Preparation of Poly-(GMA-EDA-β-CD-co-TMPTMA) Monolith as High-Performance Liquid Chromatography Chiral Stationary Phase Column

Stevin Carolius Angga[1,2,3,*], Dias Septiana[1,2], Suci Amalia[1,2,4], Warsito[1,2], Elvina Dhiaul Iftitah[1,2], and Akhmad Sabarudin[1,3]

[1]*Department of Chemistry, Brawijaya University, Jl. Veteran, Malang 65145, Indonesia*

[2]*Essential Oil Institute, Brawijaya University, Jl. Veteran, Malang 65145, Indonesia*

[3]*Research Center for Advanced System and Material Technology, Brawijaya University, Jl. Veteran, Malang 65145, Indonesia*

[4]*Department of Chemistry, Maulana Malik Ibrahim State Islamic University, Jl. Gajayana No. 50, Malang 65144, Indonesia*

* ***Corresponding author:***

email:
stevin.carolius.angga@protonmail.com

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Abstract: *An enantiomer molecule consisted of the chiral atom has different structure conformations, which exhibit different activities as well. Yet, its separation considerably difficult since ordinary separation could not separate both molecules. One of the popular enantioseparations which are often used was using organic polymer monolithic column modified by ethylenediamine-β-cyclodextrin (EDA-β-CD) as the enantioseparations site. The aim of this research was to produce chiral stationary phase column for enantioseparations of (±)-citronellal. It was conducted by preparing monolithic column using monomer glycidyl methacrylate (GMA), trimethylolpropane trimethacrylate (TMPTMA) as crosslinker, 1-propanol, 1,4-butanediol, and water as pore-forming agents (porogens) in the presence of α,α'-azoisobutyronitrile (AIBN) as radical initiator inside polyetheretherketone (PEEK) tubing. It was then modified with EDA-β-CD synthesized from β-CD. Finally, it was installed as a high-performance liquid chromatography column. The result shows the produced chiral stationary phase column could separate (±)-citronellal at a retention time of 44.76 and 45.71 min.*

Keywords: *enantioseparations; cyclodextrin; organic polymer monolith; methacrylate; high-performance liquid chromatography*

■ INTRODUCTION

Cyclodextrin (CD) has gained popularity in the past decades, especially β-CD. It has 7 D-glucopyranosyl units linked by α-1,4 glycosidic bonds resembles a cone-like structure leaving cavity on the center of it. Meanwhile, the β-CD could be derived into ethylenediamine-β-cyclodextrin (EDA-β-CD) which consists of the amine with higher nucleophilicity than a hydroxyl group. It has been widely used in enantioseparations by bonding it with a monolithic column such as an organic polymer monolithic column [1-4].

According to Nema et al. [5], the monolith generally means “a single large block of stone". The word was derived from the Greek, “monolithic” with monos meaning "single", and lithos which means "stone". Meanwhile, in chromatographic, it represents a continuous single unit material with pores. It has high permeability due to uniform distribution of macropores which provides permeability for solvents to flow through and mesopores which provides a high surface area for the separation process [6-8].

The organic polymer monolithic columns could be prepared by in situ copolymerizations of monomers inside the column tubing. The polymerization reaction mixtures consist of a combination of monomers, crosslinkers, porogens, and radical initiator [9-11]. Among various kind of monomers used in the organic polymer monolith, the methacrylate-based polymer has advantages, such as high pH stability around 2-12, followed by simple preparation and modification [12-13].

In addition, Vidič et al. [14] reported that the methacrylate monolith has high mechanical and chemical stability to withstand the harsh conditions required during its utilization. Therefore, methacrylate-based monolith such as glycidyl methacrylate (GMA) and trimethylolpropane trimethacrylate (TMPTMA) can be considered as the monomer and crosslinker. GMA was the most commonly used as a monomer in organic polymer monolith preparation since it has highly reactive epoxy groups which could be easily modified leaving the monolith column itself with various uses [9,15-17].

In this work, a chiral monolithic as the stationary phase column was prepared by post modification of poly-glycidyl methacrylate-co-trimethylolpropane trimethacrylate (poly-(GMA-co-TMPTMA)) monolithic column. It was conducted by immobilizing EDA-β-CD (poly-(GMA-co-TMPTMA)) monolithic column. Later on, it was installed onto HPLC and applied to separates (±)-citronellal. Meanwhile, the poly-(GMA-co-TMPTMA) itself was prepared by in situ copolymerizations of GMA as a monomer, TMPTMA as crosslinker, and 1-propanol, 1-4-butanediol, and water as the ternary porogen in the presence of AIBN radical initiator.

■ EXPERIMENTAL SECTION

Materials

All chemicals purchased from different sources were used without further purification. H_2SO_4, methanol, acetone, ethanol were purchased from Smart Lab Indonesia. Ethylenediamine (EDA), β-cyclodextrin (β-CD), *p*-toluenesulfonyl chloride (Ts-Cl), glycidyl methacrylate (GMA), 1-propanol, 1,4-butanediol, and (±)-citronellal were purchased from Sigma-Aldrich (Singapore). Na_2CO_3 and pyridine from Merck (Indonesia). Meanwhile, trimethylolpropane trimethacrylate (TMPTMA) from Tokyo Chemical Industry (Japan), water (aqua pro injection) from Ikapharmaindo (Indonesia), and α,α'-azobisisobutyronitrile (AIBN) from Himedia were used. Polyetheretherketone tubing (PEEK) as column housing (1.00 mm i.d. and 1/16" o.d.) was purchased from Supelco (Canada).

Instrumentation

All LC experiments were performed using HPLC unit Prominence 20 from Shimadzu (Japan) equipped with Shimadzu's workstation for system control and data acquisition. The system was composed by communication bus module (CBM-20A), HPLC pump (LC-20AD), UV/Vis detector (SPD-20A), and Rheodyne 8125 injector with custom-made 2 μL PEEK sample loop. Meanwhile, other instruments used for supporting data were SEM-EDX (FEI Inspect-S50), FT-IR (Shimadzu), ^{1}H-NMR and ^{13}C-NMR (JNM-ECZ500R, 500 MHz Super Conductive Magnet).

Procedure

The procedures consisted by few steps, which are pretreatment of PEEK as column housing, preparation of monolith column, synthesis of EDA-β-CD as chiral separating site, and immobilization of EDA-β-CD synthesized through monolith column to produce chiral stationary phase, and finally its application for enantioseparations of (±)-citronellal.

Vinylization of column inner surface

To provide covalent attachment of monolith to the column inner surface, PEEK was pretreated through sulfonation and vinylization similarly to the method described by Shu et al. [18] with minor modification. The PEEK was pretreated firstly by filling it with H_2SO_4 50% (v/v) and placed at room temperature for 6 h. After the washing out water almost reached pH 7, 1 M GMA in acetone filled into sulfonated PEEK. Both ends were sealed and placed at 60 °C for 4 h. Finally, the column was rinsed with acetone leaving the column with reactive vinyl groups, which serve as attachment of the monolith to the inner surface during the polymerization.

Preparation of poly-(GMA-co-TMPTMA) monolith column

The monolith column was prepared by the procedure as described by Sabarudin et al. [15] also with minor modification. Monomer mixture consisted of 24% GMA, 6% TMPTMA, 70% porogens 1-propanol/1,4-butanediol/water (7:4:1), and addition of AIBN 1% (w/v) towards total monomers. Initially, the

mixture was prepared inside microtube. The mixture was homogenized by using vortex for 15 min, shook for 5 min, and then sonicated for 15 min before filling the mixture into the pretreated PEEK. Both column ends were sealed, aligned vertically and polymerization was allowed to continue for 12 h at 60 °C. Subsequently, the obtained column was washed thoroughly with ethanol and water for 6 h at 10 µL/min respectively to remove the unreacted monomers and the remaining porogen present in the column.

Synthesis of mono-6-ethylenediamine-6-deoxy-β-cyclodextrin (EDA-β-CD)

Synthesis of EDA-β-CD was conducted through 2 steps, which are a synthesis of mono-6-(*p*-toluenesulfonyl)-6-deoxy-β-cyclodextrin (Ts-β-CD) and it's derivating into EDA-β-CD. It was conducted several times to obtain the necessary amount of products. The synthesis of Ts-β-CD was conducted according to Tang and Ng [19]. A solution of 46.7 mL pyridine was immersed into an ice-water bath, and then 3 g of β-CD (2.6 mmol) was added slowly under stirring. Afterward, 0.5 g of Ts-Cl (2.6 mmol) in 3.5 mL cold pyridine was dripped slowly and stirred vigorously for 24 h at room temperature. After the reaction was completed, most of the pyridine was removed by rotary evaporation. The syrup residue generated was dripped to 75 mL of cold acetone and stirred for 30 min. The resulted precipitate then collected, rinsed with acetone (12.5 mL × 3), and recrystallized from hot water (6.25 mL). The product was dried at 40 °C overnight to yield 29.67% of Ts-β-CD. Resulting data of Ts-β-CD: FT-IR (cm^{-1}, KBr): 3391, 2926, 1652, 372, 1297, 1162, 1073, 1038, 942, 855, 811. ^{1}H-NMR (DMSO, δ, ppm): 7.78 (2H, aromatic protons), 7.38 (2H, aromatic protons), 5.75-5.68 (14H, OH-2,3), 4.83-4.70 (7H, H-1), 4.49-4.46 (6H, OH-6), 3.68-3.53 (28H, H-3, 5, 6), 3.36-3.26 (14H, H-2,4), 2.50 (3H, -CH_3, overlaps with DMSO-d_6). ^{13}C-NMR (DMSO, δ, ppm): 149.65 (C Ts), 136.26 (C Ts), 128.10 (C Ts), 124.00 (C Ts), 101.99 (C_1), 81.57 (C_4), 73.09 (C_2), 72.44 (C_3), 72.07 (C_5), 59.94 (C_6), 21.99 (CH_3 Ts).

The EDA-β-CD was synthesized according to the previous report by Liu et al. [20]. One gram of Ts-β-CD (0.5 mmol) was reacted with EDA (6 mL, 89.8 mmol) at 75 °C for 4 h. After the reaction was completed, the solution was cooled down to room temperature, and most of the unreacted EDA was removed by rotary evaporation. Later on, it was dripped into 6 mL of cold acetone. The resulted precipitate was dissolved into 6 mL of the water-methanol mixture (1:1) and reprecipitated by 6 mL cold acetone, this step was repeated 3 times to purify the solid from remaining EDA. The product was dried at 40 °C overnight to yield 60.32% of EDA-β-CD. Resulting data EDA-β-CD: FT-IR (cm^{-1}, KBr): 3378, 2946, 2886, 1673, 1621, 1474, 1175, 1102, 1052, 957. ^{1}H-NMR (DMSO, δ, ppm): 5.70 (14H, OH-2,3), 4.82 (7H, H-1), 4.48 (6H, OH-6), 3.67-3.54 (28H, H-3,5,6), 3.37-3.28 (14H, H-2,4), 2.91 (2H, CH_2-NH-), 2.70 (2H, CH_2-NH_2), however protons bonded to N were not detected due to protons exchange with DMSO-d_6. ^{13}C-NMR (DMSO, δ, ppm): 101.99 (C_1), 81.57 (C_4), 73.11 (C_2), 72.46 (C_3), 72.09 (C_5), 59.96 (C_6), 48.61(C-NH-), 39.51 (C-NH_2).

Preparation of poly-(GMA-EDA-β-CD-co-TMPTMA) as chiral stationary phase monolith column

Preparation of poly-(GMA-EDA-β-CD-co-TMPTMA) was conducted by immobilizing EDA-β-CD towards poly-(GMA-co-TMPTMA) monolith column. Immobilization was performed similarly described by Li et al. [21]. The EDA-β-CD was dissolved into 0.1 M Na_2CO_3 (100 mg/mL), it was pumped into the column at 10 µL/min until the column is full. Both ends were sealed and placed at 60 °C for 16 h. The column was then washed with water for 6 h at 10 µL/min to remove unreacted EDA-β-CD.

Application of chiral stationary phase column

The produced column was installed to HPLC unit and pumped with ethanol at 5 µL/min until the stable response of the detector was achieved. UV absorption wavelength was set at 290 nm. Furthermore, 2.5 ppb of (±)-citronellal was then injected to demonstrate the performance of the produced monolithic column for enantioseparations.

■ RESULTS AND DISCUSSION

Preparation of Poly-(GMA-EDA-β-CD-co-TMPTMA)

The chiral monolithic column was produced using a poly-(GMA-co-TMPTMA) monolithic column and EDA-β-CD. Wherein the poly-(GMA-co-TMPTMA) was prepared as an anchorage for EDA-β-CD by utilizing the epoxy group consisted within it. Firstly, the column housing which is PEEK was pretreated by sulfonation and vinylization to leave it with alkene group as attachment of monolith towards column as Fig. 1. Sulfonation was conducted with H_2SO_4 within water which provides SO_3 as substituent towards benzene ring on PEEK. Meanwhile, the vinylization was utilizing epoxy group contained in GMA. But before vinylization conducted, the sulfonated PEEK was washed until the washing out water almost reached pH 7 so the epoxy group does not encounter ring opening by the acidic solution left inside PEEK. Instead, GMA will be attached to the sulfonated column producing vinylized PEEK which is also we called pretreated PEEK column.

Furthermore, the monolithic column was then made by in situ copolymerizations through utilizing each alkene group of pretreated PEEK (illustrated by block with alkene site), monomer GMA and TMPTMA as a crosslinker with radical initiator AIBN as in Fig. 2. The monomer mixture of GMA, TMPTMA, porogens, and AIBN was filled into the pretreated column and polymerized at 60 °C for 12 h producing poly-(GMA-co-TMPTMA) with an epoxy group.

Hereafter, the poly-(GMA-co-TMPTMA) was modified with EDA-β-CD as in Fig. 3. (β-CD illustrated as cone shape). A solution of EDA-β-CD and Na_2CO_3 in water was pumped into the monolithic column. Both ends were sealed, and immobilization conducted for 16 h at 60 °C. Afterward, the produced chiral monolithic column was washed with water to remove the remaining reagents.

Characterization of Chiral Stationary Phase Column

The poly-(GMA-EDA-β-CD-co-TMPTMA) column we prepared inside PEEK were only maintained about 9 MPa. We produced the poly-(GMA-co-TMPTMA) once more. Thereafter the produced columns were pumped with water until the monolith pushed out. Both of it were characterized using FT-IR and SEM-EDX to study the functional group by comparing nitrogen of EDA-β-CD

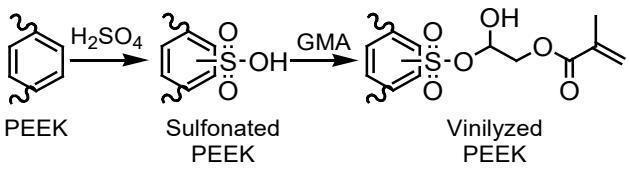

Fig 1. Pretreatment of PEEK as column housing

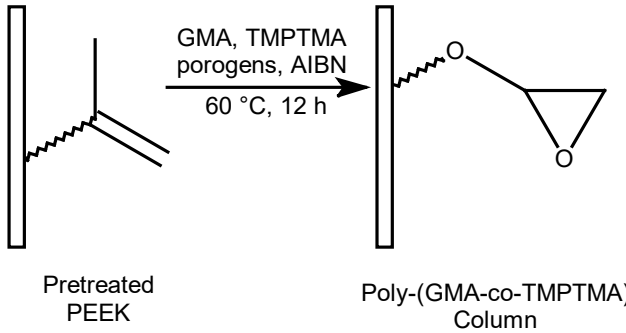

Fig 2. Preparation of poly-(GMA-co-TMPTMA) column

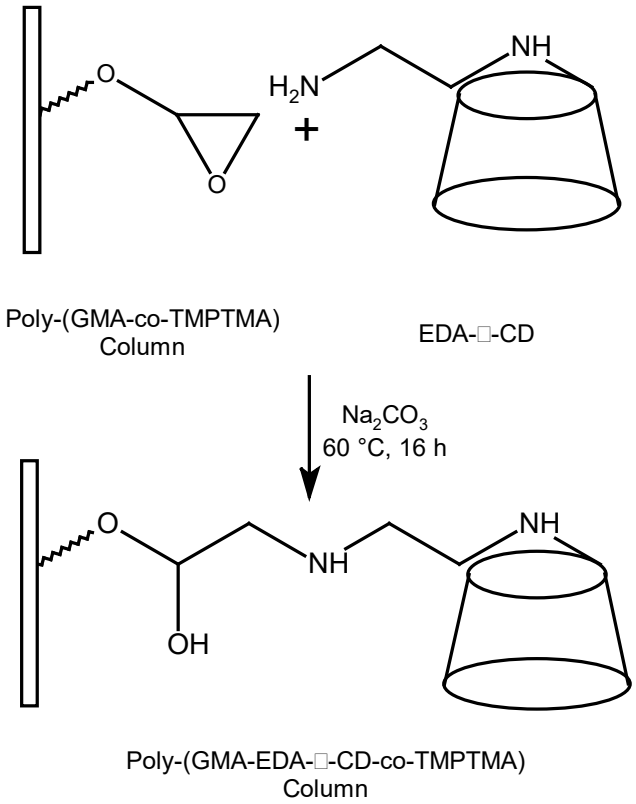

Fig 3. Preparation of poly-(GMA-EDA-β-CD-co-TMPTMA)

immobilized and observe the morphology of the monoliths.

As in the spectra presented (Fig. 4), absorption peaks of poly-(GMA-EDA-β-CD-co-TMPTMA) were slightly different than the poly-(GMA-co-TMPTMA). At ~3500 cm^{-1} shows overlapping of OH and NH stretch from EDA-β-CD anchored onto monolith, ~2900 cm^{-1} of CH_2 symmetric and asymmetric from poly-(GMA-EDA-β-CD-co-TMPTMA) become much stronger than poly-(GMA-co-TMPTMA), at ~1650 cm^{-1} and ~1500 cm^{-1} shows amine bending. Meanwhile, at ~1700 cm^{-1} shows no changes from C=O absorption of GMA and TMPTMA from the monolith itself.

As in Fig. 5, poly-(GMA-EDA-β-CD-co-TMPTMA) EDX spectra show the appearance of nitrogen bonded onto the monolith up to 5% of relative atoms ratio compared to poly-(GMA-co-TMPTMA) which means the EDA-β-CD has been anchored. However, poly-(GMA-EDA-β-CD-co-TMPTMA) ratio of C:N:O was 72:5:23 was even higher than atoms ratio in EDA-β-CD itself which are 72:3.3:55.7. We suppose it was due to analysis that was established at the part of the monolith on the rich nitrogen side. Where in analysis, we cut the monolith into 5 mm long which likely eliminate β-CD, leaving nitrogen atoms onto the monolith surface.

The morphology of monoliths was studied in Fig. 6 by comparing both of them. It shows lumps of poly-(GMA-co-TMPTMA) was modified by EDA-β-CD, leaving it with the smaller flow-through channel from 1200–2500 nm to 500–1000 nm. We also analyzed another part of poly-(GMA-EDA-β-CD-co-TMPTMA) with 50,000× magnification to compare its morphology as shown in Fig. 7. It shows even distribution of EDA-β-CD on the monolith produced.

Chiral Stationary Phase Column

The chiral monolith column, poly-(GMA-EDA-β-CD-co-TMPTMA), was produced once more to be used for enantioseparations of (±)-citronellal. Meantime, its mechanical stability was also studied by pumping ethanol thoroughly at 1–10 μL/min flow rate with 1 μL/min interval. Backpressure of each flowrate was then plotted.

As presented in Fig. 8, the mechanical stability of the

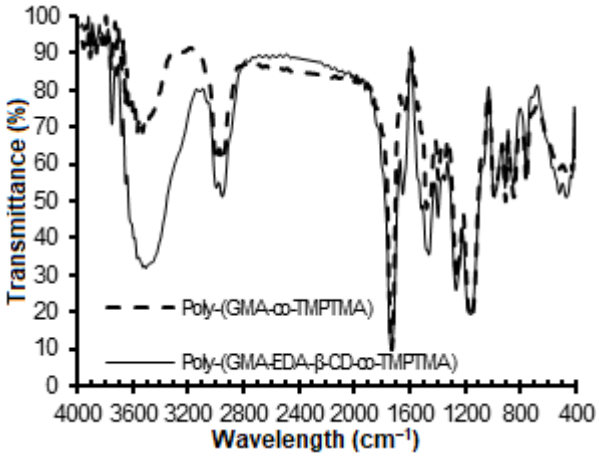

Fig 4. FT-IR spectra comparison of poly-(GMA-co-TMPTMA) and poly-(GMA-EDA-β-CD-co-TMPTMA)

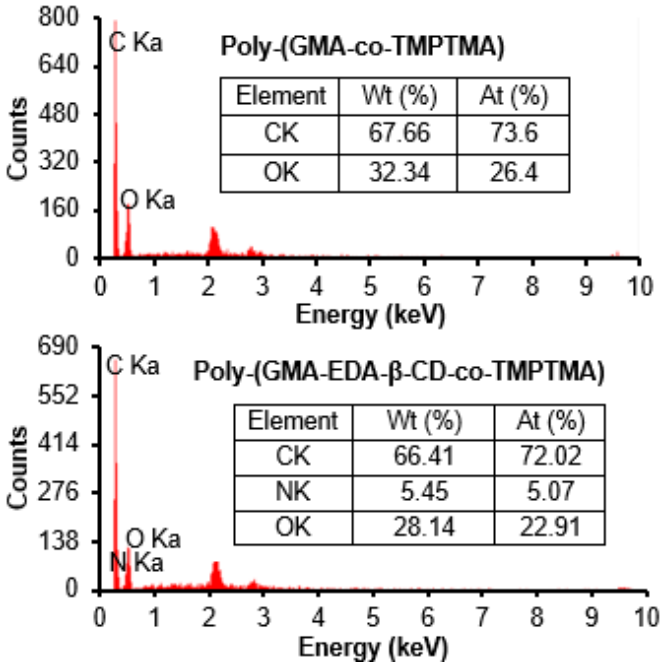

Fig 5. EDX spectra comparison of poly-(GMA-co-TMPTMA) and poly-(GMA-EDA-β-CD-co-TMPTMA)

chiral monolithic column was passably good to reached R^2 = 0.9929. Hereafter, a 2.5 ppb of (±)-citronellal was injected into the HPLC system to study the performance of the produced monolithic column for enantio separations. The separation was performed at wavelength detection of 290 nm using ethanol as mobile phase at 5 μL/min with backpressure below around 3 MPa. Wherein aforementioned, the resulted monolith column was maintained at a pressure up to 9 MPa before it was pushed out.

The chromatogram as in Fig 8, shows its potential as enantioseparation of (±)-citronellal at 44.76 and 45.71 min with relative peak area percentage of 46.91 and 49.15%, respectively. Moreover, at the retention time of 0–10 min shows negative peak and a peak with 3.94% of the area. We assume it was void time and impurity of the compound, as the purity of the sample is ≥ 95% for (±)-citronellal. Moreover, we still have no clue which citronellal enantiomers were identified in the first and second peaks (Fig. 8 right sides). The preparative analysis of (±)-citronellal using the produced chiral monolithic column is necessary to ensure the yielded separation with a polarimeter. Although the produced chiral monolithic column successfully demonstrated its potential for enantioseparation of (±)-citronellal, its chemical stability still needs improvement since it can only be used for ~250 h.

■ CONCLUSION

Based on EDA-β-CD ability to recognize chiral compounds and the flexibility of monolithic polymer with an epoxy group, a research of monolithic chiral column as enantioseparator has been developed and reported. The separation was conducted using produced column

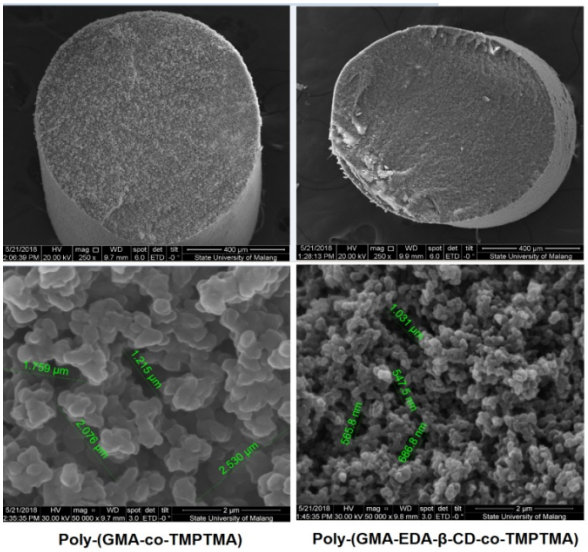

Fig 6. SEM images of poly-(GMA-co-TMPTMA) and poly-(GMA-EDA-β-CD-co-TMPTMA) at 250× and 50,000× magnification

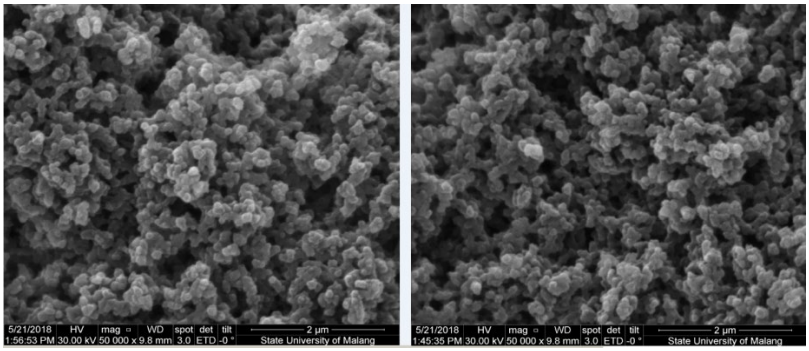

Fig 7. SEM images of poly-(GMA-EDA-β-CD-co-TMPTMA) at 50,000× magnification

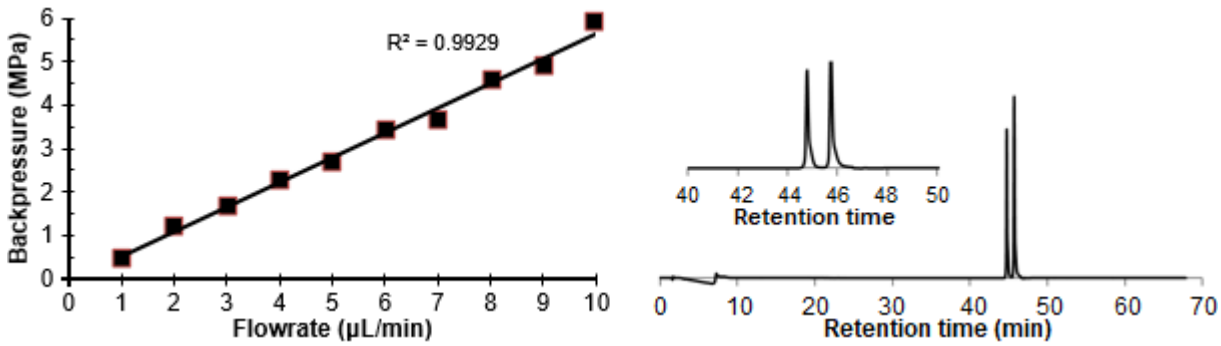

Fig 8. The plot of poly-(GMA-EDA-β-CD-co-TMPTMA) backpressure vs. flowrate using ethanol and enantioseparations of (±)-citronellal at 5 µL of ethanol at wavelength detection of 290 nm

installed into HPLC to separates (±)-citronellal with ethanol as mobile phase at 5 µL/min using isocratic mode. Chiral monolithic as HPLC stationary phase showed promising potential to be developed even further in the future.

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