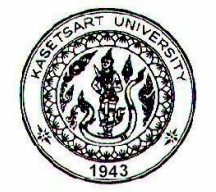

THESIS APPROVAL

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TITLE: Characterization of Silica Monolithic Column by Inverse Gas Chromatography

NAME: Mr. Anupop Sasook

THIS THESIS HAS BEEN ACCEPTED BY

_____ **THESIS ADVISOR**

(Assistant Professor Orapin Chienthavorn, Ph.D.)

_____ **THESIS CO-ADVISOR**

(Ms. Pinsuda Viravathana, Ph.D.)

_____ **DEPARTMENT HEAD**

(Assistant Professor Noojaree Prasitpan, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON _____

_____ **DEAN**

(Associate Professor Gunjana Theeragool, D. Agr.)

THESIS

CHARACTERIZATION OF SILICA MONOLITHIC COLUMN BY INVERSE GAS CHROMATOGRAPHY

ANUPOP SASOOK

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science (Chemistry)
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Silica monolithic material having single piece skeleton with large through pore size was synthesized by an in situ reaction inside capillary tubing. Surface area of the silica monolithic material was determined by using an inverse gas chromatographic technique (IGC). To be characterized, the column was used as a sample connected between the injection port and the flame ionization detector via a fused silica tubing. Parameters affecting surface area determination including operating temperature, injection volume for each column, type of probe molecules were optimized. Selected probe molecules in this work were octane, nonane, decane and undecane of which optimum operating temperatures were 100, 120, 140 and 160˚C, respectively. All probe molecules could be used to determine the surface area with reproducible results. However, as decane gave minimum relative standard deviation, it was used as a probe molecule in this work. Parameters of column fabrication affecting specific surface area of the monolith were also studied. Results showed that specific surface area decreased with increasing column diameter and length. In addition, surface areas of silica monolithic columns determined in this work varied from approximately 10 to 300 $m^2 \bullet g^{-1}$, indicating unreproducible columns. To be used for separation as a typical gas chromatographic column, the surface of silica monolithic column was coated with polyethyleneglycol (PEG) (MW 10,000 $g \bullet mol^{-1}$). Three coating methods, namely evaporation, dynamic and static coating method, were studied. Best column efficiency for separating a mixture of hexane and acetone was found for a 0.32 mm × 43 cm column coated with 30% w/w PEG in acetonitrile by a static coating method. The resolving power of the column was corresponded to ca. 40 m. commercial Carbowax column with a shorter analysis time.

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Student's signature Thesis Advisor's signature

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Anupop Sasook

October 2008

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INTRODUCTION

Chromatographic techniques are involved with separation of a wide range of compounds varying from polar to non-polar, the most important part is therefore an analytical column where separating components occurred. Efficiency of a column is generally explained by a van Deemter equation as shown below.

$$H = A + B/u + Cu \qquad \ldots(1\text{-}1)$$

where, H is plate height, u is linear velocity of mobile phase, and A, B and C are coefficients related to multiple flow paths, longitudinal diffusion and mass transfer between phases, respectively. Column efficiency is increased by decreasing plate height with decreasing A, B and C. In a chromatographic packed column, plate heights are minimized by uniformly packing of small spherical solid support, which relating to a decrease in A and B terms. However, very small particles causes a high pressure drop, besides, it is difficult to uniformly pack into a column. The performance of packed column is therefore limited by a particle size of packing material.

A monolithic column is a material having one single piece structure with interconnected skeletons and through pores. From such properties of structure, monolithic material provides high permeability, which is interesting to chromatographers. One of the most popular materials is monolithic silica that can be synthesized by an in situ polycondensation of alkoxysilane, e.g. tetramethoxysiliane (TMOS) and tetraethoxysilane (TEOS), in the presence of polyethylene glycol (PEG) inside tubing. An aqueous acetic solution is generally used as a solvent and catalyst in the reaction. By treating with ammonia or adding urea to the starting material mesopores are formed after the formation of silica structure, making the process simpler (Tanaka *et al.*, 2002).

To this decade monolithic silica structure fabricated in tubing used as a chromatographic column is receiving more attention, as it gives advantages over a classical packed column. Large through pores and small skeletons size of monolithic column provides high permeability that reduces an A-term of the van Deemter equation, so column efficiency is increased. Furthermore, diameter of monolithic column can be reduced to the range of capillary of which pressure drop was lower than that of packed column. Fast separation and low solvent consumption, which reduces analysis time, lowers cost of solvent and environmentally friendly solvent usage, are also beneficial.

Properties of material surface such as basic, acidic, pore size and surface area are important parameters to predict type and performance of reaction or interaction of monolithic materials and packing particles. In chromatography, surface of stationary phase inside column is a place where interaction between analyte(s) and stationary phase occurs. Interaction between each analyte and a stationary phase depends on types of analyte, experimental condition, performance of separation and efficiency of column, thus relating to surface properties of stationary phase. It is therefore essential to characterize surface properties of those materials.

Method for surface characterization can be classified into two main groups. First is a group of spectronic methods, i.e. scanning electron microscopy (SEM), atomic force microscopy (AFM) and transmission electron microscopy (TEM). Information obtained from these methods is involved with morphology, such as image of the surface. The other group is dealed with adsorption technique, such as gas adsorption and mercury adsorption, where information of structure of surface such as surface area and pore size are provided. A typical standard method for determination of surface area of solid is nitrogen adsorption at 77 K and fit adsorption data of nitrogen to an equation is provided. The method was named after three scientists, Brunauer, Emmett and Teller, and is called BET equation. Details in the equation will be followed accordingly.

Meanwhile the other sorption techniques including nitrogen sorption have been developing to give more information about surface of material. Based on interaction between molecules and surface of material depended on surface properties, the method provided experimental data of which mathematical equations are consequently derived to relate observed adsorption information to desired surface properties. In this research, a technique called inverse gas chromatography (IGC) was applied to determine surface area of monolithic silica column.

Inverse Chromatography is a surface characterization technique using a simple chromatographic instrument. In the technique, property of stationary phase is unknown and the stationary phase material is packed into an empty tubing and used as a chromatographic column. A compound with known properties is injected into mobile phase stream and it is called probe molecule that is utilized to determine properties of stationary phase. This means that the roles of phases are inverted from typical analytical chromatography, which is consequently named “inverse chromatography”. An inverse chromatography can be categorized into two types similarly to analytical chromatography: inverse gas chromatography (IGC) and inverse liquid chromatography (ILC) (Thielmann, 2004). In this work the first technique, inverse gas chromatography (IGC) will be focused.

When a probe molecule is injected to a chromatographic system, those molecules interact with the surface of material which acts as a stationary phase. In case of gas-solid, the interactions are adsorption and desorption. The molecules adsorb and desorb on the surface of material until they reach the detector and chromatograms are obtained. Some chromatographic parameters such as retention time, t_R, can be measured from those chromatograms. From the processes, it can be said that chromatographic parameters are functions of probe molecule-surface interaction. So measured chromatographic parameters can be converted to material surface properties, such as adsorption isotherm and surface area, by calculation.

Keys of surface area measurement are a number of molecules exclusively adsorbed one layer on the surface of material, or it is called a “monolayer capacity”

and the area that each molecule occupied, or “cross-sectional area of molecule”. If the adsorption of molecules on surface is monolayer, surface area will be calculated from amount of adsorbed molecule and cross-sectional area of molecule. In order to measure surface area of solid material using IGC technique, material is packed into a bare tubing and used as a column, then a probe molecule is injected. The amount of molecule adsorbed on material surface, or a “retention volume”, can be calculated from retention time by this equation (Zhou and Cadwallader, 2004)

$$V_N = (t_R - t_0)F_c \quad \ldots (1\text{-}2)$$

From equation (1-2) V_N is a net retention volume, t_R is a retention time of probe molecule, t_0 is a dead time and F_c is a corrected carrier gas flow rate. Flow rate of carrier gas is usually measured by a soap-bubble flow meter which is a rate of gas flow as it exits the column. The measured flow rate must be corrected to obtain an average flow rate of carrier gas in column (Zhou and Cadwallader, 2004) by equation (1-3):

$$F_c = F \times \frac{T_c}{T_f} \times \frac{P_f}{P} \times j \quad \ldots (1\text{-}3)$$

Where F is a measured flow rate of the carrier gas using flow meter, T_c is a column temperature, T_f is a flow meter temperature, P_f is a pressure in soap-bubble flow meter obtained by subtraction vapor pressure of water from ambient, P is an ambient pressure and j is a pressure drop correction factor which calculates from equation (1-4) (Skoog and Leary, 1991)

$$j = \frac{3[(P_i/P)^2 - 1]}{2[(P_i/P)^3 - 1]} \quad \ldots (1\text{-}4)$$

In equation (1-4) P_i is an inlet pressure and P is an outlet pressure, which is ambient pressure. Injection volumes of probe molecule that forms a monolayer adsorption on material surface are optimized by plotting injection volume against retention volume. Range of retention volume independent on injection volume is a volume of probe molecule formed monolayer adsorption.

After obtaining range of volume of probe molecule forming monolayer on surface, different volume of probe molecule which ranges in monolayer adsorption are injected into chromatography system. Partial pressure (p) and adsorption coefficient (a) of each injection volume of the probe are calculated from equation (1-5) and (1-6), respectively (Vega *et al.,* 2002)

$$p = \frac{m_a HRT}{FS_{peak}} \quad \ldots(1\text{-}5)$$

$$a = \frac{m_a S_{ads}}{mS_{peak}} \quad \ldots(1\text{-}6)$$

In equation (1-5) and (1-6), m_a is an amount of injected probe molecule, H is a peak height of probe molecule, R is the gas constant, T is a column temperature, F is a flow rate of carrier gas, S_{peak} is a peak area of adsorbed molecule, m is a material mass in the column and S_{ads} is the second vertically-half peak area of non-adsorbing molecule.

Monolayer capacity of probe molecule that is the key of surface area determination is estimated from adsorption coefficient and partial pressure of injected probe molecules by applying Brunauer, Emmett and Teller (BET) equation as shown below in equation (1-7)

$$\frac{p}{a(p_0 - p)} = \frac{(c-1)p}{a_m c\ p_0} + \frac{1}{a_m c} \quad \ldots(1\text{-}7)$$

This equation is similar to linear equation where p_0 is vapor pressure of probe molecule at operating temperature, a_m is a monolayer capacity and c is the BET constant. The vapor pressure of liquid at a working temperature can be calculated from Antoine equation as shown in equation (1-8) (Reid *et al.*, 1987: 214-215)

$$\log(p_0) = A - \frac{B}{T + C} \quad \ldots(1\text{-}8)$$

In equation (1-8), A, B and C are Antoine coefficients and T is a working temperature in Celsius.

By plotting $p/a(p_0 - p)$ versus p/p_0 of each injection volume of probe molecule, a_m and c constant are estimated from the slope and y axis-intercept. Finally, known monolayer capacity and cross-sectional area of probe molecule are converted to surface area following equation (1-9):

$$S = a_m N A_{cs} \quad \ldots(1\text{-}9)$$

In equation (1-9) S is the surface area, N is the Avogadro's constant and A_{cs} is the cross-sectional area of probe molecule which estimated from equation (1-10)

$$A_{cs} = 1.33 \times (M/\rho N)^{2/3} \times 10^{14} \quad \ldots(1\text{-}10)$$

In the equation, M and ρ are molecular weight and density of the molecule, respectively.

Inverse gas chromatography (IGC) is a simple technique compared with conventional nitrogen adsorption techniques. A commercial GC can be utilized without any modifications of instrument and an experiment is maintained in a common condition which easily controlled. On the other hand, nitrogen sorption technique is performed under vacuum at 77 K, which requires liquid nitrogen to cool down the system. System suitability of nitrogen sorption technique is doubtful, since error in measurement caused by too low sample weight of monolothic column in a capillary is occurred. Flow-through pore structure also makes it difficult to determine its particles and pore sizes. Advantages of IGC technique over nitrogen sorption are rapid, easy to handle and low cost experiment. A characterization of a monolithic capillary can be performed by a direct connection to a GC system without breaking solid material, which generally occurred during packing a chromatographic conventional column. Crushing material can cause a change in information of surface. IGC method is considered as an alternative way to characterize surface of material particularly for a very low-weight sample in a milligram level.

OBJECTIVES

1. To fabricate silica monolithic column.

2. To study variable factors affecting the structure of monolith.

3. To investigate factors affecting surface area determination by using inverse gas chromatography technique.

4. To modify the surface of synthesized monolithic column by a liquid stationary phase and study the chromatographic parameters of the modified column by gas chromatography.

LITERATURE REVIEW

Monolithic material is one piece solid with interconnected skeletons and interconnected flow path. As it provides high permeability and large surface area, the material was applied in many areas,e.g. analytical column in liquid chromatography and capillary electrochromatography and support material in solid phase micro extraction. Synthesis of some metal oxide monolith and their applications were reviewed here.

Kobayashi *et al.* (2002) used a fabricated monolithic silica column in capillary electrochromatography (CEC). The monolithic silica column was prepared by mixing tetramethoxysilane, polyethylene glycol with a molecular weight of 10000 and urea in aqueous acetic acid at 0˚C for 45 min then the solution was charged into 100-200 cm length 50μm i.d. fused-silica tubing and allowed to react at 40˚C overnight and temperature was raised to 120˚C for 3 hr. Surface of monolithic silica column was then modified by feeding C_{18} ligand, using octadecyltrichlorosilane into the fabricated tubing and allowed reaction at 60˚C. The reaction time was varied from 30-240 min for changing surface coverage. The CEC experiment was performed at 20˚C at an applied voltage of up to 30 kV. Mixture of 80% acetonitrile with 20% aqueous buffer (pH 8) was a mobile phase and alkylbenzenes were used as model compound to measure column efficiency. Plate heights of column were 3-4 μm. From chromatogram it was found that low surface coverage gave fast separation and best efficiency.

Shitani *et al.*(2003) developed a monolithic silica column to be used as an in-tube solid phase microextraction (SPME) for HPLC-UV and compared it with a conventional open-tubular in-tube SPME. The C_{18}-bonded monolithic silica column was prepared by following the method described by Kobayashi *et al.* (2002). In brief, an in situ hydrolysis of alkoxysilane occurred inside a 450mm x 0.2 mm i.d.fused-silica capillary tubing,. Then the capillary was cut to 150 mm long to be used as an in-tube SPME, and it was mounted in a position of sample loop of a six-port valve. After washing and conditioning by rinsing with methanol and pure water, a sample was

injected into the SPME column. When the valve was switched to inject position, the sample was desorbed with mobile phase and transported to HPLC-UV system. For chromatographic conditions, 0.3×150 mm Inertsil ODS-3 column with 3 μm particle sizes was used as a analytical column with (75 : 25) acetonitrile : water mobile phase at a flow rate of 5μL/min. The results showed that using monolithic column as an in-tube SPME, peak height and performance of separation of biphenyl extract were higher than using a conventional open-tubular column.

Joung *et al.(*2004) fabricated a monolithic silica column from a mixture of tetramethoxysilane (TMOS), polyethylene glycol (PEG) and urea in 0.01 N. aqueous acetic acid. Composition of the reagent was varied to improve column efficiency. After mixing the reagents at 0˚C for 30 min, the reaction mixture was filled into 0.5 mm i.d. stainless steel tubing and placed in an oven at 40˚C for 24 h, then the temperature was raised to 110˚C for 24 h to form the silica monolith. The surface of monolithic silica column was attached to dimethylchlorooctadecylsilane or C_{18} ligand, by filling the reagent into the column and kept at 110˚C to perform the reaction. The column was also end-capped by a reaction with trimethylchlorosilane at 110˚C. Finally, the column was washed and used as a HPLC column. A mixture of benzene, toluene and ethylbenzene was used as model compounds to test column efficiency. A mixture of 60% methanol-water with 0.1% trifluoroacetic acid (TFA) was a mobile phase at a flow rate of 0.001 mL/min. Chromatogram of the model compounds showed that the column fabricated from mixture of 1.0 g TMOS, 0.220 g PEG and 0.225 g urea in 2.5 mL aqueous acetic acid gave the best efficiency.

Hoth *et al.* (2005) synthesized two types of metal oxide monolithic column, namely zirconia and hafnia, by an in situ polycondensation of metal chloride inside a capillary tubing. Either zirconium tetrachloride or hafnium tetrachloride was used as a starting material in order to fabricate zirconia or hafnia monolith, respectively. An aqueous metal oxide was mixed with N-methylformamide and propylene oxide then the mixture was injected into 50 μm i.d. fused-silica capillary tubing and kept at 50˚C. Skeleton structure and through-pore of both types of monolith were observed by SEM. Suitability of hafnia monolithic capillary column was also tested by using CEC

and liquid chromatography (LC) to separate pyridine, pyrazole and imidazole. For CEC, the three components were separated in less than 8 min. For LC, analysis time was longer than that found in CEC with a 6.9 bar (690 kPa) pressure to drive the mobile phase.

Randon *et al.* (2006) synthesized a zirconia monolithic column and used it in a nano-LC. Starting material of the monolith was zirconium alkoxide mixed with a solution containing acetic acid, PEG with a molecular weight of 10000, water and n-butanol. Composition of reaction solution was characterized by several parameters, namely alkoxide concentration, hydrolysis ratio (or a number of water molecule over alkoxide molecule), complexation ratio (or a number of complexant, acetic acid, molecule over alkoxied molecule) and PEG concentration. Typical condition used in this work was 1 M of alkoxide with a hydrolysis ratio of 1, a complexation ratio of 3, and 5×10^{-2} M PEG. After mixing, the mixture was filled into 26 cm x 75 μm i.d. fused-silica tubing and kept at 30˚C for 24 h then heated at 150˚C for 6 h and used as a chromatographic column. A mixture of three amines, namely naphthalene, orthotoluidine and aniline, was used to test column efficiency with hexane mobile phase at a velocity of 0.09 cm/s. SEM images of monolithic column showed that the skeleton of zirconia was close to 2 μm. Specific surface area of the monolith measured by BET method was 60 m^2/g. However, a chromatogram showed that monolithic zirconia column had poor efficiency.

Inverse gas chromatography (IGC) is a useful technique for surface characterization. By using a simple gas chromatographic system with a principle that chromatographic parameters depend on adsorption of probe molecules on surface of stationary phase, both inorganic such as catalyst and organic compounds including some polymers can be characterized. Here are reviews of some applications of the IGC technique.

Vega *et al.* (2002) determined specific surface area of two types of polyarylamide fiber, namely non-crystalline fiber A and crystalline fiber B, using IGC method compared with BET method with a nitrogen adsorption at -196 ˚C and

geometrical method obtained from SEM. For IGC technique, chromatographic columns were prepared by packing each fiber into 5.3 mm i.d. stainless steel tubing. The column length was 112.5 cm for fiber A that equivalent to a total weight of 10.0010 g and 75.9 cm for fiber B that equivalent to 9.5935 g. a gas chromatography with flame ionization detection (GC-FID) system with He carrier gas was used to determine the adsorption chromatogram. N-Alkanes (C_9-C_{11}) were used as probe molecules and GC oven temperature was maintained at 40-80˚C isothermally. Peak area and peak height of each probe molecule were collected and related to adsorption parameters. In this research it was showed that both fibers obtained symmetrical peak using n-decane at 50˚C. Injection volume was optimized by plotting injection volume versus specific retention volume and the retention volume was independent on the amount of probe molecule. The specific surface area was obtained by fitting adsorption parameters that was determined at optimized condition to BET plot. Comparison of surface area measured from different methods was revealed that the values obtained by IGC were slightly lower than the nitrogen adsorption values with geometrical values being the lowest.

Singh *et al.* (2004) determined thermodynamic parameters and pore size of active carbon spheres (ACSs) by IGC technique and compared with a classical method using nitrogen adsorption at -196 ˚C. For IGC experiment, three ACSs prepared under different conditions were used as stationary phase. Each ACSs material was packed into 15 cm x 2 mm i.d. stainless steel tube then it was connected to GC-FID system using nitrogen carrier gas. GC oven was set at 30-300 ˚C isothermally. Thermodynamic parameters of ACSs surface were calculated from retention volume of probe molecules which were a series of n-alkane (C_2-C_7). Pore sizes of the materials were approximated from molecular sieving behavior of branched alkane probe molecules, namely n-alkanes, 2-methylalkanes and 2,2-dimethylalkanes. In molecular sieving behavior consideration, molecules having greater size than pore of material were not adsorbed and completely excluded, this was obtained from free energy of adsorption. In this research it was observed that thermodynamic parameters depended on microporous character of ACSs and pore size measured by IGC technique differed from that obtained by nitrogen adsorption

technique. The variations were probably due to different analysis conditions, which mainly were temperature and amount of injection. In IGC, small amount of probe molecules was injected and they were insufficient to cover the surface of material. Thus, the measured parameters represented the most active sites which retained probe molecules whereas nitrogen adsorption method represented total surface of material.

Zhou and Cadwallader (2004) applied IGC technique to study binding interactions between volatile flavor compounds and soy protein isolate (SPI) under controlled relative humidity. IGC column was prepared by packing 0.76 g SPI into 17.8 cm x 4 mm i.d. deactivated glass tubing and it was conditioned under carrier gas stream overnight before used. GC-FID was used with helium carrier gas to perform the experiment and oven temperature was controlled at 30, 35 and 40 ˚C isothermally. Relative humidity was controlled by mixing a proper ratio of dry and humidified carrier gas, which obtained by passing dry helium through a bottle filled with distilled water.. Volatile flavor compounds namely hexane, 1-hexanol and hexanal were used as probe molecules. Enthalpy of adsorption and sorption isotherm of each probe molecule at 0% and 30% relative humidity were calculated from retention volume. From results, it was showed that at 0% relative humidity binding efficiency of hexanol and hexanal were higher than that found for hexane due to H-bond interaction but binding efficiency of volatile flavor compounds decreased with increasing relative humidity.

Katarzyna and Adam (2006) determined solubility parameter of polyethylene glycols (PEG) with molecular weights of 2000 10000 and 35000 in different organic solvent by IGC technique. The IGC column was prepared by dissolving PEG in methanol, mixing it with inert solid support, then evaporating the solvent, and filling into a 2 mm i.d. × 30 cm glass column. The column was connected to GC-FID using helium carrier gas at a flow rate of 20mL/min. The temperature was controlled at 85, 95, and 105˚C isothermally. Different organic solvents with different intermolecular interactions, such as n-alkane, acetonitile, chloroform and ethanol, were used as probe molecules and retention data were collected. The solubility parameter and interaction parameter were calculated from Hansen’s equation, resulting that the solubility

parameter of PEG 2000 decreased with increasing temperature, while those for PEG 10000 and 35000 increased with raising temperature. At the same temperature the solubility parameter of PEG 2000 was higher than that found for PEG 10000 and 35000.

Baoli *et al.* (2007) measured Lewis acid-base properties of polymer surface using inverse gas chromatographic technique. Seven polymers were treated then packed into stainless steel columns and used as gas chromatographic columns with nitrogen carrier gas and flame ionization detection. Five polar solvents, namely chloroform, acetone, diethyl ether, tetrahydrofuran and ethyl acetate, were used as probe molecules. The oven temperature was set at 40, 50, 60 and 70˚C isothermally. The adsorption enthalpy and entropy by Lewis acid-base interaction at various temperatures were calculated from a retention volume of probe molecule. Then Lewis acid (Ka) and base constant (Kb) were achieved from a slope and an intercept of graph plotting between enthalpy/acceptor numbers of probe molecule versus donor numbers/acceptor numbers of probe molecule. From those Ka and Kb, the polymers were grouped into two regions by plotting Ka versus Kb. Region I, the value of Ka/Kb were 1.4 – 2.1 and the others were region II where the polymers had higher basicity than that found in region I.

Cava *et al.* (2007) characterized surface properties of biodegradable polymers, namely polylactic acid, (aliphatic polyester) and polycaprolactone (semi-crystalline polyester) by IGC technique. The polymers' pellets were packed into 30 cm length, 3 mm i.d glass columns, then conditioned at appropriate temperature, and used as chromatographic columns. Measurements were carried out using GC-FID where helium was used as carrier gas at 25, 30 and 35˚C isothermally. In order to measure dispersive component of surface energy, n-alkane series (C_5-C_7) was selected as probe molecules. To determine specific interaction, electron acceptors, namely carbon tetrachloride and chloroform; and electron donors, namely acetonitrile and 1,4-dioxan, were used. Retention volume were determined then converted to desired parameters. In this research, values of dispersive component of surface energy of both materials

were small and close to those reported in literature. The analysis of specific interactions described the surface of both polymers as neutral.

Arp H. *et. al.* (2008) determined gas – particle equilibrium partition coefficient (Kip) of organic compounds using gas chromatography. Tefoln coated glass fiber filter (TGFF) were silylated then placed in stainless steel tubing and used as chromatographic column to measure Kip of polar compounds. And TGFF with deceasing silylation degree was used as a column to measure Kip of non-polar compounds. The column was connected to GC-FID system which experimental condition were set at the ambient then semi-volatile organic compounds (SVOCs) such as pesticides, PAHs, and n-alcohol was injected. Retention volume were determined then converted to Kip by dividing with mass of particle on filter (Mp). From the results, this technique gave reliable Kip compared with classical technique, sample-and-extract.

Zang *et al.* (2008) prepared catalyst containing rhodium (Rh) using H-β-zeolite support by impregnation method then it was characterized by using IGC technique. For characterization, crumbled Rh/ H-β-zeolite with 60-80 mesh size was used as a stationary phase for IGC technique, compared with crumbled H-β-zeolite. IGC column was prepared by packing a 0.2 g of zeolite into 30 cm length, 2 mm i.d.stainless steel, then plugging both ends by glass wool. Chromatographic experiments were performed with GC-FID using nitrogen carrier gas and oven temperature was controlled at 200-240˚C isothermally. Three alkanes (C_5-C_7), namely cyclohexane, benzene, trichloroethylene and tetrachloroethylene, were used as probe molecules. Surface properties of zeolite, such as standard surface free energy and enthalpy of adsorption, and specific components of surface free energy of those probe molecules were calculated from retention data. From the results, it was found that surface area obtained from scanning electron microscope, enthalpy of adsorption, and surface free energy, decreased after the impregnation rhodium and rhodium had special adsorption for benzene, that was useful for making catalyst for a reaction involving benzene.

Apart from inverse gas chromatography, several methods were utilized to characterize properties of surface of monolithic material. Some conventional methods used to study surface area and porosity of the material were listed below.

Urban *et al.* (2008) prepared polymer-based monolithic capillary columns and determined pore size of the column using inverse size exclusion chromatography (ISEC) compared with mercury intrusion porosimetry (MIP). The monolithic columns were fabricated using butyl methacrylate (BMA) and ethylene dimethacrylate (EDMA) as monomers, either 1,4-butanediol or 1-propanol as a porogen, and AIBN as an initiator. The mixture was filled into 250-μm i.d. fused-silica capillary tube, and simultaneous polymerization occurred in the tubing. Polymerization was carried out in vial for a determination of pore size with MIP. Monolithic material prepared in a glass vial was crushed and cut into a small pieces and its pore size was determined by MIP technique. For ISEC, monolithic column was connected to a HPLC-UV system using THF as a mobile phase and polystyrene standards with molar masses ranged from 92 to 2950000 were used as probe molecules. Retention volume were collected and correlated to the pore size. From the results it was found that porosities and pore size depended on the composition of polymerization mixture. In addition, pore volume measured with ISEC was 1 to 2.8 times greater than values determined by using MIP method because of limitation of accessibility of mercury into the small pores.

Wieder *et al.* (2008) had optimized concentration of monomer, crosslinker and porogen using as starting materials in fabrication of monolithic column. The monomer was p-methylstyrene and it was copolymerized with a crosslinker namely bis(p-vinylbenzyl)dimethylsilane (BVBDMS). Toluene and 2-propanol were used as porogen. Monolithic columns were prepared by an in situ polymerization of starting materials inside 200 μm i.d. fused-silica capillary tubing. Measurements of porosity were performed by using mercury intrusion porosimetry (MIP). Specific surface area was obtained by MIP and BET sorptiometry. Column efficiencies were also studied by using the synthesized monolithic column as an analytical column to separate a mixture of 9 proteins using HPLC-UV. From the results it was found that high total

monomer content led to a constriction of average pore size and implemented surface area value. Increasing porogen content improved surface area value but an average pore size was decreased due to a long precipitation time in polymerization process. Although specific surface area value was higher by raising concentration of porogen, column efficiency was reduced when the concentration of porogen was more than 14%.

For In implementing a fused-silica gas capillary column, liquid stationary phase may be coated onto the inner wall. Two coating methods, namely physical and chemical coating, were typically applied. Physical coating is a technique in which a stationary phase is dissolved in an organic solvent, and directly filled into the capillary. The solvent was subsequently vacuumed drawn out of the tubing by using a pump. Chemical method was performed by an in situ reaction of stationary phase and solid support or wall of capillary tubing. Stationary phase with an active functional group was dissolved in a solvent, and filled into column tubing. Then the tubing was conditioned to allow the reaction occurred. A number of applications of coating methods of polyethylene glycol liquid stationary phase were given here.

Shende *et al.* (2003) developed a method of bonding PEG to an inner wall of fused-silica capillary tubing and used as GC column. The PEG stationary phase was prepared through sol-gel process starting from methyltrimethoxysilane (MTMOS) and bis(trimethoxylsilylethyl)benzene as sol-gel precursor, PEG with one and two active ends, trifluoroacetic acid and ammonium fluoride as catalyst. The sol-solution was filled into 10 m x 250 μm i.d. fused-silica capillary tube to attach to the inner wall of tubing at a controlled condition. Various polar and non-polar solutes were injected to test chromatographic performance. The results showed that the column had high reproducibility, which was represented as RSD of chromatographic parameters, i.e. retention time, separation factor, and theoretical plates of run-to-run and column-to-column, ranged from 0.5-1.37% (n = 5). Stability of the fabricated sol-gel PEG column was examined by comparing retention times for Grob test mixture of pre-and post-rinsing column with those of solvent. RSDs of all compounds were less than 0.5% and low bleeding of column occured at a temperature of 320˚C.

Lambertus *et al.* (2004) fabricated square-spiral column on a silicon wafer for a conventional benchtop GC-FID instrument. Each column was fabricated by using an etch, resulting in 3-m-long square spiral channels with a 150-μm-wide by 240-μm deep on a silicon wafer. For a standard 4-inch silicon wafer, 4 columns were fabricated simultaneously. Then the open surface of silicon wafer was sealed with Pyrex glass. Inner surface of the columns was firstly coated with SiO_2, and finally coated with dimethyl polysiloxane for non-polar stationary phase and trifluoropropylmethyl polysiloxane for polar stationary phase. Coating method was performed by static coating of which a solution of stationary phase was filled into the column, then sealed at one end and vacuum was applied to the other end. In studying column efficiency, a mixture of 20 n-alkanes was injected with both isothermal and temperature program. All chromatograms showed symmetrical peaks, indicating that active surface of silica was not a significant problem for the relatively thick stationary phase used in this study. Theoretical plates ranged from 4600 to 8200 plates for dimethyl polysiloxane and from 3500 to 5500 plates for trifluoropropylmethyl polysiloxane columns.

MATERIALS AND METHODS

1. Methods

Bared fused – silica capillary tubing with different internal diameters of 0.25, 0.32 and 0.53 mm obtained from SGE (Victoria, Australia) was pretreated with sodium hydroxide purchased from Merck (Darmstadt, Germany). Monolithic silica column was fabricated from a mixture of an alkoxysilane using an analytical reagent grade of either tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS) purchased from Merck (Darmstadt, Germany) and polyethylene glycol (PEG) with a molecular weight of 10,000 g mol^{-1} obtained from Fluka (Seelze, Germany) in aqueous acetic acid solution, which was prepared by dissolving glacial acetic acid obtained from BDH (Poole, England) in deionized water.

The mixture was filled into a pretreated fused-silica tubing by blowing oxygen free nitrogen gas obtained from Plexair (Bangkok, Thailand) into reaction mixture.

In washing step, one end of a F-243X tubing sleeve obtained from Upchurch Scientific (Oak Harbor, WA, USA) was connected to a newly fabricated monolithic silica column and the other end was connected to a 500 μL gas tight syringe obtained from SGE (Victoria, Australia). A HPLC grade methanol was purchased from Fisher (Leicestershire, UK) and used as a solvent to remove any contaminants and unreact reagent.

Four straight chain alkanes, namely octane purchased from Carlo erba (Milan, Italy), nonane from Sigma (Steinheim, Germany), decane and undecane obtained from fluka (Buchs, Switzerland), were of analytical grade used as probe molecules to determine surface area.

Surface of a newly fabricated monolithic silica was coated with polyethylene glycol (PEG) with a molecular weight of 10,000 g mol^{-1} obtained from Fluka (Seelze,

Germany) by dissolving it in HPLC grade acetonitile purchased from Fisher (Leicestershire, UK).

Hexane and acetone of analytical reagent grade were obtained from BDH (Poole, England) were used as model compounds to test column performance.

After washed and dried, the monolithic column was connected to a gas chromatographic system by using a glass connector obtained from Alltech (Illinois, USA).

2. Reagent preparation

2.1 0.01 M Aqueous acetic acid

A 1.00 M stock aqueous acetic acid solution was prepared by weighing 6.00 g of glacial acetic acid and dissolving in a 100.00 mL deionized water and it was diluted ten times with deionized water.

2.2 1.00 M Sodium hydroxide solution

A 2.00 g of sodium hydroxide was dissolved in 50 mL deionized water.

2.3 Polyethylene glycol solution at a concentration between 1, 5, 10, 20, 30, 40, and 50 % w/w in acetonitrile

PEG with a molecular weight of 10,000 g mol^{-1} was weighted to 0.010, 0.050, 0.100, 0.200, 0.300, 0.400, or 0.500 g and dissolved in acetonitrile, and the final weight was adjusted to 1.0000 g by adding acetonitrile. The solution was sonicated in an ultrasonic bath until PEG was completely dissolved and used as a coating solution.

3. Column preparation

3.1 Pre-treating a fused silica capillary

To be used as a column, fused-silica tubing was pre-treated with NaOH by aspiring 1.0 M NaOH solution into the tubing, sealed at both end, and placed in an oven at 40°C for 6 h. The seal was subsequently removed and the tubing was washed by deionized water, and dried by blowing with nitrogen gas.

3.2 Fabrication step

Monolithic silica column was prepared following a modification of Kobayashi (2002) Ref. method described as follows.

In a 1 dram vial, 0.09 g of urea was dissolved in 1 mL of 0.01 M of acetic acid and added 0.088 g of PEG in the mixture. After homogeneously mixing, the vial was placed in an ice bath where 1 to 2 g of salt was added. A 0.4 mL of TMOS was added into the mixture drop by drop using a pasteur pipette and it was stirred for 45 min using a magnetic stirrer. Stirring rate was controlled between 700 to1000 rpm. The reaction mixture was filled into a NaOH pre-treated fused-silica capillary tubing by pressure as shown in Figure 1. A nitrogen gas stream was flowed into the vial to push the mixture into the capillary and a space was leaved at both ends. The capillary was tightly sealed with GC septum and it was placed in an oven at 40°C isothermally for 24 h, and then the oven temperature was raised to 120°C isothermally for 6 h for a reaction to occur.

Finally, the fabricated capillary column was washed with methanol using syringe pump by connecting the column to a 500 μL SGE gas tight syringe (Victoria, Australia) via a sleeve. The solvent was driven at a flow rate of 10 $\mu L \cdot min^{-1}$ for 50 min, then the column was dried by a gentle heat at 60°C for 3 h.

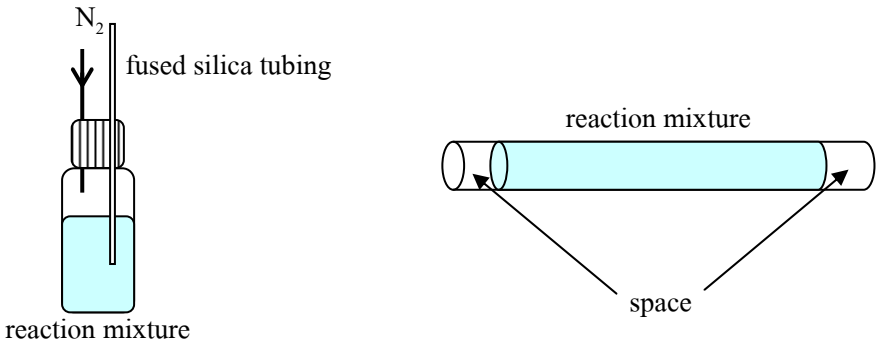


Figure 1 Schematic of filling a reaction mixture into a capillary by pressure

3.3 Refabrication

A doubly fabricated monolithic silica capillary was constructed by refilling a dried firstly fabricated monolithic silica column with the reaction mixture and reacted following section 2.2. In this work the refabrication was performed on a 10 cm x 0.53 mm i.d. monolithic capillary.

3.4 Column coating

After forming monolithic structure, a fabricated silica capillary was coated with polyethylene glycol by using a solution prepared following section 1.3. Surface of the monolithic capillary was coated by static coating method described below.

3.4.1 Coating step

A coating solution was filled into a monolithic silica capillary by a syringe pump. In order to fill the solution into the column, a gas tight syringe containing a coating solution was connected to the column by using a tubing sleeve as shown in Figure 2 and delivered the solution at a flow rate of 100 $\mu L \cdot h^{-1}$.

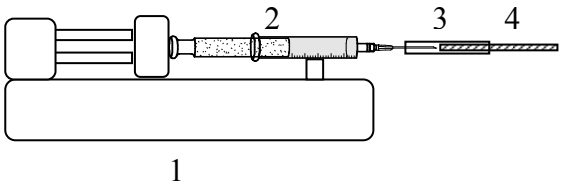


Figure 2 Schematic of coating method: 1 = syringe pump, 2 = gas tight syringe, 3 = tubing sleeve and 4 = monolithic silica column

3.4.2 Evaporation step

After the monolithic silica capillary was completely filled with the coating solution, acetonitrile solvent was evaporated to leave a thin film of PEG solute on its surface. To evaporate the filled capillary was sealed at one end with a GC septum and the other end was penetrated into a vial through a line septum. A hypodermic needle connecting to a vacuum pump via a plastic tubing was also inserted into the same vial (Figure 3). When the vacuum pump was switched on the air in a vial was evacuated to perform a vacuum. The evaporating step had been performed for 3 h to remove a solvent of coating solution. Finally, the coated monolithic column was removed and placed in an oven at 60˚C for 3 h to ensure that solvent was completely removed.

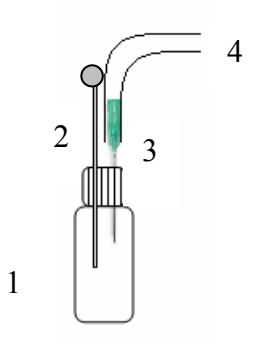


Figure 3 Schematic of solvent evaporation under vacuum: 1 = vial, 2 = a one end sealed monolithic column, 3 = hypodermic needle and 4 = plastic tubing connected to vacuum pump.

3.5 Coating a bare fused-silica tubing

Because the length of fabricated monolithic column was not enough to connect between injection port to detector, a 2 m length × 0.32 mm i.d. bared fused-silica tubing was connected from a monolithic column by a glass connector to FID detector. Surface of the bare tubing was also coated with PEG solution using a dynamic technique following these steps. A 5% w/w PEG solution was injected into a bare tube by purging nitrogen gas into a vial in the same way as described in section 2.2 to create a plug of solution with 20 cm long at the beginning of tubing. The plug was then forced to pass through the tube at a rate of 2 to 3 mm·min^{-1} by a controlled flow of nitrogen gas purging as shown in Figure 4. Two controlled valves were arranged in series in order to reduce the nitrogen gas flow, as well as to maintain flow continuity.

When the plug was passed throughly, the tubing was placed in an oven at 60˚C for 3 h to remove solvent.

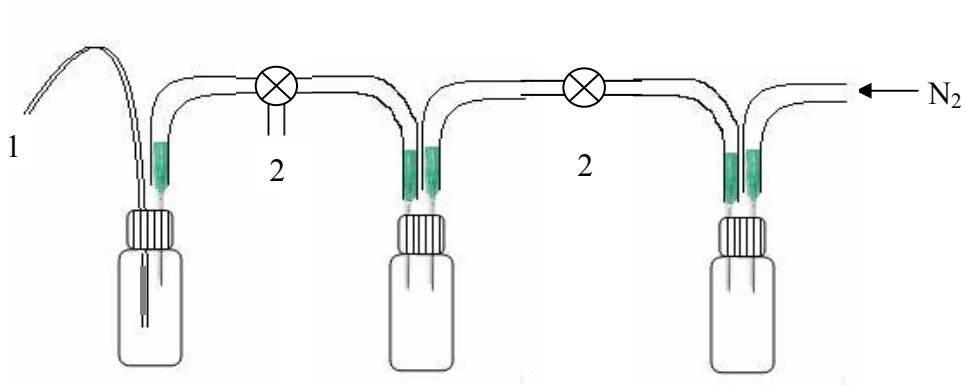


Figure 4 Schematic of dynamic coating: 1 = bare fused-silica tubing with a plug of solvent and 2 = controlled valve

After fabrication, the fabricated silica monolith column would be a sample subjected to an inverse gas chromatographic determination. To reduce any confusion the might occur during experiment, each column was given a code, i.e. a 10 m × 0.32

mm i.d. bared fused silica tubing was coded as a 32-10 sample. The number “32” was represented the internal diameter of column, and the number “10” was for its length.

4. Surface characterization

4.1 Inverse gas chromatography

Surface area of fabricated monolithic silica column was determined using inverse gas chromatographic technique following these steps.

Monolithic silica column was connected to an injector of a HP-5890 series II GC (Hewlett-Packard, Polo Alto, CA, USA) at one end and to a 10 m × 0.32 mm i.d. bared uncoated fused silica tubing (SGE, Victoria, Australia) via a glass connector at the other end. The bared fused silica tubing was inserted to a flame ionization detector (FID) and nitrogen was used as carrier gas as a schematic diagram shown in Figure 5.

Gas flow rate was controlled at 2.3 to 2.5 mL min^{-1}. Both injection port and detector temperature were maintained at 250°C.

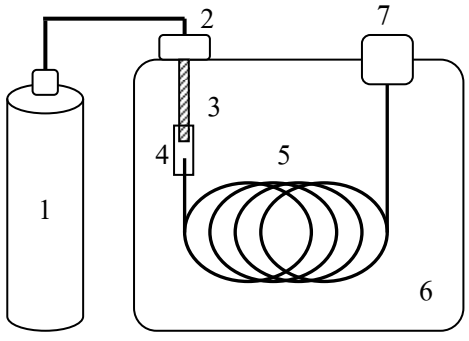


Figure 5 Schematic of modified gas chromatograph: 1 = carrier gas, 2 = injection port, 3 = fabricated monolithic silica column, 4 = glass connector, 5 = bared fused silica tubing, 6 = GC oven and 7 = FID detector

4.1.1 Optimization

Conditions for a determination of surface area of monolithic silica column using IGC technique were optimized. The experiments were performed on a single column, which was a 0.53 mm i.d. × 10 cm long monolithic silica column. In this research, experimental factors namely temperature, injection volume of each probe molecule for this column and probe molecules were optimized. Reproducibility of the technique was also studied.

4.1.1.1 Operating temperature

The operating temperature was studied in order to obtain the best peek shape of each probe molecule. The oven temperature was varied from 80 to 160°C isothermally for each injection of 1 μL of probe molecule.

4.1.1.2 Injection volume

An injection volume for each monolithic silica column was also studied to optimize the volume range that probe molecule caused monolayer adsorption. The amount of probe molecule injected was varied from 0.2 to 2.0 μL under the optimized temperature.

4.1.1.3 Probe molecules and reproducibility

A day-to-day variation, which related to reproducibility of the method, and effect of probe molecules were studied using a randomized block design. Numbers of experiment and order of measurements were given in Table 1. All experiments were studied under the optimized temperature and injection volume.

Table 1 Orders of measurement of experiment

Day	Probe molecule			
1	decane	nonane	octane	undecane
2	octane	nonane	decane	undecane
3	octane	decane	undecane	nonane

4.1.2 Optimization of column fabrication

Surface area of fabricated monolithic silica columns were determined under optimized conditions to study some effects of column fabrication on surface area, such as refabrication and column diameter.

4.1.2.1 Refabrication

Surface area of synthesized monolithic silica structure in 0.53 mm × 10 cm capillary tubing was determined by using IGC technique under optimized conditions. The same column was refabricated in the same way as described in section 2.3 then surface area of the refabricated column was determined using the same IGC technique and both measured surface areas were compared.

4.1.2.2 Inner diameter and column length

Silica monolithic columns were fabricated in 0.25 and 0.32 mm i.d. fused-silica tubing with ~10 cm and ~ 25 cm length in order to study the effect of inner diameter and length of silica monolithic column on surface area by using IGC technique under optimum conditions. Each column was given a number and the order of measurement was randomized as shown in Table 2.

Table 2 Order of surface area measurement for each monolithic silica column

Order of measurement	Column
1	0.32 mm × 10 cm
2	0.25 mm × 20 cm
3	0.25 mm × 10 cm
4	0.32 mm × 20 cm

4.1.3 Repeatability of column fabrication

Reproducibility of surface area of monolithic silica columns was studied by measuring surface area of the columns prepared from different batches. Three batches of monolithic silica column were fabricated with different time and three columns of each batch were selected to determine surface area by using IGC method under optimized conditions. All columns were synthesized in 0.32 mm i.d. fused-silica capillary tubings and the length of columns was 10 cm for first two batches and 20 cm for the last batch.

4.2 Brunauer, Emmett and Teller (BET) method

Before determined surface area, monolithic column was conditioned at 90˚C and evacuated to eliminate any contaminant gases. The column was evacuated with a rate of 50 mmHg · s^{-1} for 60 min, then the temperature was raised to 150 ˚C for 300 min.

Surface area was determined from nitrogen adsorption by using Micromeritics ASAP 2020 surface area and porosity analyzer (Particle and surface sciences, New South Wales, Australia) at -195.7 ˚C

4.3 Scanning electron microscope

To investicate the morphology of silica monolithic column, the column was cut at the end and coated with gold by using an EIKO IB-2 ion coater (Eiko engineering, Ibaraki, Japan) with a current of 5 mA for 5 min. Then, the column was scanned by using a JEOL JSM 5600LV scanning electron microscope (JEOL,Tokyo, Japan) at 20 kV.

Homogeneity of PEG coated silica monolithic column was also investigated by determining amount of carbon at both ends using a JEOL JSM 5410 scanning electron microscope (JEOL, Tokyo, Japan) coupled with an Oxford Inca 300 energy dispersive spectrometry (Oxford, Oxfordshire, UK) or SEM-EDS. The current was set at 37 μA with 100 s acquisition time.

5 Chromatographic properties of PEG coated monolithic silica column

Monolithic silica columns were coated with PEG with an average molecular weight of 10,000 solution in acetonitrile to be used as an analytical column. The coated column was connected to the injection port at one end and the other end was connected to a 2 m wall coated fused-silica capillary tubing of which preparation method was previously given in section 2.5. The instrument setup was similar to that shown in Figure 4. GC conditions were as follows: nitrogen was used as a carrier gas with a flow rate of 2.3 to 2.5 $mL \cdot min^{-1}$, injection volume of model compound was 1 μL, both injection port and detector temperatures were 250˚C and oven temperature was kept at 60 ˚C isothermally. The effect concentration of coating solution and column length was studied by measuring chromatographic parameters of model compound, which was a mixture of n-hexane and acetone.

RESULTS AND DISCUSSION

Part I Fabrication of silica monolithic columns

Monolithic silica structure was synthesized in a fused silica capillary tubing to be used as a chromatographic column. It was fabricated by modifying a method from Kobayashi *et al.* (2002) which was given in Section 3.

Initially, alkoxy silane used in the reaction was tetraethoxysilane (TEOS) and the capillary tubing was made of glass with 1 mm i.d. It was found that mixture was evaporated when placing in an oven and the reaction was not occurred. The glass tubing was consequently sealed at both ends to prevent the evaporation. However, monolithic silica structure was not formed when reacting under the same condition as expected. It might be a result of inertness of ethoxy leaving group of silane. When changing the ethoxy to methoxy leaving group by replacing TEOS with tetramethoxysilane (TMOS) and the mixture in the tubing was subjected to the same conditions, it was found that monolithic silica structure was finally formed.

However, the monolithic silica structure in the glass tubing could not be used as a chromatographic column because cracking and shrinkage of the structure occurred. Some reports have demonstrated that the silica fracture could be lessened by decreasing the internal diameter of the capillary (Nunez *et al.,*2008). To proof the size of tubing was then reduced to capillary range. Scanning electron micrographs of the cross-sectional silica monolithic in a capillary tubing with 0.53 and 0.32 mm i.d. were shown below.

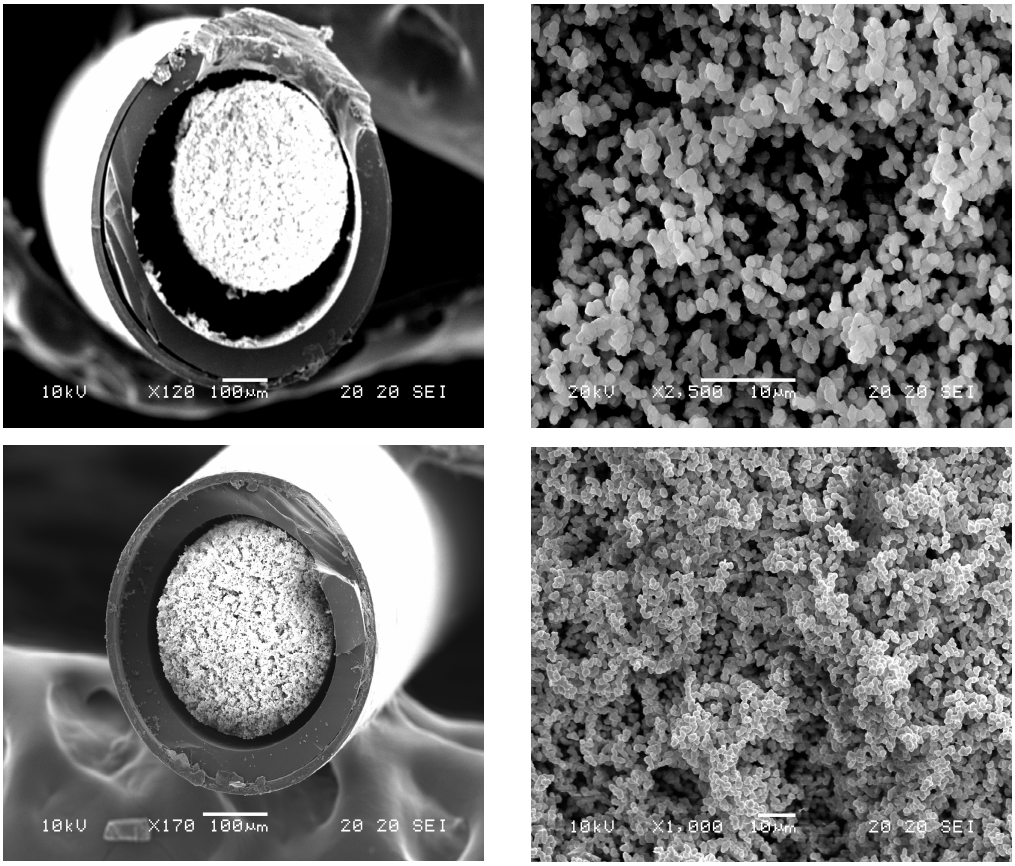


Figure 6 Scanning electron micrographs of silica monolithic column with 0.53 mm i.d. (above) and 0.32 mm i.d. (below)

As seen from Figure 6, the void between the monolith structure and the wall of tubing was a result of shrinkage of silica structure. The shrinkage of small tube was less than the big one, the void was therefore reduced by decreasing the internal column diameter. Silica skeletons of for both 0.53 and 0.32 mm i.d. were similar in shape and size of which was around 1 μm and its through pore was approximately 10 μm.

Although a reduction of column diameter decreased shrinkage of silica skeleton, its structure cracked when rising the reaction temperature from 40 to 120˚C, which might be a result of bubble formation during preparation of the reaction mixture. To homogenize the reactant mixture, it was stirred. Too low stirring rate caused the solution separated into two phases when adding TMOS. On the other hand,

with too high rate, the bubbles were formed, thus column was cracking in final. After many trials, the suitable stirring rate was found between 700 to 1000 rpm to obtain homogeneous solution with no bubble formation, thus minimizing the cracking.

Not only stirring rate, but the bubble could occur in the solution at high temperature. To reduce or eliminate those bubbles, after filling the reaction mixture into the tubing, both ends of the fused-silica tubing were needed to leave gaps before sealing with GC septum.

Part II Inverse gas chromatography

After fabrication, surface area of silica monolithic columns was measured by using IGC technique by connecting the column to a typical gas chromaograph with flame ionization detector. Overall procedures were summarized step by step as a flowchart shown in Appendix B.

In this work, four n-alkanes, namely octane, nonane, decane, and undecane were selected as probe molecules to determine surface area by IGC technique according to their weak interaction to silica surface.

1 Optimization

In this work, surface area of silica monolithic column was determined by using IGC technique. Some variables, i.e. temperature, injection volume, type of probe molecule, etc., affected chromatographic parameters and interaction of probe molecule on surface were studied.

1.1 Operating temperature

An operating oven temperature was an importance factor, since it affected the adsorption of molecule on monolithic silica surface. Too low temperature resulted in not only broader peak of probe molecule, but also long retention time.(Theilmann, 2004). At too high temperature, molecule might be eluted before reaching the equilibrium of interaction between gas mobile phase and liquid stationary phase.

An appropriate temperature for each probe molecule was studied by injecting 1 μL of probe molecule into the GC-FID coupled with a test monolithic silica column at 80, 100, 120, 140 and 160˚C isothermally. The same monolithic silica column was used for all other conditions. Chromatograms of each probe molecule at different temperatures are shown in Figure 7 and chromatographic parameters of these chromatograms were summarized in table 3 to 6. Examples, equations and

calculations of all chromatographic parameters, namely capacity factor, tailing factor, and theoretical plates, were given in Appendix A

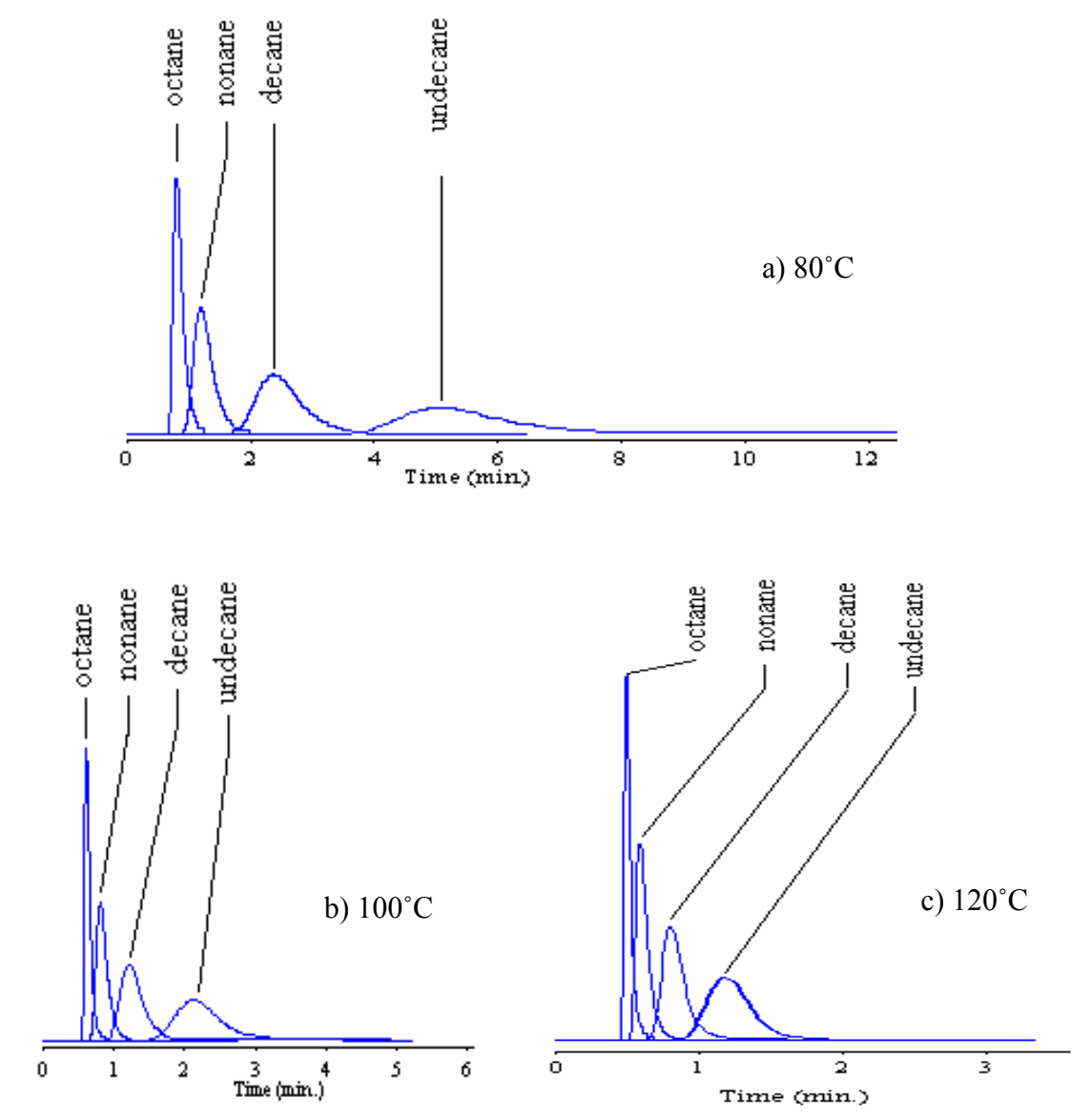


Figure 7 Chromatograms for octane, nonane, decane, and undecane on silica monolithic column at an isothermal temperature of (a) 80˚C, (b) 100˚C, (c) 120˚C, (d) 140˚C and (e) 160˚C

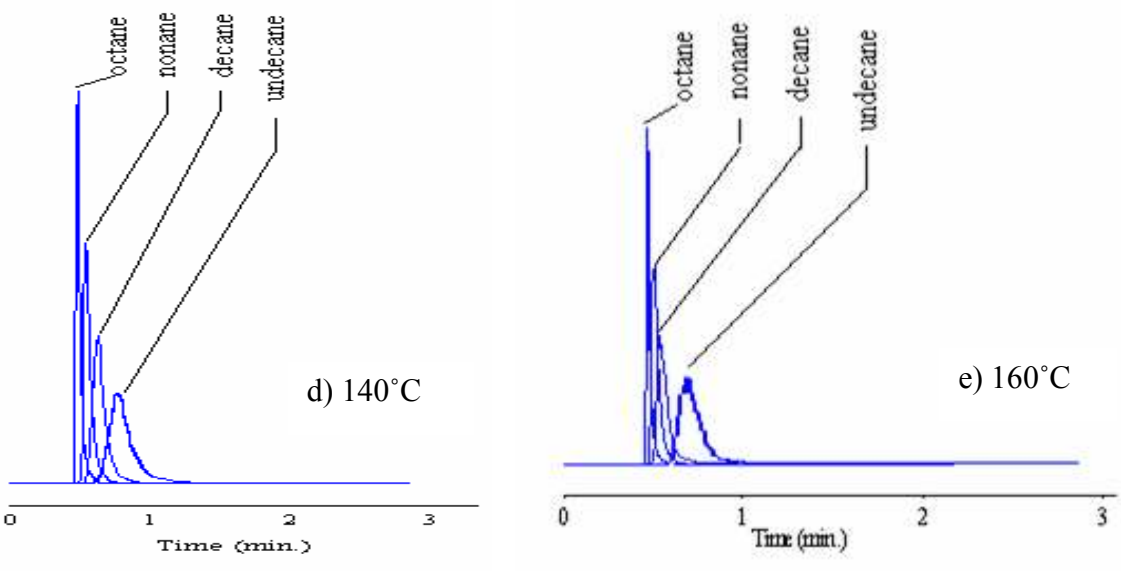


Figure 7 (continued)

Table 3 Chromatographic parameters of 8 cm × 0.32 mm i.d. monolithic silica using octane as probe molecule at various temperatures.

Octane	Capacity factor (k')	Tailing factor	Peak width	Theoretical plates (plates per meter)
80	1.025	2.167	0.166	3,857
100	0.561	2.250	0.086	8,541
120	0.378	2.536	0.050	19,681
140	0.308	2.882	0.032	43,328
160	0.300	2.667	0.024	76,050

Table 4 Chromatographic parameters of 8 cm × 0.32 mm i.d. monolithic silica using nonane as probe molecule at various temperatures.

Nonane	Capacity factor (k')	Tailing factor	Peak width	Theoretical plates (plates per meter)
80	2.417	2.088	0.354	2,415
100	1.161	2.018	0.175	3,953
120	0.669	2.224	0.094	8,176
140	0.472	2.606	0.057	17,291
160	0.397	3.350	0.040	31,626

Table 5 Chromatographic parameters of 8 cm × 0.32 mm i.d. monolithic silica using decane as probe molecule at various temperatures.

Decane	Capacity factor (k')	Tailing factor	Peak width	Theoretical plates (plates per meter)
80	5.661	2.064	0.792	1,833
100	2.450	1.841	0.349	2,533
120	1.239	1.915	0.173	4,341
140	0.744	2.129	0.096	8,559
160	0.542	2.686	0.061	16,556

Table 6 Chromatographic parameters of 8 cm × 0.32 mm i.d. monolithic silica using undecane as probe molecule at various temperatures.

Undecane	Capacity factor (k')	Tailing factor	Peak width	Theoretical plates (plates per meter)
80	13.161	2.193	1.469	2,409
100	5.125	1.788	0.700	1,985
120	2.375	1.657	0.323	2,830
140	1.294	1.950	0.179	4,259
160	0.842	2.362	0.112	7,008

From the chromatographic parameters obtained from those chromatograms it was found that tailing factor of all peaks in the chromatograms were greater than unity so the peaks of four probe molecules were tailing in the range of studied temperature, it might be an effect of strong interaction of probe molecule on silica surface. The tailing peaks at increased temperature also implied a non-equilibrium interaction between two phases.

Peak width of probe molecule decreased with rising operating temperature which increased peak sharpness of probe molecule. Theoretical plates also increased with temperature as a result of a decrease in peak width. Capacity factor (k') of all probe molecules dropped when raising oven temperature because of high velocity of molecule at high temperature which reduced time of molecule to interact on monolithic silica surface, or retention time (t_R).

The optimum temperature for each probe molecule in this work was the lowest temperature that provided an equilibrated interaction of molecule on silica surface, represented by a sharp symmetrical peak for an accurate retention time measurement. Using n-octane as probe molecule, at 80˚C the peak was broad. When the temperature was risen up to 100˚C, peak was sharper than that found for lower temperature and the capacity factor decreased. Similar effect was also occurred for a

higher temperature than 100 ˚C. So oven temperature for using n-octane as probe molecule was 100˚C.

Like octane, n-nonane, n-decane and n-undecane gave similar effect, which obtained broad peak at low temperature and sharp and tailing peak at increased temperature.. The selected temperature was then 120˚C for nonane, 140˚C for decane and 160˚C for undecane. For all probe molecules, theoretical plates were approximately 8,000 plates per meter at optimized temperature.

1.2 Injection volume

Because determination of surface area using IGC technique was based on monolayer adsorption of probe molecule on material surface, amount of injected molecule was optimized by injecting 0.2 to 2.0 μL of each probe molecule and operating the GC instrument at its optimum temperature.

Retention times of n-octane at 100˚C for the column sample number 53-08 (or a 0.53 mm i.d. × 8 cm monolithic silica column) at various injection volume were shown in Table 7. For each volume, probe molecule was injected triplicately and the average retention time was converted to retention volume using equation (1-2) with a collected carrier gas flow rate of 3.34 $mL \cdot min^{-1}$, which was calculated by using equation (1-3) and (1-4).

Table 7 Retention time and calculated retention volume of octane using various injection volumes at 100˚C

injection volume	t_R (min)					Retention
(μL)	1	2	3	avg	SD	volume (V_N)
0.2	0.560	0.559	0.559	0.559	0.001	0.76
0.5	0.562	0.561	0.560	0.561	0.001	0.76
1.0	0.561	0.559	0.557	0.559	0.002	0.76
1.5	0.565	0.563	0.560	0.563	0.003	0.77
2.0	0.558	0.560	0.564	0.561	0.003	0.76

One example of calculation of retention volume (V_N) at 0.2 μL of injection volume was shown here. Some parameters were given as follows. Both injection port and detector temperature of GC instrument were 250˚C, and oven temperature (T_C) was 100˚C or 373 K isothermally. An average carrier gas flow rate (F) of 3 measurements, which was measured by a flow meter was 2.48 $mL \cdot min^{-1}$, giving a column head pressure (P_i) of 12.4 psi. Dead time (t_0) determined by an injection of methane was 0.332 min. Actual room temperature (T_F) was 20˚C or 293 K, and vapor pressure of water at room temperature was 0.3384 psi. Atmospheric pressure (P) was 14.97 psi. Under these circumstances, the pressure drop correlation factor (j) was calculated by using equation (1-4) as follows.

$$j = \frac{3[(P_i/P)^2 - 1]}{2[(P_i/P)^3 - 1]}$$

Substituted variables with those values giving

$$j = \frac{3[(12.4/14.97)^2 - 1]}{2[(12.4/14.97)^3 - 1]}$$

$$= 1.09$$

Corrected flow rate (F_C) was calculated by using equation (1-3) as follows.

$$F_c = F \times \frac{T_c}{T_f} \times \frac{P_f}{P} \times j$$

Replaced the variables giving

$$F_c = 2.48 \times \frac{373}{293} \times \frac{(14.97 - 0.3384)}{14.97} \times 1.09$$

$$= 3.34\ mL \cdot min^{-1}$$

Retention volume was then obtained from equation (1-2) as follows.

$$V_N = (t_R - t_0)F_c$$
$$= (0.559 - 0.332) \times 3.34$$
$$= 0.76 \text{ mL}$$

From the retention volume, optimized injection volume for the column sample number 53-08 was obtained by plotting injection volume (x-axis) against retention volume (y-axis) as shown in Figure 8.

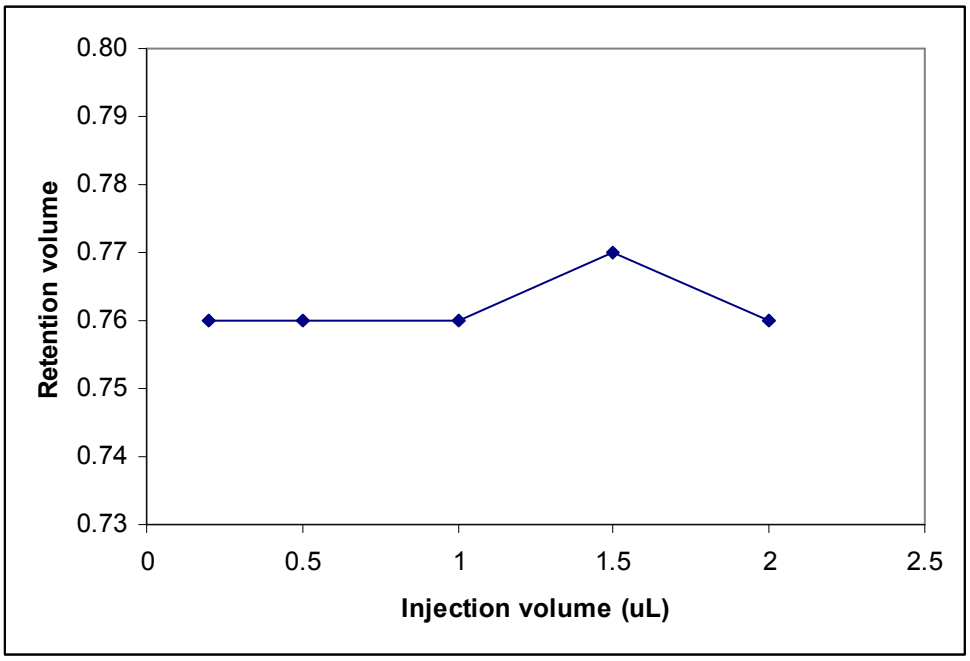


Figure 8 Relationship between injection volumes against retention volumes for octane at 100˚C for the column sample number 53-08.

As shown in Figure 8, retention volumes of octane at optimized temperature for the column were almost constant with an increase in injection volume from 0.2 to 2.0 µL. This meaned that retention volume were independent on sample size of octane, or in other word, adsorption feature of octane on this monolithic column surface was monolayer. Optimum injection volume of octane for the column was therefore chosen between 0.5 to 2.0 µL.

Similar to octane probe molecule, nonane, decane and undecane were also examined. Retention time and retention volume for each injection volume of nonane at 120˚C, decane at 140˚C, and undecane at 140˚C in the same column sample were studied and calculated as shown in Table 8, 9 and 10, in addition to their relationship between injection volume and retention volume illustrated in Figure 9, 10 and 11.

Table 8 Retention times and calculated retention volumes of nonane for five injection volumes at 120˚C

Injection volume (μL)	t_R (min)					Retention volume (V_N)
	1	2	3	avg	sd	
0.2	0.587	0.592	0.591	0.590	0.003	0.91
0.5	0.594	0.591	0.592	0.592	0.002	0.92
1.0	0.590	0.591	0.599	0.593	0.005	0.92
1.5	0.597	0.594	0.598	0.596	0.002	0.93
2.0	0.600	0.604	0.609	0.604	0.005	0.96

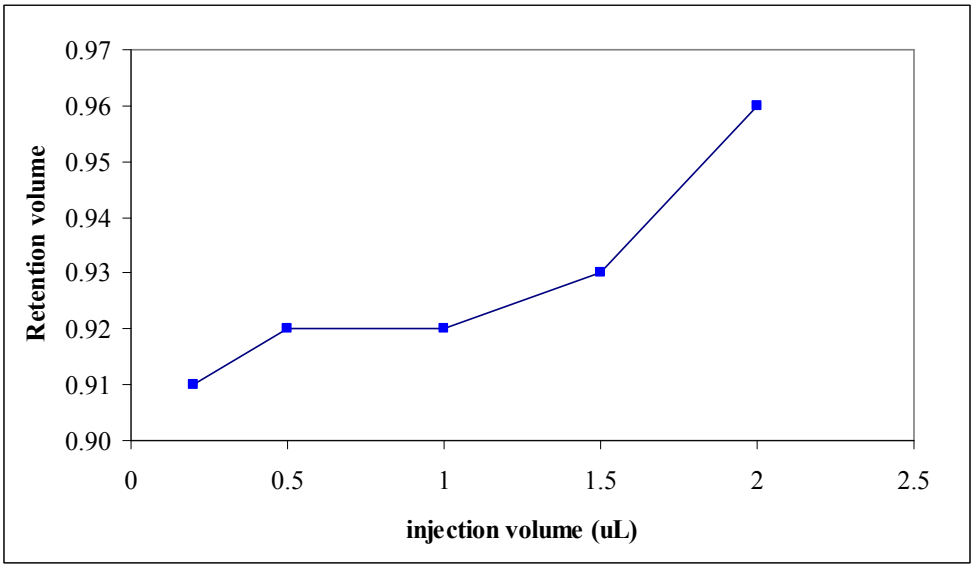


Figure 9 Relationship between injection volumes against retention volumes for nonane at 120˚C for the column sample number 53-08.

Table 9 Retention times and calculated retention volumes of decane for five injection volumes at 140˚C

Injection volume (µL)	t_R (min)					Retention volume (V_N)
	1	2	3	avg	sd	
0.2	0.609	0.606	0.608	0.608	0.002	1.02
0.4	0.614	0.614	0.614	0.614	0.000	1.04
0.6	0.621	0.610	0.613	0.615	0.006	1.05
1.0	0.621	0.626	0.625	0.624	0.003	1.08
1.5	0.633	0.635	0.634	0.634	0.001	1.12

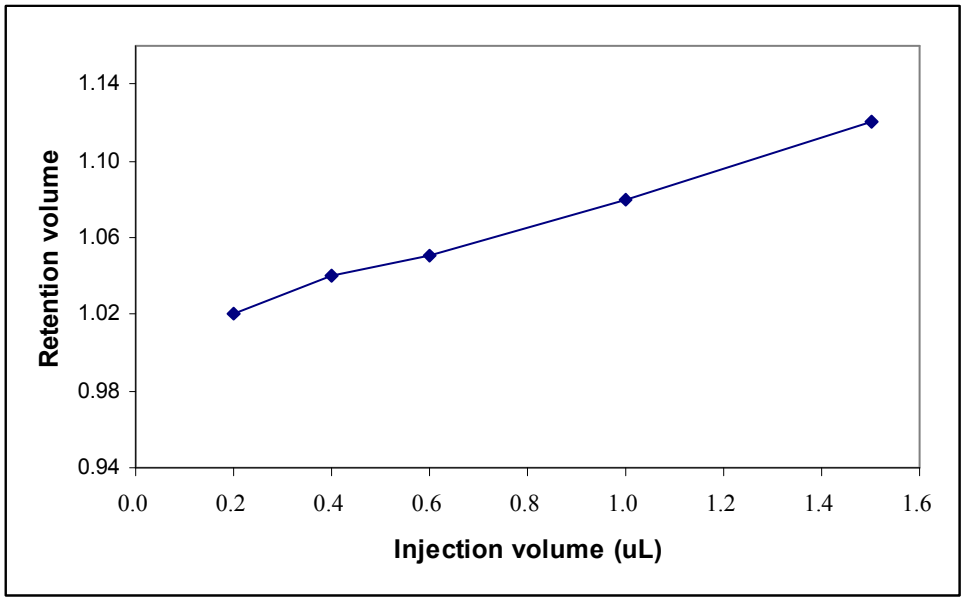


Figure 10 Relationship between injection volumes against retention volumes for decane at 140˚C for the column sample number 53-08

Table 10 Retention times and calculated retention volumes of undecane for five injection volumes at 160˚C

Injection volume (µL)	t_R (min)					Retention volume (V_N)
	1	2	3	avg	sd	
0.2	0.625	0.621	0.624	0.623	0.002	1.13
0.5	0.636	0.638	0.634	0.636	0.000	1.18
1.0	0.644	0.644	0.641	0.643	0.006	1.21
1.5	0.638	0.644	0.639	0.640	0.003	1.20
2.0	0.657	0.656	0.663	0.659	0.001	1.24

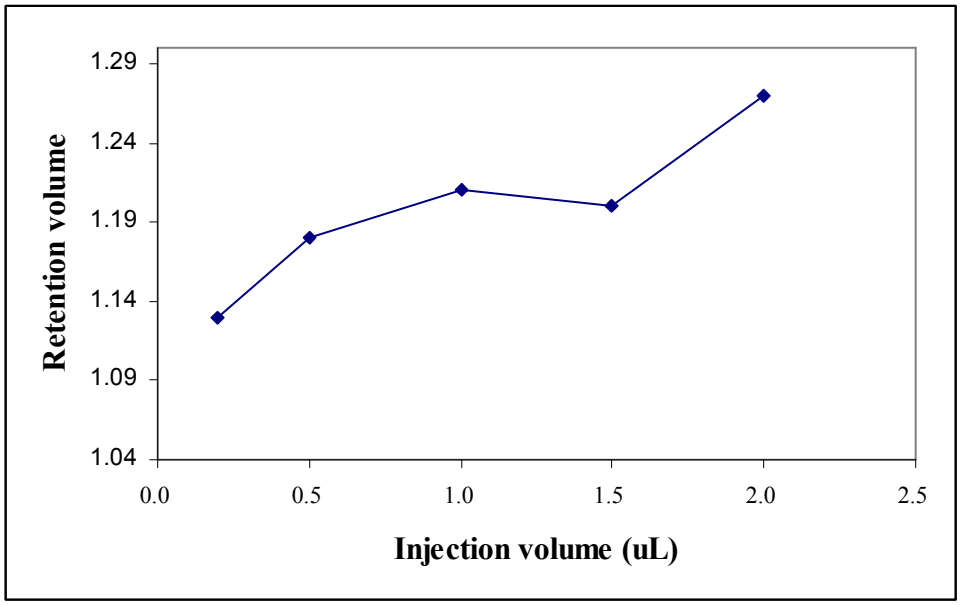


Figure 11 Relationship between injection volumes against retention volumes for undecane at 160˚C for the column sample number 53-08.

When plotted injection volume against retention volume of nonane under its optimized temperature at 120˚C (Figure 9), it was found that graph can be divided into 3 regions. First region was range between 0.2 to 0.5 µL of injection volume where the retention volume increased with g injection volume. Second region were 0.5 to 1.5 µL, where the retention volume was independent on injection volume because of monolayer adsorption. In the last region at 1.5 to 2.0 µL the retention

volume was slightly increased with increasing injection volume. Thus, provided that nonane was used as a probe molecule, the volume of between 0.5 to 1.5 μL was chosen in order to determine surface area of the column.

With either decane or undecane as a probe molecule, its relationship between injection volume and retention volume was similar to that of nonane. The volume of monolayer adsorption was found in a range of 0.4 - 0.8 μL for decane at 140˚C, and 0.6 - 1.2 μL for undecane at 160˚C.

1.3 Types of probe molecule and reproducibility of the method

In this experiment, four non-polar compounds, namely octane, nanane, decane and undecane, were used as probe molecules to adsorb on polar surface of monolithic silica structure using IGC technique. Surface area of the same column obtained from different probe molecules was examined. Day-to-day variation, which related to reproducibility of the method, was also studied simultaneously using randomized and blocking design. An order of experiment was shown in Section 4.1.13.

Four volumes in an appropriate range of the probe molecule were introduced to GC where the column number 53-08 was coupled. Triplicate injection was performed for each volume. A number of parameters of each injection required for BET plot were calculated and given in Table 11.

Table 11 Chromatographic parameters and other calculated factors required for BET plot of octane at 100˚C

Injection volume (μL)	m_a (μmol)	Average peak area (S_{peak}) (%RSD)		Average peak height (H) (%RSD)		a	p	$p/a(p_0 - p)$	p/p_0
0.6	3.69	90942567	(1.58)	15574367	(0.65)	97.68	575	0.025864	0.002698
0.8	4.92	113619333	(2.13)	19482600	(0.54)	104.32	768	0.031697	0.003351
1.0	6.15	135924667	(5.23)	23408600	(2.42)	109.00	964	0.036767	0.003833
1.2	7.39	165497333	(0.34)	27131633	(0.42)	107.43	1102	0.041337	0.004383

Adsorption coefficient (a) and partial pressure (p) were calculated from equation (1-5) and (1-6).

Example of calculation for 0.6 μL of octane was given. Amount of injected molecule (m_a) for octane at 0.6 μL calculated from its density and molar mass which were 0.703 $g \cdot cm^{-3}$ and 114.23 $g \cdot mol^{-1}$, respectively.

$$m_a\ (\mu mol) = 0.6\ \mu L \times \frac{0.703\ g}{1\ mL} \times \frac{1\ mL}{1000\ \mu L} \times \frac{1\ mol}{114.23\ g} \times \frac{1 \times 10^6\ \mu mol}{1\ mol}$$

$$= 3.69\ \mu mol$$

The gas constant (R) is 8.314 $J \cdot K^{-1} \cdot mol^{-1}$, the optimum temperature (T) for octane was 373 K, and the corrected flow (F) was 3.41 $mL \cdot min^{-1}$. The second vertically-half peak area of methane that was a non-adsorbing molecule (S_{ads}) was 8907385. Mass of monolithic silica material, measured by subtracting weight of tubing from weight of fabricated monolithic silica in tubing, was 0.0037 g. When these values were substituted into equation (1-5) and (1-6), the results were shown below.

$$p = \frac{m_a HRT}{FS_{peak}}$$

$$= \frac{3.69\ \mu mol \times 15574367\ \mu A \times 8.314\ J \cdot mol^{-1} \cdot K^{-1} \times 373\ K}{3.41\ mL \cdot min^{-1} \times 90942567\ \mu A \cdot min^{-1}}$$

$$= 575\ Pa$$

$$a = \frac{m_a S_{ads}}{mS_{peak}}$$

$$= \frac{3.69\ \mu mol \times 8907385\ \mu A \cdot min^{-1}}{0.0037\ g \times 90942567\ \mu A \cdot min^{-1}}$$

$$= 97.68\ \mu mol \cdot g^{-1}$$

In general the vapor pressure of probe molecule at the operating temperature, p_0 is calculated from Antoine equation as shown in equation (1-8)

$$\log(p_0) = A - \frac{B}{T + C}$$

Where A, B and C are Antoine coefficients, which were 6.90940, 1349.820 and 209.385 for octane, respectively, p_0 is vapor pressure in mmHg, and T is temperature in Celsius. When replaced the values into the equation, giving p_0 equaled 351 mmHg which was 46822 Pa. To obtain a BET curve, p/p_0 was plotted against $p/a(p_0 - p)$ as shown in Figure 12.

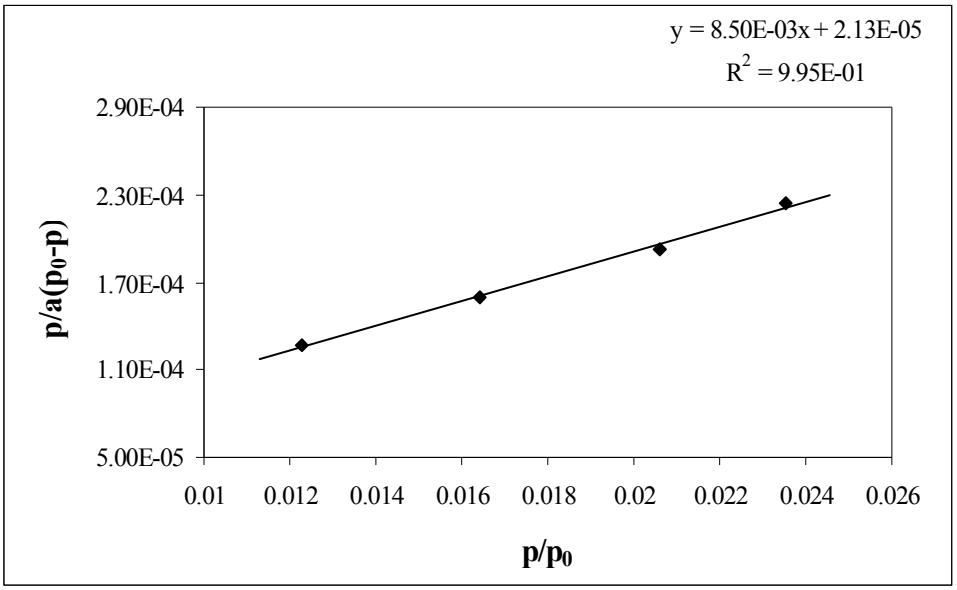


Figure 12 BET plot of n-octane on monolithic silica column number 53-08 at 100˚C

From equation (1-7) or BET equation, it was found that $(c - 1)/a_m c$ and $1/a_m c$ can be estimated from slope and y axis-intercept of a BET curve, respectively. In Figure6, the linear equation of octane at 100˚C was

$$y = 8.5 \times 10^{-3}x + 2.13 \times 10^{-5}$$

Therefore, $(c - 1)/a_m c = 8.5 \times 10^{-3}$, and $1/a_m c = 2.13 \times 10^{-5}$.

By calculation, BET constant (c) and monolayer capacity (a_m) equaled to 400 and 1.17×10^2 μmol·g^{-1}, respectively.

When monolayer capacity was known, the surface area (S) of material can be estimated from equation (1-9).

$$S = a_m N A_{cs}$$

Where N is the Avogadro's constant, 6.02×10^{23}, and cross-sectional area of n-alkane molecule (A_{cs}) was calculated in nm^2 from equation (1-10)

$$A_{cs} = 1.33 \times (M/\rho N)^{2/3} \times 10^{14}$$

In the equation, M is molecular weight and ρ is the density of octane, which was 114.23 g·mol^{-1} and 0.703 g·(cm^3)$^{-1}$, respectively. By substituting the values in the equation, the result was

$$A_{cs} = 1.33 \times \left(\frac{114.23}{0.703 \times 6.02 \times 10^{23}} \right)^{2/3} \times 10^{14} \text{ nm}^2\cdot\text{molecule}^{-1} \times \frac{1 \text{ m}^2}{1 \times 10^{18} \text{ nm}^2}$$

$$= 5.55 \times 10^{-19} \text{ m}^2\cdot\text{molecule}^{-1}.$$

Replaced calculated A_{cs} and a_m in the equation (1-8) above,

$$S = 1.17 \times 10^2 \text{ μmol}\cdot\text{g}^{-1} \times \frac{1 \text{ mol}}{1 \times 10^6 \text{ μmol}} \times 6.02 \times 10^{23} \text{ molecule}\cdot\text{mol}^{-1}$$

$$\times \ 5.55 \times 10^{-19} \text{ m}^2\cdot\text{molecule}^{-1}.$$

$$= 38.86 \text{ m}^2\cdot\text{g}^{-1}$$

The surface area of monolithic silica column sample number 53-08 determined by IGC using n-octane as a probe molecule at 100˚C on the first day was 38.86 m^2·g^{-1}. Necessary parameters of the other probe molecules for surface area

calculation were shown in Table 12. In addition, surface areas of the same column measured by using the other probe molecules determining on different days were summarized in Table 13 - 16, together with their relative standard deviations (%RSD).

Table 12 Some parameters of probe molecules for surface area determination

Probe molecule	Antoine coefficient[1]			Vapor pressure[2] (Pa)	Molecular weight ($g \cdot mol^{-1}$)	Density $g \cdot (cm^3)^{-1}$	Cross-sectional area ($m^2 \cdot molecule^{-1}$)
	A	B	C				
Octane	6.9094	1349.82	209.385	46821.529	114.23	0.703	5.50×10^{-19}
Nonane	6.9344	1429.46	201.820	40800.680	128.26	0.714	5.90×10^{-19}
Decane	6.96375	1508.75	195.374	38926.100	142.28	0.730	6.20×10^{-19}
Undecane	6.9722	1569.57	187.700	38160.770	156.31	0.740	6.60×10^{-19}

[1] From reference: Reid *et al.* (1987)

[2] Calculated at optimum temperature for each probe molecule.

Table 13 Parameters obtained from BET curve using octane as a probe molecule examining on inter-day.

Day	Linear equation	Correlation coefficient	Monolayer capacity ($\mu mol \cdot g^{-1}$)	Surface area ($m^2 \cdot g^{-1}$)
1	$y = 8.50 \times 10^{-3} x + 2.13 \times 10^{-5}$	0.995	117.35	38.86
2	$y = 7.62 \times 10^{-3} x + 1.92 \times 10^{-5}$	0.999	130.90	43.34
3	$y = 7.82 \times 10^{-3} x + 2.03 \times 10^{-5}$	1.000	127.55	42.23
Average surface area				41.48
Standard deviation of surface area (%RSD)				2.33 (5.63)

Table 14 Parameters obtained from BET curve using nonane as a probe molecule examining on inter-day.

Day	Linear equation	Correlation coefficient	Monolayer capacity ($\mu mol \cdot g^{-1}$)	Surface area ($m^2 \cdot g^{-1}$)
1	$y = 9.02 \times 10^{-3} x + 1.77 \times 10^{-5}$	1.000	110.65	39.30
2	$y = 8.07 \times 10^{-3} x + 1.79 \times 10^{-5}$	0.998	123.64	43.91
3	$y = 7.94 \times 10^{-3} x + 1.68 \times 10^{-5}$	0.996	125.68	44.64
Average surface area				42.62
Standard deviation of surface area (%RSD)				2.90 (6.79)

Table 15 Parameters obtained from BET curve using decane as a probe molecule examining on inter-day.

Day	Linear equation	Correlation coefficient	Monolayer capacity ($\mu mol \cdot g^{-1}$)	Surface area ($m^2 \cdot g^{-1}$)
1	$y = 8.59 \times 10^{-3} x + 2.42 \times 10^{-5}$	1.000	116.09	43.33
2	$y = 8.35 \times 10^{-3} x + 1.72 \times 10^{-5}$	1.000	119.51	44.61
3	$y = 7.90 \times 10^{-3} x + 1.80 \times 10^{-5}$	1.000	126.29	47.14
Average surface area				45.03
Standard deviation of surface area (%RSD)				1.94 (4.31)

Table 16 Parameters obtained from BET curve using undecane as a probe molecule examining on inter-day.

Day	Linear equation	Correlation coefficient	Monolayer capacity ($\mu mol \cdot g^{-1}$)	Surface area ($m^2 \cdot g^{-1}$)
1	$y = 9.25 \times 10^{-3} x + 1.64 \times 10^{-5}$	0.999	107.92	42.88
2	$y = 8.32 \times 10^{-3} x + 1.75 \times 10^{-5}$	0.999	119.94	47.65
3	$y = 8.26 \times 10^{-3} x + 1.49 \times 10^{-5}$	0.998	120.85	48.02
Average surface area				46.18
Standard deviation of surface area (%RSD)				2.87 (6.21)

From Table 13 to 16, it was shown that the values of surface area determined from different block were close, ranging between 38 to 48 $m^2 \cdot g^{-1}$, and the standard deviations of measurement for all selected probe molecules were less than 3 $m^2 \cdot g^{-1}$ with 4.31-6.79 %RSD, meaning that deviation of measurement using different molecule was quite small.

Determined surface areas were analyzed by two-way ANOVA method in order to study effect of probe molecules and day-to-day analysis and the result was given in Table 17.

Table 17 Two-way ANOVA result for different probe molecules and inter-day (P=0.05)

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between - days	45.62	2	22.81	22.81	39.33
Between - probes	41.94	3	13.98	13.98	14.73
Error	5.99	6	1.00		
Total	93.55	11			

When compared the between-days mean square, 22.81, with the error mean square, 1.00, the F-value was 22.81, which was less than the critical value of F, 39.33, for a two-tailed test at 95% confidence interval. Thus, there was no difference between two variances, or in other words, day-to-day error of surface area measurement did not differ from random error, meaning that this method was reproduced. Likewise, the F-value of between-probe molecules compared with random error was less than the F-critical value, showing that no significant difference when using different probe molecules.

However, the results from Table 13 to 16 showed that standard deviation of surface area determination were smallest when decane was used as probe molecule.

Surface area determination of monolithic silica column was therefore performed by using n-decane at 140˚C.

Surface area of the column sample number 53-08 was also determined by using a nitrogen adsorption conventional method, resulting the value of 31.81 $m^2 \cdot g^{-1}$. Compared with the area of 45.03 $m^2 \cdot g^{-1}$ obtained from IGC method with n-decane probe molecule, the two values were not close. Because the mass of silica in the tubing (ca. 0.0037 g) was much lower than mass of sample required for the nitrogen adsorption technique (0.01 g), the accuracy of the later method was then suspected.

Since the fabricated monolithic silica column was not long enough to connect from injection port to detector of GC instrument, and a flow rate of carrier gas was too high to ignite flame of FID because of large through pore of the material, a 10 m × 0.32 mm bare fused-silica tubing was connected between the monolithic column and the detector. With such configuration for IGC technique, the surface area of bare tubing was also determined without a coupling with monolithic silica column. It was found that retention time of decane was 0.331 min, which was very close to the dead time of system, 0.329 min. Hence, retention volume of molecule on the bare fused silica tubing was very small and surface area of the tubing was then negligible.

2. Surface characterization of refabricated monolithic silica column

Shrinkage of silica structure was the main problem of silica monolith fabrication. To improve the silica structure, a single fabricated monolithic silica column was refabricated. Morphology of the structure by SEM was performed for both single and double fabricated monolithic column, showing in Figure 13, Surface area of the 0.53 mm i.d. × 9 cm monolithic silica column singly and doubly fabrication was determined, resulting BET equations and calculated surface areas (Table 18 and 19).

a)

b)

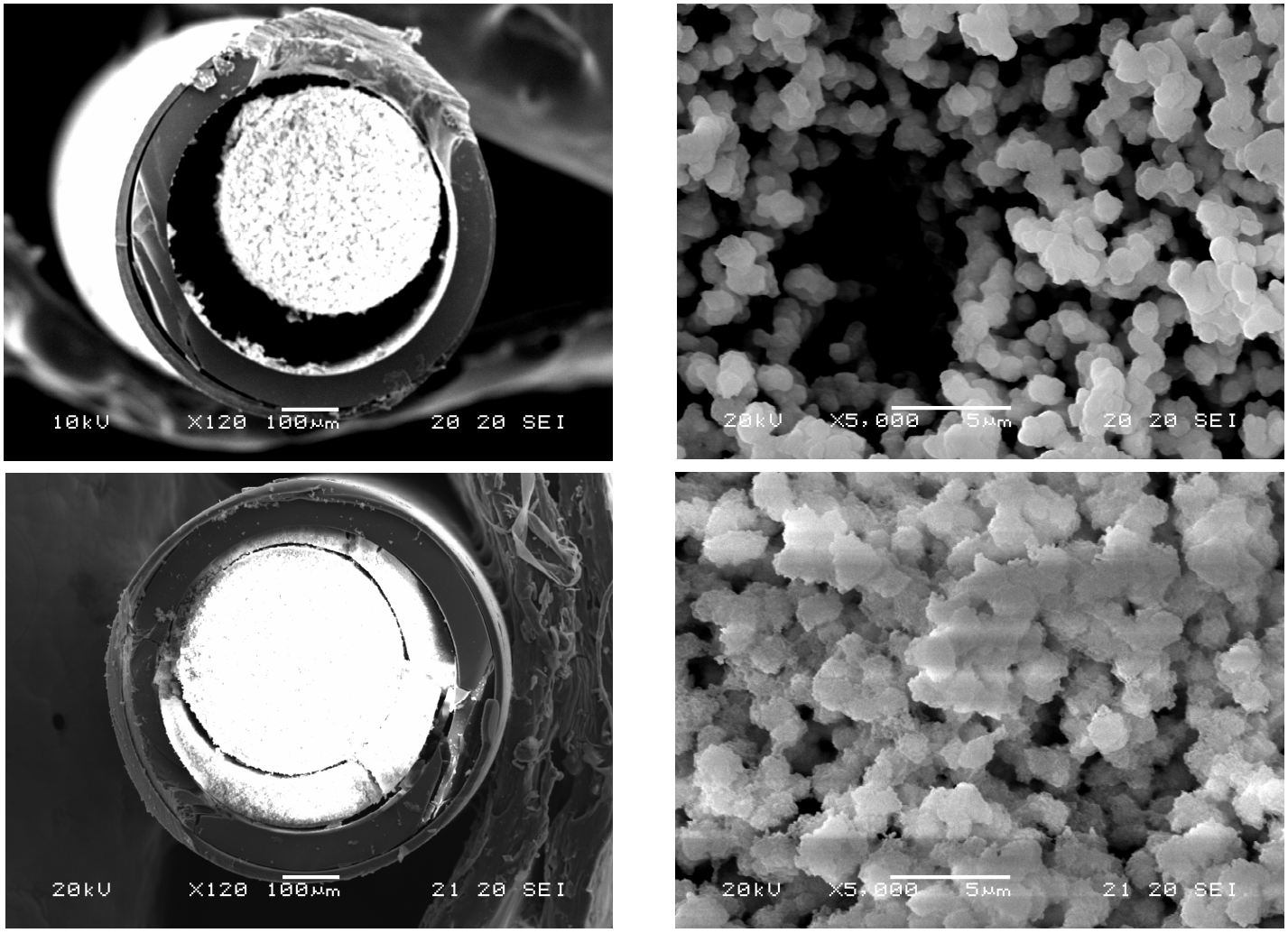


Figure 13 SEM micrographs of single fabricated (above) and refabricated monolithic silica column sample number 53-09 (below).

Table 18 Parameters obtained from BET curve using decane as probe molecule for a single fabricated column sample number 53-09.

Injection number	Linear equation	Correlation coefficient	Monolayer capacity ($\mu mol \cdot g^{-1}$)	Surface area ($m^2 \cdot g^{-1}$)
1	$y = 9.74 \times 10^{-3} x + 2.23 \times 10^{-5}$	0.998	102.43	38.23
2	$y = 9.74 \times 10^{-3} x + 2.77 \times 10^{-5}$	1.000	102.38	38.21
3	$y = 9.76 \times 10^{-3} x + 3.56 \times 10^{-5}$	1.000	102.09	38.10
Average surface area				38.18
Standard deviation of surface area				0.07

Table 19 Parameters obtained from BET curve using decane as probe molecule of for a double fabricated column sample number 53-09.

Injection number	Linear equation	Correlation coefficient	Monolayer capacity ($\mu mol \cdot g^{-1}$)	Surface area ($m^2 \cdot g^{-1}$)
1	$y = 6.97 \times 10^{-3} x + 3.45 \times 10^{-5}$	1.000	142.77	53.29
2	$y = 7.06 \times 10^{-3} x + 3.46 \times 10^{-5}$	0.999	140.95	52.61
3	$y = 7.06 \times 10^{-3} x + 3.39 \times 10^{-5}$	0.999	140.97	52.61
Average surface area				52.84
Standard deviation of surface area				0.39

From Figure 13a), it was obviously seen that the structure of single fabricated monolithic silica column shrank. The monolithic framework did not attach to the tubing wall, leaving a big gap between the monolith and the wall. The through pore size of the monolith was around 5 μm, and the silica skeleton was approximately 1 μm. When the column was refabricated by subjecting to the same reaction, the gap between the structure of monolith and tubing wall was filled with a new structure and the single fabricated silica skeleton was wrapped with a new one. The size of skeleton remained 1 μm but its surface was rougher. The double fabricated structure was unattached to the primary structure, so as to the wall, thus affecting homogeneity of the column. Furthermore, the primary through pore was also filled with refabricated silica, which reduced pore size and permeability of the column.

Surface areas of single and double fabricated monolithic silica column sample number 53-09 were determined by using IGC method, and the results were compared by using one-way ANOVA, given in Table 20.

Table 20 One-way ANOVA test at 95% confidence interval of surface area of monolithic silica column sample number 53-09 singly and doubly fabrication.

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between Groups	322.23	1	322.23	4027.88	12.22
Within Groups	0.32	4	0.08		
Total	322.54	5			

In Table 20, variation of between groups represented variation of surface area between singly and doubly fabrication, while variation of within groups was a variation of surface area of 3 replicates measurement in each group, singly and doubly fabrication, which represented a random error of measurement. From the results it was found that F-value obtained from mean square of between and within groups was

40527 and critical value of $F_{1,4}$ (two-tailed, P = 0.05) was 12.22. Greater F-value than the critical F-value meant that variation of surface area from refabrication was larger than that found for random error. Thus, surface area between singly and doubly fabrication was totally different. Surface area of double fabricated monolithic silica column was significantly higher than that found for single fabricated monolithic silica column.

Although the double fabricated monolithic silica column possesses higher surface area than the single one, the homogeneity and through pore size decreased, which might reduce efficiency of the column. The refabrication process therefore seemed not suitable for improving the monolithic structure.

3. Effect of inner diameter and length of tubing on surface area of monolithic silica

Shrinkage of monolithic silica structure fabricated in capillary can be reduced by decreasing inner diameter of fused-silica tubing (Tanaka *et. al*., 2002). As described in Section 1, shrinkage of silica structure was occurred when increasing tubing length. Such factors were therefore studied by surface characterization.

Surface area of four different synthesized columns was determined in random order of measurement, as shown in Section 4.1.2.2. Some information of four columns was summarized in Table 21, and BET equation and surface area of each column were given in Table 22.

Table 21 Information of four different monolithic silica columns.

Column number	Inner diameter (mm)	Length (cm)	Mass of monolithic silica material (mg)
32-09	0.32	9	0.6
32-22	0.32	22	2.7
25-02	0.25	8	0.2
25-21	0.25	21	1.4

Table 22 Parameters obtained from BET curve of the column samples.

Column number	Injection number	Linear equation	Correlation coefficient	Monolayer capacity ($\mu mol \cdot g^{-1}$)	Surface area ($m^2 \cdot g^{-1}$)
32-09	1	$y = 1.28 \times 10^{-3} x + 2.65 \times 10^{-5}$	0.991	765.40	285.68
	2	$y = 1.22 \times 10^{-3} x + 3.31 \times 10^{-}$	0.957	798.02	297.85
32-22	1	$y = 1.03 \times 10^{-2} x - 1.47 \times 10^{-4}$	0.987	98.49	36.76
	2	$y = 1.11 \times 10^{-2} x - 2.43 \times 10^{-4}$	0.988	92.11	34.38
25-08	1	$y = 4.46 \times 10^{-4} x - 2.76 \times 10^{-7}$	0.975	2243.54	837.38
	2	$y = 3.69 \times 10^{-4} x + 1.95 \times 10^{-}6$	0.965	2695.78	1006.17
25-21	1	$y = 1.90 \times 10^{-3} x - 1.57 \times 10^{-5}$	0.991	527.90	197.03
	2	$y = 1.87 \times 10^{-3} x - 1.32 \times 10^{-5}$	0.984	538.56	201.01

To test an effect of surface area of monolithic column depended on column internal diameter and length, two-way ANOVA was performed for two-tailed test at 95% confidence interval, resulting data shown in Table 23.

Table 23 Two-way ANOVA test for different diameters and lengths of the column.

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Column length	479172	1	479172	133.77	12.22
Column diameter	314789	1	314789	87.88	12.22
Interaction	108839	1	108839	30.38	12.22
Within	14330	4	3582		
Total	917130	7			

In Table 23, F-value for column lenght was 133.77, and for internal diameter was 87.88, which was greater than the critical F-value, 12.22. Thus, surface area depended on both inner diameter and length of monolithic silica column. Besides, an interaction between the two factors also significantly affected the surface area. An interaction plot of the results was shown in Figure 14.

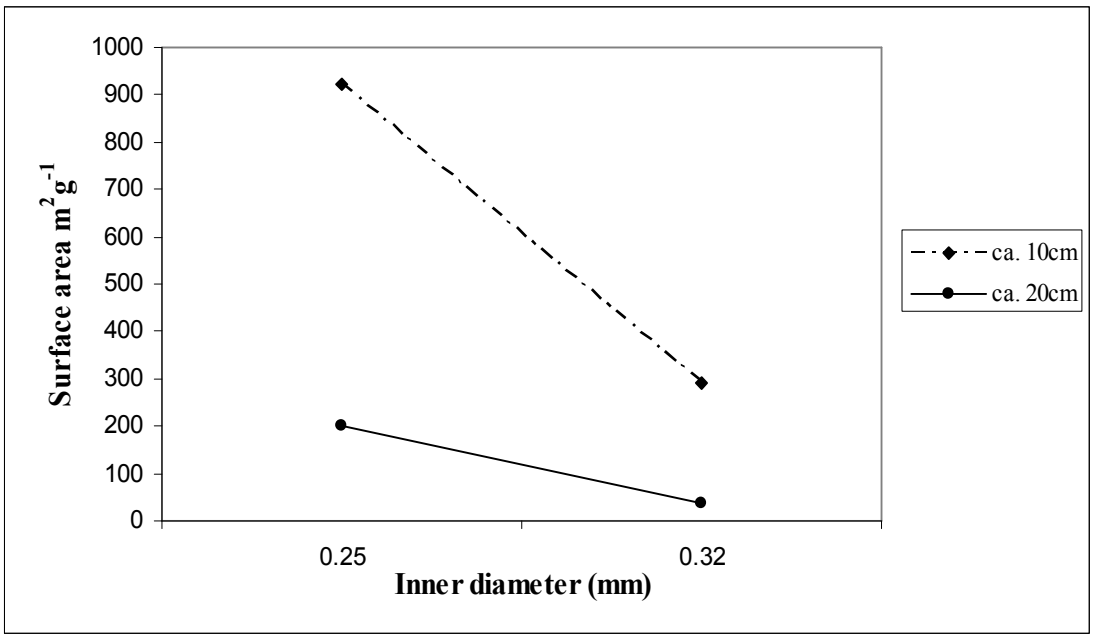


Figure 14 Relationship between inner diameters plotted against surface area of column with ca. 10 cm (above) and ca. 20 cm length (below)

In a typical analytical column, surface area of packing material increases with expanding diameter and/or length of column, however in this work the surface area mentioned since the beginning was the area per gram of sample, which meant "specific surface area". In Figure 14, the two lines were not parallel, implying an interaction between the two factors. Both negative slopes of the lines with increasing column diameter showed that specific surface area decreased with inner diameter. It was postulated that the monolithic silica synthesized in the large diameter tubing gave lower surface area because more shrinkage was occurred than that for the small diameter, therefore the void between the wall and silica structure was increased as illustrated in Figure 6. Furthermore, the two lines were located at different heights on the graph, indicating that surface area depended on column length.

However, an error of surface area determination of short columns, namely column sample number 32-09 and 25-08, could occur as a result of very small mass of synthesized monolithic silica in the tubings.

4. Reproducibility of surface area for column fabrication.

As mentioned in the previous section about several problems occurred during fabrication of monolithic silica column, e.g. cracking and shrinkage of its structure, reproducibility of fabricated monolith was then studied. Three batches of monolithic silica were fabricated in 0.32 mm i.d. fused-silica tubings. Surface area of three columns synthesized from each batch was determined. Some information of each column from the three batches was given in Table 24.

Table 24 Measured data of selected monolithic silica columns from three batches

Column number	Inner diameter (mm)	Length (cm)	Mass of monolithic silica material (mg)
Batch-1			
32-09/1	0.32	9	0.8
32-07/1	0.32	7	0.8
32-09/2	0.32	9	0.6
Batch-2			
32-08/1	0.32	8	0.4
32-07/2	0.32	7	0.8
32-09/3	0.32	9	0.7
Batch-3			
32-08/2	0.32	8	2.9
32-07/3	0.32	7	6.6
32-22	0.32	22	2.7

Surface area was determined by IGC method using decane as probe molecule at 140˚C for 3 replicates per column in a day. Results from BET plot were shown in Table 25, as well as those for column sample number 32-22 (Table 22).

Table 25 Parameters obtained from BET curve of the column samples

Batch number	Column number	Correlation coefficient		Monolayer capacity ($\mu mol \cdot g^{-1}$)		Surface area ($m^2 \cdot g^{-1}$)	
		Mean	SD	Mean	SD	Mean	SD
1.	32-09/1	0.994	0.010	897.94	47.02	335.15	17.55
	32-07/1	0.995	0.005	565.62	3.18	211.11	1.19
	32-09/2	0.996	0.001	841.80	44.58	314.19	16.64
2.	32-08/1	0.998	0.002	895.08	10.44	334.08	3.90
	32-07/2	0.980	0.018	133.63	2.16	49.88	0.80
	32-09/3	1.000	0.000	98.67	1.47	36.83	0.55
3.	32-08/2	0.999	0.002	148.20	8.06	55.32	3.01
	32-07/3	0.997	0.002	38.69	0.59	14.44	0.22
	32-22	0.990	0.005	109.30	24.46	40.80	9.13

From the results, reproducibility of surface area for column fabrication was analysed by one-way ANOVA using average surface area of each column. Results of ANOVA analysis were shown in Table 26.

Table 26 One-way ANOVA analysis of average surface area of 9 columns in 3 batches at 95% confidence interval.

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between batches	34634	2	17317.29	0.82	39.33
Within batches	126128	6	21021.33		
Total	160762	8			

From Table 26, F-value obtained from comparison of mean square of within batches with between batches was 0.82380. The critical value of two-tailed $F_{6,2}$ (P = 0.05) was 39.33. Since the F-value was less than the critical F-value, the difference in surface area between batches was obviously not significant. This indicated that surface area of monolithic silica column fabricated in different batches was reproducible.

Nevertheless, surface area shown in Table 25 varied from 10 to 300 $m^2 \cdot g^{-1}$, which was a wide range. It was hypothesized that the insignificant variation between batches might be a result of a large variation occurred in the same batch. Then, the one-way ANOVA was performed to test the difference of surface area in the same batch. Results of the analysis were shown in Table 27 to 29.

Table 27 One-way ANOVA analysis of surface area of columns in first batch at 95% confidence interval.

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	26449	2	13224	67.68	7.26
Within columns	1172	6	195.4		
Total	27621	8			

Table 28 One-way ANOVA analysis of surface area of columns in second batch at 95% confidence interval.

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	169297	2	84649	15390	7.26
Within columns	33	6	5.5		
Total	169330	8			

Table 29 One-way ANOVA analysis of surface area of columns in third batch at 95% confidence interval.

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	2576	2	1288	41.78	7.26
Within columns	185	6	30.83		
Total	2761	8			

From Table 27 to 29, between columns variance was variance of surface area of different columns in the same batch. Within columns variance represented variance of surface area of 3 replicates measurement for a single column, or it was a random error. The higher F-values of all batches than the critical value meant that specific surface area of each column in the same batch was largely different.

As seen from the results, inverse gas chromatography (IGC) was a useful technique for determination of surface area, especially for a material that packed or synthesized in a tube as silica monolithic column. However, size of through pore and micropore of the monolith cannot be determined directly from IGC method. The pore size can be estimated from molecular sieving effect by using IGC but it was different from the conventional nitrogen adsorption technique. (Singh *et al.,* 2004)

Part III Study of PEG coated monolithic silica columns

One application of using silica monolith is its utilization in GC as an analytical column. Typical PEG coated fused silica GC capillary column is commercially available in trade names of Carbowax, HP-Wax, BP20, etc. and used for separation of intermediately polar volatile compounds, i.e. alcohol, ketone, aldehyde. This experiment was therefore aimed to apply the synthesized monolithic silica to separate those volatile components. Since its surface is very polar, it was coated with polyethylene glycol (PEG) with an average molecular weight of 10000 $g \cdot mol^{-1}$. Three methods for coating PEG on silica monolithic column were studied. Afterwards, performance of the coated columns were studied and compared to a conventional PEG coated GC capillary column or Carbowax by using chromatographic parameters obtained from an injection of a mixture of model compounds of n-hexane and acetone in suitable conditions.

1. Efficiency of conventional PEG coated column or Carbowax 20M

As the aim of this work was to fabricate PEG coated on surface of silica monolithic column, therefore, efficiency of fabricated column must be compared with a standard PEG coated column. The standard column used in this work was 60 m × 0.32 mm × 3 μm Carbowax 20M.

To test the efficiency of the Carbowax 20M, the column was connected to GC-FID and 1 μL of model compound was injected. Chromatogram of a model compound for Carbowax 20M at 60˚C was shown in Figure 15. Some chromatographic results, namely retention time and peak width were taken to calculate the other parameters, i.e. retention factor (k'), selectivity factor (α), resolution (Rs), and plate number (N), of the compounds as shown in Table 30.

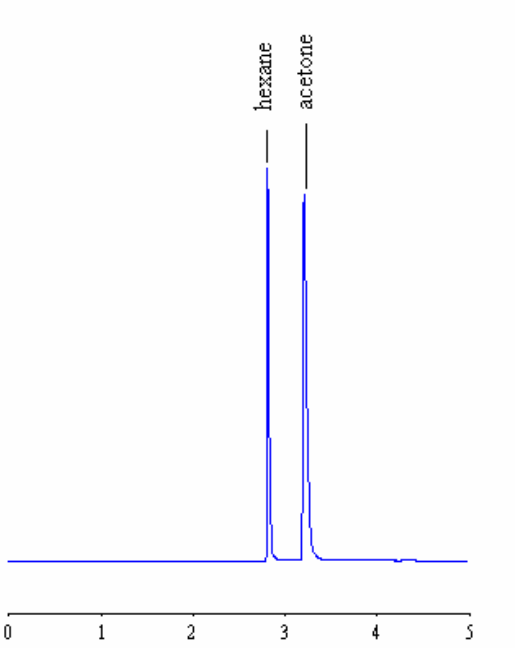


Figure 15 Chromatogram of model compounds separated by using Carbowax 20M at 60˚C isothermally

Table 30 Chromatographic parameters of model compounds for Carbowax20M

Compound	t_R (min)	W (min)	k'	α	Rs	N (plates·m^{-1})
hexane	2.823	0.041	0.024	6.950	9.020	1458
acetone	3.216	0.046	0.166			

In Table 30, the chromatographic parameters for separation on Carbowax 20M were obtained from the chromatogram giving the selectivity factor of 6.950 and a resolution of 9.020, which obviously shown excellent separation between two components.

2. Effect of a fused-silica tubing

Because the length of syntheisized silica monolithic column was too short to connect from injection port to detector, a 2m × 0.32 mm bare fused-silica capillary tubing was connected between the silica monolithic column and the detector as previously shown in Section 3. As chromatographic efficiency can be changed by the

connection, effect of inserted tubing on separation and efficiency of the column was therefore primarily studied. However, because the inserted tubing was fused-silica of which wall is polar according to the silanol group, polar compound gives a broad tailing peak. Thus, the wall of tubing must be coated to deactivate the reactive surface. In this work, surface of bared fused-silica tubing was coated by using a dynamic method with PEG solution. A 1 μL of polar acetone was injected to test the coated tubing. Chromatograms of acetone for non-coated and coated tubing connected from a newly synthesized monolithic column at 60˚C were shown in Figure 16.

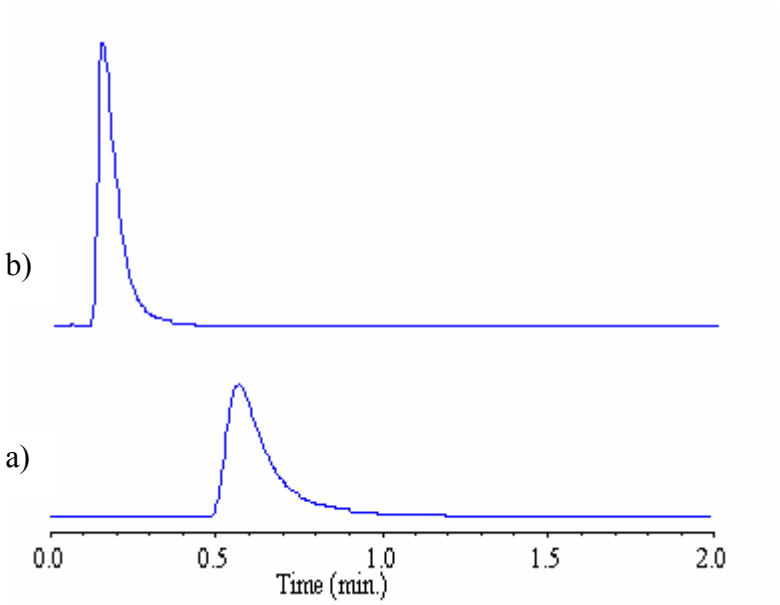


Figure 16 Chromatograms of acetone for a coated silica monolithic column connected with a) non-coated and b) PEG coated on the wall of bare tubing.

It was found that peak of acetone for coated monolithic column connected with non-coated tubing was very broad and tailing (Figure 16(a)). Coating with PEG improved the efficiency of separation, as the peak shape of acetone was sharper and its tailing was reduced (Figure 16(b)).

However, efficiency of separation of model compound might be a result of coated PEG on the tubing. To test if PEG significantly affected the separation, the silica monolithic column was removed, leaving only the coated tubing connected

between injection port and detector. Then 1 μL of model compound mixture was injected, obtaining a chromatogram shown in Figure 17.

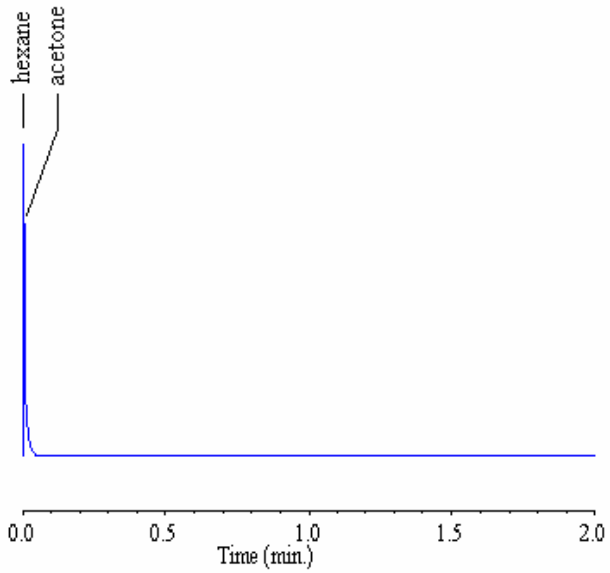


Figure 17 Chromatogram of model compound mixture for 2m × 0.32 mm coated tubing at 60˚C

Figure 17 obviously showed that two peaks of hexane and acetone were fused as a single peak with virtually no retention. It indicated that efficiency and resolving power of separation on the 2m × 0.32 mm PEG coated tubing was negligible. The PEG coated tubing could be used as a connector without disturbing a separation.

3. Study of coating methods.

To coat PEG on the surface of silica, PEG was prepared as a solution and filled into the monolithic column then the solvent was eliminated to leave a film of PEG on the surface. It was hypothesized that method of filling solution and elimination of solvent affect the homogeneity of coated column, therefore three methods of coating were studied.

3.1 Solvent evaporation method

Firstly, PEG in methanol was filled into a newly synthesized silica monolithic column then it was connected to GC-FID with an oven temperature of 60˚C and the carrier gas flow rate was 2.40 $mL \cdot min^{-1}$. By the flow of carrier gas and heat, solvent was evaporated. To ensure a complete elimination of methanol, the instrument was operated during conditioning the column until the baseline was stable. Afterwards the model compound mixture was injected to test the efficiency of column.

Concentration of PEG solution to be coated on surface of monolithic column was calculated from the amount of PEG on Carbowax 20 M surface. For a 60m × 0.32 mm x 3 μm Carbowax column, the calculated volume of PEG coating was 0.018 cm^3. The density of PEG with a molecular weight of 20000 $g \cdot mol^{-1}$ was 1.2 $g \cdot cm^{-3}$, then the mass of PEG was totally 0.022 g. Finally, the amount of PEG per area of column was calculated by dividing with surface area of the column and the result was 0.37 g PEG per square meter.

Since the molecular weight of PEG used in this work was 10,000 $g \cdot mol^{-1}$, the amount of PEG coated on surface must be halved, then it was 0.18 $g \cdot m^{-2}$. The silica monolithic tubing number 32-07 had a surface area of 211.11 $m^2 \cdot g^{-1}$ and total silica monolith mass of 0.8 mg and an internal surface area of bare tubing of 0.17 m^2. To coat PEG it required the mass of PEG of 0.031 g on 0.8 mg silica. It was obviously seen that amount of PEG was too concentrate to prepared. The amount of PEG was then reduced to 0.0031 g to be coated on the surface.

Silica monolithic column number 32-09 having surface area of 335.15 $m^2 \cdot g^{-1}$ was also coated with PEG solution on surface. Amount of PEG on surface and coating method was similar to that for the column number 32-07.

Chromatograms of model compounds for a column number 32-07 before and after PEG coating and a coated column number 32-09 were shown below in

Figure 18. Chromatographic parameters obtained from the chromatograms were summarized in Table 31.

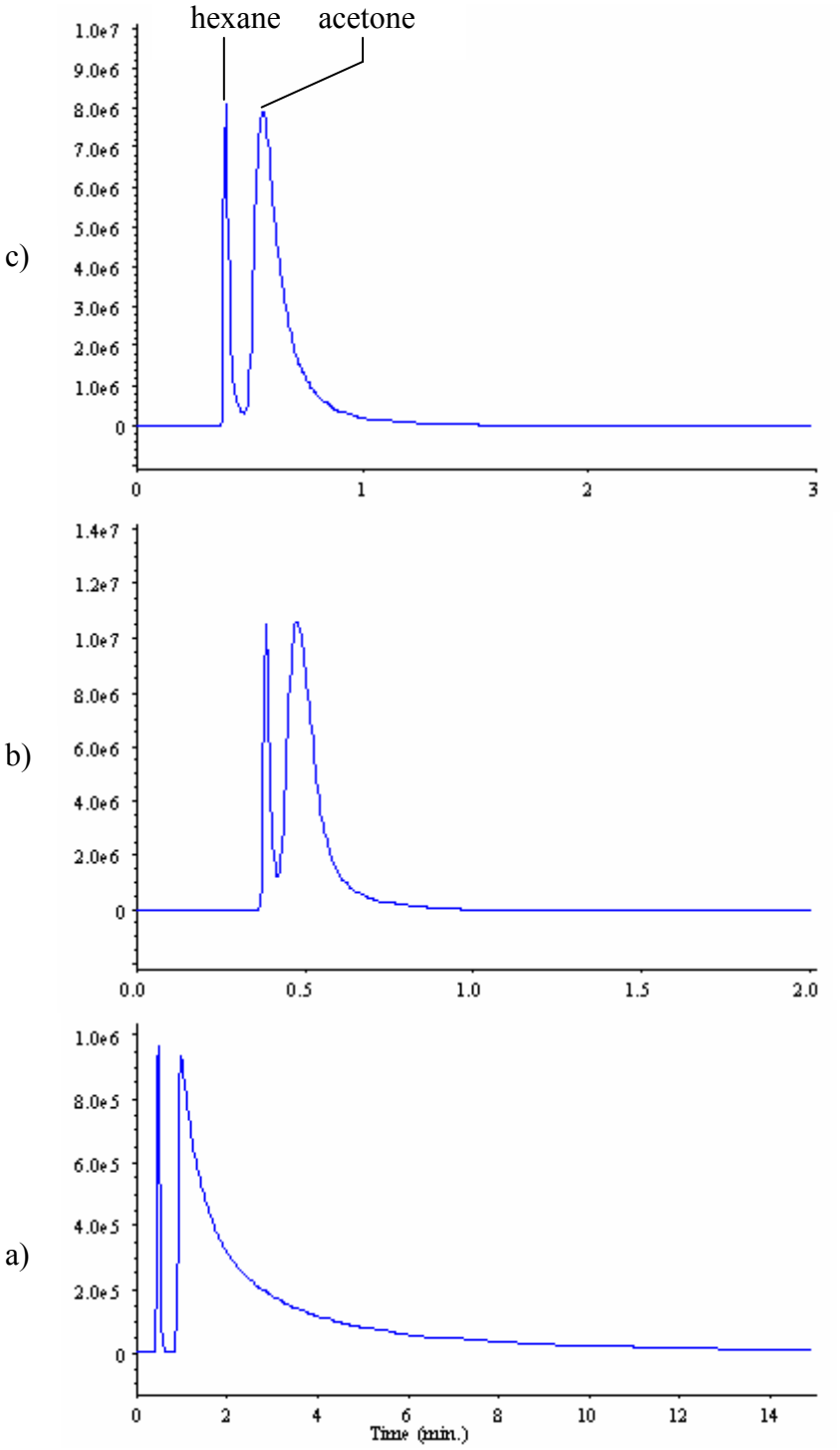


Figure 18 Chromatograms of model compound for a) non-coated and b) PEG coated silica monolithic column number 32-07 and c) PEG coated silica monolithic column number 32-09

Table 31 Chromatographic parameters of model compounds for non-coated and PEG coated silica monolithic column.

Compound	t_R (min)	W (min)	k'	α	Rs	N (plates·m^{-1})
Non-coated column number 32-07						
hexane	0.471	0.063	0.281	5.997	1.007	851
acetone	0.988	0.962	1.686			
PEG coated column number 32-07						
hexane	0.386	0.023	0.048	6.324	1.587	14941
acetone	0.480	0.096	0.303			
PEG coated column number 32-09						
hexane	0.396	0.026	0.077	6.854	2.111	9117
acetone	0.562	0.131	0.527			

From the chromatographic parameters obtained from these columns, selectivity factor resolution and theoretical plates of the column were compared by using one-way ANOVA to test whether chromatographic parameters of non-coated and two coated columns were different or not. Results of the ANOVA analysis were shown in Table 32 to 34 for selectivity factor, resolution and theoretical plates testing respectively.

Table 32 Results of one-way ANOVA calculation to test the difference of **selectivity factor (α)** of the columns (P = 0.05)

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	1.12157	2	0.560785	11.68636	39.33
Within columns	0.287918	6	0.047986		
Total	1.409488	8			

Table 33 Results of one-way ANOVA calculation to test the difference of **resolution (Rs)** of the columns (P = 0.05)

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	1.830139	2	0.91507	1305.561	39.33
Within columns	0.004205	6	0.000701		
Total	1.834345	8			

Table 34 Results of one-way ANOVA calculation to test the difference of **theoretical plates (N)** of the columns (P = 0.05)

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	3.01×10^8	2	1.5×10^8	2322.896	39.33
Within columns	388454.1	6	64742.34		
Total	3.01×10^8	8			

From Table 32, the less experimental F-value than the critical value meaned that the selectivity factors of non-coated, coated column number 32-07 and of coated column number 32-09 were not different. Meanwhile, the greater experimental

F than the critical value in Table 33 and 34 indicated that the resolution and theoretical plates of those columns were significantly different.

To test the difference between resolutions of the two columns, the least significant difference was calculated. The resolution difference between non-coated and coated column number 32-07 was 0.58, and that between coated column number 32-07 and 32-09 was 0.52. The least significant difference obtained from calculation, which equation and example of calculation was showed in appendix A, was 0.053. As both values were greater than the least significant difference value, it indicated that the resolution of columns were completely different from each other.

Similar to resolution, when comparing the least significant difference value of 509 for theoretical plates with the difference between each value, it was found that theoretical plates obtained from the columns differed significantly from each other.

In summary, selectivity factors of model compounds for these columns were not different, meaning that resolving power of all columns for the compounds was similar. Both resolution and theoretical plates of the columns were absolutely different, which increased after coating with PEG. However, resolution values of both PEG coated silica monolithic column were small. Thus, the separation was not good. In an addition, the PEG had bled and contaminated the detector after using for 2 to 3 times. Thus, the PEG coating method was not satisfactory.

Two methods for conventional coated stationary phase on GC column namely dynamic and static coating method were also applied to coat PEG on surface of fabricated silica monolithic column.

3.2 Dynamic coating method

Dynamic coating method was performed by filling solution into a tubing to create a plug of solution, and then the plug was pushed from one end of column to the

other, leaving a thin film on the wall. The schematic of the method was shown in Section 3.5

The method was performed by coating PEG on the surface of silica monolithic column. After a plug of PEG solution was created in ca. 10 cm monolithic column, it was pushed by purging nitrogen gas to one end of column. It was found that by using too low flow rate of nitrogen gas of 0.8 $mL \cdot min^{-1}$ the plug did not move. When increased the flow rate to about 0.9 $mL \cdot min^{-1}$, the plug moved slowly and evaporated before passing through the other end, so the column was not completely coated. However, the same effect was not observed when a bare capillary tubing was tested. The evaporation of plug before passing through the column might be a result of high suface area of silica monolithic material in the tubing. The dynamic coating was then not a suitable method of coating viscous liquid stationary phase on surface of packing material readily packed inside tubing.

3.3 Static coating method

To coat the surface of silica monolithic column, the column was fully filled with PEG solution and the solvent was evaporated under vacuum, leaving film of PEG on the surface of silica earlier described in Section 3.4.

Concentrations of PEG solution to give an efficient coated column were studied. Firstly, three silica monolithic columns of about 10 cm long were coated with 1, 5 and 10% w/w PEG in acetonitrile by using the static method. To test the efficiency, each coated column was connected to GC-FID and the model compounds were injected, obtaining chromatograms as shown in Figure 19.

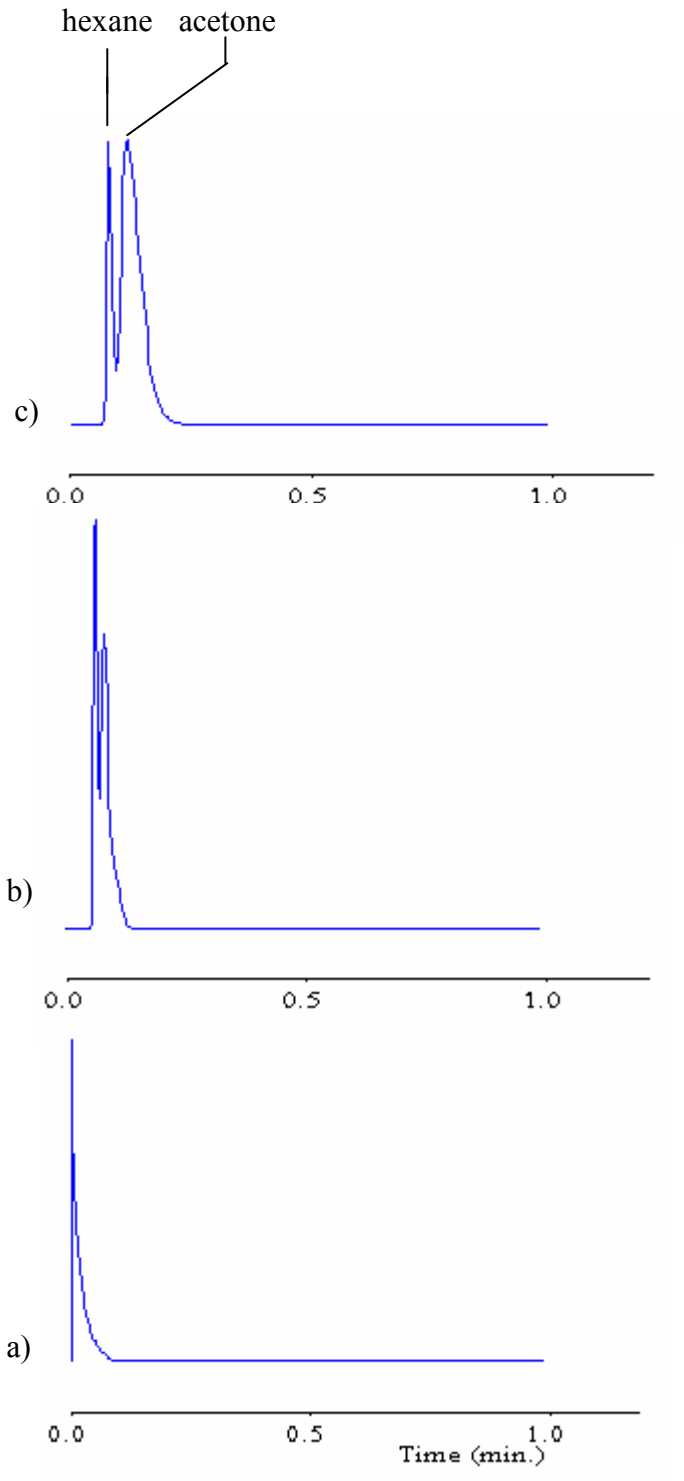


Figure 19 Chromatograms of model compounds for ~10 cm × 0.32 mm silica monolithic column coated with a) 1%, b) 5% and c) 10% w/w PEG solution.

At too low PEG concentration, silica monolithic column could not separate the compounds, hexane and acetone were co-eluted into one peak (Figure 19(a)). In the second column, a separation of both model compounds were observed when increasing the concentration of PEG to 5%, meaning that resolving power of the column increased with concentration as shown in Figure 19(b). With an increased PEG concentration of 10% w/w, two components in the mixture were clearly separated, giving better resolving power (Figure 19(c)).

However, as the components were incompletely separated, to improve the efficiency, the length of silica monolithic columns was extended from ca. 10 cm to about 20 cm and the concentrations of PEG solution for coating on silica surface were re-examined. Chromatograms of the model compound mixture for 10, 20, 30 and 40% w/w PEG in acetonitrile to be coated on monolithic columns were shown in Figure 20. Chromatographic parameters of the chromatograms were summarized in Table 35.

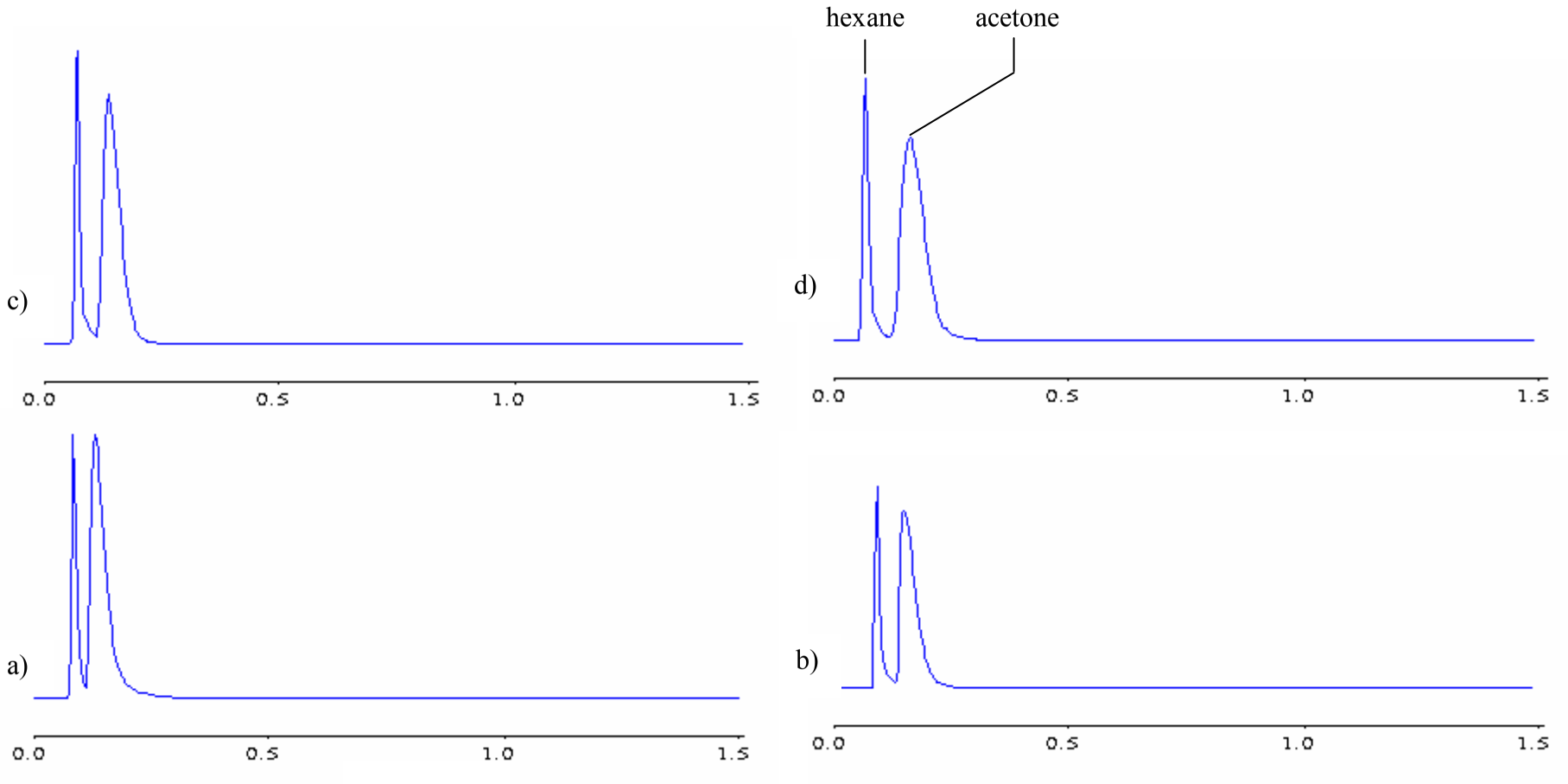


Figure 20 Chromatograms of model compound for a) 10% b) 20% c) 30% and d) 40% PEG solution coated on surface of silica monolithic columns.

Table 35 Chromatographic parameters of model compound for coated silica monolithic column with 10, 20, 30 and 40%w/w PEG solution.

Compounds	t_R (min)	W (min)	k'	α	Rs	N (plates·m^{-1})
10% PEG						
hexane	0.087	0.011	0.922	2.085	1.910	1855.57
acetone	0.132	0.036	1.922			
20% PEG						
hexane	0.092	0.011	0.082	9.709	2.560	2517.40
acetone	0.152	0.036	0.782			
30% PEG						
hexane	0.072	0.011	0.254	5.521	2.569	1925.51
acetone	0.137	0.040	1.404			
40% PEG						
hexane	0.070	0.015	0.429	5.452	2.579	1205.61
acetone	0.164	0.058	2.337			

A column coated with 50% PEG solution on surface of silica monolithic column was also studied, but the PEG solution was too viscous, resulting in inhomogeneity of filling the solution into the column, and its solvent could not be completely evaporated in a desired time. Then the column could not be used as a GC column.

From the chromatographic parameters, one-way ANOVA was performed to test significant difference of chromatographic performances of the columns coated with various concentrations of PEG solution on surface at 95% confidence interval. Results of ANOVA analysis were shown in Table 36 to 40 for the difference of

capacity factor of hexane, capacity factor of acetone, selectivity factor, resolution and theoretical plates, of five columns, respectively.

Table 36 Results of one-way ANOVA calculation to test the difference of **capacity factor of hexane** of the columns (P = 0.05)

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	0.78744	3	0.26248	1549.414	9.979
Within columns	0.000678	4	0.000169		
Total	0.788118	7			

Table 37 Results of one-way ANOVA calculation to test the difference of **capacity factor of acetone** of the columns (P = 0.05)

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	2.706517	3	0.902172	3165.688	9.979
Within columns	0.00114	4	0.000285		
Total	2.707657	7			

Table 38 Results of one-way ANOVA calculation to test the difference of **selectivity factor** of the columns (P = 0.05)

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	58.46568	3	19.48856	18.19052	9.979
Within columns	4.285433	4	1.071358		
Total	62.75111	7			

Table 39 Results of one-way ANOVA calculation to test the difference of **resolution** of the columns (P = 0.05)

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	0.65283	3	0.21761	48.02316	9.979
Within columns	0.018125	4	0.004531		
Total	0.670955	7			

Table 40 Results of one-way ANOVA calculation to test the difference of **theoretical plates** of the columns (P = 0.05)

Source of Variation	Sum of squares	Degree of freedom	Mean square	F	F crit (two-tailed)
Between columns	1727384	3	575794.7	152.8188	9.979
Within columns	15071.31	4	3767.827		
Total	1742455	7			

The ANOVA calculations showed that F-values for all cases were absolutely greater than the critical value. Thus, chromatographic performance of each column was different.

From the results of capacity factor of hexane the least significant difference obtained was 0.036. From Table 35, the difference of capacity factor between each column was larger than the significant value, meaning that capacity factor of hexane obtained from five columns differed from each other. Similar to hexane, the capacity factor difference of acetone between the columns was greater than that for least significant value, which was 0.047. Then the capacity factor of acetone differed from each other. From the table the capacity factors of both hexane and acetone were mostly increased with increasing concentration of PEG.

For selectivity factor, the least significant difference was 2.877. Again from Table 35, the differences of selectivity factor from each column were greater than the least significant difference except the value between 30% and 40% PEG, which was 0.069. It indicated that the selectivity factors of the model compound obtained from the columns were different, except those between 30% and 40% PEG showed no difference.

For resolution, the least significant difference was 0.187. The differences of resolution from Table 35 were smaller than the significant value, meaning that resolution obtained from each column were not different, except that for 10% PEG. Resolution of model compound obtained from 10% PEG was smallest and significantly differed from the others (0.650 or more). Thus, it provided the lowest resolving power.

From the results, it can be concluded that for 10% PEG the efficiency of the column was low as a result of poor resolution of model compounds, while the resolution of other columns were not different significantly. As seen in Table 35, 20% PEG coated column gave the best theoretical plates and resolution, but the capacity factors were significantly low, comparing to the other columns, resulting less accurate retention time determination. Although selectivity factor and resolution for 30% and 40% PEG coated column was not different, the theoretical plate of 30% PEG was greater than that found for 40% PEG. Thus, the suitable concentration of PEG in acetonitrile to be coated on surface of silica monolithic column was 30% w/w.

However, because two peaks of the model components were incompletely separated despite of the optimized concentration of PEG solution on 20 cm silica monolithic column. Therefore, the length of fabricated column was increased from about 20 to 40 cm and kept the PEG coating concentration at optimum.

The column number 32-43 was then coated with 30% w/w PEG solution in acetonitrile by using a static coating The efficiency of the coated column was tested

and a chromatogram of model compounds was shown in Figure 21, together with chromatographic parameters shown in Table 41.

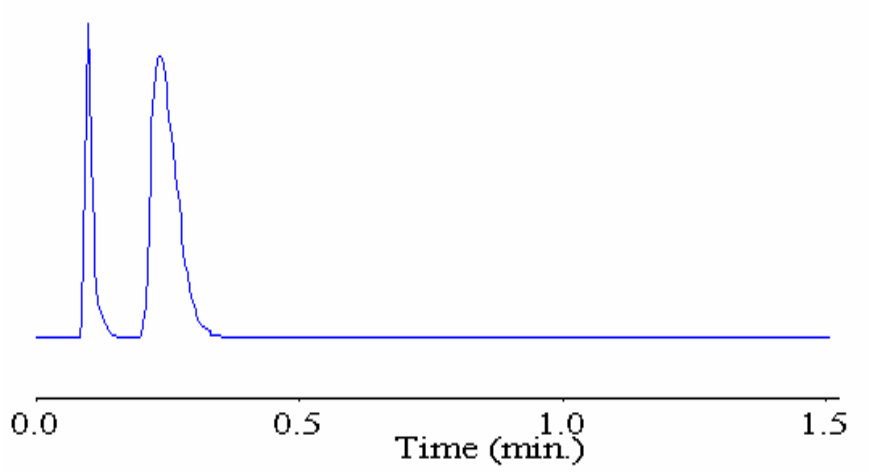


Figure 21 Chromatogram of model compounds for 43 cm × 0.32 mm 30% PEG coated silica monolithic column.

Table 41 Chromatographic parameters of model compounds obtained from 43 cm 30% PEG coated silica monolithic column

Compound	t_R (min)	W (min)	k'	α	Rs	N (plates·m^{-1})
hexane	0.101	0.017	0.485	5.152	3.808	1629.34
acetone	0.238	0.055	2.500			

To compare the efficiency between ~20 cm and ~40 cm column, the difference in standard deviation of two columns must be initially tested by using a two-tailed F-test at 95% confidence interval. The results of the difference in standard deviation of selectivity factor, resolution and theoretical plates of two columns were shown in Table 42.

Table 42 Results of two-tailed F-test for the difference in standard deviations of chromatographic parameters of 20 cm and 40 cm of 30% PEG coated silica monolithic columns

	Selectivity factor		Resolution		Theoretical plates	
	20 cm column	40 cm column	20 cm column	40 cm column	20 cm column	40 cm column
Mean	5.521	5.152	2.569	3.808	1925.51	1629.34
Variance	0.02949	0.001837	0.001893	0.01217	4493.52	11794.36
Degree of freedom	1	1	1	1	1	1
F	0.0623		6.425		2.625	
F Critical	647.8		647.8		647.8	

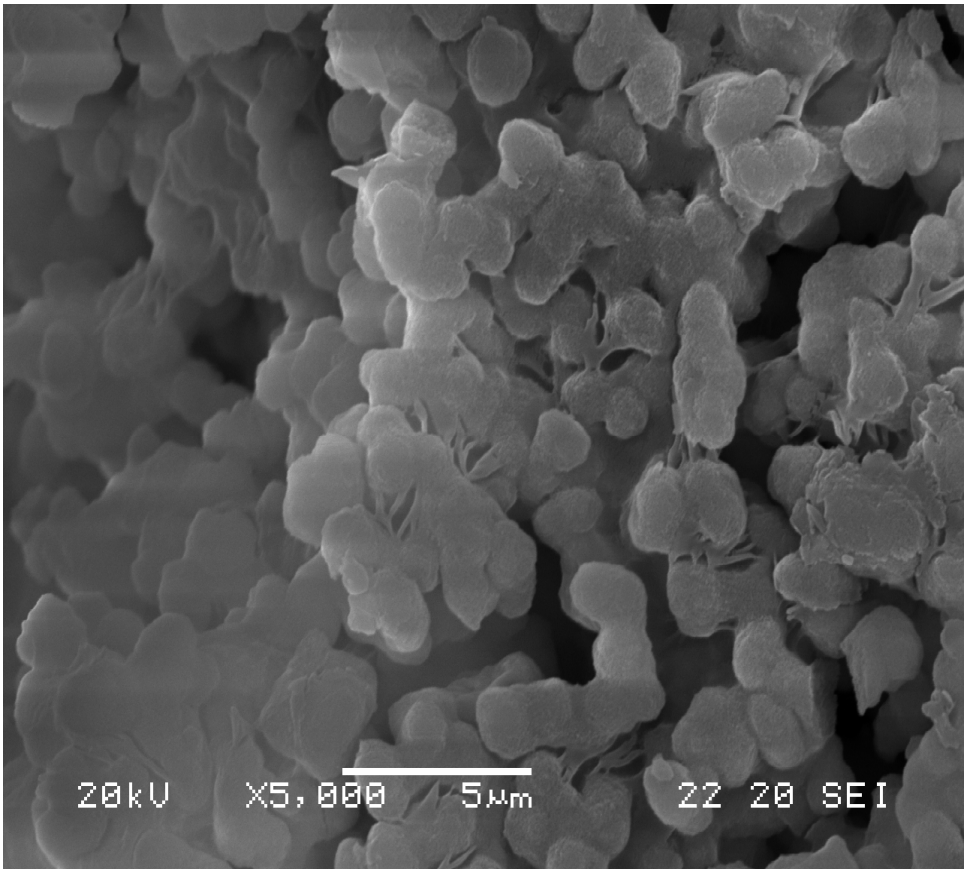
From the results shown in Table 42, F-values of all chromatographic parameters were less than that found for the critical value of $F_{1,1}$ (two-tailed, P = 0.05), which was 647.8, meaning that two standard deviations of each parameter were not significantly different. Therefore, it was assumed that the two means of each parameter had similar varience. Afterwards, the t-test was analysed for the two means to compare column efficiency and the results were shown in Table 43.

Table 43 Results of two-tailed t-test analysis of performance of 20 cm and 40 cm of 30% PEG coated silica monolithic columns

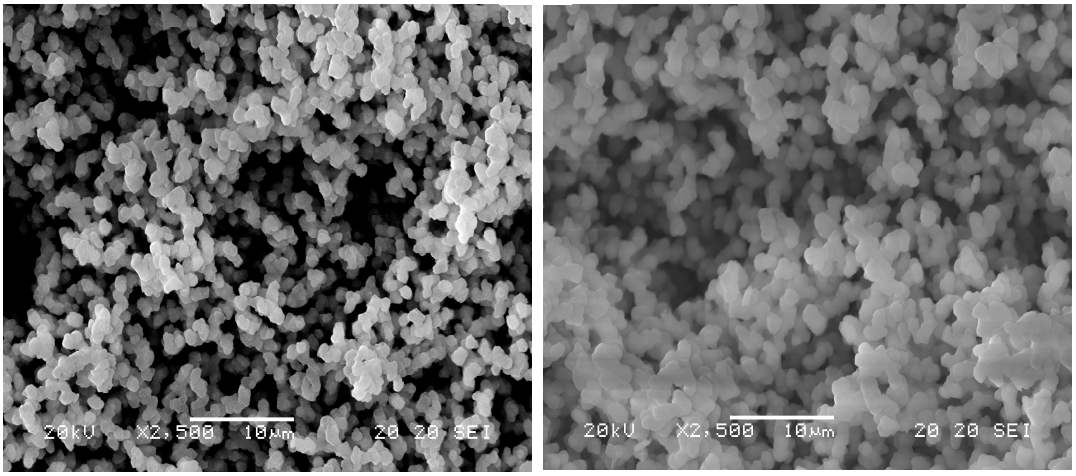
	Selectivity factor		Resolution		Theoretical plates	
	20 cm column	40 cm column	20 cm column	40 cm column	20 cm column	40 cm column
Mean	5.521	5.152	2.569	3.808	1925.51	1629.337
Variance	0.02949	0.001837	0.001893	0.01217	4493.52	11794.36
Pooled Variance	0.015663		0.00703		8143.938	
Degree of freedom	2		2		2	
t-value	2.956		14.772		3.282	
t-critical	4.303		4.303		4.303	

In Table 43, t-value of selectivity factor and theoretical plates were smaller than the critical value, which meant that the selectivity factor and theoretical plates of the two columns were not significantly different. However the t-value for resolution was greater than that of critical value, which meant that resolutions of the compounds for 20 cm and 40 cm PEG coated silica monolithic column differed significantly. The results were indicated that resolution of the long column was greater than that of the shorter one. Thus, increased column length resulted in higher resolution, then improving resolving power as expected.

For homogeneous coating along the column from one end to the other, both ends of the column was cut, scanned by using SEM micrograph and determined amount of carbon on the surface of the monolithic structure by using a Scanning Electron Microscopy-Energy Dispersive Spectrometry (SEM-EDS) as earlier described in Section 3. The images obtained from SEM were shown in Figure 22. The results of scanning both ends of the column obtained from SEM-EDS and amount of carbon coated on surface of silica monolith were shown in Figure 23 and Table 44, respectively.



c)



a) b)

Figure 22 SEM micrographs of a) non-coated and b) coated with 30% w/w PEG solution on 43 cm × 0.32 mm silica monolithic column with 2500 magnification and c) coated silica monolithic column with 5000 magnification.

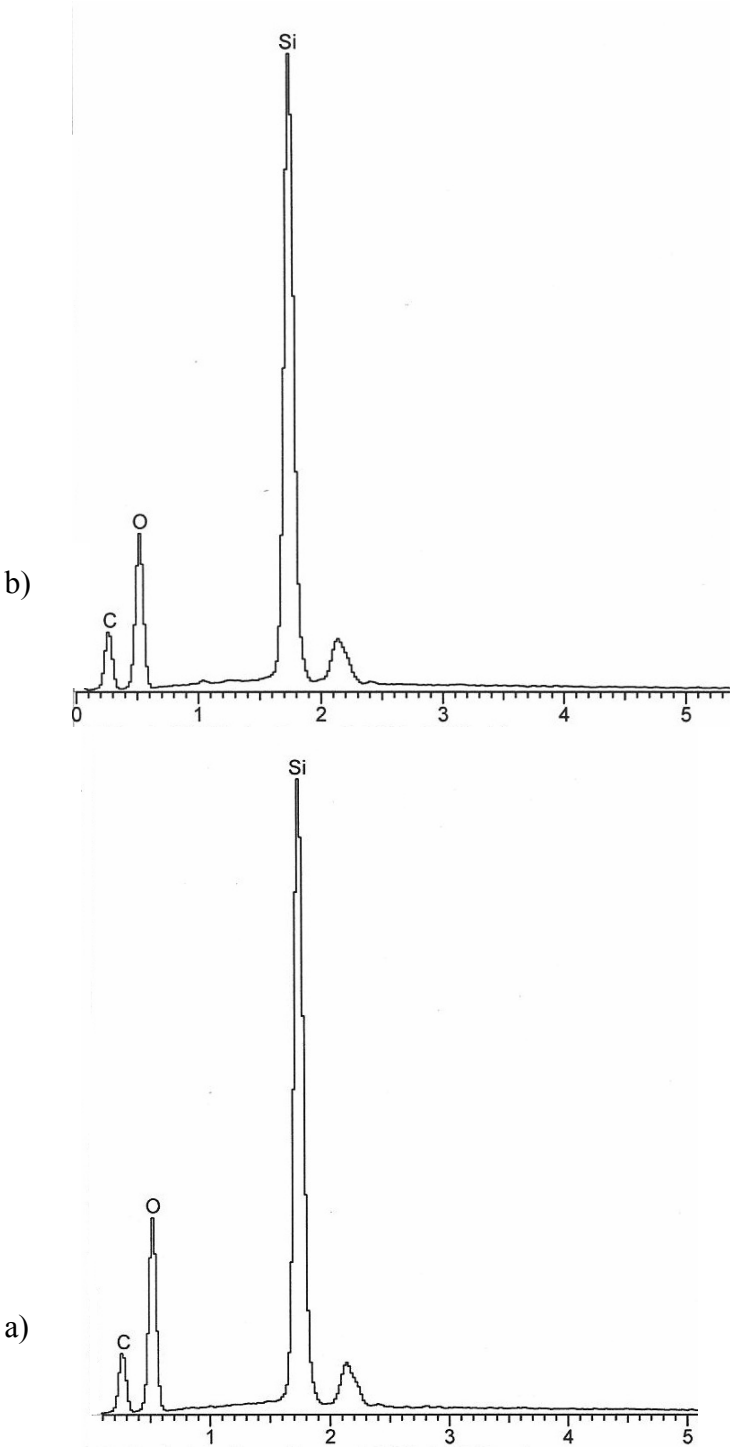


Figure 23 Results of carbon determination at both ends (a and b)of 43 cm × 0.32 mm PEG coated silica monolithic column obtained by using SEM-EDS.

Table 44 Amount of carbon at both ends on the surface of coated monolithic column

Column end	Amount of carbon (% weight)					Mean	SD
	1	2	3	4	5		
1	30.32	29.04	28.20	28.38	29.29	29.05	0.843
2	32.75	30.66	31.18	32.46	-	31.76	1.003

As shown in Figure 22 (a) and (b), the surface of silica skeleton changed after coating, as it was less rough and its particle slightly increased. By visual observation, the polymer was homogeneously covered the silica surface. To ensure the homogeneity of coating, SEM-EDS were used to determine the amount of carbon of PEG coated on surface at both ends of column. F-test was performed to examine the difference of standard deviation of the means of the carbon amounts at both ends and the two-tailed t-test analysis was conducted at 95% confidence interval. Results of F-test and t-test were shown in Table 45 and 46, respectively.

Table 45 Results of two-tailed F-test at 95% confidence interval for a comparison of the standard deviations of carbon determination at both ends of 43 cm × 0.32 mm PEG coated silica column

	First end	Second end
Mean	29.05	31.76
Variance	0.710	1.005
Observations	5	4
Degree of freedom	4	3
F-value	0.706	
F-critical value	9.979	

Table 46 Results of two-tailed t-test at 95% confidence interval for a comparison of carbon amount on the surface at both ends of 43 cm × 0.32 mm PEG coated silica column

	First end	Second end
Mean	29.05	31.76
Variance	0.710	1.005
Pooled Variance	0.837	
Degree of freedom	7	
t-value	4.427	
t-critical value	2.365	

From Table 45, calculated F-value was 0.706, which was smaller than the critical value for two-tailed testing, indicating no difference in standard deviation of amount of carbon at both ends. However, when assuming similar variance given for both ends and examining for the significance for the means of carbon amounts at both ends by t-test analysis, the calculated t-value shown in Table 46 was greater than that found for the critical value, meaning that amount of carbon obtained from both ends were different significantly. Thus, the column was not homogeneously coated with PEG.

Although the PEG coating on the surface of column number 32-43 was not homogeneous, the column provided good efficiency. When comparing the selectivity factor of model compounds separated on a 60m standard PEG column (Carbowax 20 M) (α = 6.950) (see Section 3.1) to that for PEG coated silica monolithic column number 32-43 (α = 5.152) it was found that the resolving power of the latter column were approximately 43 m of standard column. In addition, analysis time of the model compounds for the home-made monolithic column was reduced from that of Carbowax 20M approximately three times with an acceptable resolution.

CONCLUSION AND FUTURE WORKS

Conclusion

In this work, silica monolithic material was fabricated in a fused-silica capillary tubing filled with a mixture of alkoxysilane and polyethylene glycol (PEG) with a molecular weight of 10000 $g \cdot mol^{-1}$ in an 0.01 M aqueous acetic acid in present of urea. Two types of alkoxysilane, namely tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS), were used. From the experiment, skeleton of silica monolithic material formulating from TMOS was ~1 μm with ~5 μm through pore while the monolithic structure prepared from TEOS was not formed. However, the main problems faced during column fabrication were cracking and shrinkage of the structure. They were solved by carefully controlling some conditions and technique, e.g. temperature and stirring rate, and sealing at both ends of tubing to prevent solvent evaporation. Refabrication of silica monolithic column was also proposed to fill up the void of tubing caused by the shrinkage. However, the SEM micrograph of cross-sectional tubing indicated that silica monolithic structure of first and second fabrication did not attach to each other.

Specific surface area of fabricated silica monolithic structure in tubing was determined by using an inverse gas chromatography or IGC technique. The fabricated silica monolith was connected to GC-FID and used as a sample column. Several parameters to determine specific surface area, i.e. operating temperature, type of probe molecule and range of injection volume, were optimized. Precision of the method was simultaneously studied. From the results, optimum temperature for selected probe molecules, namely octane, nonane, decane and undecane, were 100, 120, 140 and 160˚C, respectively. Specific surface area of a single silica monolithic column obtained from the four probe molecules showed no significant difference between probe molecules at 95% confidence interval. Relative standard deviation (%RSD) of surface area of obtained from octane, nonane, decane and undecane were 5.63, 6.79, 4.31 and 6.21, respectively. Thus, decane with minimum %RSD was used

as probe molecule in this work. A day-to-day variation for the probe molecule was not different meaning that the IGC technique for a determination of specific surface area of silica monolithic column was very precise. The IGC method was also compared with classical nitrogen adsorption method. It was found that specific surface areaobtained from two methods were different. However, the accuracy of the latter method was suspected because of small amount of monolithic material in the column sample. Then the determinations of specific surface area of silica monolithic column in this work were preferably performed by using IGC technique.

Variable factors of column fabrication affecting specific surface area of silica monolithic column, i.e. refabrication of silica monolith, column diameter and column length, were studied. The results showed that surface area of a silica monolithic column increased after refabrication but through pore and homogeneity of silica skeleton obtained from SEM micrograph decreased as mentioned above. Thus, the refabricated column was not suitable to be used as chromatographic column. Both column diameter and column length also affected the specific surface area of silica monolith. The results showed that specific surface area decreased with increasing diameter and length of fused-silica tubing. Besides, surface area of columns synthesized in the same batch differed from each other.

The fabricated silica monolithic column was coated with PEG with a molecular weight of 10000 $g \cdot mol^{-1}$ on silica monolithic surface and used as a GC column. Methods for coating, namely solvent evaporation, dynamic and static coating, and PEG concentration in acetonitrile were studied. It was found that 43 cm × 0.32 cm silica monolithic column coated with 30% w/w PEG solution by a static coating method gave the best resolving power for a model compound mixture of hexane and acetone. Despite of homogeneity in coating from one end to the other, the variation was not too high. By comparison, the resolving power of the column was corresponded to a conventional Carbowax 20 M column of ca. 40 m long.

Future works

From the results, shrinkage of monolithic structure was a main problem in column fabrication and also affected specific surface area of silica monolithic column. The fabrication method might be improved by using a mixture of methyl trimethoxysilane (MTMS) and tetramethoxysilane (TMOS) as a starting material which minimized the shrinkage of the silica. (Nunez *et al.,* 2008)

In this work, stationary phase coated on surface of silica was polyethylene glycol (PEG) with molecular weight of 10,000 $g \cdot mol^{-1}$. Other types e.g. poly(phenylmethyl)siloxane might be coated on the column and used for separation of other compounds i.e. alkyl benzene and chlorinated aromatics.

Method for deposit stationary phase on surface of silica monolithic column was a physically method namely static coating. As seen from the results, homogeneity coated was hardly control. Chemically coated, which bonded the stationary phase to silica skeleton, will possibly give more homogeneous surface coating with a high stability than that found for static coating method.

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APPENDICES

Appendix A

Calculation of statistic and chromatographic parameters

Calculation of chromatographic parameters, namely capacity factor, tailing factor, and theoretical plates, of octane at 80˚C were examined below.

Capacity factor or k' was calculated from equation (A-1) as shown below.

$$k' = \frac{t_R - t_m}{t_m} \quad \ldots(A\text{-}1)$$

Where t_R was retention time of solute and t_m was dead time of the column. Retention time of octane was 0.729 min for column sample number 32-08. Dead time of the column was 0.360 min. When substituted the values into the equation giving

$$k' = \frac{0.729 - 0.360}{0.360}$$

$$= 1.025$$

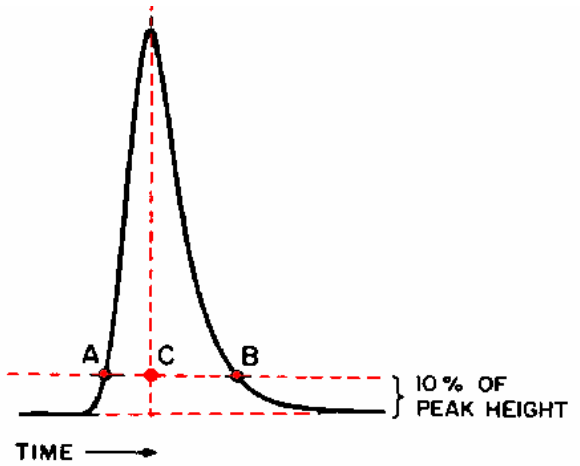
Tailing factor was a ratio of tail (b) and front (a) of the peak which determined at 10% of peak height. For octane at 80˚C, a and b values were 0.102 and 0.221, respectively. Then the ratio of b/a was 2.167.

Theoretical plates (N) were estimated by using equation (A-2) as shown below

$$N = 16\left(\frac{t_R}{W}\right)^2 \quad \ldots(A\text{-}2)$$

In equation (7-2), W was peak width which was 0.166 min using octane for the column sample. Substituted the values to the equation, the result was 308.49 plates. Then, dividing the theoretical plates with length of the column sample, which was 0.08 m, to obtain the theoretical plate per meter of the column, the result was 3,857 plates per meter.

Tailing factor was a ratio of tail to front a peak which measured at 10% of peak height as shown in appendix figure A1.



Appendix Figure A1 Measurement of a tailing factor of a peak .

Tailing factor was calculated from equation (A-3) as shown below

$$TF = \frac{BC}{AC} \qquad \ldots(A\text{-}3)$$

For octane at 80˚C, AC was 0.102 and BC was 0.221. Substituted the values into the equation, result was 2.167.

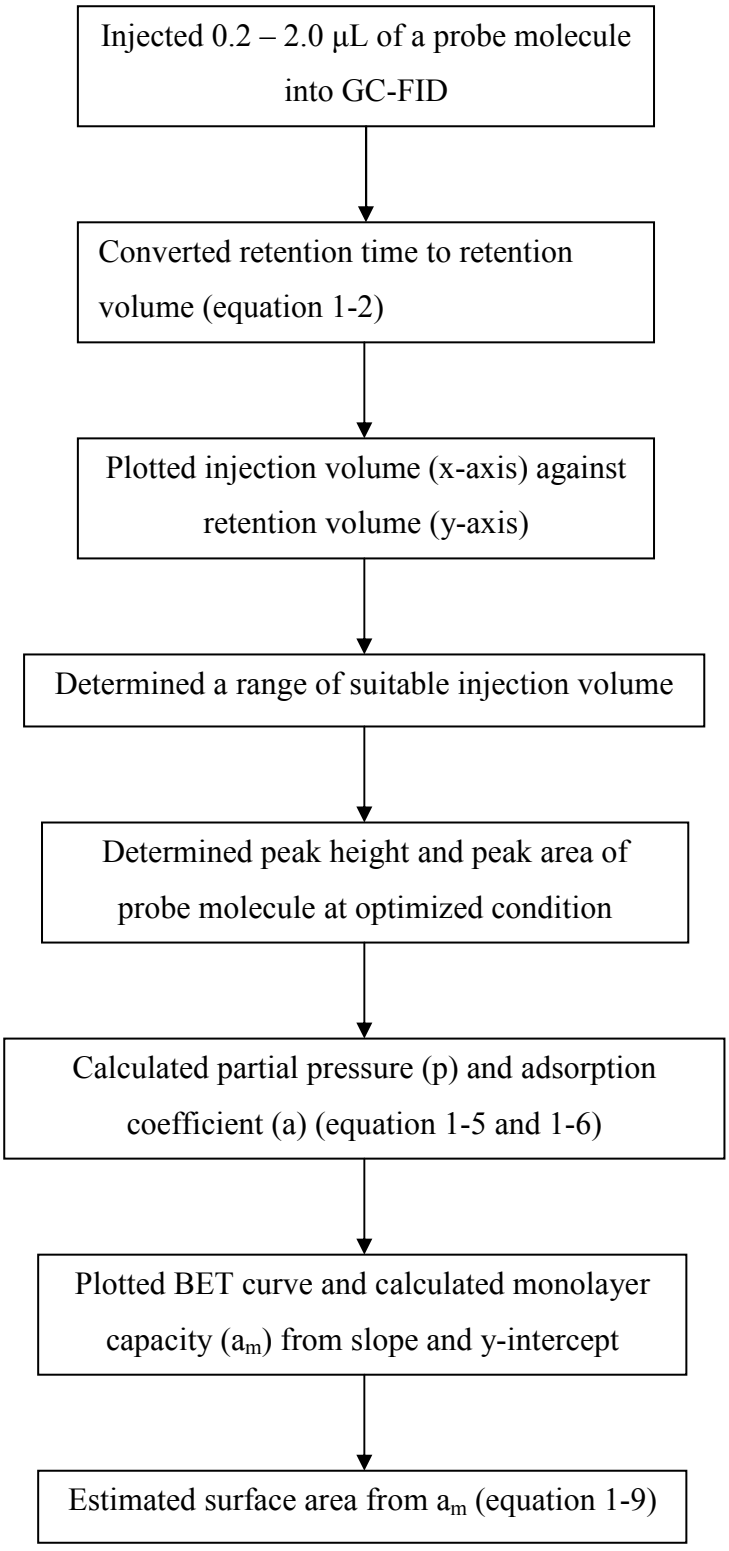
Calculation of least significant difference value was examined from one-way ANOVA test for difference between resolutions of non-coated, PEG coated column sample number 32-07 and PEG coated column sample number 32-09. The least significant difference was estimated from equation (A-4) as shown below.

$$\text{Least significant difference} = s\left(\frac{2}{n}\right)^{\frac{1}{2}} \times t_{h(n-1)} \qquad \ldots(A\text{-}4)$$

Where s was within-sample estimate of σ0 n was number of replicates which was $\sqrt{0.000701}$ and 3, respectively. And $t_{h(n-1)}$ was t-value at h(n-1) which was number of degrees of freedom of within-sample. In this case, h(n-1) was 6, then t-value which was t_6 at 95% confidence interval was 2.45. Substituted all variables to the equation, result was 0.053.

Appendix B

Summarization of procedures of surface area determination



Appendix Figure B1 Summarized step of surface area determination by using IGC technique