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Preparation and characterization of alkyl methacrylate-based monolithic columns for capillary gas chromatography applications

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ABSTRACT

Gas chromatography (GC) is considered the least common application of both polymer and silica-based monolithic columns. This study describes the fabrication of alkyl methacrylate monolithic materials for use as stationary phases in capillary gas chromatography. Following the deactivation of the capillary surface with 3-(trimethoxysilyl)propyl methacrylate (TMSM), the monoliths were formed by the co-polymerization of either hexyl methacrylate (HMA) or lauryl methacrylate (LMA) with different percentage of ethylene glycol dimethacrylate (EDMA) in presence of an initiator (azobisisobutyronitrile, AIBN) and a mixture of porogens include 1-propanol, 1,4-butanediol and water. The monoliths were prepared in 500 mm length capillaries possessing inner diameters of 250 μm. The efficiencies of the monolithic columns for low molecular weight compounds significantly improved as the percentage of crosslinker was increased, because of the greater proportion of pores less than 50 nm. The columns containing lower percentages of crosslinker were able to rapidly separate a series of 8 alkane members in 0.7 min, but the separation was less efficient for the light alkanes. Columns prepared with the lauryl methacrylate monomer yielded a different morphology for the monolith-interconnected channels. The channels were more branched, which increased the separation time, and unlike the other columns, allowed for temperature programming.

1. Introduction

Although initially used for gas chromatography (GC) [1–3], monolithic columns have been extensively studied for use in high performance liquid chromatography (HPLC) [4–8]. Crowley [1] first introduced the use of monoliths for gas chromatography in 1969. However, the simultaneous emergence of open capillary columns with monolithic columns detracted from the attention that was owed to the introduction of monolith [9]. A considerable number of monolithic materials developed for HPLC have been described in several outstanding reviews [10–13], yet only one review for monoliths in GC has been published in 2008 by Svec and Kurganov, and it is titled, "Less common applications of monoliths III. Gas chromatography" [14]. Recently in 2013 another review by Kurganov became available on line, and it is titled, "monolithic column in gas chromatography" [15].

Monolithic columns are constructed of a single continuous piece of polymeric material that fills the entire length and width of the column. This piece of adsorbent material contains two interconnected networks of pores, the macropores and the mesopores. The macropores, also called the through-pores, have dimensions in the 1.5–4 μm range. Their network provides flow paths through and along the column and ensures access of the sample molecules to the entire network of mesopores. The density of the macropores network causes the monolithic columns to contain a high external porosity. This high porosity combined with the relatively large average pore size causes monolithic columns to be highly permeable [16–19].

There is a consistent agreement in the literature suggesting that the macropores network accounts for approximately 80% of the total porosity. The mesopores network represents 10–15% of the total porosity. The average sizes of the mesopores are generally between 10 and 20 nm [20,21].

Briefly, monolithic materials can be divided into two general categories, silica sol–gels and porous organic polymers [14]. Because of their simple preparation process and the simple adaptability of column selectivity, the organic polymeric approach exhibits more potential advantages compared to silica-based monoliths. In the early 1970s, two research groups independently experimented with polyurethane foams prepared in situ for use as gas chromatographic monolithic columns [3]. A second publication followed in 1973 that fully revealed the specifics of the technology

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[22]. By today's standards, the performances of the monolithic polyurethane columns were poor. However, they were comparable with the packed columns of the early 1970s. In fact, one of the reports [22] indicated that these monolithic columns were commercially available from Analabs (North Haven, CT, USA) [14].

Monolithic polymers and copolymers of divinylbenzene (DVB) were fully studied for liquid chromatography [23–28]. The use of a poly(divinylbenzene) monolith in GC was initially introduced in Berkeley in 2000 [29]. The thermal stability of poly(divinylbenzene) is an important feature that allow elevated temperatures to influence the separation rate in GC. DVB monolith does not undergo thermal degradation until 380 °C. Korolev et al. have performed extensive studies on poly(divinylbenzene) as a separation medium for GC using a modified gas chromatography instrument that works under high pressure [30–32]. Peroni et al. have published in 2012 one of the few studies concerning monolithic column for GC using divinylbenzene under the title "*Macroporous polymer monoliths as second dimension columns in comprehensive two-dimensional gas chromatography: A feasibility study*" [33].

Methacrylates are one of the most widely used monoliths, originally introduced in 1978 by Svec et al. [34], but they were almost exclusively utilized for HPLC in the 1990s by Svec and Fréchet [35,36]. Our group has published several articles on methacrylate monoliths for HPLC [37–41]. Before this current work very few researchers had examined the potential of polymethacrylate monoliths for gas chromatographic separation [42–44], may be due to its low thermal stability. According to our results, we believe that methacrylate-based monoliths are quite valid for numerous GC applications, especially for light hydrocarbons and gases, using a conventional GC without modification. However, further studies are needed to evaluate all of the criteria.

2. Experimental

2.1. Materials

Polyimide-coated 250 μm i.d. fused silica capillaries were purchased from Restek (Bellefonte, USA). Sodium hydroxide, 3-(trimethoxysilyl) propyl methacrylate (TMSM), hydrochloric acid and azobisisobutyronitrile (AIBN) were purchased from Fluka (Buchs, Switzerland). Uracil, 1-propanol, 1,4-butanediol, lauryl methacrylate, hexyl methacrylate and ethylene dimethacrylate (EDMA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile was purchased from BDH (Lutterworth, UK). Purified water was prepared with a Millipore system (Milli-Q Advantage Elix, Millipore S.A.S. 67120 Molsheim, France) and then filtered through a 0.2 μm nylon Whatman membrane (Maidstone, UK). Various alkanes (pentane, hexane, heptane, octane, nonane, decane, undecane and dodecane) and alkylbenzenes (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene, hexylbenzene, heptylbenzene and octylbenzene), all of the highest purity grade, were obtained from Merck (Darmstad, Germany). All gases (methane, helium, hydrogen, nitrogen and air), of high-purity grade (99.9999%), were purchased from SIGAS (Riyadh, Saudi Arabia). For the comparative study, TR-5 MS column, 30 m length and 250 μm inner diameters was purchased from Thermo Scientific (Waltham, MA, USA).

2.2. Preparation of monolithic polymethacrylate capillary

To clean and activate the capillary inner surface, the fused silica tubing (500 mm × 0.25 mm i.d.) was rinsed with a 1.0 mol/L NaOH solution for 5 min and soaked in the same solution for 10 min. It was then rinsed with water and dried with air for 2 min two times. The column was then flushed with 1.0 mol/L HCl for 2 min and dried with air for 5 min. Afterwards, the capillary was rinsed with toluene for 10 min and flushed with a 10% 3-(trimethoxysilyl) propyl methacrylate in toluene solution for 10 min before soaking in the same solution for 2 h. It was then rinsed with toluene for 5 min and dried with air for 5 min.

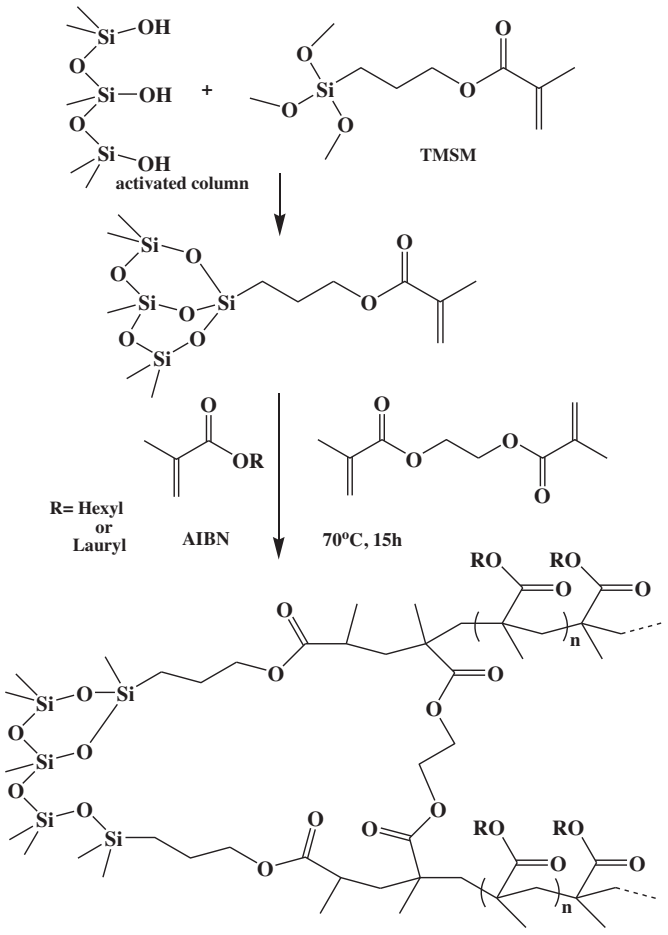

Fig. 1. Schematic representation of the preparation procedure of monolithic columns.

Three different batches of columns have prepared to examine the effect of crosslinker percentage and monomer chain length. Each batch contained at least three identical columns from which one column was chosen. Column one (A1C6) was prepared using 12% hexyl methacrylate (w/w) as the monomer, 12% ethylene dimethacrylate (w/w) as the crosslinker and 1% AIBN as the initiator. For the second column (A2C6), the effect of crosslinking was examined by decreasing the percentage of crosslinker to 10%. For the third column (A2C12), the influence of monomer chain length was tested using lauryl methacrylate as the monomer (12-carbon atoms chain) instead of hexyl methacrylate (6-carbon atoms chain). The schematic procedure for the preparation of the monolithic columns is shown in Fig. 1.

The porogen mixtures were 76% of the total polymerization mixture for columns A1C6 and A2C12 and 78% for column A2C6 and were prepared as follows (wt%): 60% 1-propanol, 30% 1,4-butanediol and 10% water. The monomer mixture and the porogen solvents were mixed into a homogenous solution, sonicated and purged with helium gas for 3 min. The capillary columns were then filled with the reactant solution, and both ends were plugged with a piece of rubber. The polymerization was performed in a water bath at 60 °C for 20 h. After the polymerization was completed, the seals were removed, and the prepared columns were connected to an

HPLC pump and washed with acetonitrile to remove the unreacted materials and porogenic solvents.

2.3. Characterization of the monolithic columns

After the chromatographic experiments were finished, the monolithic rods in the tubes were washed and cut into small pieces and dried. The dried columns and monolithic materials were characterized by scanning electron microscopy (SEM) and thermogravimetric analysis (TGA). The pore properties and microscopic morphologies of the polymers were characterized using a JEOL (JSM-6380LA) analytical scanning electron microscope (Tokyo, Japan) at 5 kV without further coating. TGA was conducted on the monolithic material, prepared in the same way as described above, with a Mettler-Toledo TGA/DSC Stare system (Schwerzenbach, Switzerland). The sample was heated from 25 to 400 °C at a heating rate of 10 °C/min.

2.4. Instrumentation

All experiments were performed using a commercial gas chromatograph (Thermo Scientific – Trace GC Ultra, USA). The system contained a split/splitless injector, an oven with a temperature range of 50–400 °C, a heating rate of up to 14.5 °C/sec (870 °C/min), programmability of 3 ramps/4, a flame ionization detector and an acquisition rate of 300 Hz. The sample was introduced manually into the instrument. The peak integration was performed using a Chrom-Card data handling software package. Dried high-purity helium was used as the carrier gas, nitrogen was the makeup gas and a 1:10 hydrogen/air mixture was used as the flame fuel.

2.5. Methods

The columns were fixed into a U-shaped fitting before polymerization to avoid any distortion of the polymer inside the columns while connecting them to the GC, considering the lengths of the columns inside the injector and inside the detector. To experimentally determine the dead time, methane gas was used as an unretained material. Methane was injected at a constant temperature (200 °C) with varying pressures (600, 700, 800, 900 and 1000 kPa) and at constant pressure (1000 kPa) with different temperatures (160, 170, 180, 190 and 200 °C).

The prepared columns were tested to separate a mixture of linear alkanes (pentane, hexane, heptane, octane, nonane, decane, undecane and dodecane), and a mixture of alkyl benzenes (benzene, toluene, ethyl benzene, propyl benzene, butyl benzene, pentyl benzene, hexyl benzene, heptyl benzene and octyl benzene). The separation was performed at isobaric conditions (1000 kPa) with different temperatures (160, 170, 180, 190 and 200 °C), and at isothermal conditions (200 °C) with varying pressures (600, 700, 800, 900 and 1000 kPa). Sample volumes of 0.5 μL were injected for all experiments with a split ratio of 1:500. Both the injector and detector were adjusted to 200 °C. The exact mobile phase flow-rate was measured using a stopwatch and a calibrated 100 μL microburette for each experiment. All chromatographic experiments were repeated in triplicate.

HPLC was used to determine the column's porosity (ε_T) with uracil as an unretained material. The calculation of ε_T in this work was based on the following equation:

$$\varepsilon_T = \frac{F \cdot t_0 - V_e}{V_g} \quad (1)$$

where F is the volumetric flow-rate, t_0 is the retention time of an unretained marker, V_e is the extra-column volume (void volume) and V_g is the geometrical volume. V_g was calculated using the equation $V_g = \pi r^2 L$, where r is the column inner diameter, and L is the column length.

The extra-column volumes (void volume) corresponding to the connections between either the injector and column or the column and detector, were accurately measured and estimated to be 8.845 μL for both A1C6 and A2C6 columns and 4.989 μL for A2C12 column. The retention times of uracil (dead time) at a constant flow-rate of 6 μL/min using acetonitrile/water as mobile phase (50/50, v/v %) on the three columns A1C6, A2C6 and A2C12 were 4.9, 4.7 and 4 min, respectively.

The permeability of the gas chromatographic column was defined by a modified Darcy's equation [14]:

$$K^\circ = \frac{u\eta L}{\Delta P j'} \quad (2)$$

where $\Delta P = P_i - P_o$ (P_i and P_o are the pressures of the carrier gas at the inlet and outlet of the column, respectively), j' is the Halasz compressibility correlation factor ($j' = 3(P^2-1)(P+1)/4(P^3-1)$, where P equals P_i/P_o), L is the column length, η and u are the carrier gas viscosity and the mean velocity, respectively, and K° is the column permeability.

The Hagen–Poiseuille equation is a physical law that calculates the pressure drop through a long cylindrical pipe [45,46]:

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

where R is the average pore size of monolith channels (macropores).

To examine the efficiency of our columns while separating a real sample, a gasoline sample from the local market was injected onto the A2C12 column. The chromatographic conditions were optimized after several experiments at 40 °C for 3 min, and the temperature was increased at a rate of 25 °C/min to 170 °C and held for 15 min. A constant pressure of 1000 kPa was used with a split ratio of 1/500, and the sample volume was 0.5 μl. The alkanes and alkyl benzenes mixtures were separated using the same chromatographic conditions for qualitative analysis.

3. Results and discussion

3.1. Characterization

TGA of the polymethacrylate monoliths (Fig. 2) indicated that they did not undergo any significant thermal degradation until the temperature reached 215 °C. Although this temperature is relatively low for GC separations, it is still sufficient for numerous applications involving volatile compounds.

To characterize the prepared monolithic materials, the morphologies of the polymers were examined by SEM. Several representative SEM images are shown in Fig. 3, and they demonstrate that the preparation procedure for all columns rendered a permeable monolith with a uniform porosity. Moreover, the well-developed macroporous structure, typical for monolithic supports, is clearly illustrated. Monoliths typically contain two types of pores, classically referred to as mesopores (between 2 and 50 nm) and macropores (>50 nm). The former allow for diffusive mass transport and interactions, whereas the latter pores allow for mobile phase flow and convective mass transport. The SEM images of the capillary cross sections illustrate a uniform structure of the monolith bed with the presence of interparticle spaces. The monolithic bed of our columns represented a continuous and relatively dense polymeric phase with low interstitial porosity.

The figures illustrate that the synthesized monoliths were composed of spherical microglobules with an average diameter of 1–2 μm that were linked together to form a skeleton around the macropores. Compared to the other two columns, larger

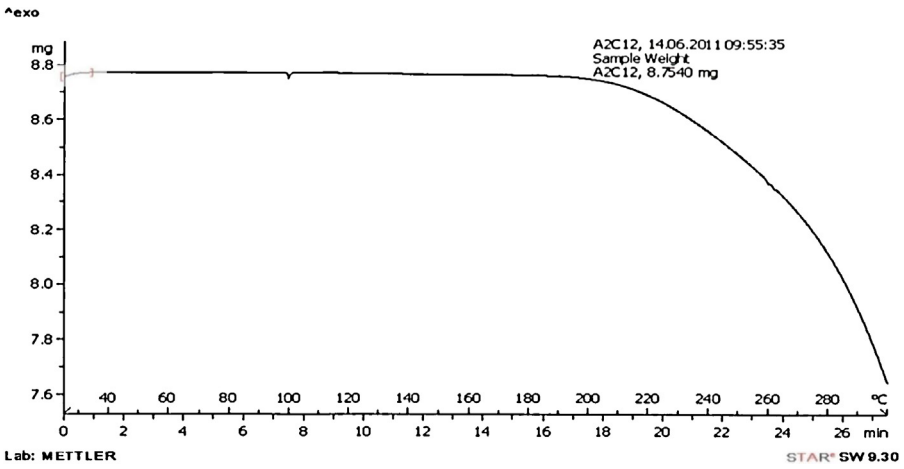

Fig. 2. TGA for poly lauryl methacrylate corresponding to column A2C12.

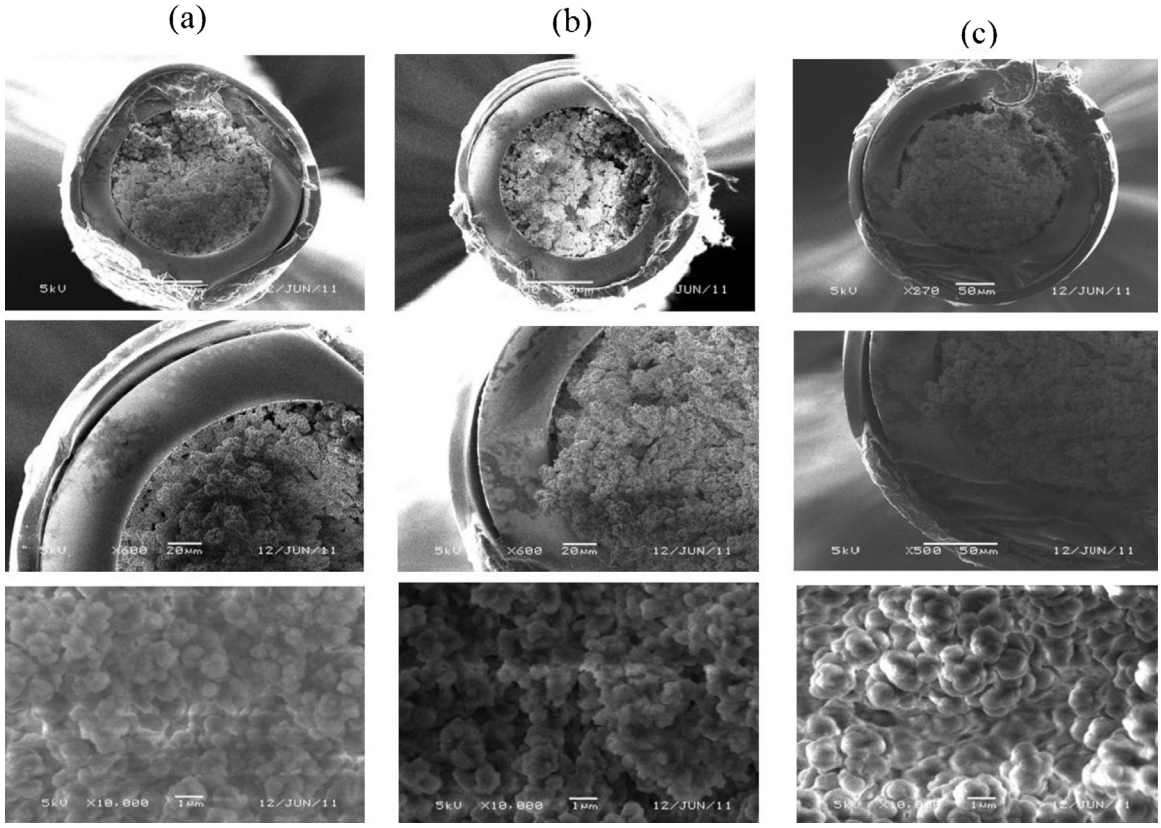

Fig. 3. Microphotographs of monolithic capillary columns prepared with (a) 12% hexyl methacrylate + 12% ethylene dimethacrylate (column 1), (b) 10% hexyl methacrylate + 10% ethylene dimethacrylate (column 2), and (c) 12% laurryl methacrylate + 12% ethylene dimethacrylate (column 3).

macropores and microglobules were obtained with column A2C12 (Fig. 3c), which was characterized by a relatively high polymer density. In addition to the macropores, larger channels were observed in the bulk material, resulting in higher permeability but a lower mass transfer rate. This macroporous structure facilitated the mobile phase flow, promoted effective solute/stationary phase interactions and allowed for high permeability of the prepared capillary columns.

3.2. Hydrodynamic properties of the column

In the present work HPLC was used to determine the porosity of our columns using uracil as an unretained material. The application of Eq. (1) revealed that the highest porosity corresponded to column A1C6 ($83.7 \pm 0.91\%$), followed by A2C6 ($79.3 \pm 1.14\%$) and A2C12 ($77.5 \pm 0.75\%$) (Table 1). We noticed that the longer lauryl methacrylate chain column (A2C12) exhibited a lower porosity than the shorter hexyl methacrylate chain column (A1C6), which was expected because as the length of the monomer chain increases, the volume of the polymer increases while the pore volume decreases.

In addition, we found that the porosity of the A1C6 column was higher than that of the A2C6 column. The higher percentage of crosslinker in A1C6 (12%) than in A2C6 (10%) led to a more crosslinked polymer that contained a higher percentage of mesopores, leading to a higher total porosity than A2C6.

Table 1
Porosity and hydrodynamic flow properties of monolithic columns.

Column		A1C6	A2C6	A2C12
ε_T (%)		83.7 ± 0.91	79.3 ± 1.14	77.5 ± 0.75
R (μm)	200 °C	2.726 ± 0.004	2.772 ± 0.001	2.014 ± 0.001
	160 °C	2.701 ± 0.008	2.763 ± 0.003	2.002 ± 0.002
K° (m^2)		3.29×10^{-16}	3.39×10^{-16}	1.78×10^{-16}

ε_T – total porosity; R – average pore size; K° – permeability

Table 2
Dead time (min) practically through unretained methane gas retention time using different three columns A1C6, A2C6 and A2C12, at constant pressure (1000 kPa) and different temperatures (200, 190, 180, 170 and 160 °C) and at constant temperature (200 °C) and different pressures (1000, 900, 800, 700 and 600 kPa).

Pressure (kPa)	1000	900	800	700	600
A1C6	0.136	0.147	0.160	0.178	0.200
A2C6	0.132	0.141	0.154	0.172	0.195
A2C12	0.251	0.272	0.297	0.331	0.368
Temp (°C)	200	190	180	170	160
A1C6	0.136	0.132	0.128	0.127	0.124
A2C6	0.132	0.128	0.127	0.124	0.123
A2C12	0.251	0.247	0.242	0.238	0.230

The Hagen–Poiseuille equation (Eq. (3)) was used to calculate the average pore size of the macropores at 1000 kPa at two different temperatures (200 and 160 °C) to study the effect of temperature on the average pore size. The ΔP term in the equation refers to the pressure drop between the inlet (10^6 Pa) and the outlet of the columns, which corresponded to atmospheric pressure (1.01325×10^5 Pa). The viscosity of the carrier gas (helium) was 2.739×10^{-5} kg/m.s at 200 °C and 1000 kPa and 2.533×10^{-5} kg/(m s) at 160 °C and 1000 kPa [47]. The average velocity of the mobile phase over the channel section, the linear velocity – (u), was obtained from the retention time of the unretained gas (dead time) which was methane in this work (Table 2).

The average pore size for A1C6, A2C6 and A2C12 were 2.726 ± 0.004, 2.772 ± 0.001 and 2.014 ± 0.001 μm, respectively, at 200 °C and 2.701 ± 0.008, 2.763 ± 0.003 and 2.002 ± 0.002 μm, respectively, at 160 °C (Table 1). We noticed that the lower crosslinker percentage in A2C6 led to a less crosslinked polymer with larger macropores. In the lauryl methacrylate column (A2C12), the long polymer chain led to a bulkier polymer with smaller macropores and low porosity than the hexyl methacrylate columns, A1C6 and A2C6. In addition, we found that the pore size increased as the temperature was increased, which indicated the same effect for the mesopores. The effect of temperature on the pore sizes may have affected the separation velocity for bulky compounds.

The permeability of a column determines how much pressure is needed to achieve a given flow-rate [48]. From the above results, we expected that the permeability should increase as the porosity increased, but the pore size also greatly influences the permeability. In our work, we found that A1C6 had a higher porosity but smaller macropores size than A2C6. The average pore size, which represented the macropore size, appeared to play a larger role with regard to permeability, as A2C6 had a higher permeability than A1C6.

Using the modified Darcy Law (Eq. (2)) to calculate the permeability of our columns, the permeability of the largest pore size column, A2C6, was 3.39×10^{-16} m^2; while the permeabilities of A1C6 and A2C12 were 3.29×10^{-16} and 1.78×10^{-16} m^2, respectively (Table 1).

3.3. Fast isobaric and isothermal separation of alkanes and alkylbenzenes

Two different mixtures were injected onto the columns to evaluate and compare their chromatographic properties. The first sample was a series of linear alkane (pentane, hexane, heptane, octane, nonane, decane, undecane and dodecane) and the second sample was a series of alkyl benzenes (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene, hexylbenzene, heptylbenzene and octylbenzene).

To evaluate the three columns in terms of their chromatographic performance, we separated the two samples under the optimum isothermal and isobaric conditions (200 °C and 1000 kPa) that yielded the best performance for the three columns with regard to separation efficiency and total separation time.

For the alkane series, fast base line separation was obtained in less than two minutes using A1C6 column. A2C6 provided the fastest separation of 0.7 min among the three columns for the eight *n*-paraffin members because of its higher permeability and larger macropore size, but, unfortunately, the separation of the first four members (pentane, hexane, heptane and octane) was not as good as the other heavier homologues (nonane, decane, undecane and dodecane). This result confirmed the observation that A2C6 had a lower percentage of mesopores than A1C6 because of its decreased crosslinker percentage, which predominantly affected separation efficiency. Based on the results of the alkane separation with A2C6, it was hypothesized that this column could be efficiently used to quickly separate heavier hydrocarbons. To separate the light alkanes with A2C6, it required a lower separation temperature, which tested at 160 °C using the same pressure (Fig. 4). These conditions allowed for the efficient and complete separation of the alkanes in 1.5 min, which was faster than the separation time of A1C6 at 200 °C.

Using A2C12, pentane, hexane and heptane were not completely separated. The overall separation time was relatively long (2.1 min), and the elution of the first compound (pentane) was delayed to 0.37 min compared to A1C6 (0.2 min) and A2C6 (0.21 min). The longer lauryl methacrylate chain gave the polymer in A2C12 a higher polymeric volume than in A1C6 or A2C6, despite the same column volume. This polymer also led to a lower porosity and smaller pore size with more branched channels and a longer path, which was clearly observed by the dead time determination (Table 2). The narrower branched channels of A2C12 induced a higher diffusion of the analytes than in the other two columns, which led to broader peaks and increased retention compared to A1C6 due to the longer path. Peak broadening was clearly evident due the overlapping of the peaks of the light alkanes and from their large peak width values at half height (Fig. 5). Lower separation temperature was required to increase the efficiency of A2C12 for separating of the lighter alkanes. Therefore, the same experiment was repeated at 160 °C and the same pressure. A better and more complete separation was obtained, but it required a longer total separation time of 5.3 min. This longer run time at 160 °C could be decreased by programming the oven temperature to begin at 160 °C and increase to 200 °C at a fast rate. From these results, it was expected A2C12 to be the best column for the separation of a real sample, as it had the best capability for temperature programming.

For the *n*-alkylbenzene series, the complete separation of the homologues was achieved in a relatively short time on the three columns. The separation times were 8.82, 3.51 and 8.79 min on A1C6, A2C6 and A2C12, respectively. The separation of the alkyl benzene mixture using A2C6 provided the fastest separation among the three columns without any peak overlapping (Fig. 4). The higher boiling points of the alkyl benzenes compared with those of the corresponding *n*-alkanes prevented peak overlapping when separated

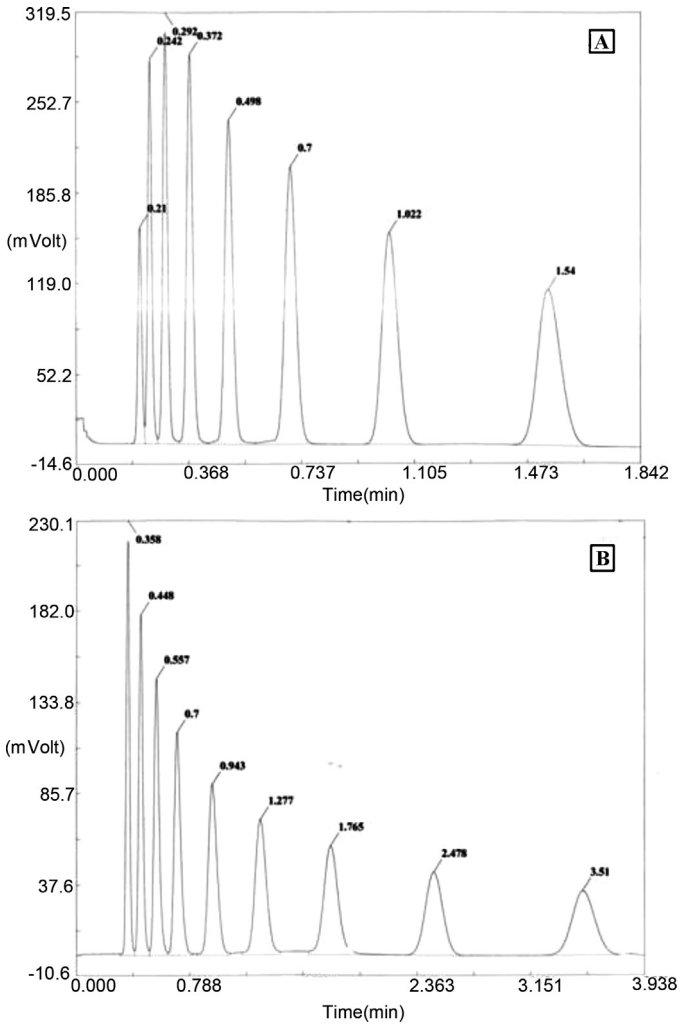

Fig. 4. Fast and complete separation at isothermal and isobaric conditions of, (A) alkanes sample (pentane, hexane, heptane, octane, nonane, decane, undecane, dodecane) at 1000 kPa, 160 °C, and (B) alkyl benzenes sample (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene, hexylbenzene, heptylbenzene, octylbenzene) at 1000 kPa, 200 °C separated on A2C6, as an example of separation chromatograms.

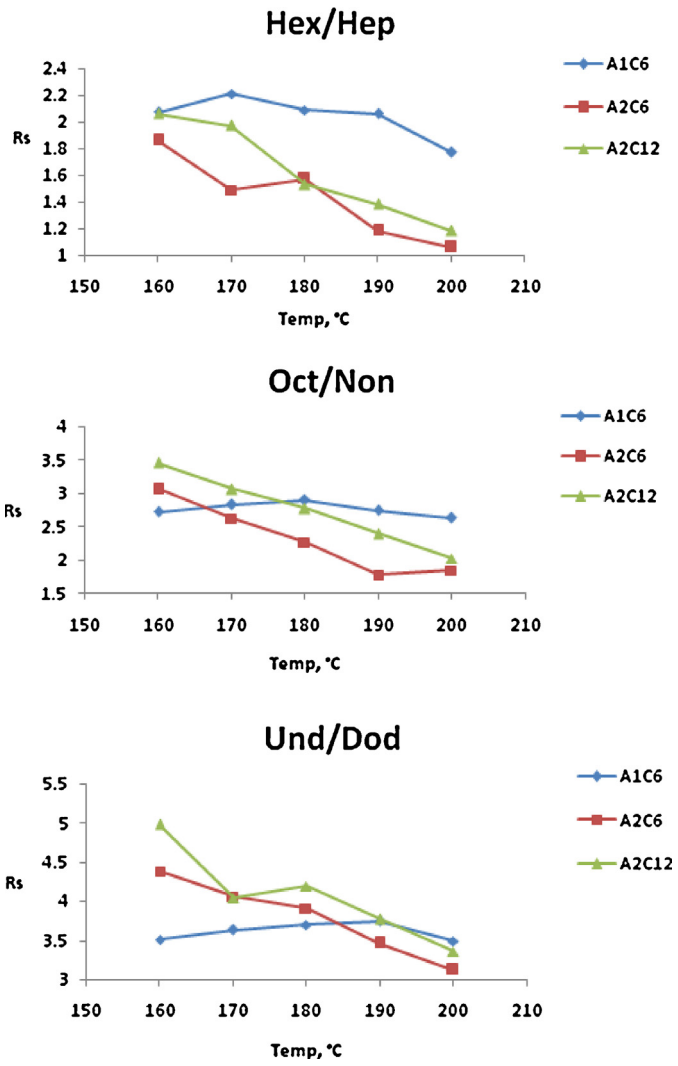

Fig. 5. Resolution correlated with temperature for three different pairs of alkanes (Hex/Hep), (Oct/Non) and (Und/Dod) separated on the three monolithic columns (A1C6, A2C6, A2C12) comparing the effect of temperature on resolution for different molecular weight pairs at constant pressure.

on A2C6 at the same temperature and pressure conditions used to separate the alkanes, despite the higher permeability and larger pore size of the column.

Again, a marked peak broadening was noticed in the chromatograms from A2C12 column. Additionally, a longer retention time of the first analyte (benzene) using A2C12 was observed (0.64 min) compared with the other columns (0.38 and 0.35 min for A1C6 and A2C6, respectively), and this result was explained by the column's lower porosity and permeability and its long, narrow and branched channels. Hence, it was concluded that A2C12 was not the optimal choice for the rapid separation of light *n*-alkanes and alkylbenzenes.

3.4. Resolution

The resolution (R_S) was calculated to describe the degree of separation between the neighboring peaks for in the alkane sample (8 members) and in the alkylbenzenes sample (9 members). The separation was performed at a constant pressure (1000 kPa) with different temperatures (200, 190, 180, 170 and 160 °C), for the three columns.

Resolution is defined as the ratio of the distance between two peaks to the average width of these peaks (at baseline or at half height), and this descriptor encompasses both the efficiency and selectivity

$$R_S = 2\frac{t_{R2} - t_{R1}}{w_1 + w_2} = 1.18\frac{t_{R2} - t_{R1}}{w_{(1/2)1} + w_{(1/2)2}} \quad (4)$$

where t_R is the total retention time, w is the peak width at baseline and $w_{1/2}$ is the peak width at half height.

Typically, an R_S value greater than 0.8 required for accurate quantification of two peaks. A value of 1 for two equally sized peaks indicates an overlap of approximately 2%. Complete separation requires an $R_S > 1.2$ units. In general, all the separations between the alkylbenzenes on the three columns were complete with resolution values greater than 1.2. The resolution results for the separation of the alkanes were also greater than 1.2 except for the lightest analyte couples, pentane/hexane and hexane/heptane, on A2C6 and A2C12 (Fig. 5).

At constant pressure, as the temperature increased, the peaks became sharper. A2C12 produced wider peaks than A1C6, but the A1C6 column produced wider peaks as the temperature decreased and the molecular weight of the probes increased. This result

confirmed the previous conclusions that A1C6 had the highest percentage of mesopores and that the sizes of the mesopores increased with temperature. Therefore, with the A1C6 column, as the temperature decreased, the sizes of the mesopores also decreased, inducing longer retention times for the heavier alkanes and alkyl benzenes and wider peaks than observed with A2C12. The A2C6 column produced the narrowest peaks because of its high permeability and large pore size.

We analyzed the results of the resolution based on the $w_{1/2}$ results. The effect of the temperature on the resolution under isobaric conditions was compatible with the effects observed for the width at half height: the resolution decreased for the same column as the temperature was increased at constant pressure. In comparing the three columns at constant pressure and different temperatures, it was found that A2C6 had the lowest resolution for alkanes, even though it produced the narrowest peaks, but it had the lowest retention factors and the lowest total separation time. Therefore, the peaks were closer together, and the resolution was poorer. As the $w_{1/2}$ values with A1C6 were larger than those with A2C12 for alkanes; therefore, A1C6 showed lower resolutions than A2C12 (Fig. 5). For the alkylbenzenes, the effect of temperature on $w_{1/2}$ had little effect on the resolution because of the long total separation time.

3.5. Column efficiency

To evaluate the efficiencies of our columns, van Deemter curves were plotted that correlated the height equivalent of the theoretical plate (HETP) to the flow-rate of the carrier gas (helium) for the alkane and alkylbenzene samples separated on the prepared columns at a constant temperature (200 °C) (Fig. 6). The flow-rate of the carrier gas was determined using a flowmeter over a range of different pressures (600, 700, 800, 900 and 1000 kPa) for each column.

For a typical van Deemter curve and at flow-rates below the optimum, the overall efficiency is dependent on diffusion effects (the *B* term). At higher flow-rates, the efficiency decreases because the mass transfer, or *C* term, becomes more significant. The minimum of the van Deemter curve represents the ideal or optimum flow velocity, which corresponds to the maximum column efficiency. It is a compromise between the *B* and *C* terms. In contrast to diffusion in particulate separation media, interphase mass transfer in monoliths is governed by convection, and the total pore volume is utilized [49,50]. This effect allows for a dramatic reduction in the time required for mass exchange between the mobile and stationary phases, i.e., the *C* term is reduced in the van Deemter equation, and the separation efficiency improves.

Many studies on monolithic columns [51–53] have reliably demonstrated that the column efficiency and the dynamic binding capacity are not affected by flow. In other words, there is no significant change in HETP when the flow-rate through the monolithic column is increased to greater than the optimum value.

At low flow-rates, the van Deemter curves began at high HETP, as shown in Fig. 6. As the flow-rate increased, the HETP decreased rapidly at the beginning and then stabilized as a straight line with no optimum flow-rate as the effect of the longitudinal diffusion decreased (*B* term) and with the reduced *C* term for monolithic stationary phase. At the same flow-rate, we found that the lighter alkanes and alkylbenzenes had higher HETP values than the heavier ones.

The value of the flow-rate that yielded the lowest HETP was 0.61, 0.5 and 0.3 mL/min for A1C6, A2C6 and A2C12, respectively. The HETP ranged from 0.44 to 0.82 mm for A1C6, 0.24 to 1.09 mm for A2C6 and 0.35 to 2.3 mm for A2C12 for alkanes separation. For alkylbenzenes, the HETP values ranged from 0.25 to 0.88 mm for A1C6, 0.22 to 0.87 mm for A2C6 and 0.33 to 1.69 mm for A2C12.

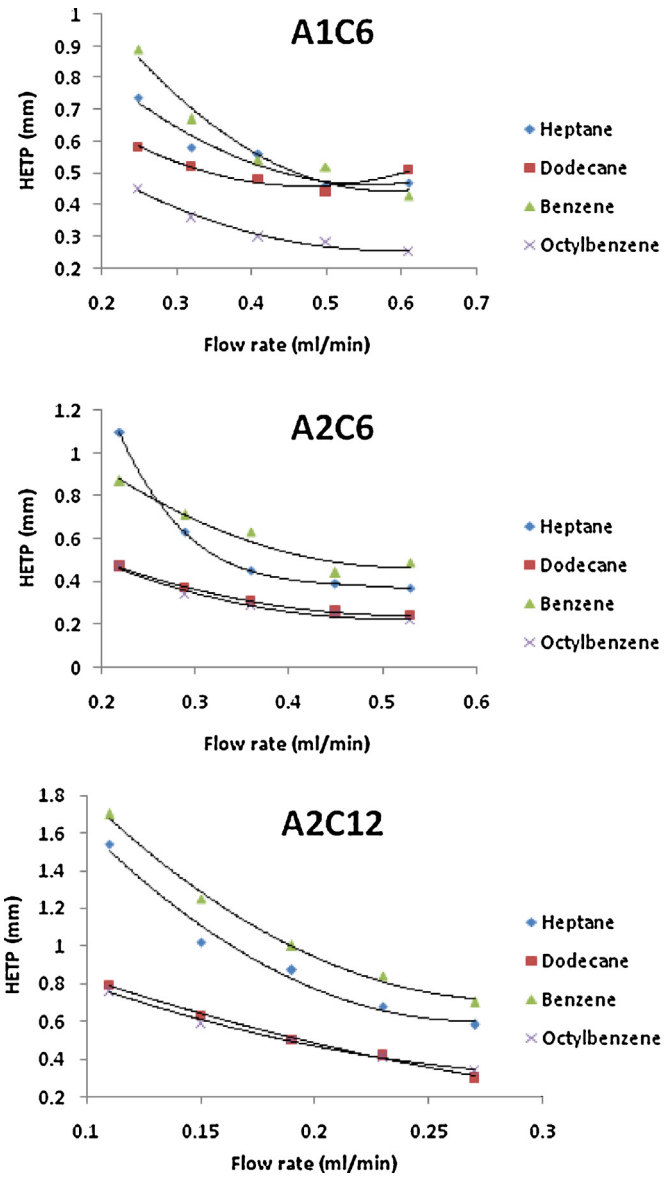

Fig. 6. Van Deemter plots relating height equivalent of theoretical plate HETP and flow rate *F* of the carrier gas (helium) for the three prepared monolithic columns (A1C6, A2C6, A2C12). Solutes: heptane, dodecane, benzene and octylbenzene.

3.6. Comparison with an open tubular commercial column

A comparison of the separation efficiency between the prepared monolithic columns and a commercial open tubular column was performed. A sample of alkanes (pentane, hexane, heptane, octane, nonane, decane, undecane and dodecane) was injected onto our columns using the optimum flow-rate derived from the van Deemter curves.

For the three prepared monolithic columns A1C6, A2C6 and A2C12 (250 μm × 0.5 m) the separations were performed under isothermal conditions at 200 °C, using the optimum flow-rate for each column, which corresponded to 0.6, 0.5 and 0.3 mL/min for columns A1C6, A2C6 and A2C12, respectively. Separations of the same sample with the same flow-rates were performed using the TR-5 MS (250 μm × 30 m) column as an example of a commercial

Table 3
Comparison between the efficiency of separation at the optimum conditions of an open tube commercial column (TR-5 MS 30 m), and the monolithic columns (A1C6, A2C6 and A2C12), through the values of retention time (t_R), number of theoretical plates (N), and the height equivalent of theoretical plate (HETP) of light alkane (pentane), and heavy alkane (dodecane), at the same flow rates.

Flow rate (ml/min)	Column	Pentane			Dodecane		
		t_R min	N	HETP mm	t_R min	N	HETP mm
0.6	TR-5 MS (30 m)	2.60	52,462	0.57	3.44	236,009	0.13
	A1C6 (0.5 m)	0.20	1246	0.40	1.96	1129	0.44
0.5	TR-5 MS (30 m)	3.08	62,607	0.48	4.86	209,507	0.14
	A2C6 (0.5 m)	0.21	1120	0.45	0.78	2079	0.24
0.3	TR-5 MS (30 m)	4.69	40,197	0.75	6.73	144,388	0.21
	A2C12 (0.5 m)	0.38	486	1.03	2.18	1407	0.35

Table 4
Run-to-run (a), day-to-day (1–7) and column-to-column (a–c) repeatability of chromatographic properties on A1C6 monoliths.

Poly. mixture	Run to run				Day	Day to day				Batch	Column to column			
	Benzene		Octylbenzene			Benzene		Octylbenzene			Benzene		Octylbenzene	
	k	H (mm)	k	H (mm)		k	H (mm)	k	H (mm)		k	H (mm)	k	H (mm)
a	3.53	0.41	280.74	0.23	1	3.51	0.41	280.64	0.23		3.51	0.41	279.97	0.23
	3.52	0.41	280.74	0.23	2	3.54	0.41	280.66	0.23	a	3.51	0.41	280.21	0.24
	3.54	0.41	280.73	0.23	3	3.52	0.41	280.64	0.22		3.53	0.42	280.05	0.26
	3.52	0.42	280.73	0.23	4	3.52	0.41	280.64	0.22		3.60	0.43	280.22	0.26
	3.53	0.41	280.74	0.24	5	3.53	0.4	280.61	0.23	b	3.58	0.44	279.95	0.27
	3.52	0.42	280.75	0.23	6	3.52	0.41	280.65	0.23		3.58	0.43	280.19	0.26
	3.52	0.43	280.74	0.24	7	3.52	0.41	280.65	0.23		3.52	0.42	280.13	0.23
	3.53	0.41	280.73	0.24						c	3.51	0.41	280.10	0.24
	3.52	0.41	280.74	0.24							3.51	0.41	279.98	0.23
	3.53	0.41	280.75	0.24										
Average	3.53	0.414	280.74	0.235		3.52	0.409	280.64	0.227		3.54	0.420	280.09	0.247
RSD% (%)	0.6	0.6	0.7	0.5		0.9	0.3	1.5	0.4		3.6	1.1	10.6	1.6

k – retention factor; H – height equivalent to a theoretical plate; RSD, relative standard deviation.

open tubular column. The optimum conditions for the TR-5 MS column were:

- Temperature programmed at 100 °C for 1 min was increased at a rate of 50 °C/min to 280 °C and steady for 5 min at an optimum flow-rate of 0.6 mL/min to compare the column to A1C6.
- Temperature programmed at 90 °C for 1 min was increased at a rate of 50 °C/min to 280 °C and steady for 5 min at an optimum flow-rate of 0.5 mL/min to compare the column to A2C6.
- Temperature programmed at 70 °C for 1 min was increased at a rate of 50 °C/min to 280 °C and steady for 5 min under the optimum flow-rate of 0.3 ml/min to compare the column to AC12.

Table 3 shows the results obtained using the three prepared monolithic columns and the commercial TR-5 MS column at the optimum flow-rates of each column. At 0.6 mL/min, A1C6 separated the alkane sample in 1.96 min with a mean HETP value of 0.42 mm, while TR-5 MS separated the same sample in 3.44 min with a mean HETP value of 0.35 mm. At 0.5 mL/min, A2C6 separated the alkanes sample in 0.78 min with a mean HETP value of 0.35 mm, while TR-5 MS separated the sample in 4.86 min with a mean HETP value of 0.31 mm. At 0.3 mL/min, A2C12 separated the alkanes sample in 2.18 min with a mean HETP value of 0.69 mm, while TR-5 MS separated the sample in 6.73 min with a mean HETP value of 0.48 mm.

This comparison shows that the monolithic columns separated the mixtures faster than the open tubular columns using GC but with an acceptable lower efficiency due to the higher HETP values. According to this comparison, A1C6 saved 48% of the separation time and carrier gas, A2C6 saved 84% of the separation time and carrier gas and A2C12 saved 67% of the separation time and carrier gas.

3.7. Repeatability, reproducibility and stability

The isothermal-isobaric separation of the alkylbenzenes (160 °C–1000 kPa) showed very good repeatability on a single column. Table 4 shows the characteristics of ten repeated separations of benzene and octylbenzene on the A1C6 column, with average run-to-run errors of 0.6% (benzene) and 0.7% (octylbenzene) in terms of the retention factor and 0.6% (benzene) to 0.5% (octylbenzene) for the efficiencies in terms of HETP. Standard deviations of 0.9% (benzene) and 1.5% (octylbenzene) for the retention factor and 0.3% (benzene) and 0.4% (octylbenzene) for the efficiency revealed very good repeatability on a single column and for the day-to-day separations. Separation of the alkylbenzenes on nine individual columns prepared from three separate polymerization mixtures shows good column-to-column reproducibility, with standard deviations of 3.6% (benzene) and 10.6% (octylbenzene) for the retention factor and 1.1% (benzene) and 1.6% (octylbenzene) for the efficiency. These results demonstrated that the preparation of monolithic polymethacrylate columns was reliable using the present approach.

3.8. Qualitative analysis of a gasoline sample

Fig. 7 demonstrates the successful separation of a real gasoline sample purchased from the local market using column A2C12 at optimum conditions. We determined that the A2C12 column was the most suitable column for real sample separations. Although it had the lowest porosity, A2C12 contained the longest and the most branched pathways and the narrowest pore size. These features allowed for the temperature programmed separation to be applied to A2C12 compared to the other columns, which were more suitable for the rapid separation of light hydrocarbons. After injecting synthetic mixtures of *n*-alkanes and alkylbenzenes as references,

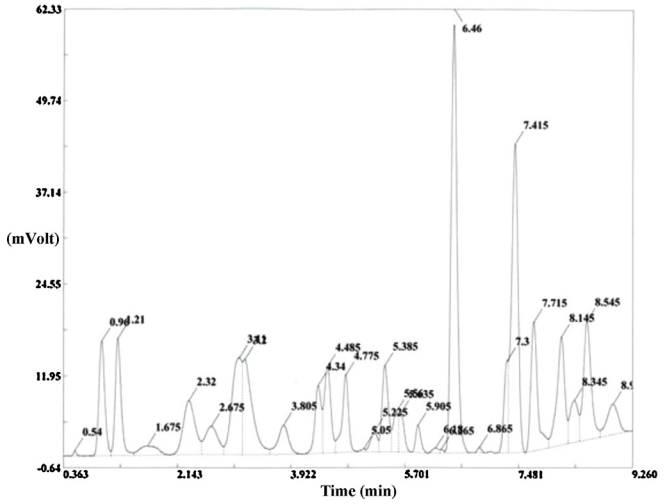

Fig. 7. Qualitative analysis of a gasoline sample using A2C12 monolithic polymeric capillary column (500 mm length × 250 μm i.d.) with lauryl methacrylate (12%) as monomer and ethylene dimethacrylate (12%) as crosslinker. Temperature program: 40 °C (3 min), rate of 25 °C/min, 170 °C (5 min), under 1000 kPa pressure.

the qualitative analysis with the area percent results of the gasoline sample yielded the following: pentane – at 1.21 min (4.31%), hexane – at 3.20 min (5.72%), heptane – at 4.78 min (3.11%), benzene – at 5.38 min (3.41%), octane – at 5.90 min (0.87%), toluene – at 6.46 min (16.14%), nonane – at 6.87 min (0.15%), ethylbenzene – at 7.30 min (2.43%), decane – at 7.71 min (5.46%), propylbenzene – at 8.15 min (5.29%), undecane – at 8.55 min (5.74%) and butylbenzene – at 8.96 min (2.16%).

4. Conclusions

Efficient separations of alkane and alkylbenzene samples were successfully achieved using polymethacrylate based monolithic columns and normal gas chromatography device with low pressure. The separation efficiency and the time of separation could be further improved using a temperature program rather than isothermal conditions. The poor thermal stability of the methacrylate polymer limited the temperatures that could be utilized. To solve this problem, a more thermally stable polymer could be used in the future works, such as poly(divinyl benzene), which remains stable up to 400 °C.

Finally, we found that each column contained unique advantages. For example, A1C6 was suitable for the separation of light alkanes and gases, A2C6 was suitable for rapid separations and A2C12 was better suited to programming than the other columns.

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