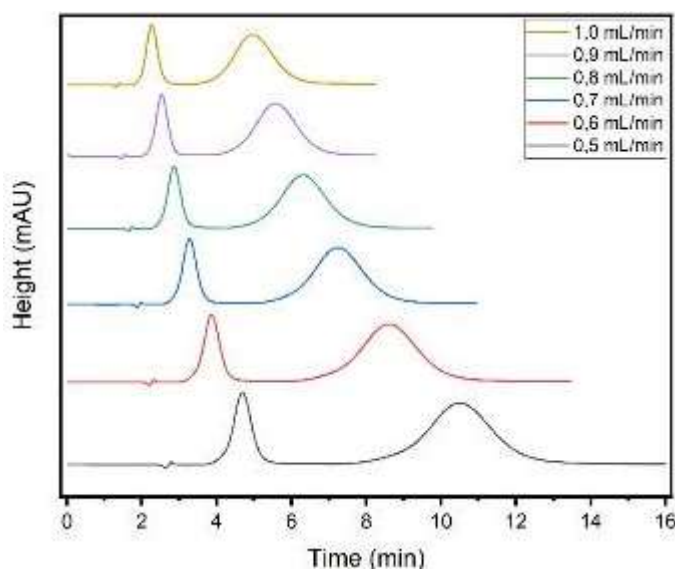


Fig. 9: Optimization of flow rate

Table 3. Flow rate variation

Flow rate (mL/min)	Solvent retention time (minutes)	Retention time (minutes)		Width		Rs
		Peak 1	Peak 2	Peak 1	Peak 2	
0.5	2.79	4.69	10.5	0.55	2.18	4.26
0.6	2.32	3.87	8.61	0.49	1.86	4.03
0.7	1.98	3.28	7.24	0.44	1.43	4.24
0.8	1.74	2.86	6.31	0.4	1.32	4.01
0.9	1.55	2.53	5.57	0.36	1.2	3.90
1.0	1.47	2.46	5.51	0.36	1.22	3.86

Wavelength variation was examined using the 3D plot for each detected peak. For peak 1 (paracetamol), the maximum absorbance was observed at 246 nm, while for peak 2 (caffeine), it was at 274 nm. Additional wavelengths tested included 202, 250, and 280 nm. The optimal results, including the highest area and peak height values, were obtained at 274 nm, where peaks 1 and 2 were well-separated. Other wavelengths (202, 246, 250, and 280 nm) showed lower area and peak height values and exhibited less stable peak heights between peak 1 and peak 2, as shown in **Table 4**. **Fig.10** and **11** display the 3D plots for peak 1 and peak 2, respectively.

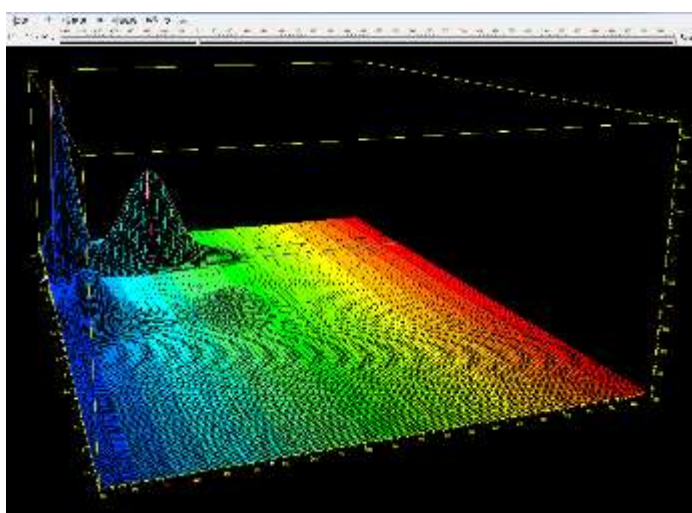


Fig. 10: Paracetamol wavelength (246 nm)

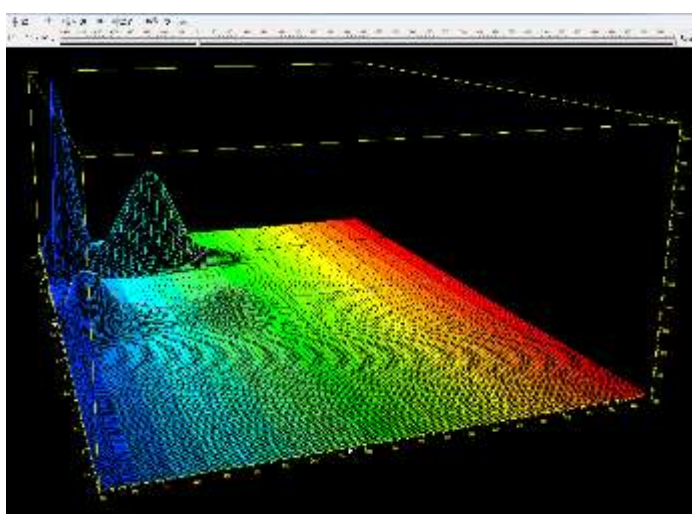


Fig. 11: Caffeine wavelength (274 nm)

Important parameters in reversed-phase HPLC include the mobile phase and its polarity. Polarity significantly influences separation and is indicated by the continuity between the mobile phases. A decrease in polarity can lead to a shorter retention time for polar analytes in the stationary phase. Conversely, increasing the polarity of the mobile phase will result in a longer retention time for the analyte in the stationary phase [17]. Based on the optimization results, the optimal solvent composition was found to be

60:40 methanol: water, with a flow rate of 1.0 mL/min and a detection wavelength of 274 nm. These conditions were then used for method validation.

Table 4. Wavelength variation

Wavelength (nm)	Solvent ret. time (min)	Retention time (minutes)		Width		Area (mAU)		Rs
		Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2	
202	1.47	2.46	5.51	0.42	1.22	127.00	9449.12	3.72
246	1.47	2.46	5.51	0.36	1.04	5616.48	1008.81	4.36
250	1.47	2.46	5.51	0.38	1	5934.97	1175.81	4.42
274	1.47	2.46	5.51	0.36	1.22	1241.66	3560.75	3.86
280	1.47	2.46	5.51	0.36	1.19	1029.14	2973.61	3.94

4. Conclusion

The manufacture of column fillers was successfully achieved through a polymer synthesis method, using methacrylate as a spherical core, which was then coated with silica and phenyl modifications. The resulting material was packed into a column and optimized to separate paracetamol and caffeine using CKT. The optimization yielded an optimal mobile phase composition of methanol: water at 60:40, a flow rate of 1.0 mL/min, and a detection wavelength of 274 nm. Under these conditions, paracetamol and caffeine were successfully separated, detected at peak retention times of 2.46 and 5.51 minutes, respectively, with a resolution value (Rs) of 3.86.

5. Acknowledgment

The author would like to thank Mr. Handajaya Rusli of Chemistry Department for the materials support.

6. References

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