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Tea Safety Control

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Research Article

Monolithic metal–organic framework MIL-53(Al)-polymethacrylate composite column for the reversed-phase capillary liquid chromatography separation of small aromatics

A monolithic capillary column containing a composite of metal–organic framework MIL-53(Al) incorporated into hexyl methacrylate-*co*-ethylene dimethacrylate was prepared to enhance the separation of mixtures of small aromatic compounds by using capillary liquid chromatography. The addition of 10 mg/mL MIL-53(Al) microparticles increased the micropore content in the monolithic matrix and increased the Brunauer–Emmett–Teller surface area from 26.92 to 85.12 m^2/g. The presence of 1,4-benzenedicarboxylate moieties within the structure of MIL-53(Al) as an organic linker greatly influenced the separation of aromatic mixtures through π–π interactions. High-resolution separation was obtained for a series of alkylbenzenes (with resolution factors in the range 0.96–1.75) in less than 8 min, with 14 710 plates/m efficiency for propylbenzene, using a binary polar mobile phase of water/acetonitrile in isocratic mode. A reversed-phase separation mechanism was indicated by the increased retention factor and resolution as the water percentage in the mobile phase increased. A stability study on the composite column showed excellent mechanical stability under various conditions. The higher resolution and faster separation observed at increased temperature indicated an exothermic separation, whereas the negative values for the free energy change of transfer indicated a spontaneous process.

1 Introduction

Metal–organic frameworks (MOFs) are considered the newest class of porous materials [1–3]. They consist of an organic ligand and an inorganic metal-containing unit that self-assemble and yield a wide variety of highly ordered frameworks. MOFs can be precisely designed through the choice of appropriate components and preparation methods. MOFs are attractive materials for chromatographic applications owing to their high diversity, high surface area, high porosity, chemical stability, and designable structure [4–6]. In HPLC, MOFs have been used mostly as a packing material, but they

suffer from low efficiency owing to their irregular particles with a wide size distribution [7].

The incorporation of nano- or microparticles of MOFs into an organic polymer monolith to form a monolithic composite column is a successful strategy to overcome the above-mentioned problems and combine the advantages of MOFs and monoliths [8–13]. Organic polymer monoliths are usually used to separate macromolecules such as proteins and nucleotides, but they exhibit poor behavior in separating small molecules. To increase the efficiency of the organic polymer monoliths for separating small molecules, several efforts have been made, including techniques that incorporate porous [8–14] or nonporous particles [15–20].

Fu et al. presented a monolith prepared in a conventional stainless-steel column (7 cm long × 4.6 mm i.d.) of methacrylic acid-*co*-ethylene dimethacrylate (MAA-*co*-EDMA) incorporated with UiO-66 for HPLC [8]. The surface area of the UiO-66(MAA-*co*-EDMA) composite column increased by approximately 67% compared with the control column without UiO-66, and a good efficiency of 28 000 plates/m (for 2,6-dimethylphenol) was achieved. MIL-101 (Cr) incorporated into a butyl methacrylate-*co*-EDMA monolith was introduced by Huang et al. as the first capillary MOF-monolith composite

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Abbreviations: **ACN**, acetonitrile; **BDC**, 1,4-benzene-dicarboxylate; **cLC**, capillary liquid chromatography; **HMA-*co*-EDMA**, hexyl methacrylate-*co*-ethylene dimethacrylate; **MOF**, metal–organic framework; **MAA-*co*-EDMA**, methacrylic acid-*co*-ethylene dimethacrylate; **TGA**, thermogravimetric analysis
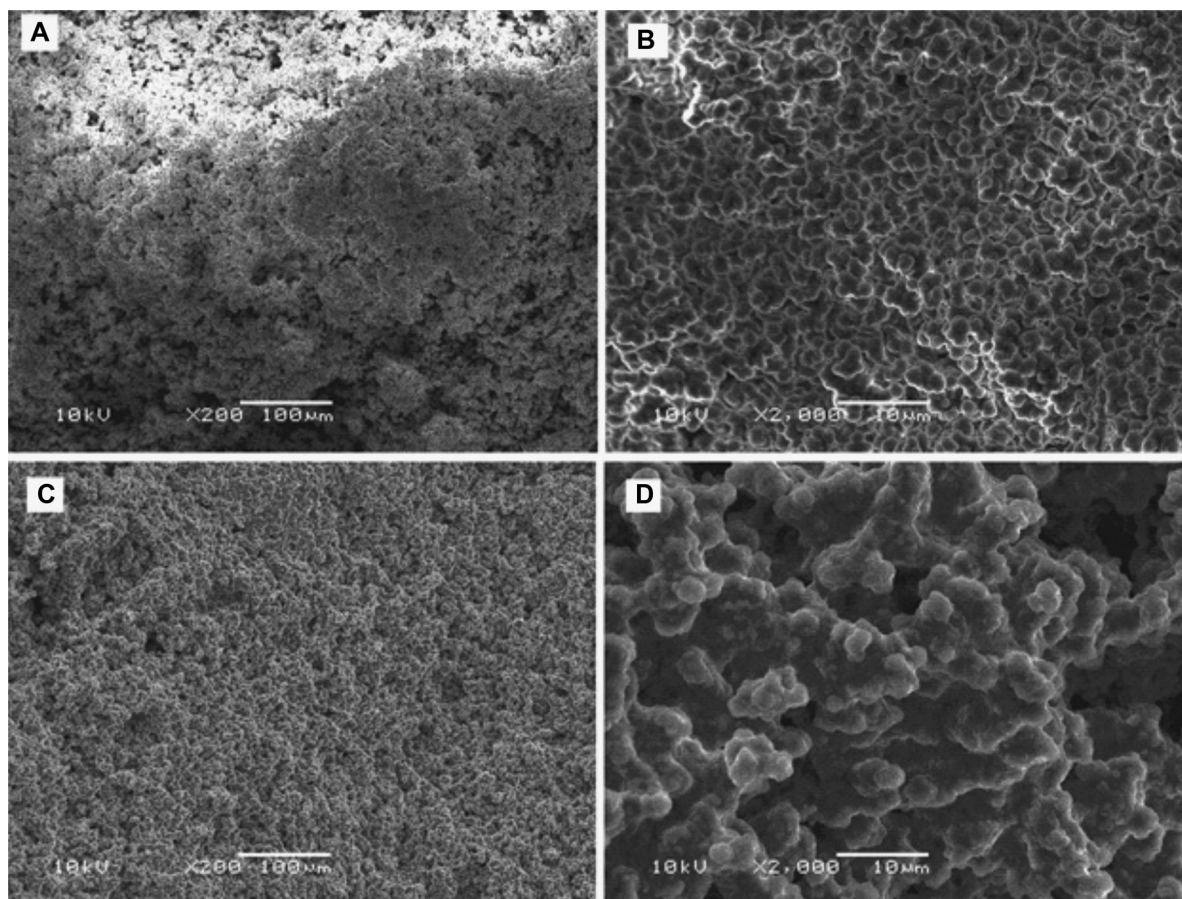
Figure 1. SEM images of (A, B) poly (HMA-*co*-EDMA) monolith and (C, D) MIL-53(Al)-(HMA-*co*-EDMA) composite monolith with incorporation of 10 mg/mL MIL-53(Al).

column [9]. The prepared column exhibited a satisfactory performance for CEC (52 000 plates/m) and for nano-LC (24 000 plates/m). The use of HKUST-1 nanoparticles to enhance the performance of a glycidyl methacrylate-*co*-EDMA monolith in a capillary column also led to a high efficiency, ranging from 16 300 to 44 300 plates/m [10]. Recently, the same research group examined the in situ grafting of HKUST-1 instead of incorporation [11]. The technique of in situ synthesizing HKUST-1 in a MAA-*co*-EDMA monolith in a capillary column resulted in good performance and resolution, with the efficiency increasing with the HKUST-1 density.

In this study, we report the exploration of incorporating MIL-53(Al) microparticles in combination with a hexyl methacrylate-*co*-ethylene dimethacrylate (HMA-*co*-EDMA) monolith as a stationary phase for capillary liquid chromatography (cLC). MIL-53(Al) [Al^{III}(OH)(BDC)] (BDC is 1,4-benzenedicarboxylate) is a 3D framework of lozenge-shaped pores (8.5 Å in diameter) and it consists of Al^{III} octahedral vertices linked by terephthalate and hydroxyl linkers [4]. The high surface area (940 m^2/g) of MIL-53(Al) and its flexible structure due to the presence of OH groups make it one of the most utilized MOF materials for chromatographic separation, mostly in the form of packed columns. Methacrylate

monoliths are a widely used organic monolithic material [4]. However, their low surface area and lack of micropores lead to poor efficiency for the separation of small molecules when used without further modifications (e.g. by hypercrosslinking or by the incorporation of various particles). To examine the performance of MIL-53(Al)-(HMA-*co*-EDMA), different percentages of MIL-53(Al) microparticles were incorporated into an HMA-*co*-EDMA monolith, and the separation efficiency of the resulting composites for various aromatic mixtures was investigated. A stability study was then performed to monitor the column performance over time, using different percentages of water in the mobile phase. Finally, the thermodynamic parameters of interaction were measured to better understand the separation mechanism.

2 Materials and methods

2.1 Reagents and materials

Polyimide-coated 250 μm i.d. fused-silica capillaries were purchased from Restek (Bellefonte, USA). Aluminum terephthalate-based MOF (MIL-53(Al)) was purchased from
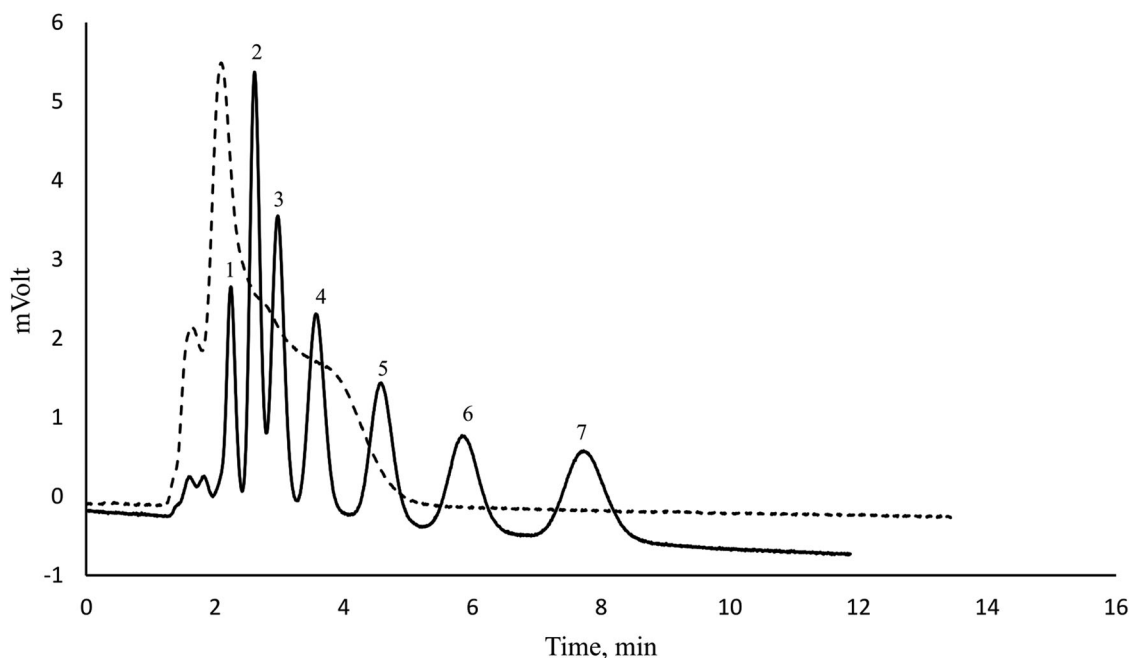
Figure 2. Chromatogram of alkyl benzenes separated on MIL-53(Al)-(HMA-*co*-EDMA) composite column (continuous line) and control monolithic column (dashed line). Separation conditions: mobile phase, 50% water/50% ACN v/v, flow rate at 5 μL/min, detection wavelength, 260 nm, injection volume, 5 nL, effective length, 20 cm, column temperature, 50°C. 1 (benzene), 2 (toluene), 3 (ethylbenzene), 4 (propylbenzene), 5 (butylbenzene), 6 (pentylbenzene), 7 (hexylbenzene).
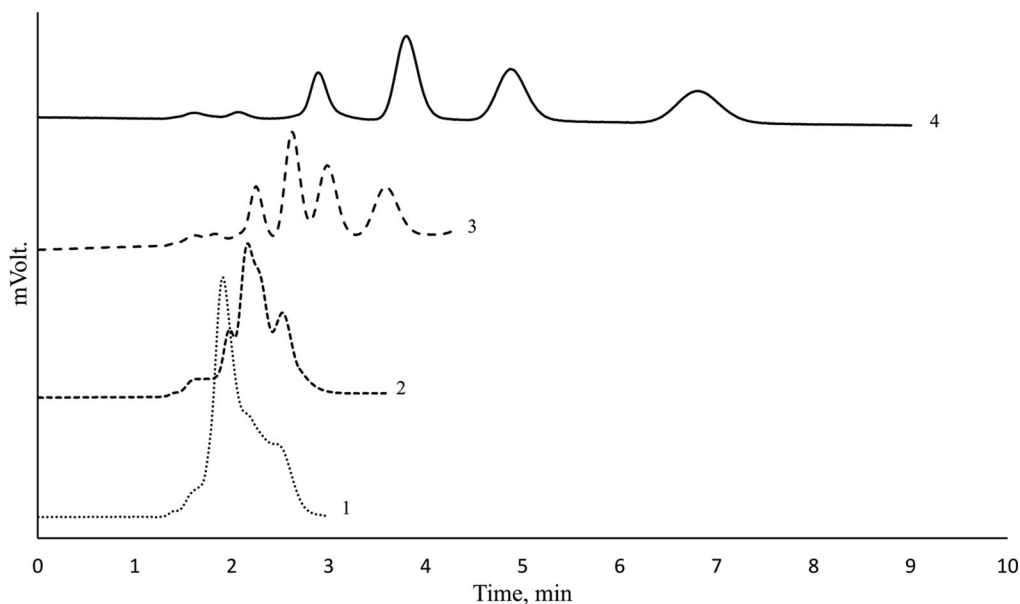

Figure 3. Chromatograms of alkyl benzenes (benzene, toluene, ethylbenzene, propylbenzene) separated on MIL-53(Al)-(HMA-*co*-EDMA) composite column showing the effect of water percentage in the mobile phase; 1 (30% water), 2 (40% water), 3 (50% water), 4 (60% water). Separation conditions: flow rate at 5 μL/min, detection wavelength, 260 nm, injection volume, 5 nL, effective length, 20 cm, column temperature, 50°C.

Sigma–Aldrich (St. Louis, MO, USA) and produced by BASF under the commercial name of Basolite® A100. Sodium hydroxide, hydrochloric acid, 3-(trimethoxysilyl) propyl methacrylate, and azobisisobutyronitrile were obtained from Fluka (Buchs, Switzerland). Thiourea, 1-propanol, 1,4-butanediol, HMA, and EDMA were purchased

from Sigma–Aldrich. High-purity analytes (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene, hexylbenzene, aminophenol, phenol, *m*-cresol, nitrophenol, *p*-xylene, 1,2,4-trimethylbenzene, and naphthol) were obtained from Merck (Darmstadt, Germany). Acetonitrile (ACN; HPLC grade) was purchased from BDH (Lutterworth,
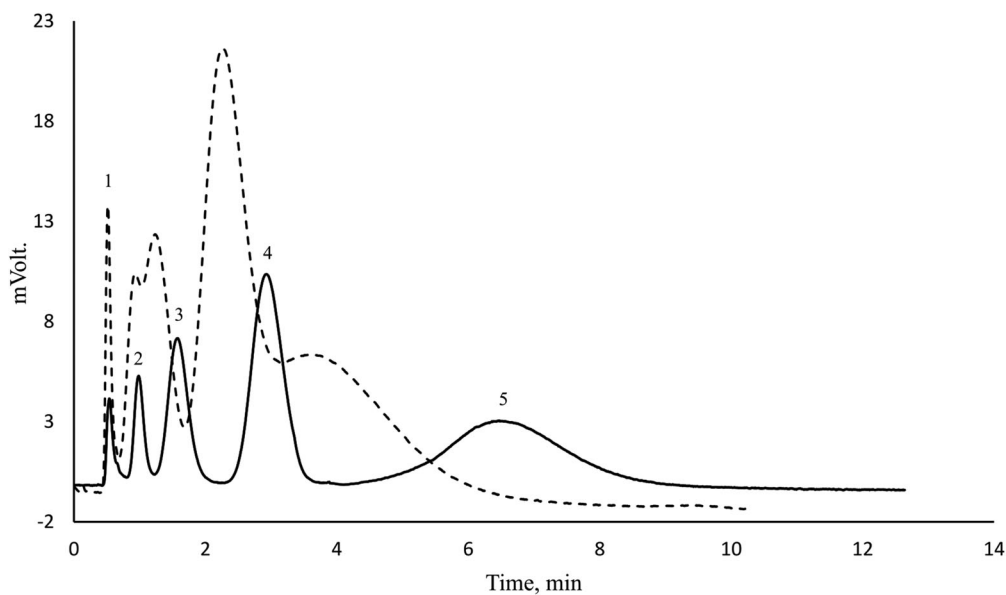
Figure 4. Chromatograms of phenolic compounds separated on MIL-53(Al)-(HMA-*co*-EDMA) composite column (continuous line) and control monolithic column (dashed line). Separation conditions: mobile phase, 85% water/15% ACN v/v, flow rate at 15 μL/min, detection wavelength, 220 nm, injection volume, 5 nL, effective length, 20 cm, column temperature, 60°C. 1 (aminophenol), 2 (phenol), 3 (*m*-cresol), 4 (nitrophenol), 5 (naphthol).
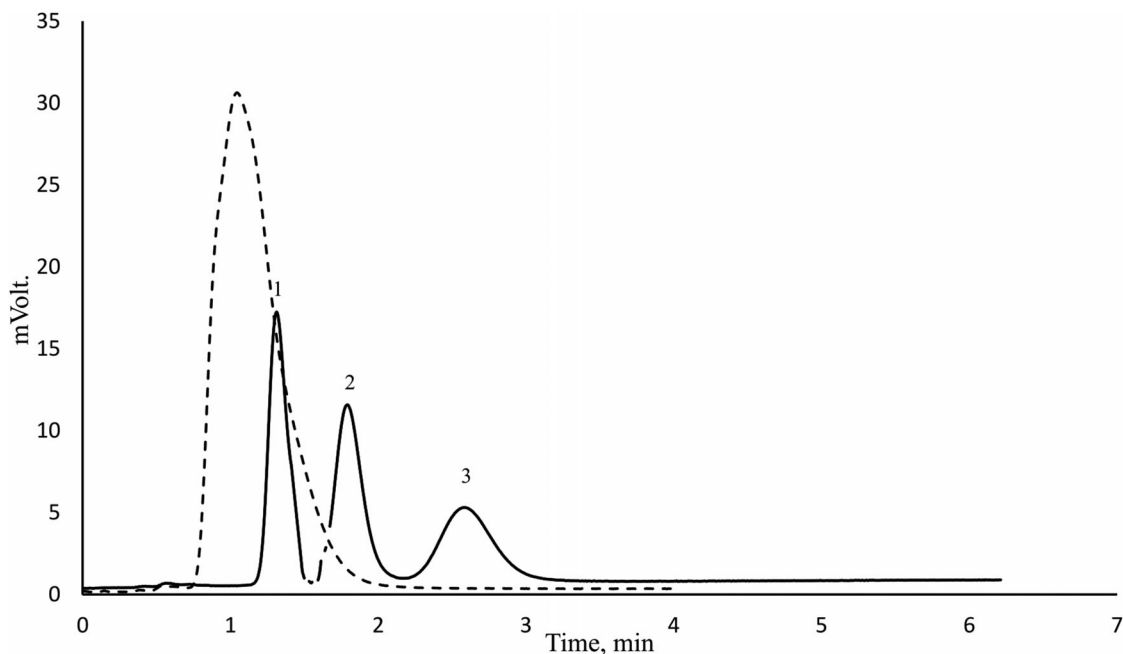

Figure 5. Chromatogram of 1 (toluene), 2 (*p*-xylene), 3 (1,2,4-trimethylbenzene) separated on MIL-53(Al)-(HMA-*co*-EDMA) composite column (continuous line) and control monolithic column (dashed line). Separation conditions: mobile phase, 60% water/40% ACN v/v, flow rate at 15 μL/min, detection wavelength, 254 nm, injection volume, 5 nL, effective length, 20 cm, column temperature, 60°C.

UK). Purified water was prepared with a Millipore system (Milli-Q Advantage Elix, Millipore, Molsheim, France) and then filtered through a 0.2 μm Nylon Whatman membrane (Maidstone, UK).

2.2 Instrumentation

cLC experiments for all analytes were carried out using a Dionex Ultimate 3000 RSLC nanosystem equipped with an

Ultimate 3000 RS autosampler and an Ultimate 3000 variable wavelength detector (Sunnyvale, CA, USA). The Chromeleon 7.2 data package was used to control the nano-UHPLC system and to acquire the chromatographic data.

The microscopic morphology of the composite material was characterized using a JEOL (JSM-6380LA) SEM (Tokyo, Japan) at 5 kV without further coating. The thermal stability of the composite material was measured using thermogravimetric analysis (TGA) with a Mettler-Toledo TGA/DSC Stare system (Schwerzenbach, Switzerland). The sample was heated from 25 to 400°C at a rate of 10°C/min.
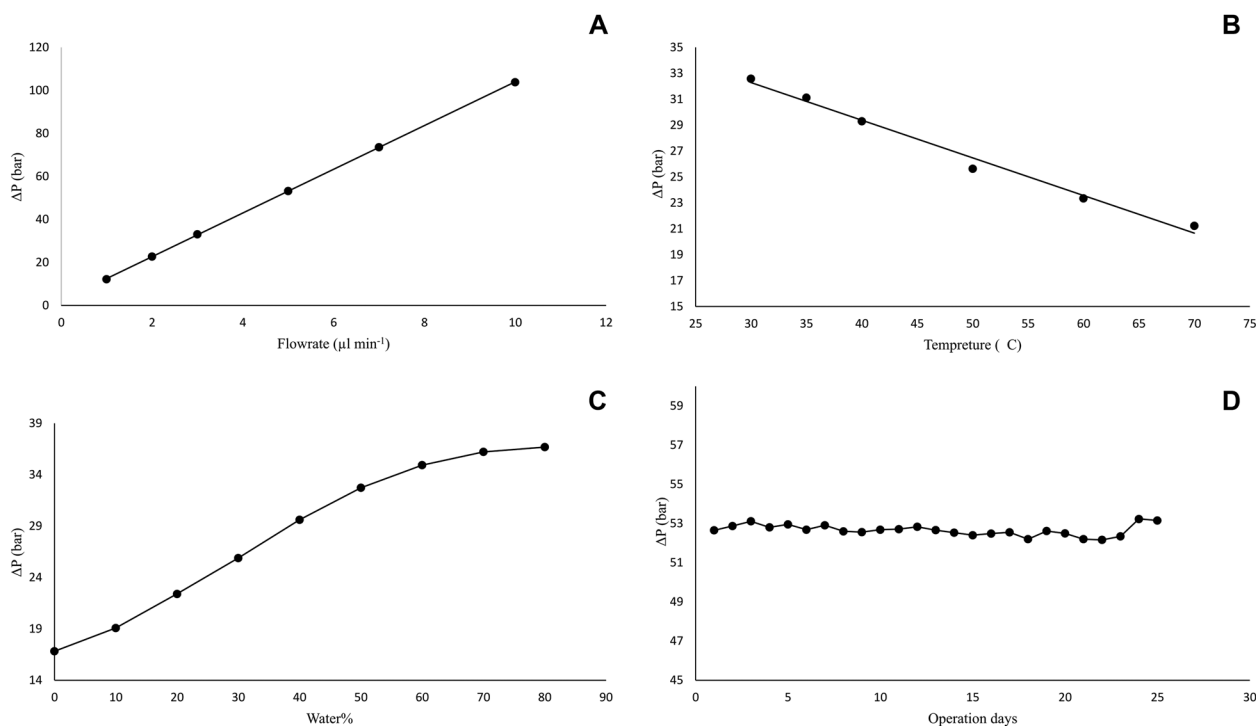
Figure 6. Column backpressure as a measure of stability versus (A) flow rate, (B) temperature, (C) water%, and (D) operation days for MIL-53(Al)-(HMA-*co*-EDMA) composite capillary column.

2.3 Preparation of MIL-53(Al)-HMA monoliths

The monolithic capillaries were prepared according to a previously described method [21] with some modifications. The polymerization mixture consisted of 30% monomers (50% HMA, 50% EDMA) and 70% porogen (50% 1-propanol, 50% 1,4-butandiol). MIL-53(Al) microparticles were dispersed and homogenized in the polymerization mixture under sonication for 10 min, and the mixture was then purged with helium for 5 min. After activation and modification with 3-(trimethoxysilyl) propyl methacrylate, empty capillaries (20 cm long × 250 μm i.d.) were filled with the polymerization mixture and thermally polymerized at 70°C for 16 h. Two batches of columns were prepared: a control batch without MIL-53(Al) microparticles and a batch with the addition of 10 mg/mL MIL-53(Al), which is the maximum concentration that provided a homogenous dispersion of the MIL-53(Al) in the polymer monolith precursor monomer/porogen mixture.

2.4 Calculations

The column's porosity (ε_T) was determined using the following equation:

$$\varepsilon_T = \frac{F \cdot t_0}{V_g} \tag{1}$$

where F is the volumetric flow rate, t_0 is the retention time of an unretained marker (thiourea), and V_g is the geometric column volume.

Darcy's equation was used to calculate the permeability ($K°$) of the prepared columns [22]:

$$K° = \frac{u \eta L}{\Delta P} \tag{2}$$

where ΔP is the pressure drop, L is the column length, η and u are the mobile phase viscosity and mean velocity, respectively.

The average pore size of the monolith channels (R) was estimated using the Hagen–Poiseuille equation [22, 23]:

$$u = \frac{\Delta P R^2}{8 \eta L} \tag{3}$$

The Gibbs free energy change of transfer (ΔG, kJ/mol), the partial molar enthalpy change of transfer (ΔH, kJ/mol), and the partial molar entropy change of transfer (ΔS, J mol^{-1} K^{-1}) were measured to evaluate the thermodynamics of the transfer of the solutes from the mobile phase to the composite stationary phase [24, 25]. A series of alkylbenzenes (from toluene to hexylbenzene) were used over different temperatures in the range of 40–70°C at the same chromatographic conditions. The Van't Hoff equations were then used to calculate ΔG, ΔH, and ΔS (according to Eqs. 4 and 5):

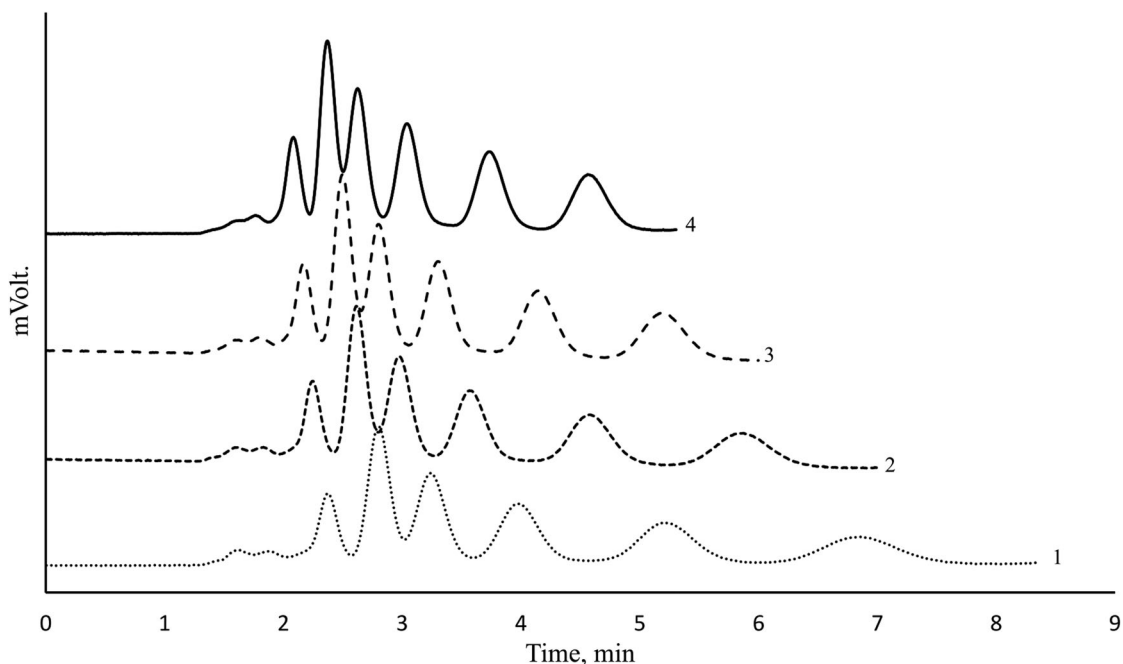$$\ln k\prime = -\Delta H/(RT) + \Delta S/R + \ln \Phi \tag{4}$$

Figure 7. Chromatograms of alkyl benzenes (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, and pentylbenzene) separated on MIL-53(Al)-(HMA-*co*-EDMA) composite column showing the effect of column temperature. Separation conditions: mobile phase, 50% water/50% ACN v/v, flow rate at 5 μL/min, detection wavelength, 260 nm, injection volume, 5 nL, effective length, 20 cm, column temperature at 1 (40°C), 2 (50°C), 3 (60°C), 4 (70°C).
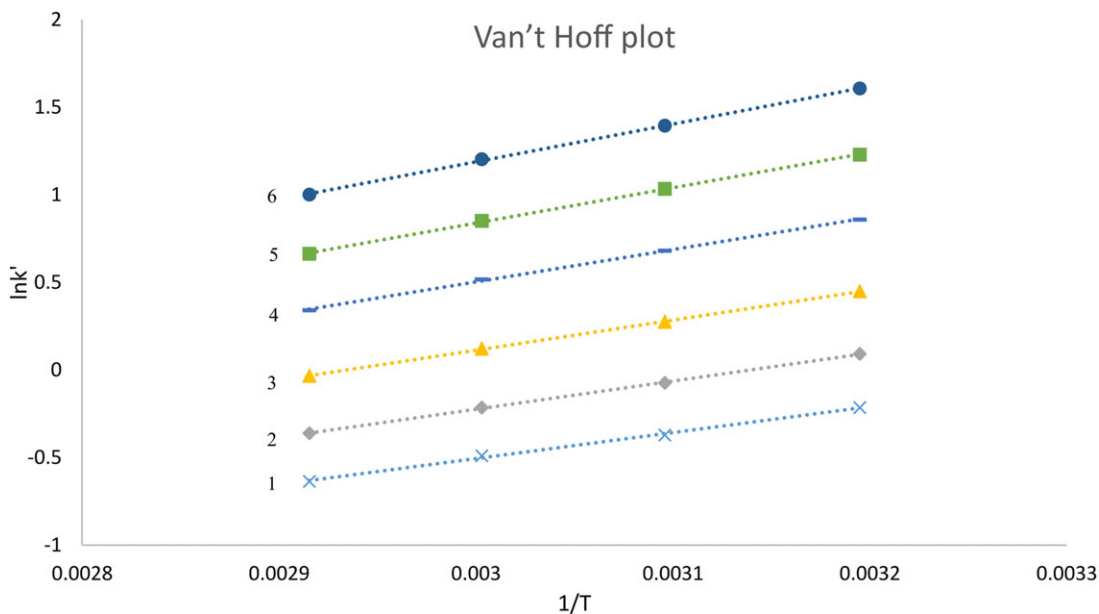

Figure 8. Van't Hoff plots for 1 (toluene), 2 (ethylbenzene), 3 (propylbenzene), 4 (butylbenzene), 5 (pentylbenzene), and 6 (hexylbenzene) separation on MIL-53(Al)-(HMA-*co*-EDMA) composite column. Separation conditions: mobile phase, 50% water/50% ACN v/v, flow rate at 5 μL/min, detection wavelength, 254 nm, injection volume, 5 nL, effective length, 20 cm, column temperature, 40–70°C.

$$\Delta G = \Delta H - T\Delta S \qquad (5)$$

where k' is the retention factor, R is the gas constant, T is the absolute temperature, and Φ is the phase ratio.

3 Results and discussion

3.1 Characterization

The dispersibility of MIL-53(Al) into the polymerization mixture was investigated visually by adding different amounts

of MIL-53(Al) microparticles (2, 5, 10, 15, and 20 mg/mL) to 1 mL of polymerization mixture and incubating the mixture for 6 h. While 2, 5, and 10 mg/mL MIL-53(Al) were well dispersed after 6 h, the suspensions including 15 and 20 mg/mL of MIL-53(Al) showed a tendency to precipitate. Accordingly, 10 mg/mL was chosen as the maximum stable amount of MIL-53(Al) in 1 mL polymerization mixture, therefore, all of the tested columns, other than the control columns, contained 10 mg/mL MIL-53(Al) microparticles.

The addition of 10 mg/mL MIL-53(Al) microparticles changed the morphology of the monolithic matrix, as shown in the SEM images (Fig. 1). The surface of the composite monolith incorporated with MIL-53(Al) became rougher, with larger clusters than the control, neat monolith. The MIL-53(Al) microparticles also affected the pore size distribution without affecting the total porosity, the total porosity was similar for the composite column (73%) and the neat monolithic column (74%), while the average channel pore size changed from 0.63 μm in the control column to 0.48 μm in the composite column, indicating the increase in the micropore percentage in the composite column. The permeability decreased from 4.93×10^{-14} for the control column to 2.85×10^{-14} for the composite column, confirming the previous conclusion.

The addition of 10 mg/mL^{-1} MIL-53(Al) increased the Brunauer–Emmett–Teller surface area of the neat HMA-*co*-EDMA monolith from 26.92 to 85.12 m^2/g, while TGA measurements showed no difference in the thermal stability, as the degradation began at approximately 210°C for both columns.

3.2 Chromatographic performance and efficiency

To explore the effect of adding MIL-53(Al) microparticles to a methacrylate monolith, a comparative study was performed between the control column (HMA-*co*-EDMA) and the composite column MIL-53(Al)-(HMA-*co*-EDMA) with 10 mg/mL MIL-53(Al)) to separate various aromatic mixtures. A mixture of alkylbenzenes (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene, and hexylbenzene) was separated with high resolution (Rs = 0.96–1.75) in less than 8 min using isocratic conditions with a 50% water/50% ACN v/v mobile phase at 50°C on an MIL-53(Al)-(HMA-*co*-EDMA) column, while the control column failed to separate this mixture under the same conditions (Fig. 2). The elution order obeys the hydrophobicity order increasing from benzene to hexylbenzene. The enhanced separation is suggested to be due to the nanosized pores introduced by the incorporated MIL-53(Al) microparticles and the higher surface area of the composite matrix over the neat monolith. The presence of BDC units as organic linkers within the structure of the MIL-53(Al) also had a significant role in the separation of the tested aromatic mixture through the strong π–π interactions. Additionally, it was found that the content of water in the mobile phase had a great effect on the separation, as increasing the water content enhanced both the retention factor and res-

olution, indicating a reverse phase separation mechanism (Fig. 3).

The chromatogram in Fig. 4 shows the separation of a mixture of phenolic compounds with different substituents (aminophenol, phenol, *m*-cresol, nitrophenol, and naphthol) using isocratic conditions with a 85% water/15% ACN v/v mobile phase at 60°C. A high resolution of 1.4–1.99 was observed for the phenolic mixture using the MIL-53(Al)-(HMA-*co*-EDMA) composite column. An excellent separation (Rs = 1.62–1.67) of a mixture of mono-, bi-, and tri-substituted benzenes (toluene, *p*-xylene, and 1,2,4-trimethylbenzene) was also achieved using the composite column in approximately 25 min (Fig. 5), while the control column did not give a satisfactory separation for the tested mixtures. The order of elution confirmed the reverse phase mechanism of separation. The composite stationary phase was found to have a particular separation enhancement with high efficiency for aromatic electron-rich compounds (e.g. $N = 14\,710$ plates/m for propylbenzene) owing to the presence of the aromatic organic linker within the MOF structure.

3.3 Stability study

A stability study was performed on an MIL-53(Al)-(HMA-*co*-EDMA) composite capillary column to investigate its repeatability and durability over 25 successive days under various conditions (Fig. 6). Figure 6A shows a directly proportional linear relationship between the flow rate (μL) and pressure drop (bar) using 50% water/50% ACN v/v as a mobile phase at 30°C. The RSD% values range from 0.26 to 0.73, indicating the excellent mechanical stability of the tested column at flow rates up to 10 μL/min. An inversely proportional linear relationship was obtained from the temperature versus the pressure drop curve (RSD% = 0.39–0.75) using 50% water/50% ACN v/v as mobile phase at a 3 μL/min flow rate over a temperature range from 30 to 70°C (Fig. 6B). The significant reduction in the column backpressure with the increasing temperature is due to the reduction of the mobile phase viscosity [26].

A linear relationship between the water percentage in the binary mobile phase and the composite column backpressure was observed in the range of 10–50%, and the relationship deviates from linearity beyond 60% and before 10% (Fig. 6C). The viscosity of the mobile phase is not a linear function of the water percentage so that a nonlinear dependence of pressure upon water percentage is observed at high compositions of water or organic solvent. The composite column also exhibits a very good pressure drop stability over the operating days at the same flow rate of 6 μL/min using 60% water/40% ACN v/v as a mobile phase at 50°C, with the RSD% equal to 0.54 (Fig. 6D).

Column-to-column reproducibility was also studied for an MIL-53(Al)-(HMA-*co*-EDMA) composite column using nine columns prepared from three different polymerization batches. Toluene and ethylbenzene were separated using nine columns, the retention factor RSD% was 1.36 for toluene and

Table 1. Values of ΔH, ΔS, ΔG, and R^2 (R^2 refers to the linear correlation coefficient of ln k' -1/T plot) for the separation of toluene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene, and hexylbenzene on MIL-53(Al)-(HMA-*co*-EDMA) composite column

Analytes	ΔH (kj/mol^{-1})	ΔS (j mol^{-1} K^{-1})	ΔG (kj/mol^{-1})	R^2
Toluene	−12.34	5.96	−11.82	0.998
Ethylbenzene	−13.41	7.88	−13.30	0.999
Propylbenzene	−14.29	10.16	−14.75	0.999
Butylbenzene	−15.32	12.79	−16.44	0.999
Pentylbenzene	−16.78	15.09	−18.35	0.999
Hexylbenzene	−17.67	17.46	−19.85	0.999

Separation conditions: mobile phase, 50% water/50% ACN v/v; flow rate at 5 μL/min; detection wavelength, 254 nm; injection volume, 5 nL; effective length, 20 cm; column temperature, 40–70°C.

3.31 for ethylbenzene, and the Height Equivalent of Theoretical Plates (HETP) was 1.76 mm for toluene and 0.97 mm for ethyl benzene.

3.5 Thermodynamic parameters

The separation of a series of alkylbenzenes (toluene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene, and hexylbenzene) was performed using an MIL-53(Al)-(HMA-*co*-EDMA) monolithic composite capillary column in the temperature range of 40–70°C to investigate the thermodynamics of separation.

As the temperature increased, the diffusion coefficient of the separated compounds increased, improving the mass transfer and leading to an increased linear velocity and a reduced retention time [27, 28] (Fig. 7), which indicated an exothermic separation. Van't Hoff plots of the examined series' separation show a linear relation between ln k' and 1/T (Fig. 8), indicating no change in the separation mechanism over the studied temperature range. The values of ΔH and ΔS obtained from the Van't Hoff plots are listed in Table 1. Negative values of ΔG indicate a spontaneous process of solute transfer from the mobile phase to the stationary phase. The more negative ΔG values give an evidence of stronger retention, therefore, longer alkyl chains exhibit a greater probability of transferring from the mobile phase to the stationary phase.

4 Concluding remarks

In conclusion, the addition of 10 mg/mL MIL-53(Al) microparticles to HMA-*co*-EDMA monolithic column led to a high permeability column with higher micropores content than the neat monolith. The presence of BDC moieties within the structure of MIL-53(Al) gives the composite column a specific separation efficiency toward small aromatic compounds through the strong π–π interactions.

The thermodynamic evaluation of the column spotted an exothermic separation and the negative values for the free energy change of transfer indicate a spontaneous process. A variety of stability studies showed an excellent mechanical and thermal stability of MIL-53(Al)-(HMA-*co*-EDMA) composite column for cLC separations as well as a very good reproducibility with high percentages of water in the mobile phase over successive runs and for 25 successive operation days.

Regardless of the relatively low efficiency of the MOF-monolith composite columns compared with the hypercrosslinked monolithic columns, this kind of columns gives a new challenge for the development of monolithic columns especially for organic monoliths due to its unlimited probabilities and as it combines the advantages of both MOF particles and the polymeric monolithic matrix.

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