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Characterizing synthetically prepared packing materials and standard monolithic columns by inverse size exclusion chromatography

Majed AL-Bokari
University of Tennessee

To the Graduate Council:

I am submitting herewith a thesis written by Majed AL-Bokari entitled "Characterizing synthetically prepared packing materials and standard monolithic columns by inverse size exclusion chromatography." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.

Georges Guiochon, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Characterizing Synthetically Prepared Packing Materials and Standard Monolithic Columns by Inverse Size Exclusion Chromatography

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Majed AL-Bokari
August 2002

Dedication

This thesis is dedicated to my

Mother, sisters, brothers, wife, and daughter.

Without their prayers, support, and love, this work would not have been finished.

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Abstract

Characterizing the analytical column is the first step in determining whether the column is suitable for the work's aim, or not. This work will be devoted to characterizing two kinds of columns: the normal practical packing column and the monolithic column. The Inverse Size Exclusion Chromatography (ISEC) method has been applied to study and characterize these columns by using a long series of standard polystyrene samples of narrow molecular weight distribution. These samples have been carefully chosen to cover a wide range of molecular weight between 5×10^2 up to 2×10^6 Dalton. The former columns were packed with synthesized and prepared materials that were made at Oak Rage National Laboratory (ORNL) by Dr. S. Dai and his student Mr. C. Liang. The monolithic columns were given to Prof. G. Guiochon as a generous gift from MERCK Company. Our work on these columns will be focused on five important characteristics: efficiency, pore-size diameter, pore–size distribution, porosity, and excluded molecular weight. In fact, by applying ISEC for these columns once by CH_2CL_2 and another by THF, we should acquire more or less the same results (except the efficiency). One of the prepared packing materials that investigated has a reasonable efficiency, as a prepared material, around 3200 plate; the other characters reveal very consistent values in both runs. The other four materials have poor efficiency (less than 2000), very different values when the run is shifted from CH_2CL_2 to THF. We conclude that these prepared materials either cannot fit and achieve the boundaries and the necessary conditions to be characterized by ISEC, or that they need to be improved in some points concerning their structure. The monolithic columns showed rather excellent and reliable values for SD's and RSD's, as well as, very high efficiency over 4500 plate comparing with the normal

packing materials. We have to be very careful when we deal with the monolithic columns, especially, regarding the purity grade of the sample and the solvents. Dealing with unfiltered reagents causes a kind of contamination, which affects the characters of the columns.

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List of Symbols and Abbreviations

Symbols

1 Standard Packing Material; LUNA PREP Silica (2)

2 Standard Packing Material; LUNA PREP Silica C18

A Prepared Packing Material; Silica 64Å

B Prepared Packing Material; Silica SBA-15 (1)

C Prepared Packing Material; Silica SBA-15 (2)

D Prepared Packing Material; Silica SBA-15 CH_3Si

E Prepared Packing Material; Silica SBA-15 C_6H_5Si

N Number of Theoretical Plate or Efficiency

R^2 R Square for the Linear Regression

r^2 Radius of the Column

Mw Molecular Weight (g/mol)

dp Particle Diameter (μm)

t_0 Retention Time (min)

L (*l*) Column Length

V_z Interstitial Volume (ml) or **V_e** External Volume (ml)

V_p Pore Volume (ml) or **V_i** Internal Volume (ml)

V_e Retention Volume (ml)

V_e Excluded Volume (ml)

V_g Geometrical Volume

V_s Skeleton Volume

V_c Closed or Inaccessible Pore Volume (ml)

V_a Layer of C18 Bounded Chains Volume.

V_u Summation of Three Volume (V_s, V_c, and V_a)

V_k Empty Separation Volume (ml)

m Mass of the Packing Material (g)

P_s True Density of the Packing Material (g/ml)

Greek Symbols

$\Delta\varepsilon$ Difference in the Porosity

$\Delta\phi$ Difference in the Pore-size Diameter

ΔM_w Difference in the Excluded Molecular Weight

ε_T Total Porosity

ε_e External Porosity

ε_i Internal Porosity

ϕ Pore-Size Diameter (Å)

Abbreviations

ISEC Inverse Size Exclusion Chromatography

HPLC High Performance Liquid Chromatography

ORNL Oak Rage National Laboratory

HETP Height Equivalent Theoretical Plate

SEM	Scanning Electron Microscopy
TEM	Tunneling Electron Microscopy
SD	Standard Deviation
RSD	Relative Standard Deviation
AV	Average Value

CHAPTER 1

PRINCIPLES

1.1. Introduction

The physico-chemical properties and pore size distributions of packing materials have been studied for a long time by many classical methods. The importance of porous materials in chromatography comes from the need for achieve good separations. Pharmaceutical companies pay millions of dollars to support and sponsor people whose work and researches are focused upon making new materials or developing and modifying existing ones in order to obtain a satisfactory separations.

The main aim for this work is the investigation of the properties of a novel series of porous silica gel materials prepared of group of Dr. Sheng Dai in Chemical and Analytical Division, the Oak Ridge National Laboratory (ORNL).

Most popular methods applied to measure porosity and pore size distributions due capillary condensation, Mercury Porosimetry, and Size Exclusion Chromatography.

By studying the sorption of nitrogen on a solid at the temperature of its atmospheric boiling point *(Halász and Martin, 1978),* the pore diameter (ϕ) can be determined by assuming that capillary condensation takes place. The principal of these methods is relate the pore size and/or the adsorbed mass (volume) of N_2 corresponding to the relative pressure P/P_0, neglecting the layer adsorption,

$$\phi(\Delta) = \frac{8.28}{\log p_o/p}$$

where P_0 is the vapor pressure and **P** is the equilibrium pressure of nitrogen *(Halász and Martin, 1978)*. The disadvantage of this method is the limitation of the pore diameter range, which is approximately between 20 and 200Å.

On the other hand, there is a relationship between the pore diameter (**ϕ**) volume of liquid forced into the pore and the applied pressure, using an empirical approximation. This is the mercury porosimetry,

$$\phi(\Delta) = \frac{150{,}000}{P}$$

In this method, the mercury can penetrate into the solid under pressure. Also, here, the pore diameters cannot be determined if it is less than 70Å. The weakest point regarding this method is the results would be falsified if the material collapses while increasing the pressure up to 2000 atm, which is required for the measurements.

To have completed and corrected pore size distribution (PSD) for the same fine material, the results should be overlapped from these methods: the mercury porosimetry and the capillary condensation *(Halász and Martin, 1978)*. Because of the long time, approximately 35 hours, determine the pore diameter of a single sample through the above methods, the high cost of the apparatus, as well as, of the maintenance, there is a need to have and utilize a simple, fast, and accurate method to determine pore-size diameters and pore-size distributions.

Recently, methods have been proposed to study and determine the properties of porous packing materials, such as small-angle X-ray scattering, neutron scattering, nuclear magnetic resonance, coulometric measurement, and MRI. However, but these methods are still developing and in the early stages *(Guan and Guiochon, 1995)*.

The most practical method to determine the properties of porous materials is the Inverse Size Exclusion Chromatography (ISEC). Going from the logical point of the research, which is the simplicity and rapidity coupled with accuracy and cheapness, this work will be done by using Inverse Size Exclusion Chromatography to investigate the prepared packing materials and monolithic columns.

1.2. Theory of Inverse Size Exclusion Chromatography

Inverse Size Exclusion Chromatography (ISEC) is one of the liquid chromatography methods, which separates a mixture of compounds. Nevertheless inverse size exclusion chromatography differs from all the other Liquid Chromatography methods. The fundamental principle of ISEC that makes it different is that the separation does not depend on the chemical attractions or interactions, but depends on the physical sieving process (molecular volume).

Originally, the birth of the Inverse Size Exclusion Chromatography arose from two various groups of researchers. One utilized the principle, where separation of biochemical polymers occurs by using aqueous solution as a mobile phase and dextran gels as a stationary phase. This method was called "Gel Filtration Chromatography". Later, a group of polymer chemists used polystyrene gels and non-aqueous mobile phase to achieve the separation between the synthetic organic polymers. They used a term of Gel Permeation Chromatography. Later, some researchers used other names in the field. Recently, the term Size Exclusion is applied to both categories *(Miller, 1988)*. In addition to others as long as the base of the separation is Physical sieving.

Thus, a mixture of compounds is separated by its molecular size (weight) or what is called "hydrodynamic volume" in solution, as the solvent elutes through a packed column *(Provder, 1986)*. In other words, the molecules are separated according to their size and by their ability to penetrate a sieve-like structure in the stationary phase. Consequently, the large molecules will pass faster (shorter retention time) through the column and remain in the mobile phase, while the smaller molecules will get caught in the stationary and will pass slower (longer retention time) through the column and remain in the stationary phase longer.

This simple principle has been employed to determine pore-size diameters and pore size distributions for packing materials by injecting a long series of standard polymer (like Standard Polystyrene) samples of narrow molecular weight distribution. The relationship between the retention volumes of these samples and logarithms of their average molecular weight will reveal a few of the properties of the porous for studied material. Figure (1) illustrates the exclusion and the permeation in Size Exclusion Chromatography where the logarithmic of the molecular weight of the samples is plotted versus their retention volumes. The same figure can discern that large molecular weight compounds are eluted first because of the capability of pore size discrimination.

Many scientist and researchers such as J. Knox, I. Halász, and K. Martin in their work have examined Inverse Size Exclusion Chromatography. Whereupon, they established the essential conditions and the boundaries, which control and make ISEC one of the best standard methods to determine pore size distributions.

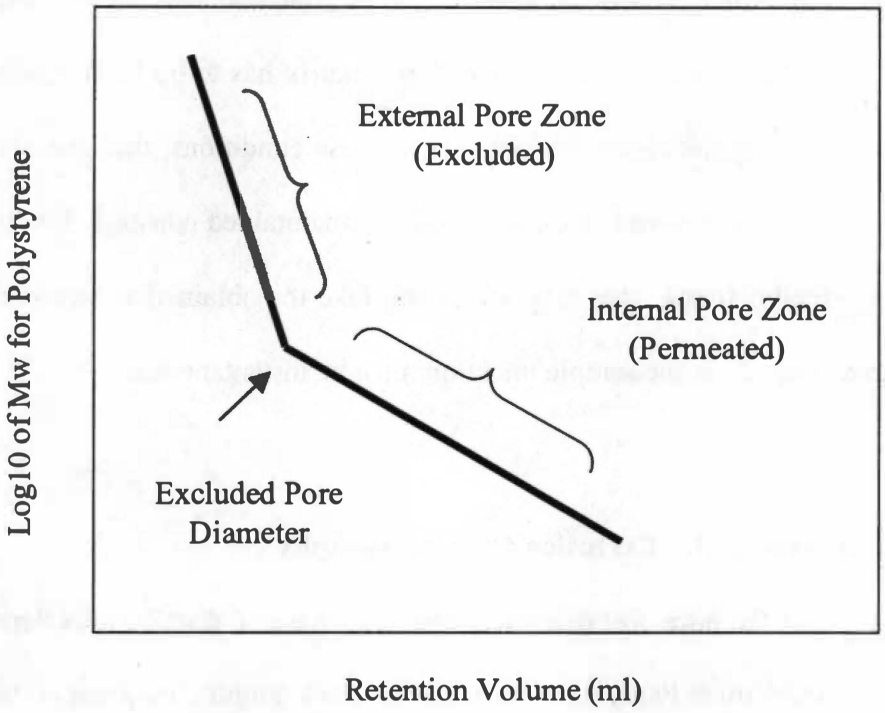


Figure 1. The Ideal Inverse Size Exclusion Chromatography Calibration Curve.

The conditions that must be accomplished are: the samples must not be adsorbed on the solid surface; instantaneous equilibrium should be established between the eluent held within the pores and the flowing eluent the column should not be overloaded with the test material; the elution peak should be similar to Gaussian peak, with an asymmetry factor smaller than 2; and the matrix of the solid has to be rigid and should not shrink or swell. According to *I. Halazs and K. Martin, 1978*, the matrix has to be hard enough to not be affected by changing the eluent. In addition to these conditions, the temperature and the flow rate of the eluent have to be controlled and maintained constant during the whole experiment. Finally, simple step may affect and fake the obtained values such as injecting the sample. Therefore, the sample injection must be instantaneous.

1.3. Calculations of Inverse Size Exclusion Chromatography

Determination of the pore size diameters and the pore size distributions depends on knowing the molecular mass (weight) of polymer standard samples and their retention time (volume). By getting these variables while keeping temperature and flow rate constant and coming with the essential conditions, the rest is a matter of calculations.

According to the theory, the voids between the internal wall of the column and the packing material are going to be filled in by certain volume from the eluent (mobile phase or the solvent), which is called the "interstitial volume" ($\mathbf{V_z}$) (*Halász and Martin, 1978*) or "external volume" ($\mathbf{V_e}$) (*Guan and Guiochon, 1995*). The pores of the test

material will be filled in by a fraction from the eluen, which is called the "pore volume" (V_p) (*Halász and Martin, 1978*) or "internal volume" (V_i) (*Guan and Guiochon, 1995*). Here, it is better pay an attention not to confuse among V_e (external volume), V_e (elution volume), and V_e (excluded volume) to calculate the external porosity, which will be discussed latter. Also, the internal porosity (ε_i) is not calculated depends on V_i "internal volume" like the case for the external porosity (ε_e), which will be calculated depends on the "excluded volume"(V_e). The large molecules (high molecular weights) which have diameters (ϕ) bigger than the packing material's pores cannot penetrate the pores of the packing material (stationary phase); they will pass axially through the column with the moving eluent by a specific volume called "elution volume" (V_e). The largest molecular weight has the minimum retention volume ($V_{e,min}$) and the maximum diameter (ϕ_{max}). The smaller molecular weights, whose their diameter smaller than that of the pores of the packing material, have the more retention volumes ($V_{e,max}$) and the smaller diameters (ϕ_{min}). These retention volumes while the column having the packing material. But when the column is empty, we have the geometrical volume (V_g) (*Halász and Martin, 1978*), which is the sum of the "solid skeleton volume" (V_s), the "pore or internal volume" (V_p or V_i), the "interstitial or external volume" (V_z or V_e), and the volume of the pores inaccessible to the eluent (V_e)

$$V_g = V_p + V_z + V_s + V_e$$

Geometrically, we can calculate (V_g) from the following geometrical equation since the column as a tube shape

$$V_g = \pi r^2 l$$

where, l and r are the column length and the radius, respectively. Logically, the pore volume (V_p) equals the difference between the maximum volume and the minimum volume, which is

$$V_p = V_{e,max} - V_{e,min}$$

By the definition, (V_z) equals ($V_{e,min}$)

$$V_z = V_{e,min}$$

The skeleton volume, (V_s), can be calculated directly through the following equation

$$V_s = \frac{m}{\rho s}$$

where m and ρ_s are the mass of the packing material and its true density, respectively. The mass should be known when we prepare the material through the process of the packing and its true density can be determined by many methods such as X-ray diffraction (*Halász and Martin, 1978*).

The last volume, which is volume of the closed pores, (V_c), can be easily calculated from the previous equation. In (*Guan et al, 1995 and 1997*), the empty separation volume (V_k) can be calculated by

$$V_k = V_e + V_i + V_u$$

Where V_u is the sum of three volumes: (V_s), the skeleton or stationary-phase solid volume; (V_c), the closed or inaccessible pore volume; and (V_a), the layer of C_{18} bounded chains volume.

The porosity is one of the most essential considerations, which shouldn't be forgotten during the study of a new packing material through size exclusion chromatography. Simply, the porosity is a ratio of the volumes in place of the volumes

themselves (*Halász and Matrin, 1978*). As a result of that definition, we have the external porosity (ε_e) or the interstitial porosity (ε_z), the internal porosity (ε_i) or the pore porosity (ε_p), and the total porosity (ε_T). These porosities can be calculated by:

$$\varepsilon_e = \frac{V_e}{V_k} \text{ or } \varepsilon_z = \frac{V_z}{V_k}$$

It should be mentioned here that Halász and Martin (1978) used the minimum retention volume ($V_{e,min}$) in order to calculate the external porosity while Guan and Guiochon (1995) used the excluded retention volume ($V_{excluded}$), which is the retention volume of an interception point of two linear regressions for the inverse size exclusion chromatography calibration curve as it is mentioned later.

$$\varepsilon_p = \frac{V_p}{V_k} \text{ or } \varepsilon_i = \frac{V_i}{V_k(1-\varepsilon_e)}$$

Once again, (*Halász and Martin, 1978*) calculated the internal porosity depends on, as it is mentioned above, the difference between maximum and minimum elution (retention) volume, which represents the chromatographic picture. On the other hand, (*Guan and Guiochon,1995 and 1996*) calculated it as the actual fraction volume of the particles that accessible to the mobile phase, excluding the external porosity, which represents the chemical engineering picture. This definition or concept is applied to eliminate any influence of the external porosity due to the packing (*Guan and Guiochon 1995 and 1996).*

$$\varepsilon_T = \frac{V_T}{V_k} \text{ or } \varepsilon_T = \varepsilon_e + \varepsilon_i \text{ or } \varepsilon_T = \varepsilon_i(1-\varepsilon_e) + \varepsilon_e$$

The last definition is used when the three porosities are obtained from different and independent measurements in order to have an idea about the consistency of the result (*Guan et al , 1996, 1996, and 1997*).

In this work the following definitions will be considered as long as the same packing procedure is applied for the all the packing columns:

$$\varepsilon_T = \frac{V_T}{V_k}$$ (V_T is the total volume for the unretained compound)

$$\varepsilon_e = \frac{V_e}{V_k}$$ (V_e is the excluded volume for the excluded Mw at he interception point)

$$\varepsilon_i = \varepsilon_T - \varepsilon_e$$

Once again, the maximum diameter (ϕ_{max}), the minimum diameter (ϕ_{min}), and the rest of the diameters between (ϕ_{max}) and (ϕ_{min}) can be determined depending on nature of the solvent as well as the taken considerations for the whole standard polymer in the sample, which means what the configuration that the polymer will take in the solvent. One of the best materials and polymers, which have been used in the inverse size exclusion chromatography, is the standard polystyrene. Polystyrene has characterization, which yield the best results to study the pore-size diameter and pore-size distributions for the packing material. For instance, polystyrene does not adsorb on the packing material and does not agglomerate in the solvent where it is still discrete (*Guan and Guiochon, 1996*).

In a good solvent, like tetrahydrofuran (THF) or methylene chloride (CH_2CL_2), a linear polymer, such as standard polystyrene, keeps its polymeric chains in the

conformation of a random coil (*Guan and Guiochon, 1996*). That behavior has been discovered and realized by many polymeric chemists. According to the experiments and results of Kreveld and Van den Hoed using light-scattering and to consideration of polymer-statistic, there is a strong empirical relationship between the pore size diameter (ϕ) and the molecular weight (**Mw**) of the polymer. The empirical relationship wholly depends on nature of the solvent and how it is good in keeping the polymer in the random coil structure. Viscosity and refractive index are the most important features of the solvent, which give the "function" to the solvent. THF and CH_2CL_2 are considered good solvents due to their excellent refractive index at 25 ^{0}C 1.404 and 1.420, respectively. Since THF has been widely used as a polymer solvent, it is useful to mention more important features. Depending on the low refractive index, the polymer solute can be detected by HPLC's detector without any correction for the resolution. Not only can it swell the packing material, but it can also neutralize most of the active sites in the organic and inorganic packing materials (*Yau , 1979*).

In studying the pore size distributions in methylene chloride or tetrahydrofuran, they are good solvents, the relationship is

$$\mathbf{Mw = 2.25\ (\phi)^{1.7}} \quad \text{Or} \quad \mathbf{Mw = 10.87\ (\phi_r)^{1.7}}$$

where Mw is the average of molecular weight of the standard polystyrene, (ϕ) is the pore size diameter of the sample in the angstrom, and (ϕ_r) is the coil diameter of polystyrene. Both of the average molecular weights equations should give similar diameter. The polymeric chemists use the coil diameter to achieve instantaneous equilibrium by stating that the whole pore is occupied by the standard polystyrene samples. This depends on the nature of the polystyrene, which is linear polymer and it might act as random coil in the

solution. The chromatographic chemists use the approximated pore diameters (**ϕ**), which have been assigned experimentally and laid on the calibration curve (*Halász and Martin, 1978*).

It is better to mention some basic equations in High Performance Liquid Chromatography (**HPLC**) to explain what the basics that the calculations of HP chemstation depend on.

- **Number of theoretical plate (N).** This is a chromatographic terminology that gives an indication about the efficiency and performance of the column. It can be calculated from the following equation

$$N = 5.54 \left(\frac{t_R}{W_{0.5}} \right)^2$$

where t_R is the retention time of the sample and $w_{0.5}$ is the band width at the half of the peak).

- **High Equivalent of a Theoretical Plate (HETP).** This expression exhibits the efficiency of the column by clarifying how many plate exist in the column, in centimeters or millimeters

$$\mathbf{HETP} = \frac{L}{N}$$

where L and N are length of the column and number of theoretical plate, respectively.

- **Reduced High Equivalent of Theoretical Plate (HETP).** It is the **HETP** divided by the diameter of the practical d_p in μm to have a general prospective about performance of the practical

Reduced HETP = $\frac{\text{HETP}}{\text{dp}}$

- Linear Velocity of the Mobil Phase e (u). Sometimes, there is a need to calculate this term in order to precisely know the velocity of the mobile phase, in centimeter per second

$$\mathbf{U} = \frac{\mathbf{L}}{\mathbf{t_0}}$$

where, **L** and $\mathbf{t_o}$ are length of the column and the retention time of an unretained solute, respectively.

CHAPTER 2

EXPERIMENTAL

2.1. Equipment

The equipment is divided into two instruments: the packing equipment and measuring (analytical) equipment.

The column packing equipment is home made system. It has a reservoir to have the slurry, which is connected at the bottom end to the column to pack, while the top end is connected to an air-driven fluid pump (Haskel, Burbank, CA). The function of this pump is to deliver and push the solvent from the pushing solvent bottle under a pressure up to 15,000 psi generated by a powerful compressor (Campbell Hansfeld, Harrison,OH). The pressure can be adjusted and controlled by employing a control knob whereas the pressure can be monitored by a pressure gauge. Figure (2) illustrates the packing system.

The analytical equipment carried by the new series of Hewlett-Packard (Palo Alto, CA, USA) HP 1100 liquid chromatography is equipped with manually sample injection system, reservoir mobile phase bottle, degasser, quatpump, column compartment condensation, and diode-array UV detector. The feature of this series is a high stability and accuracy for flowing the mobile phase at a constant rate. The equipment is connected to a computerized data acquisition system supported by ChemStation software.

2.2. Columns

In this work, two kinds of columns are investigated: normal columns packed with standard or prepared packing material and monolithic columns or rod columns, which have been packed depending on a special (undivulged) process set up by the source.

Seven stainless-steel packing columns are purchased from Altech (Deerfield, IL, USA). One of the columns is 25 cm length x 0.46 cm I.D. and the others are 10 cm length x 0.46 cm I.D. They were packed with a standard or prepared packing materials as it is mentioned later. In order to simplify and follow the packing materials easliy, they are divided into two groups: the standard packing materials were given a sequence of numerical arrangement and the prepared packing materials were given a sequence of alphabetic arrangement. So, an alternative codes are applied to the original codes as follow:

Material	Original Code	Alternative Code
Standard	LUNA PREP Silica (2)	1
Standard	LUNA PREP Silica C18	2
Prepared	Silica 64Å	A
Prepared	Silica SBA-15 (1)	B
Prepared	Silica SBA-15 (2)	C
Prepared	Silica SBA-15 CH_3Si	D
Prepared	Silica SBA-15 C_6H_5Si	E

Six monolithic columns (serial # UM 19-24, 10 cm length x 0.46 cm I.D.) were given as gift from (Merck KgaA, Darmstadt, Germany). These columns have been filled with a porous silica-monolith wrapped inside a PEEK tube using a proprietary process that avoids leaking between the tube wall and the monolith. The silica surface in this

column is covered with a monomeric C_{18} layer, bounded from monofunctional octadecylsilanes, using a proprietary in-situ surface-modification process.

It is important to mention and state a brief introduction about the monolithic column as a new technique in the chromatography field. Monolithic columns, also referred to in literature as "rod columns", are one of the most interesting innovations in column-manufacturing technology. They can be classified as silica-based or organic polymer-based, depending on the nature of the material from which they are composed. The procedure used for preparing monolithic columns varies significantly from author to author, and from one company to another, and is usually patented and/or confidential. These columns share one common characteristic: they are made of one single piece of an adsorbent material (silica or polymer) that fills the entire length of the column.

The microscopic structure of monolithic columns has been characterized in detail in many expanding literature (*Minakuchi et al, 1998, Tanaka et al, 1998 and Ishizuka et al, 2000*), which have been carried out by chromatographic chemists specializing in this field. The single piece of adsorbing material is porous and composed of two interconnected networks of pores. A first network of macropores, the so-called through-pores whose dimensions are in the 1.5-2 μm range, provides flow paths through and along the column. The size and density of the macropores network cause the monolithic column to have a high external porosity and, consequently, a large permeability and a low column hydraulic resistance. A second network of mesopores with an average size of about 10-20 nm is responsible for the large specific surface area of the monolith, hence for the retention volumes observed for most analytes. For these reasons, monolithic

columns are efficient at high flow rates and can also be used in long connected series, enabling achievement of very high efficiencies.

Numerous research groups and commercial companies have recently developed great interest in monolithic columns. Many studies dealing with the preparation, method development and applications of monolithic columns in analytical, and preparative or semi-preparative chromatography have been published.

2.3. Chemicals

Polystyrene standards with molecular weights ranging from 2,000 to 1,860,000 were purchased from Supelco (Bellefonte, PA, USA). Polystyrene standards with molecular weights ranging from 550 to 2,000 were purchased from Scientific Polymers Products, Inc (Ontario, NY, USA). Tetrahydrofuran; as a pushing solvent in the packing process and as a mobile phase in the analytical measurements, Methylene Chloride; as a mobile phase in the analytical measurements, Acetonitrile; as a washing solvent, n-propanol and tetrachloroethylene; as a slurry solvent, and Toluene as a small molecule were HPLC grade and purchased from Fisher Scientific (Suwanee, GA, USA).

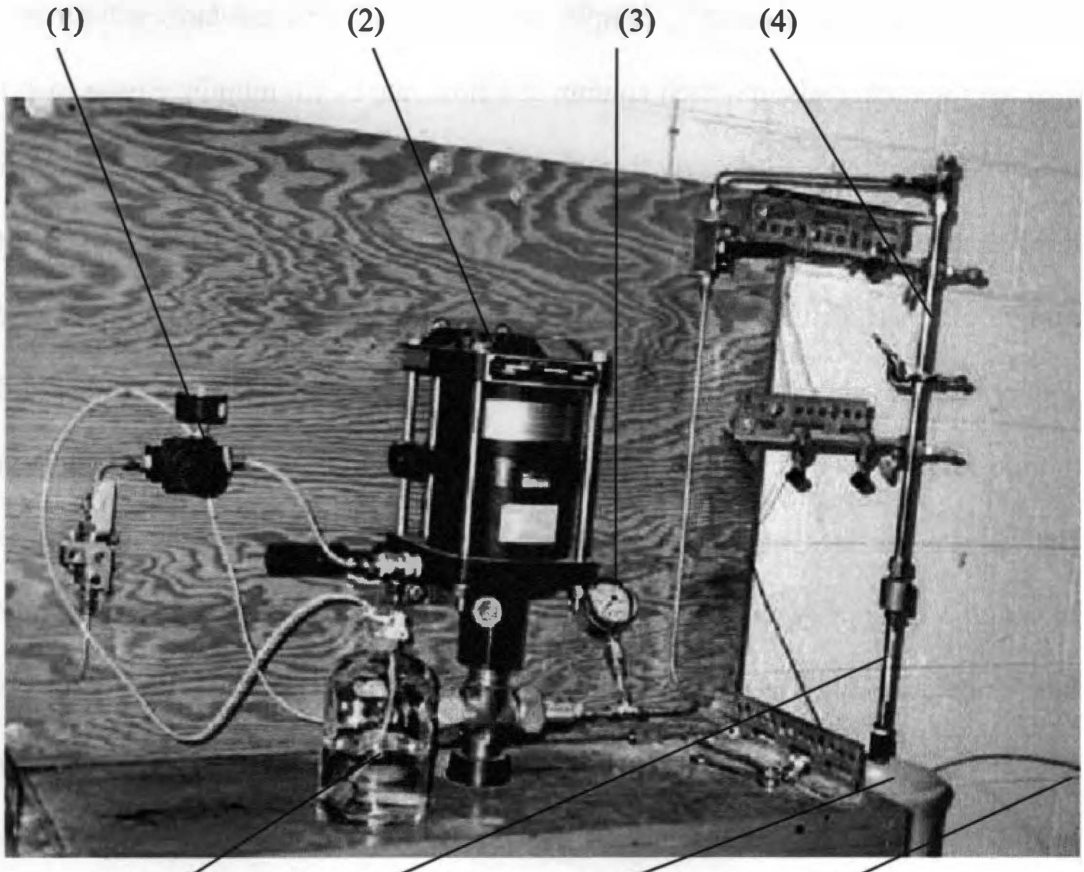
2.4. Packing Process

For the sake of testing the packing procedure, two standard packing materials have been purchased from Phenomenex® (Los Angeles, CA, USA). Column (10cm length x 0.46 cm I.D.) has been packed with LUNA 10 µm PREP SILICA (2), alternative code column (1), and column (25 cm length x 0.46 cm I.D.) has been packed with LUNA 10 µm PREP C_{18}, alternative code column (2). The other packing materials have been

prepared by Dr. Sheng Dai at the Oak Ridge National Laboratory (Knoxville, TN, USA). They are called Silica (64 Å) (alternative code column A), Silica SBA-15 (1) (alternative code column B), Silica SBA-15 (2) (alternative code column C), Silica SBA-15 CH_3Si (alternative code column D), and Silica SBA-15 C_6H_5Si (alternative code column E). The packing is done under the same conditions but with varying times according to when we received the material.

The chemical structures for the Silica (64 Å) is pure SiO_2, attached and supported by methyl group (-CH_3), as well as, Silica SBA-15 CH_3Si. But the principle of the recipe and the ratio of the ingredients for preparing those materials, which plays a major role, are completely different from each other. Silica SBA-15 C_6H_5Si, pure SiO_2, has been attached by phenyl (-C_6H_5). Silica SBA-15 (1) and Silica SBA-15 (2) are pure SiO_2, however the recipe and the ratio are different between them.

A certain amount from the available quantity was prepared as slurry according to the conventional slurry packing, which has been presented by previous authors and their work. A slurry of 1:1 of n-propanol:tetrachloroethylene was poured in the reservoir of the packing equipment. After closing the upper end of the reservoir tightly, the pump is set up to deliver the pushing solvent, (tetrahydrofuran) on 5,000 psi for 35-40 minutes. Then, the column is disconnected from the equipment. By using a sharp razor, the packed surface got flattened. Finally, by closing the column, it is ready for usage. The packing equipment is shown in figure 2.



(1) Pressure Control Knob

(2) Air Driven Fluid Pump

(3) Pressure Gauge

(4) Reservoir

(5) pushing Solvent Bottle

(6) Column

(7) Waste Container

(8) To The Compressor

Figure 2. The Column Packing Equipment

2.5. Procedure

Manually and separately, samples of 25 µl of different standards polystyrene were injected three intervals into each column at a flow rate of 1.0 ml/min. Hence each result, which has been reported directly from the HP chemstation program, is the average of the three times. All the injections were carried out at the suitable wavelength of 254 nm. A sample of 25 µl Toluene was injected to determine the total accessible porosity of the column (*Guan and Guiochon, 1996*). Each column was studied twice with a different mobile phase once by methylene chloride (CH_2CL_2) and next by tetrahydrofuran (THF). But Due to the lack of time and the need for the rod columns for other works, the monolithic columns were characterized, just, by THF as mobile phase. As a matter of fact, since column 2 for standards material yielding almost the same results for CH_2CL_2 and THF. Thus, rod columns are considered standard columns by assuming that they would give more or less the same results whether by using THF or CH_2CL_2 as a mobile phase. Retention time (volume) was determined for each injection from the peak maximum (*Halász and Martin, 1978*), which should be symmetrical, throughout the report of calculations that provided by HP chemstation. Also, the number of theoretical plate (**N**) is shown in the report of calculations, which depends on the area under the peak, and it gives an impression on how well the material or the procedure.

By plotting the logarithms of the molecular weights of the standards polystyrene versus their retention volume, it is supposed to have a curve possessing two thresholds (lines) called a bimodal pore size distribution or Inverse Size Exclusion Chromatography (ISEC) calibration curve. The two thresholds correspond to the external pore zone (higher trend) and the internal pore zone (lower trend). It is clear to observe the distribution of

polystyrene samples on both of the thresholds in order to give two straight lines intercept in a particular point called the "excluding pore diameter". This refers to the pore size diameter, which can exclude the samples. According to the work of (*Guan and Guiochon, 1996*), the excluded volume, as well as, the pore size diameter of the material could be calculated in a certain solvent by regressing the lines.

In order to estimate the efficiency of the packing and the material, a mixture of toluene and the highest molecular weight of standards polystyrene at different flow rates ranging from 0.2 to 6.0 ml/min has been injected manually.

CHAPTER 3

PACKING COLUMNS: RESULTS AND DISCUSSION

3.1. Efficiencies

Usually, the efficiency of the column is calculated by two methods: manually and instrumentally. The manual method is dependent on how accurate the person is in using a very fine ruler to perform it. That is contingent on measuring the height and half-width of the peak on the spectrum paper. By applying, the formula of the number of the theoretical plate, mentioned above, roughly estimation could be obtained for (**N**) and consequently, how good the column is. In this work, the instrumental method will be used. HPLC equipment is supported by HP chemstation software, which can give a report at the end of the run construed by a large amount of data, automatically calculated. Therefore, all (**N**) values arise directly from the provided software in the instrument.

Tables (1) and (2) show the number of the plate for the packing columns for both runs, once by CH_2CL_2 and the other by THF, respectively. It is indeed apparent for the prepared packing materials that the number of the theoretical plate (**N**) is very poor, at 1.0 ml/min, compared with the other standard materials, which have been published in literature and the company's reports and certificates at the same conditions. The efficiency at 1.0 ml/min is 4700 plate and 1200 plate for toluene and polystyrene (1,860,000 g/mol), respectively, for the column 2.

Table 1. Number of the Theoretical Plates (N) for Toluene and Polystyrene (1,860,000 g/mol). CH_2CL_2 is a mobile phase at 1.0 ml/min.

Column	No. of Theoretical plate for Toluene	No. of Theoretical plate for Polystyrene
A	3025±26	810 ± 20
B	1550 ± 30	325 ± 23
C	550 ± 26	310 ± 20
D	2150 ± 25	950 ± 22
E	4180 ± 32	1250 ± 26

Table 2. Number of the Theoretical Plates (N) for Toluene and Polystyrene (1,860,000 g/mol). THF is a mobile phase at 1.0 ml/min.

Column	No. of Theoretical plate for Toluene	No. of Theoretical plate for Polystyrene
A	3250 ± 25	1150 ± 20
B	1350 ± 24	450 ± 25
C	500 ± 20	335 ± 23
D	2360 ± 26	880 ± 21
E	3515 ± 30	1050 ± 24

In order to evaluate the overall performance of the columns, the flow rate of the mobile phase versus the Height Equivalent Theoretical Plate (**HETP**) (Van Deemter behavior) is studied. In this study, a mixture of toluene and standard polystyrene (1,860,000 g/mol) has been separated and studied, once through THF, and another through CH_2CL_2. Figure (3) reflects a nice clear Van Deemter for the standard packing material of the column 2.

On the other hand, figures (4) and (5) show bad Van Deemter curve to separate toluene and polystyrene, respectively, by CH_2CL_2 as a mobile phase for the prepared packing materials. These plots exhibit that all of the prepared materials have the same behavior. As THF is a mobile phase, figures (6) and (7) represent the Van Deemter curve for separating toluene and polystyrene, respectively. In general, these curves did give indications that the prepared packing materials have unsatisfactory efficiencies.

There are a few expected factors that could explain the deficiency of the column. Among these; the slurry of the packing material, leaking during the packing process, collapsing the packing material as a result of high compression, failure of making the particles homogeneous, and the configuration and structure (mechanical or chemical) of the material. Simply, these critical factors go under the preparation and packing process except the last factor of the structure. Since the procedure that has been followed to prepare and pack the material has been applied and tested by the people in this field for various packing materials and for a long durations of research, there is no doubt that the deficiencies of the columns are results of incorrect or bad producer. Moreover, the columns (1) and (2) show a good efficiency by using this producer.

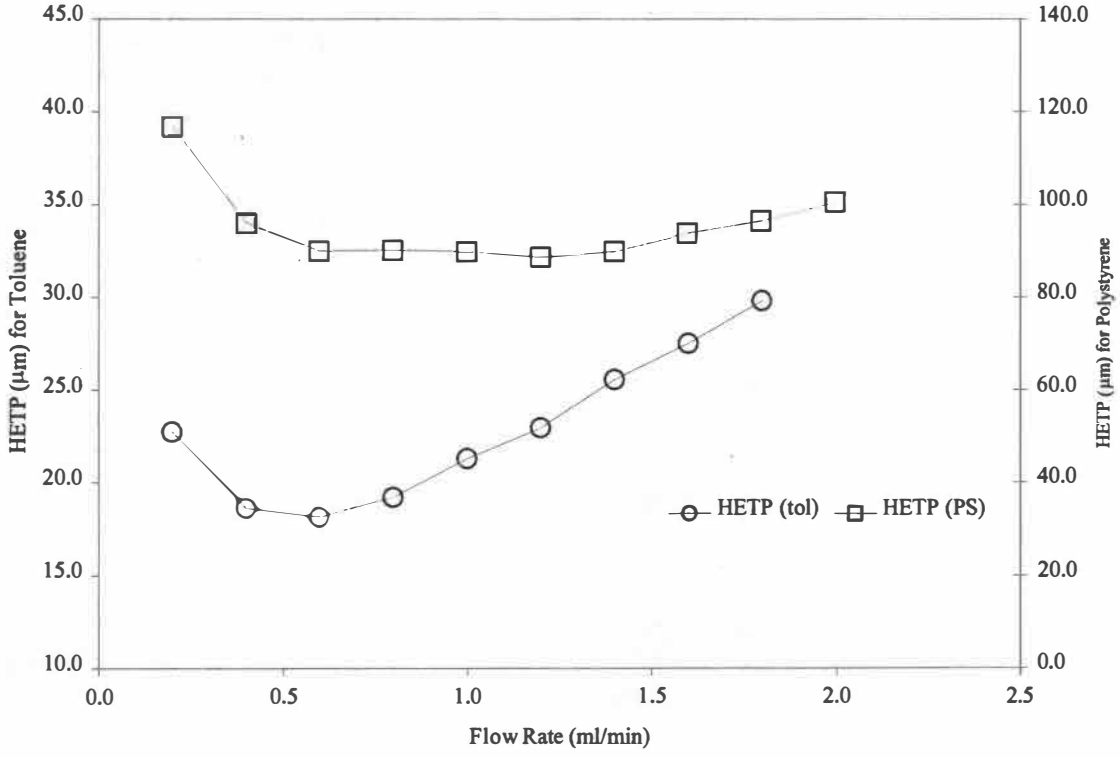


Figure 3. Van Deemter curve for the standard packing material for column (2).

THF is a mobile phase at 1.0 ml/min.

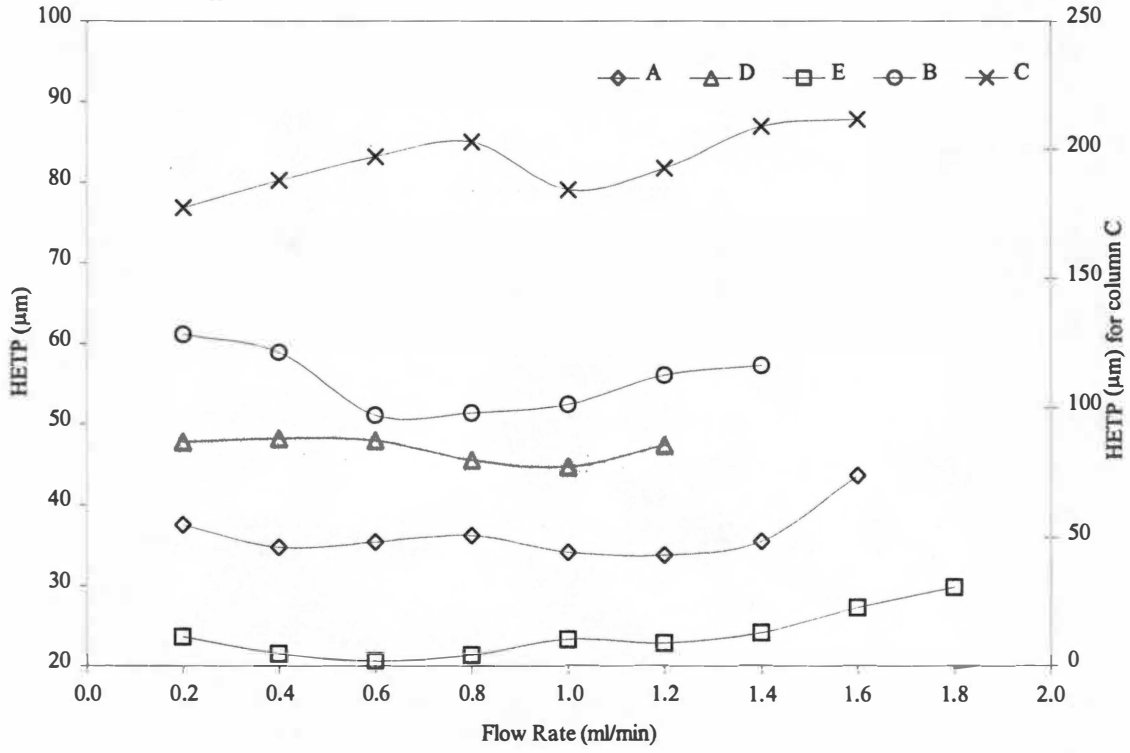


Figure 4. Van Deemter curve for the prepared packing materials.

Toluene is separated compound.

CH_2CL_2 is a mobile phase at 1.0 ml/min.

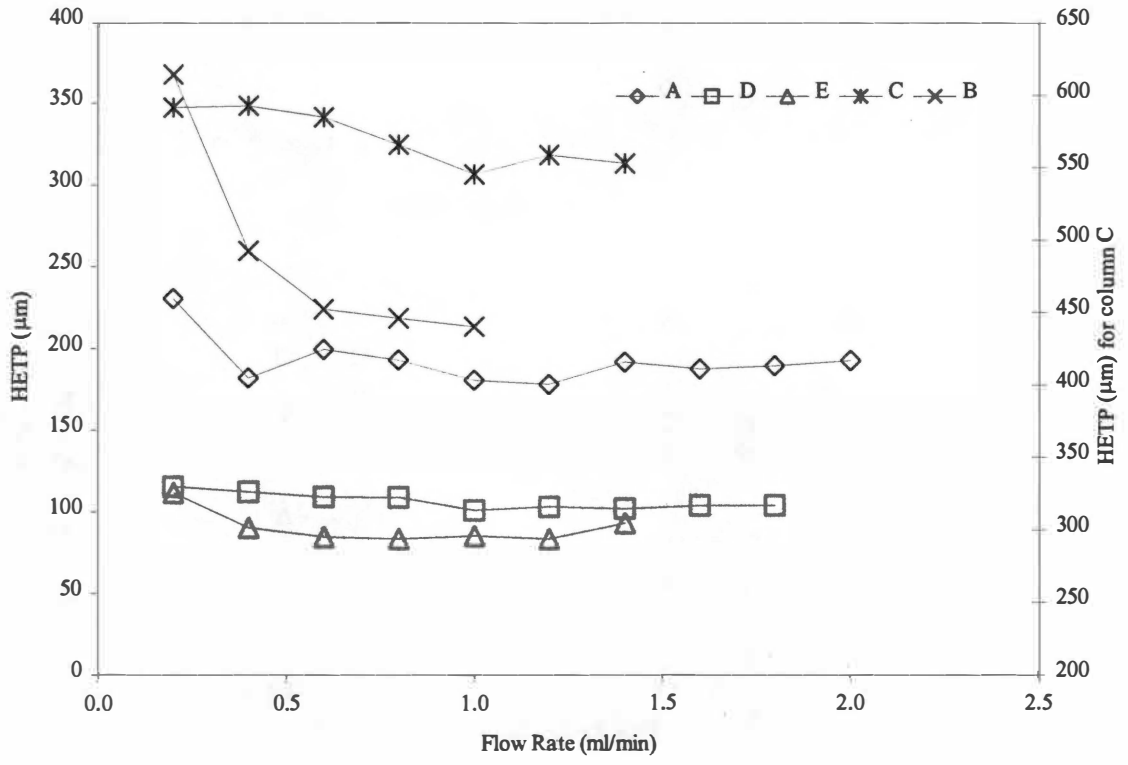


Figure 5. Van Deemter curve for the prepared packing materials.

Polystyrene (1,860,000 g/mol) is a separated compound.

CH_2CL_2 is a mobile phase at 1.0 ml/min.

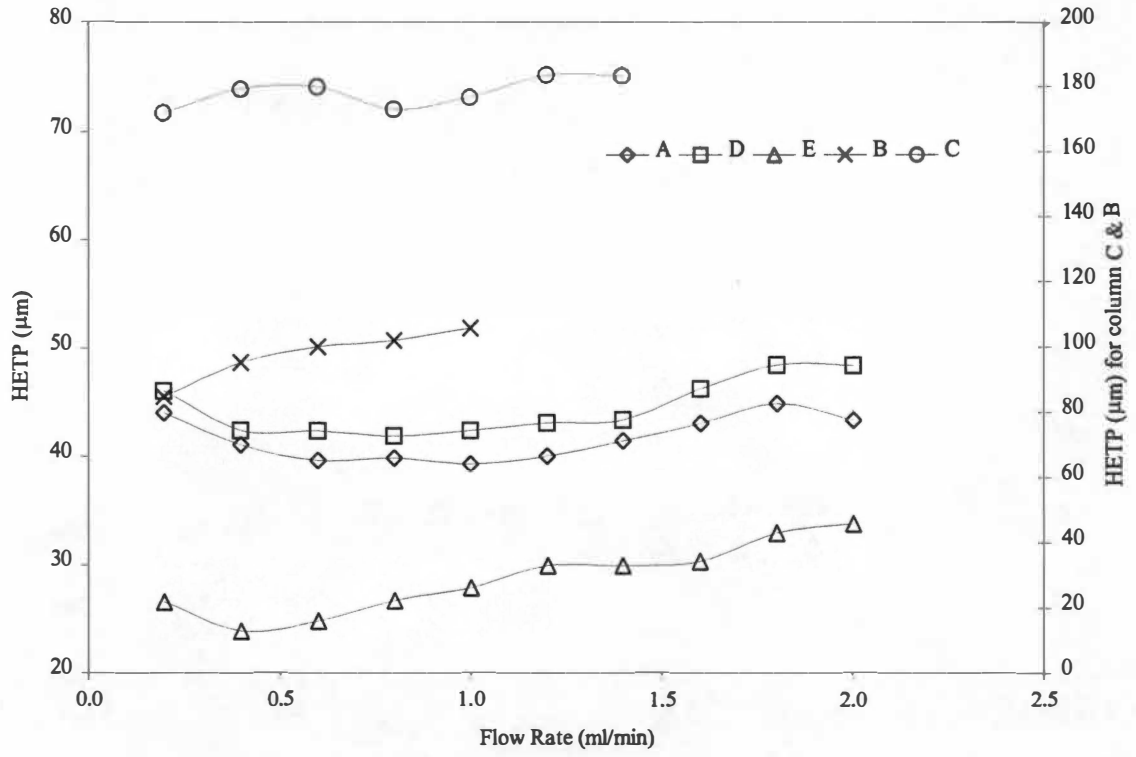


Figure 6. Van Deemter curve for the prepared packing materials.

Toluene is a separated compound.

THF is a mobile phase at 1.0 ml/min.

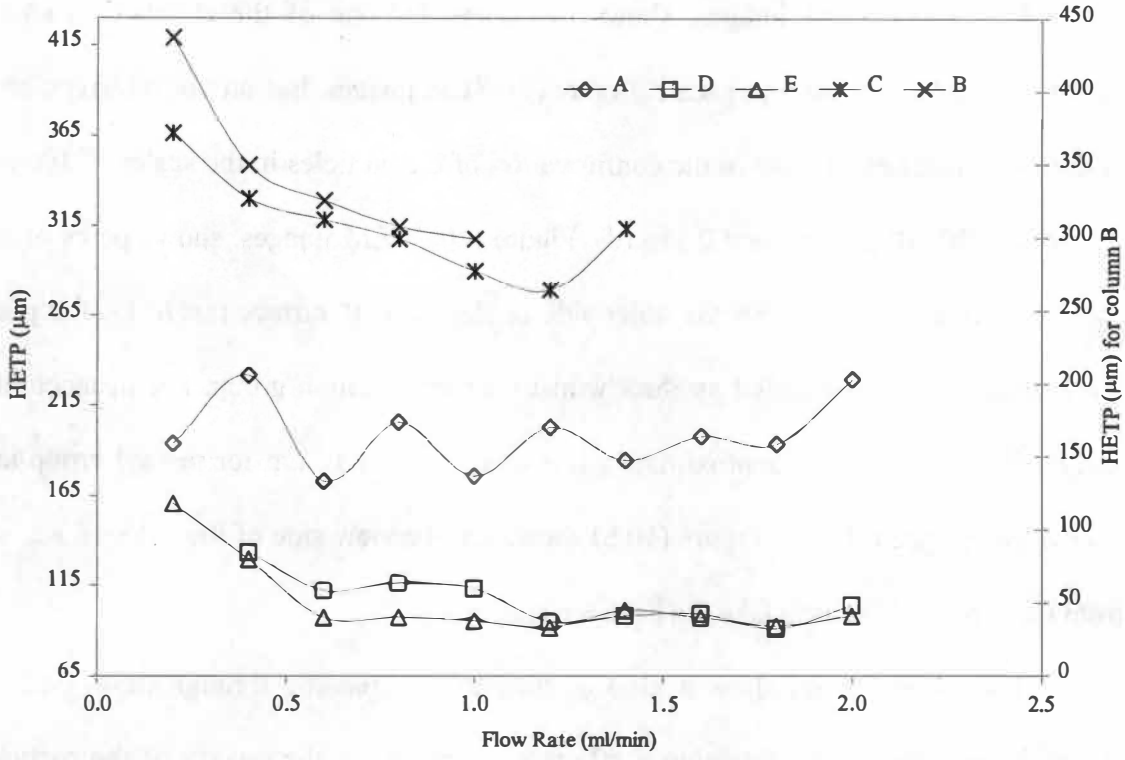


Figure 7. Van Deemter curve for the prepared packing materials.

Polystyrene (1,860,000 g/mol) is a separated compound.

THF is a mobile phase at 1.0 ml/min.

Thus, the only possible hypothesis to explain the deficiency of the column would be the particles' lacking for a regular shape, as this deficiency causes inconvenient path for the pores of the injected sample. According to this concept, we have arrived at a point necessitating our taking images for those prepared materials.

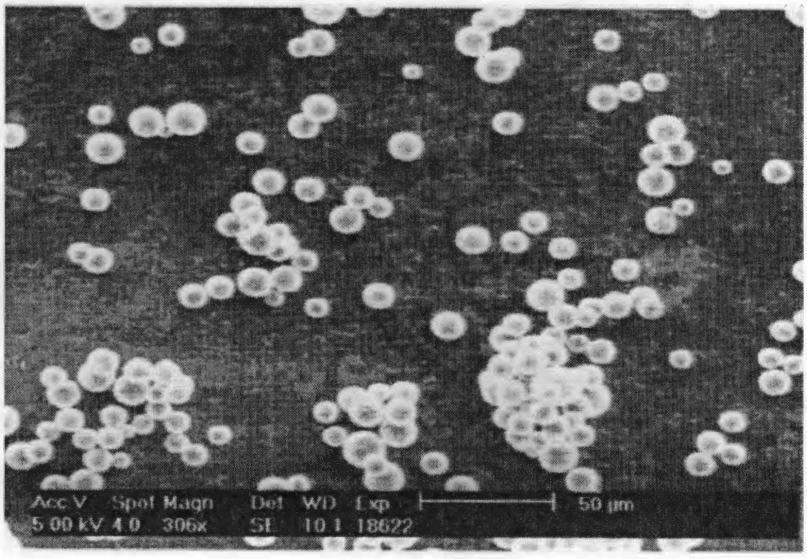
Our colleague at ORNL has taken the Scanning Electron Microscope (SEM) and Tunneling Electron Microscope (TEM) images for those materials to compare the standard packing materials with the prepared ones.

Figure (8), SEM images, shows the shape for one of the standards packing materials in a 50 μm and 5 μm scale. Figure (9), SEM images, has proved an irregularity in the shape and randomness in the configuration of the particles in the scales of 100 μm (a), 20 μm (b), 10 μm (c), and 2 μm (d). Figure (10), TEM images, shows pores of the particles. Figure (10.a) shows the inner side or the internal surface inside for the pore. These channels are controlled by the chemistry of the function group. For instance, the width of the chancels are approximately less or more than 10 nm for methyl group and phenyl group, respectively. Figure (10.b) shows an overview side of the external surface from the top. The channels take the hexagon shapes.

The taken images show a kind of difficulty of passing through those packing materials. So, the key in obtaining a efficiency depends on the quality of the particles (morphology), the recipe of synthesizing and preparing the packing material, and pore structure.

The main major role here is being the particles in a regular and convenient shape and configuration, which empower them to separate the injected sample easily. The spherical shape for the particles is the most convenient shape to have good performance

a)



b)

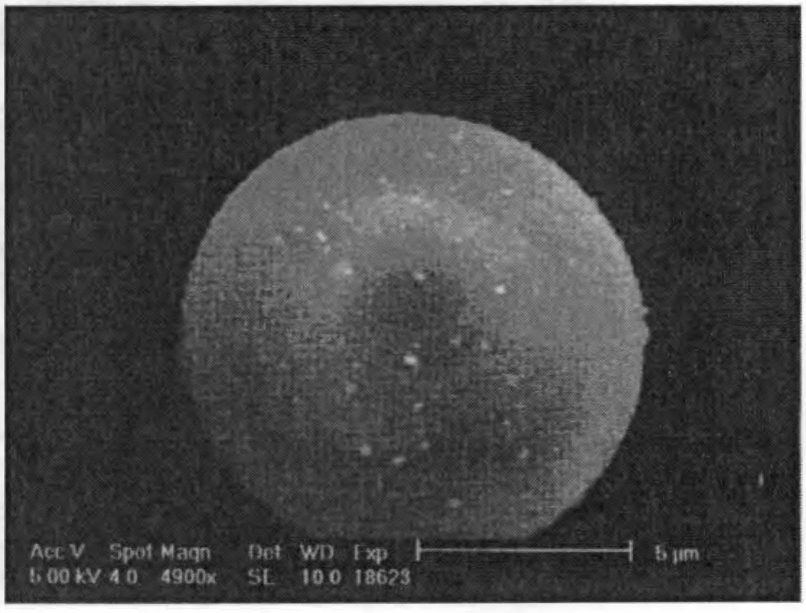
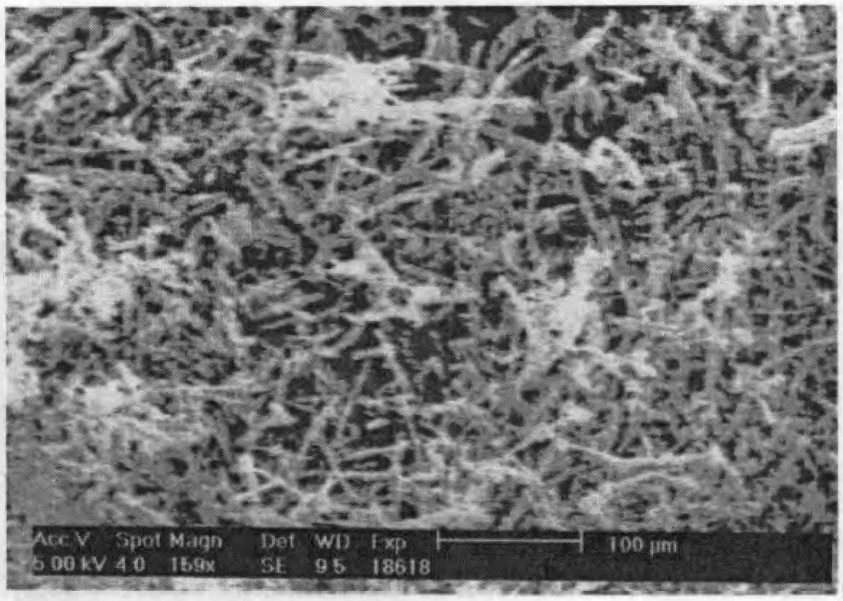


Figure 8. SEM images for the standard packing materials in scale of a) 50 µm and b) 5 µm.

a)



b)

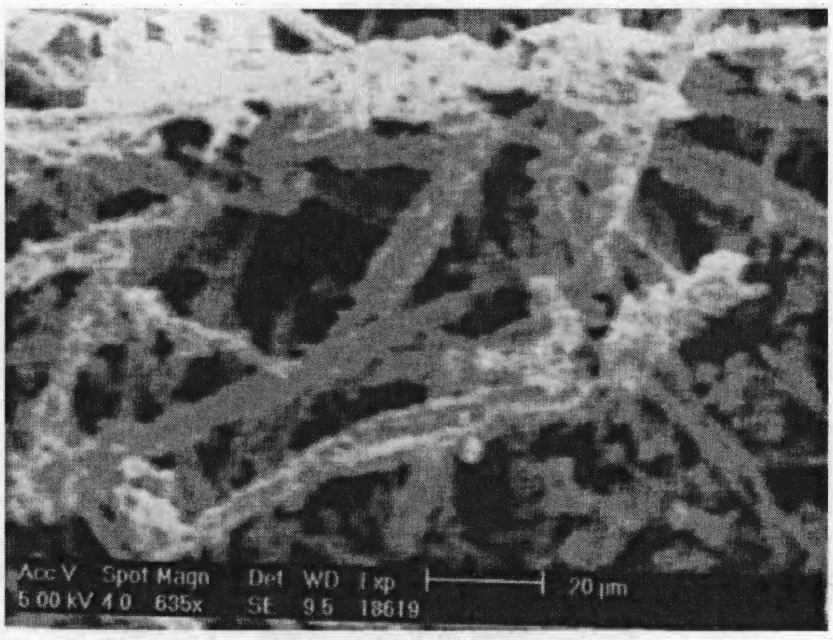
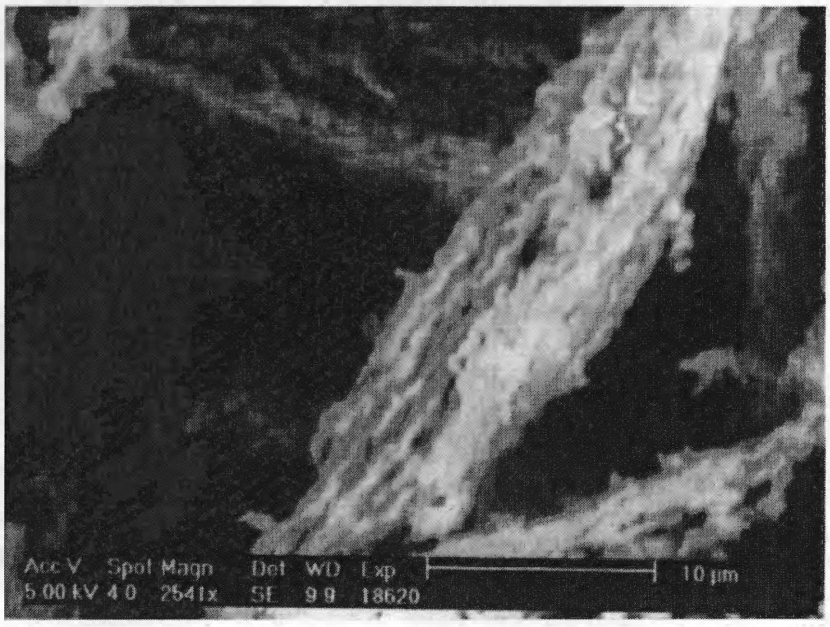


Figure 9. SEM images for the prepared packing materials in a scale of a) 100 µm, b) 20 µm, c) 10 µm, and d) 2 µm.

c)



d)

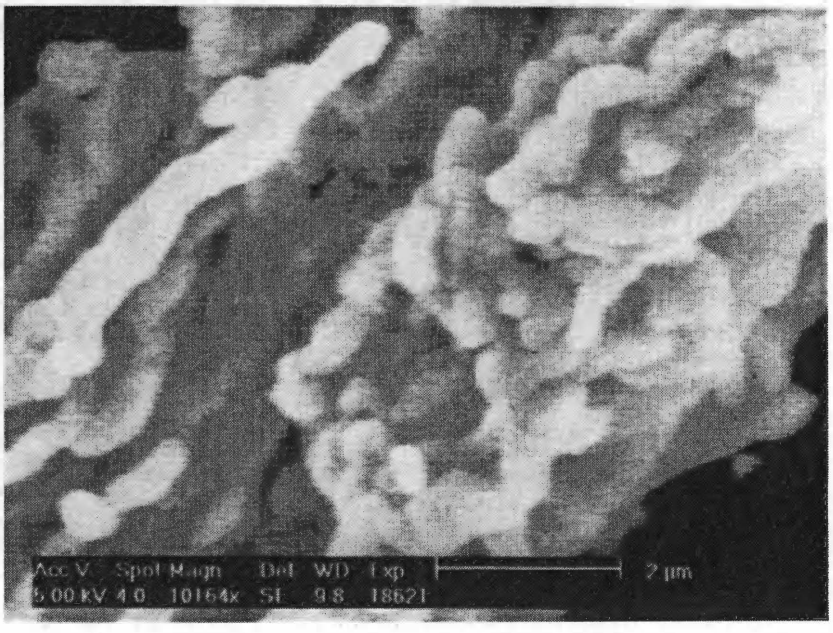


Figure 9. Continued.

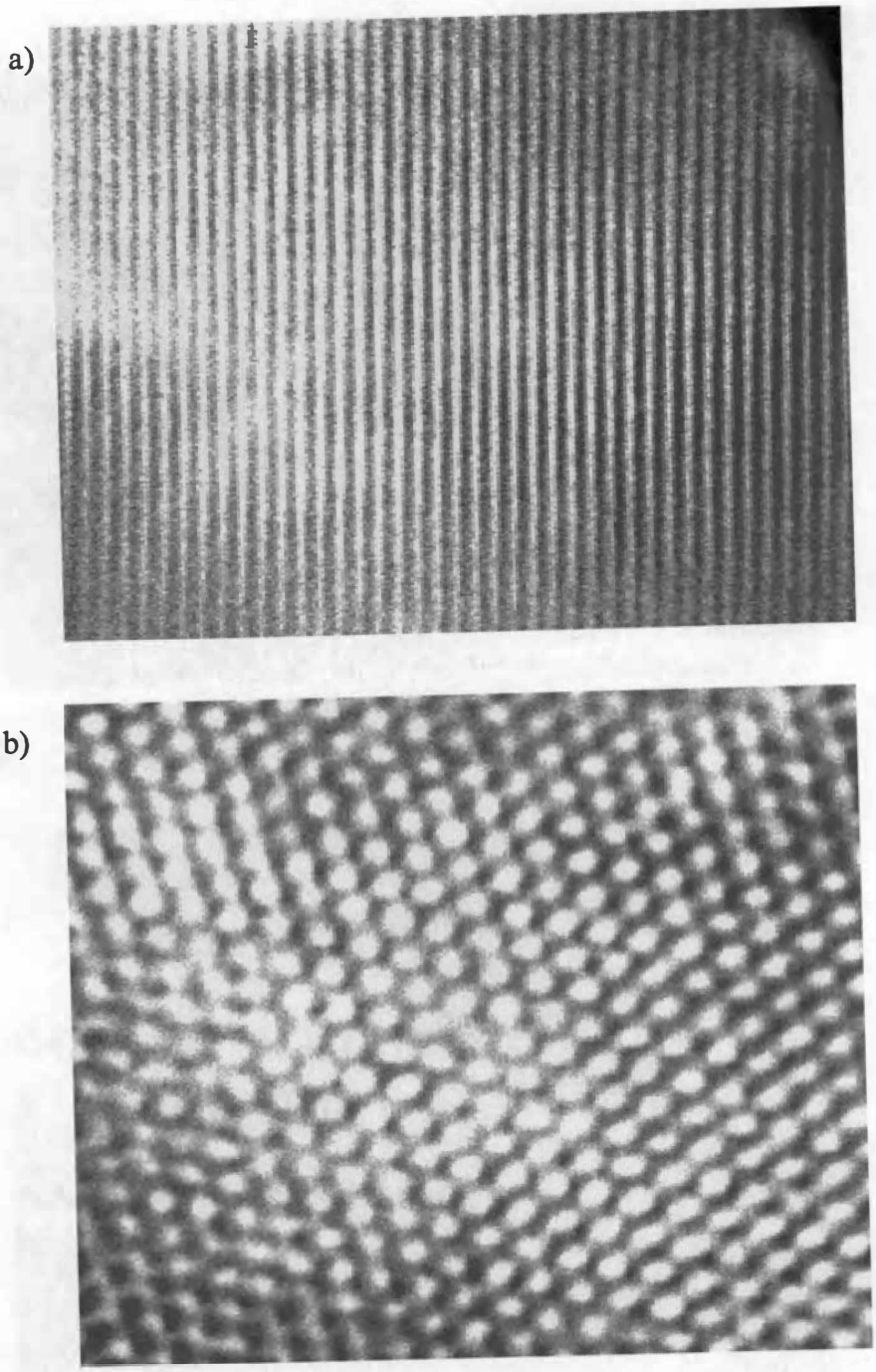


Figure 10. TEM images for a) inner side and b) external side for the pore of the particle for the prepared packing materials in a scale of 10-15Å.

in this work. On the other hand, the randomness in the prepared packing material could be effect the performance in a way or another. The internal interceptions between the particles hinder the pores from becoming separated with a good performance. Consequently, the irregular and intercepted shapes might be given a discordant ropey shape. These particles on the rope possess a certain kind of canal that leads to onerous passing and is sometimes discontinued. Therefore, a point is realized that the poor efficiency is a result of the particles' lacking of any regular shape. It should be mentioned that not only does the shape of the packing material have a tremendous influence on the efficiency, but so do the chemistries (polarity, density, interacting...etc) of the injected sample (compound). In contrast, the particles' shape of the packing material has more influence in the efficiency than the chemistry of the compound.

3.2. Pore-Size Diameters

According to (*Halász and Martin, 1978*), as mentioned above, the pore diameter of standard polystyrene and toluene could be calculated by solving the empirical equation to (ϕ) as long as the mobile phase is considered a good solvent such as methylene chloride or tetrahydrofuran.

The standard materials have reflected very superb inverse size exclusion chromatography calibration curve by using the mentioned packing procedure, which gives a good indication that procedure, is adequate.

Since the Inverse Size Exclusion Chromatography depends on the calibration curve, as it is mentioned in the theory section, it is important to show the R squared (R^2) statistical parameter for the two liner regressions data points in order to justify the

calibration curve, table (3). There is one for the internal pore zone and one for the external pore zone.

The pore-size diameters along with the statistical parameters have been tabulated in table (4). Figures (11) and (12) reveal the internal pore zone and the external pore zone, which is called inverse size exclusion chromatography calibration curve, for the standards packing material for the column (1) and (2), respectively, when the mobile phase is CH_2CL_2 or THF. In figure (11) for column 1, ISEC give a value for pore size diameter of 264Å ± 2 and with perfect curve to distinguish the external pore zone from the internal one when the mobile phase is CH_2CL_2. On the other hand, in the same figure, unusual behavior is observed. There is no inflexion point, interception point, which could be as an excluded pore size. Two majors issues could explain the unexpected curve. One by THF itself, since THF is distinguished as a highly polar solvent can act as a tensio-active agent, which results in coating the polymer molecule or/and the silica and facilitates some kind of adsorption. The other issue is concerning about the swelling in the organic solvent. According to Phenomenex®, *catalog (01/02)*, THF is classified as 70-80% swelling solvent. Consequently, the linear standard polymer samples in this case will undergo serious soaking solvation and change not only in the shape, but also in the surface polarity. Therefore, if those two issues are taken in the account, the molecule will spend more time in the column and the inflexion point in the curve will be shifted to lower masses, and that what is observed.

For column 2, while CH_2CL_2 as a mobile phase the excluded pore size diameter (Φ) is 230Å. But it is 220Å while THF as a mobile phase. As it is mentioned in the theory section, THF and CH_2CL_2 are considered good solvents and the previous experiments,

Table 3. R^2 Values for the ISEC Calibration Curve for the packing materials.

Flow rate at 1.0 ml/min.

Col	CH_2CL_2		THF	
	External Pore zone	Internal Pore Zone	External Pore Zone	Internal Pore Zone
1	0.999	0.941	---	---
2	0.996	0.961	0.967	0.960
A	0.981	0.921	0.944	0.979
B	0.995	0.968	0.962	0.989
C	0.997	0.992	0.974	0.996
D	0.993	0.998	0.992	0.927
E	0.995	0.984	0.964	0.955

Table 4. Pore-Size Diameters for the packing materials.

Flow rate at 1.0 ml/min.

Col	Pore-Size Diameter ϕ (Å)		AV of ϕ (Å)	$\Delta\phi$ (Å)
	CH_2CL_2	THF		
1	264	???	???	???
2	230	220	225	10.0
A	353	354	354	0.00
B	138	41	90.	97.0
C	179	103	141	76.0
D	148	85	117	63.0
E	167	86	127	81.0

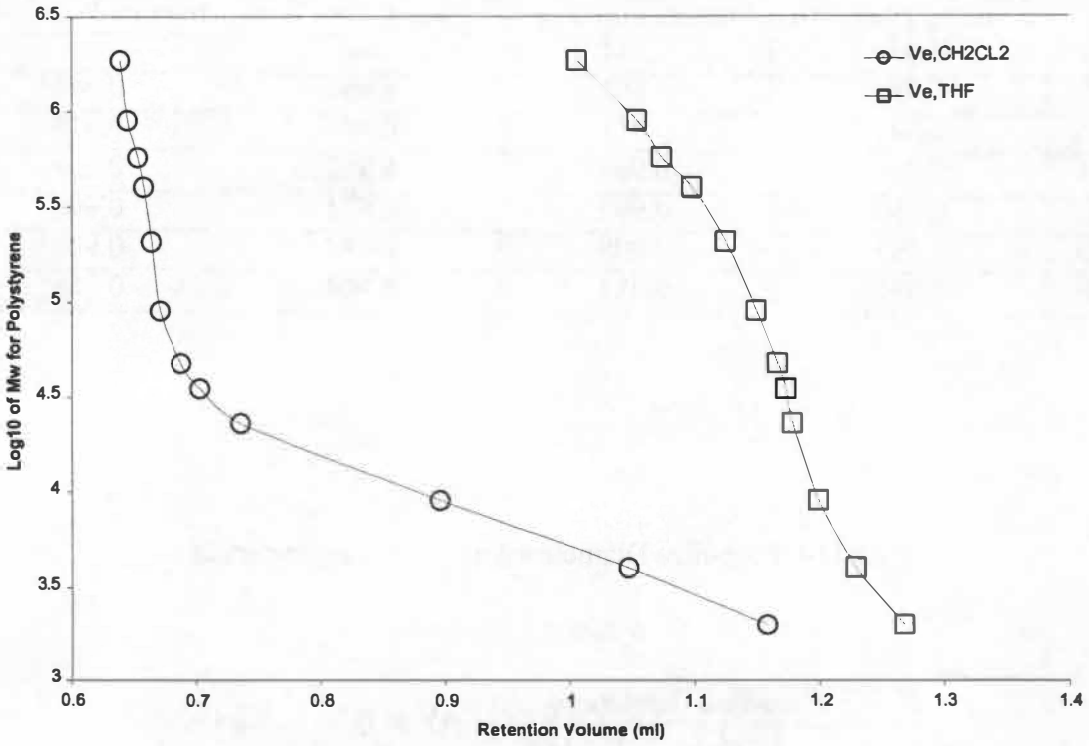


Figure 11. ISEC Calibration Curve for the standard packing material for column (1). Flow rate at 1.0 ml/min.

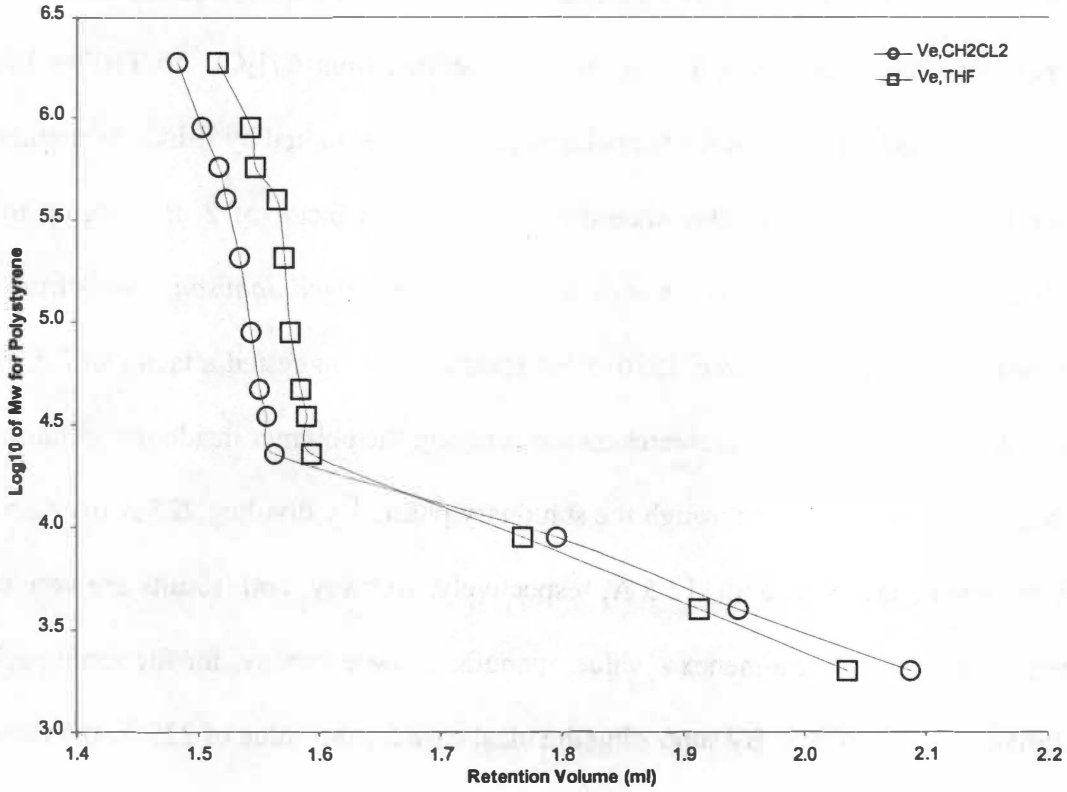


Figure 12. ISEC Calibration Curve for the standard packing material for column (2). Flow rate at 1.0 ml/min.

done by experts in this field, state that the main results such as the porosity, and the pore-size diameter by the inverse size exclusion chromatography should be, theoretically, more or less the same in the both mobile phase, individually. As a result of that assumption, the average of pore-size diameters should be taken to estimate the actual pore-size diameter with considering the difference ($\Delta\phi$) between the two values. A 225Å, is an obtained value for the pore-size diameter, (ϕ), for column 2, and the difference in the pore-size diameters when the run has been shifted from CH_2CL_2 to THF is 10.0Å. That means standard material for the column (2) could be studied by ISEC. As mentioned in the theory section, this value should be divided by a factor of 2 or more up to 2.5 (*Halász and Martin, 1978*). The works, done by (*Cantow and Johnson, 1967*) from one side and (*Verhoff and Sylvester, 1970*) from another side, suggested a factor of 2.5. Once again, this indicates how the researchers are studying the polymer inside the solution and its behavior during its path through the stationary phase. By dividing 225 Å over 2.5 and 2.0, the results are 90 Å and 112.5 Å, respectively. Anyway, both results are very close approximates from Phenomenex® value, manufacturing company, for the same packing material, which is 100 Å. By supposing the ideal case for the value of 225 Å, the factor in this case will be 2.25, which is in the range of 2.0-2.5.

According to the results for columns 1 and 2, the packing procedure, is suitable to be applied to the rest of the packing materials. In general, ISEC calibration curve of the prepared materials varies from material to another depending on factors, by a way or another related to each other.

Behavior of column A is represented through figure (13) for both CH_2CL_2 and THF, which reveals how the polystyrene pores lie on the ISEC calibration curve. The pore-size diameter is 354Å and the difference ($\Delta\phi$) between the pore-size through CH_2CL_2 and THF is almost 0.0. This means, using ISEC to estimate the parameters and characterize this packing material (column A), is preferable. Also, means this packing prepared material did achieved and approached the conditions and the boundaries to be studied by ISEC.

Figure (14) for column B, exhibits a huge $\Delta\phi$, which was 97.0 Å while the average of the pore-size diameter is 90.0Å. The same big difference was obtained for column C, figure (15), whereas an $\Delta\phi$ was 76.0Å with an average of 141.0Å. These high differences do not reflect a mistake in the method or the procedure applied for this work. On the contrary, they showed and confirmed that ISEC does not apply to these kind of prepared packing materials, which couldn't achieved the ISEC boundaries. Due to the lack of one or more of the principles of ISEC, the tremendous difference between the pore-size diameters could be explained. The chemical structures for both the packing materials for columns B and C clarify those unmatched values for pore-size diameters. Being without function groups could slightly affect the hardness of the stationary phase. Consequently, there is no a rigid matrix to perform inverse size exclusion chromatography. The last two columns D and E, shown in figures (16) and (17), respectively, have less ideal ISEC calibration curves, whereas a kind of sharp interception point could be noticed. However, the difference in the pore size diameter (ϕ) between both runs, CH_2CL_2 and THF, is still huge. Values of 117Å with 63Å difference and 127Å with 81Å difference were obtained for the columns (D) and (E), respectively.

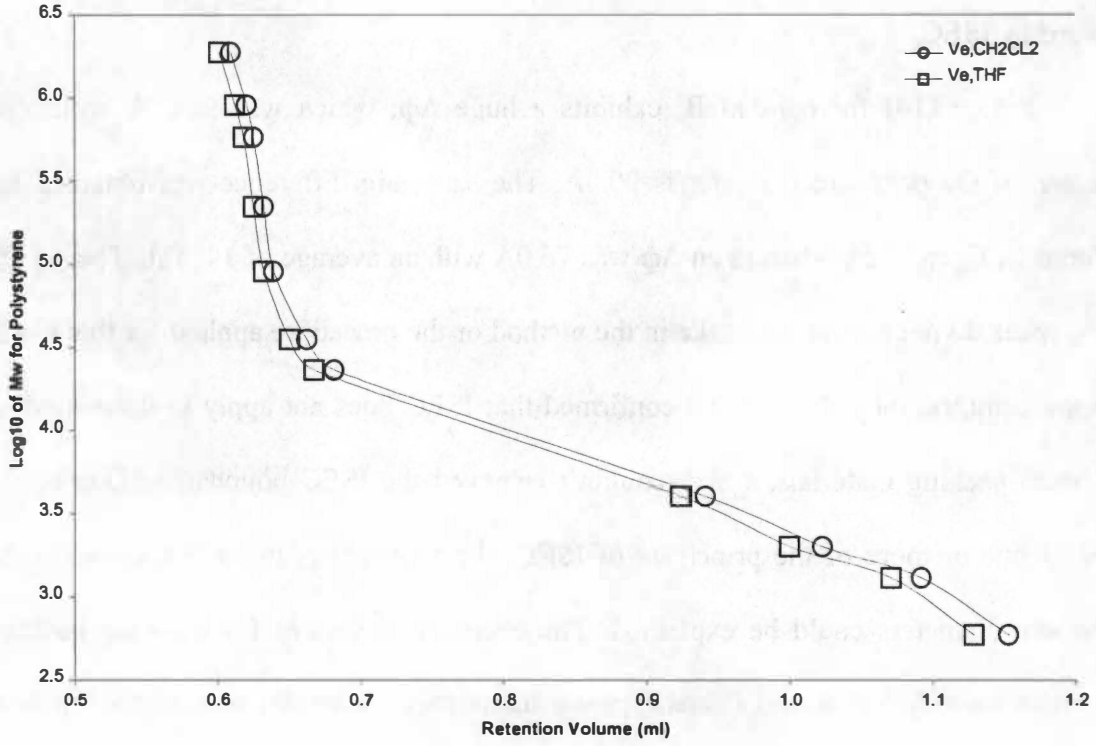


Figure 13. ISEC Calibration Curve for the prepared packing material for column (A). Flow rate 1.0 ml/min.

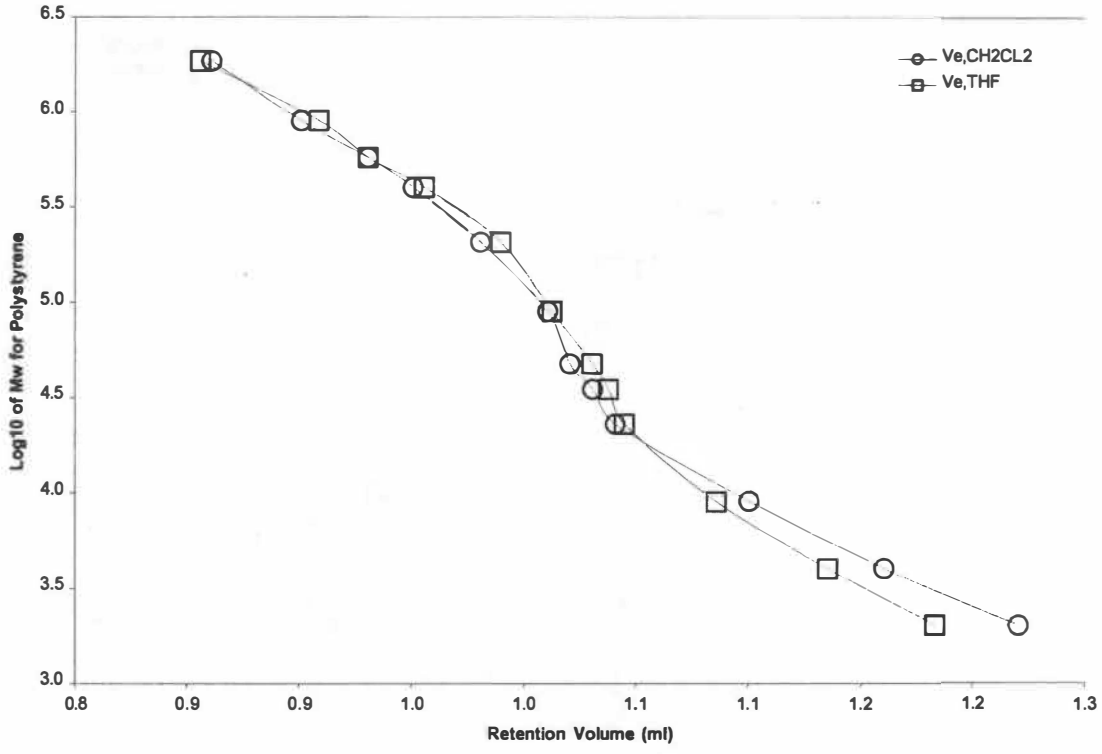


Figure 14. ISEC Calibration Curve for the prepared packing material column (B). Flow rate 1.0 ml/min.

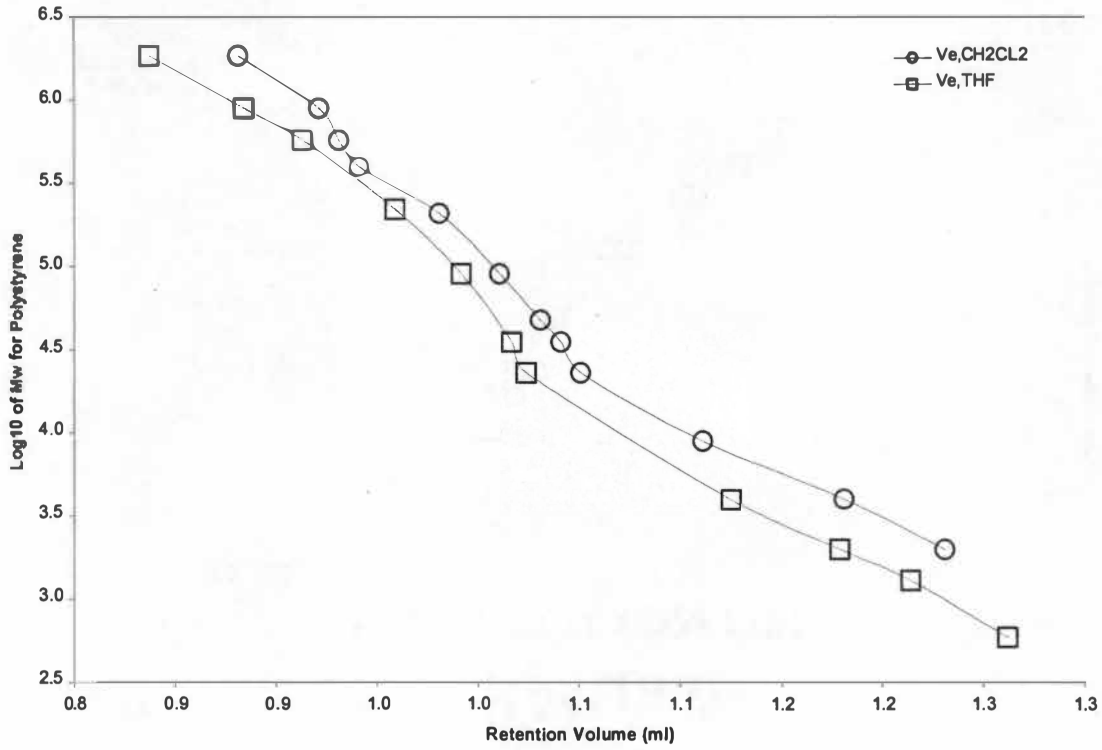


Figure 15. ISEC Calibration Curve for the prepared packing material column (C).

Flow rate 1.0 ml/min.

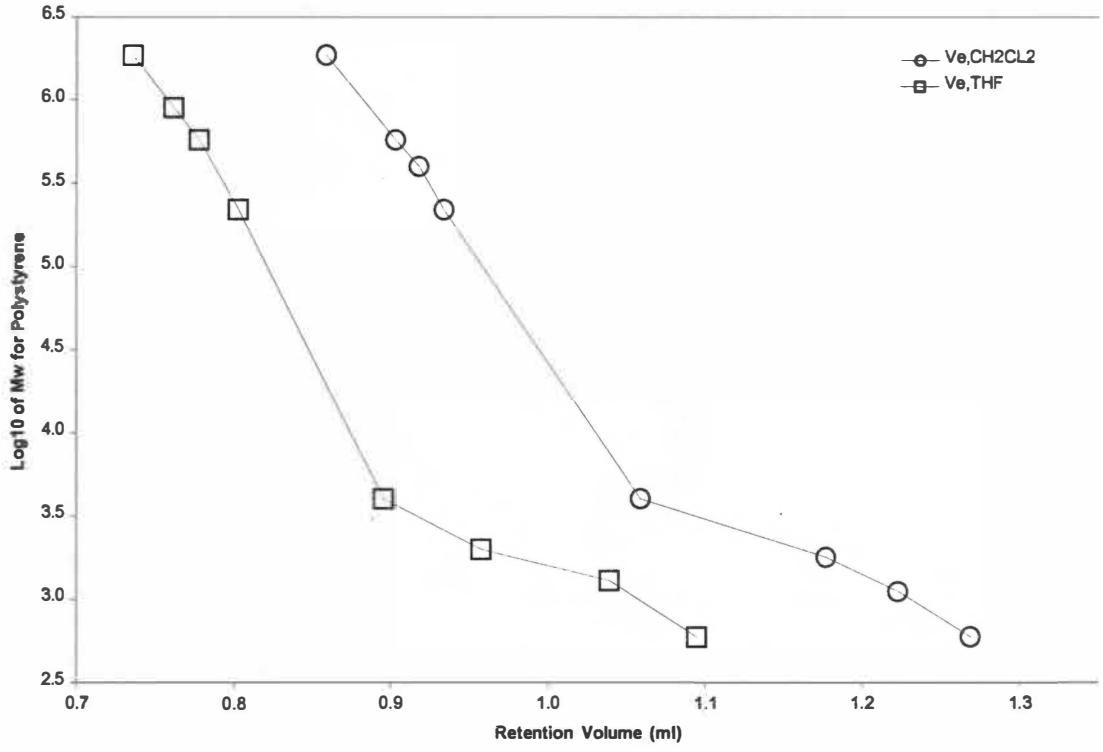


Figure 16. ISEC Calibration Curve for the prepared packing material column (D). Flow rate 1.0 ml/min.

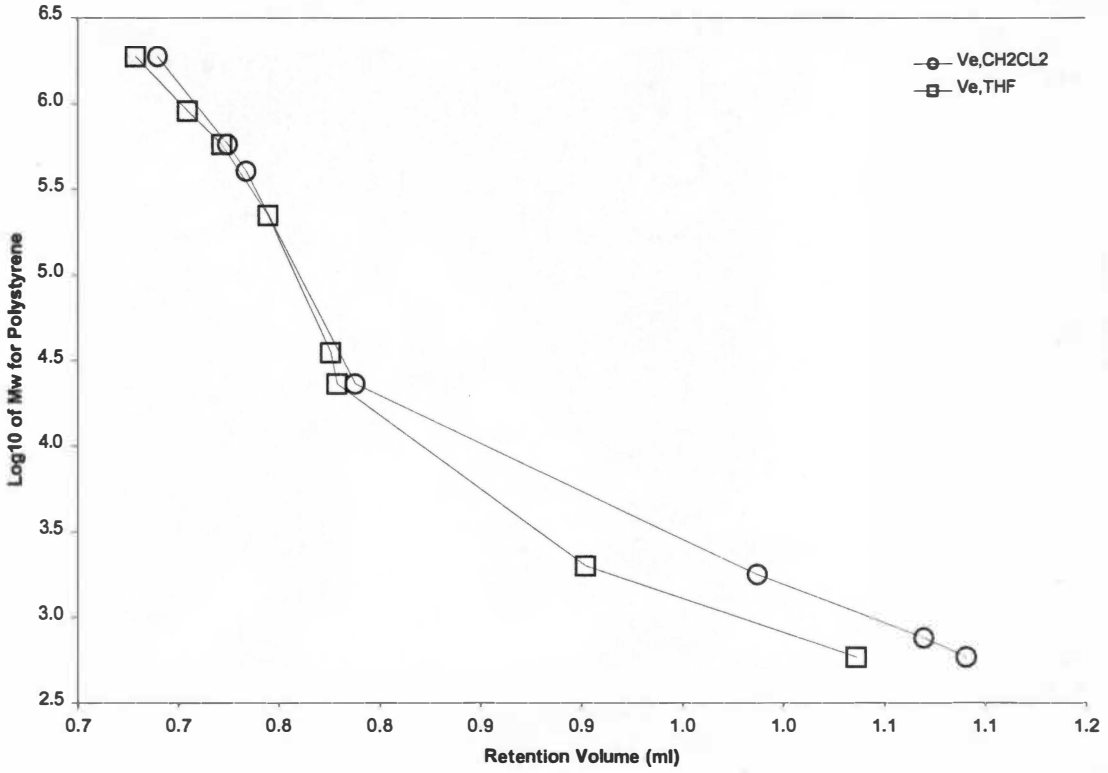


Figure 17. ISEC Calibration Curve for the prepared packing material column (E).

Flow rate 1.0 ml/min.

Thus, from these inverse size exclusion chromatography calibration curves for the prepared packing materials, we can state that not only do the ideal curves reflect the true pore size, but they also must be given more or less the same values through the solvent CH_2CL_2 and THF, individually. That means, the sharpness of the interception points should be, theoretically, identical in order to have an estimated value for the pore size diameter (ϕ) within an acceptable difference.

3.3. Porosities

As it is mentioned before, porosity is one of the most important characterizations for the column with the packing material. Therefore, as long as CH_2CL_2 and THF are good solvents with standard polystyrene for ISEC method, the total porosity (ε_t), external porosity (ε_e), and internal porosity (ε_i) should have almost the same values through both solvents for each column or within acceptable difference if the average is taken in the account. The normal porosities, which have been published in the literature, are within the range of 0.5-0.8 for the total porosity and less than 0.5 for the external and the internal porosity. In this investigation, the range is within 0.62-0.88 for the total porosity and less than 0.6 for the external and the internal porosity. The porosities values (ε_t, ε_e, and ε_i) for all the columns that have been packed are obtained and tabulated in table (5), once by CH_2CL_2 and the other by THF, with the statistical parameters. Due to the influence of THF on column 1, mentioned before, the porosities are not available. Changing the mobile phase for column 2 has almost no influence in the porosities. Theoretically, according to what is mentioned in the literature about using THF and CH_2CL_2 with the standards polystyrene, both solvents should give almost the same

Table 5. The total (ε_t), external (ε_e), and internal (ε_i) porosities for the packing materials.

Flow rate 1.0 ml/min.

Column	ε_t	AV ε_t	$\Delta\varepsilon_t$	ε_e	AV ε_e	$\Delta\varepsilon_e$
1_{CH2CL2}	0.798	????	????	0.377	????	????
1_{THF}	???			????		
2_{CH2CL2}	0.617	0.621	0.007	0.377	0.382	0.009
2_{THF}	0.624			0.386		
A_{CH2CL2}	0.757	0.744	0.026	0.399	0.396	0.007
A_{THF}	0.731			0.392		
B_{CH2CL2}	0.868	0.846	0.044	0.663	0.695	0.064
B_{THF}	0.824			0.727		
C_{CH2CL2}	0.880	0.847	0.067	0.651	0.657	0.012
C_{THF}	0.813			0.663		
D_{CH2CL2}	0.834	0.769	0.131	0.590	0.556	0.068
D_{THF}	0.703			0.522		
E_{CH2CL2}	0.765	0.732	0.066	0.484	0.493	0.017
E_{THF}	0.699			0.501		

Column	ε_i	AV ε_i	$\Delta\varepsilon_i$
1_{CH2CL2}	0.421	????	????
1_{THF}	???		
2_{CH2CL2}	0.240	0.239	0.002
2_{THF}	0.238		
A_{CH2CL2}	0.358	0.349	0.019
A_{THF}	0.339		
B_{CH2CL2}	0.205	0.151	0.108
B_{THF}	0.097		
C_{CH2CL2}	0.229	0.190	0.089
C_{THF}	0.150		
D_{CH2CL2}	0.244	0.213	0.063
D_{THF}	0.181		
E_{CH2CL2}	0.281	0.240	0.083
E_{THF}	0.198		

behavior with the same values for the porosities in ISEC experiments. That means, the ISEC method is suitable method for the standard packing material for column (2) where the average is 0.621 with a difference of 0.007 for the total porosity. Values of 0.382 and 0.239 were obtained for both the external and internal porosities, respectively.

For the prepared packing materials, column (A) has achieved a good value for the total porosity with a $\Delta\varepsilon_t$ less than 0.026 for an average of 0.744. for external and internal porosities, the average is 0.396 and 0.349, respectively.

Column (B) and (C) show more or less the same behavior due to the lack of the function groups on the silica gel. The total porosity for column (B) is slightly high, 0.846. But the external and internal porosities have various values, which are 0.695 and 0.151, respectively. This can be explained through the nature of the stationary phase, which requires a function group. Lacking the function group will affect the stationary phase by giving it the flexibility and the ability to be shrunk or swelled. This holds true for column (C). On the other hand, the values for total and external porosities are considered high for packing columns. These unexpected porosities are explained totally by rigid of the packing material cannot be stable and hard enough to perform ISEC.

Evan though column (D) shows a similar curve for both runs, it is still its values for the porosities are considered high. That is due to partially adsorption occurs when the run is changed from CH_2CL_2 to THF or the opposite. Consequently, that excludes a portion of porous from being measured. Column (E) has been faced the same problem that happened with the standard material for column (1) when it is changed to THF. But the swelling in this prepared material is not huge as in column (1), whereas a very poor inflexion point is still observed. As for column D and E, the unmatched values for the

porosities are the result of changing the mobile phase as well as the difference in their chemistry.

3.4. Excluded Molecular Weights

The interception point in the inverse size exclusion chromatography calibration curve, which is a result of extrapolating the two linear regressions, estimates the border between the internal and the external pore zone. This border, by itself, roughly reflects the excluded molecular weights from the eluting ones. Table (6) shows that the molecular weight, which is bigger than the indicated one, cannot penetrate the pores of the packing material when the mobile phase is CH_2CL_2 or THF.

The excluded molecular weight is another distinguished character for the packed column. Once again, the excluded molecular weight, regardless of whether the mobile phase is CH_2CL_2 or THF, must be more or less the same. Table (6) shows the average molecular weight with the differences (**ΔMw**) in the excluded molecular weights through CH2CL2 and THF for the packing materials.

Column (2) has given a value within an acceptable **ΔMw** of 1825 (g/mol) for an average 22737 (g/mol). Column (A), also, has been given an excellent value for the difference, 200 (g/mol) for an average of 46875 (g/mol). The rest of the columns have rather unacceptable differences between the excluded molecular weights when we shifted from THF to CH_2CL_2 for the same reasons that have been explained fro the pore size diameters and porosities.

Table 6. Excluded Molecular Weights for the packing materials.

Flow rate at 1.0 ml/min.

Column	Molecular Weights (g/mol)		AV Mw (g/mol)	ΔMw (g/mol)
	> Excluded Mw_{CH2CL2}	> Excluded MW_{THF}		
1	28448	?????	?????	???
2	23649	21826	22737	1823
A	46765	46985	46875	220
B	9526	1216	5371	8310
C	14764	5810	10287	8954
D	10740	3222	6981	7518
E	13091	2622	7856	10469

From the shown and discussed results throughout the pore size (ϕ) diameters results, the porosities (ε) results, and the excluded molecular weights (Mw) results, it has been proven that only the standard packing material (column 2) and the prepared packing material (column A) could be studied and characterized by inverse size exclusion chromatography under the conditions that have been set for this investigation. This does not indicate that the other packing materials are not well prepared or have internal obstacles preventing them from being good materials for further proposes. But indicates that these materials couldn't achieve the inverse size exclusion chromatography principles and the boundaries under the conditions that have been set up to characterize and investigate them. Nevertheless, this could mean that they might be used in the normal phase or reverse phase for the analytical separation purposes.

3.5. Pore-Size Distributions

The distributions of the pores will be applied for column 2 and A because they are the only chromatographic packed columns, which are adequate to be studied through inverse size exclusion chromatography. If the pore-size distributions apply on the rest of the columns, it will not reflect and give the true and actual distribution.

The pore-size distributions (PSD) can be expressed via a few methods, which would be preferable for the author. In this work, PSD's will be expressed by using the volume fraction percentage method (*Guan and Guiochon, 1997*). The major assumption in this distribution is that all the pores, which would pass the column and have pore-size larger than or equal to ϕ_n, have a volume V_n. Also, the pores, which having pore-size larger than or equal to ϕ_{n+1}, have a volume V_{n+1} ($\phi_{n+1} > \phi_n$). Moreover, we can determine

the volume of the pores that have a pore-size larger than ϕ_n and smaller than ϕ_{n+1} by the following equation:

$$\Delta V_{n+1,n} = V_{n+1} - V_n$$

where $\Delta V_{n+1,n}$ can be calculated from inverse size exclusion chromatography data. Here, it is crucial to indicate that more numbers of standard polymeric samples give more improvements are required to achieve a reasonable and actual pore-size distributions for the chromatographic column.

Table (7) and figure (18) reveal the PSD for the column (2) and (A). The external porosity (ε_e), which has a significant role in ISEC, has given a pore size of 225Å. This value, as PSD has revealed, is in the range of 228Å. This range occupies 13.5% of the total volume, which is a good measure through ISEC. Also, the PSD indicates that 58% of the total pores have a diameter larger than 0.3μm and around 18% possess a diameter smaller than 0.0054μm. Furthermore, the PSD can be divided to micropores (<15Å), mesopores (15Å-500Å), and macropores (>500Å). According to literature, which follows this scale, column (2) for the standees packing material has micropores with volume fraction less than 19.0 % of the total volume. The mesopores represent 21.0% of the total volume while 60.0% of the total pores are macropores. The PSD for the prepared packing material of column (A) is shown in table (8) and figure (18). The external porosity (ε_e) has given a pore size around 354Å. The value is in this range of 509Å, which presents 0.57% of the total volume. A 50% of the total pores have diameters equal to or larger than 0.3μm and 16% have a diameter less than 0.0054 μm. In the scale pores, this column has less than 16% micropores, 30% mesopores, and 54% macropores.

Table 7. Incremental pore distribution on the standard packing material for column (2).

M_w (g/mol)	ϕ (Å)	Range of ϕ	Volume Fraction %
92	9	9--54	19.0
2000	54	54--82	5.54
4000	82	82--131	5.85
9000	131	131--228	9.09
23000	228	228--292	0.23
35000	292	292--350	0.23
47500	350	350--509	0.27
90000	509	509--833	0.39
207700	833	833--1225	0.43
400000	1225	1225-1517	0.23
575000	1517	1517--1974	0.55
900000	1974	1974--3025	0.78
1860000	3025	3025--	58.0

Table 8. Incremental pore distribution on the prepared packing material for column (A).

M_w (g/mol)	ϕ (Å)	Range of ϕ	Volume Fraction %
92	9	9--54	16.0
2000	54	54--82	6.40
4000	82	82--131	7.22
9000	131	131--228	13.5
23000	228	228--292	1.15
35000	292	292--350	1.31
47500	350	350--509	0.57
90000	509	509--833	0.49
207700	833	833--1225	1.40
400000	1225	1225-1517	0.82
575000	1517	1517--1974	0.82
900000	1974	1974--3025	0.49
1860000	3025	3025--	50.0

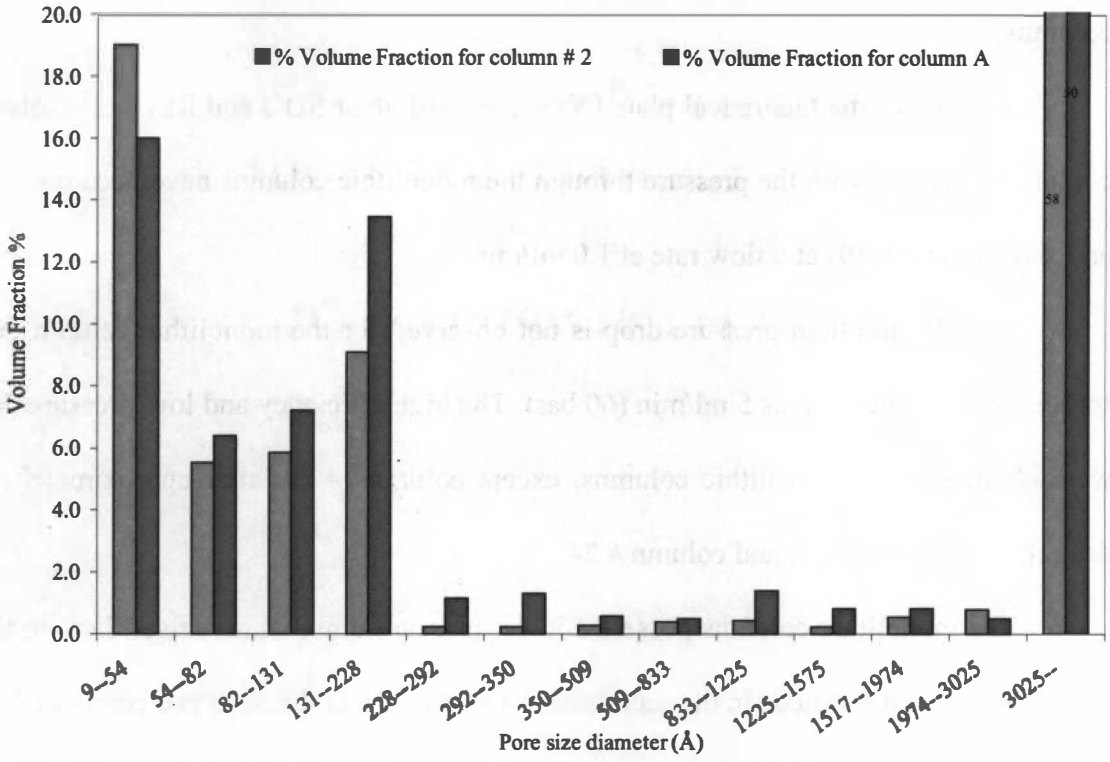


Figure 18. Average Pore-Size Distributions for the prepared packing material for column (A) and the standard packing material for column (2).

THF is a mobile phase at 1.0 ml/min.

CHAPTER 4

MONOLITHIC COLUMNS: RESULTS AND DISSCUSIONS

4.1. Efficiencies

In general, as it was mentioned before, the efficiency for any column depends on a few factors. In the monolithic column, these factors are limited as a result of enormous competition and scientific developments in the manufacturing of this kind of trustable columns.

Number of the theoretical plate (N) values with their SD's and RSD's for toluene compound parallel with the pressure through the monolithic columns have been obtained and shown in table (9) at a flow rate at 1.0 ml/min.

Usually, the high-pressure drop is not observed for the monolithic column even for a high flow rate such as 5 ml/min (60 bar). The high efficiency and low-pressure drop were obtained from monolithic columns, except columns # 20, after approximately 50 days from the first usage, and column # 24.

The monolithic columns possess a lower pressure drop, which arises them to take the advantage and privilege in the separation research field as the most preferable tool. As a result of having a lower pressure drop in the monolithic column, the high efficiency is confirmed in this work.

Table 9. Number of the Theoretical plates for the monolithic columnsin.

Mobile phase is THF at 1.0 ml/m

Column	N (plate)	RSD %	Pressure Drop (bar)
19	5213 ± 49	0.95	13
20	5432 ± 45	0.83	13
20 (After 50-d)	4252 ± 68	1.63	70
21	5714 ± 33	0.57	13
22	5822 ± 44	0.75	12
23	5723 ± 35	0.61	13
24	4301 ± 50	1.61	75

The Preeminence of having lower pressure drop and high efficiency is a result of the monolithic column possessing independent control of the size of the silica skeleton and the throughpores (macropores and mesopores) (*Cabrera et al., 2000*), which will be discussed later in Pore-Size Distributions (PSD).

Efficiency for column # 20 has been studied twice within 50 days where THF was a mobile phase. The Van Deemter curve for the height equivalent theoretical plate (**HETP**) versus flow rate (linear velocity, in some cases) is obtained in the first time with filtered chemicals figure (19, 1st). Data points for second run in the same figure (19) shows how the pressure drop varies within unfiltered chemicals. Consequently, how that behavior variance influences on (**HETP**). In other words, it indicates how the undiscerned contamination of the monolithic column changes the pressure drop.

By comparing the two sets of data, the Van Deemter curve could be obtained with a fluctuation on the (**N**) every time. As a matter of fact, that will change the efficiency.

The same tendency at the low flow rate, 0.1 – 2.0 ml/min, for both sets was obtained. At high flow rate such as over 3.0 ml/min, the efficiency fluctuates around a certain value that has a bit of a high error. (*Cavazzini et al., in press and Kale et al., in press*) observed the same behavior in their work. So, practically, the monolithic column is very sensitive toward unfiltered solutions and samples.

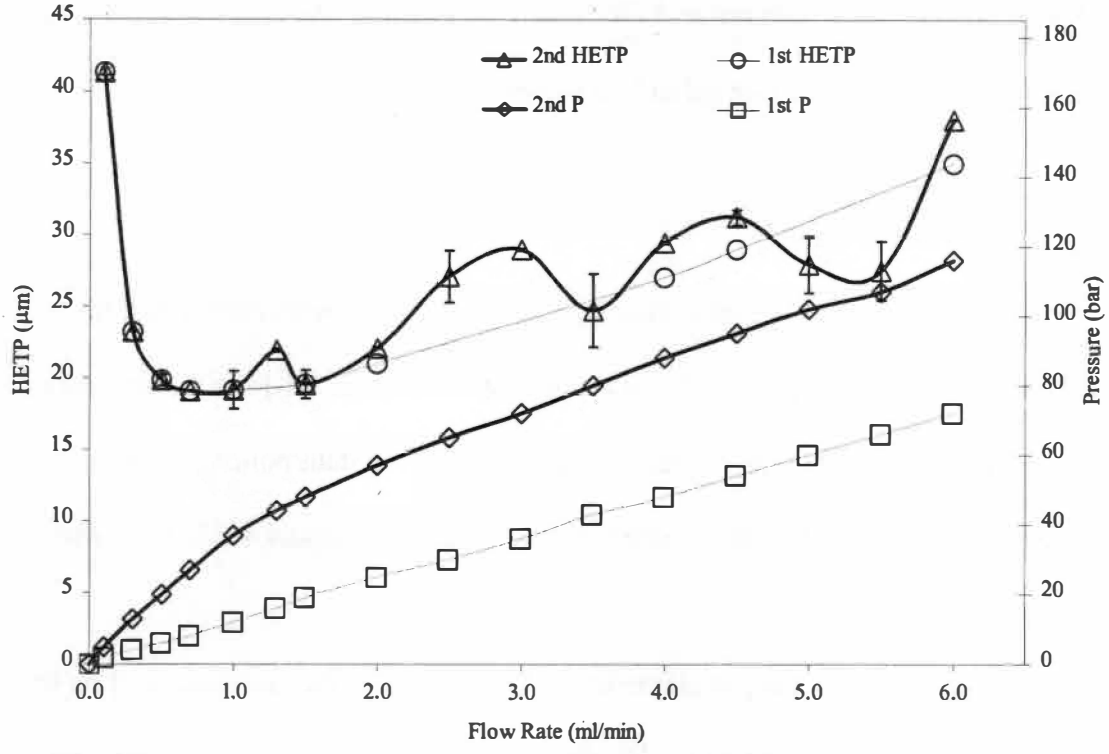


Figure 19. Van Deemter Curve for Column # 20.

THF is a mobile phase at 1.0 ml/min.

The data points for the tiny line are for the run at the beginning with filtered chemicals.

The data points for the thick line are for the run after 50 days with unfiltered chemicals.

From the work that has been done by (*Cavazzini et al., in press*) on monolithic column # 24, the initial value of the backpressure at 1.0 ml/min, when it has been used for the first time, was 28 bar. After a few weeks, the backpressure increased to more than 75 bar. Opening the inlet of the column and cleaning it by brushing the contaminated surface is the solution that has been followed in (*Cavazzini et al., in press*).

4.2. Pore-Size Diameters

Allegiant ISEC calibration curves with very sharp interception point have been obtained for all the columns. Table (10) shows R squared (R^2) for those calibration curves to give an idea how these linear regressions fit the data points. Figures (20) and (21) show these curves for the columns # 19, # 22, and #23 and # 21, # 23 and # 24, respectively.

The calculated pore-size diameters (ϕ) with their SD's values as well as RSD's have been tabulated in table (11). In general, these columns have average pore-size diameters (ϕ) in the range of 204-314Å. Since columns # 20 was studied twice within 50 days, one by filtered chemicals and another by unfiltered chemicals, there are two values for the average pore-size diameters. A value of 314 Å with error 1.91% at pressure around 13 bar and a value of 279 Å with error 5.49% at pressure around 73 bar. This difference in the pore-size diameter is due to the unfiltered chemicals, which results in high pressure. Column # 24 has a value of 255 Å at pressure around 75 bar with error 4.00%. In general, the pressure could be effect the pore-size diameter and give a fake value blocking some pores.

Table 10. R^2's value for the ISEC Calibration Curve for the monolithic columns.

THF as a mobile phase at flow rate 1.0 ml/min

Column #	THF	
	R^2 for External Pore Zone	R^2 for Internal Pore Zone
19	0.993	0.997
20	0.999	0.999
20 (after 50-d)	0.982	0.978
21	0.976	0.999
22	0.983	0.999
23	0.981	0.999
24	0.999	0.999

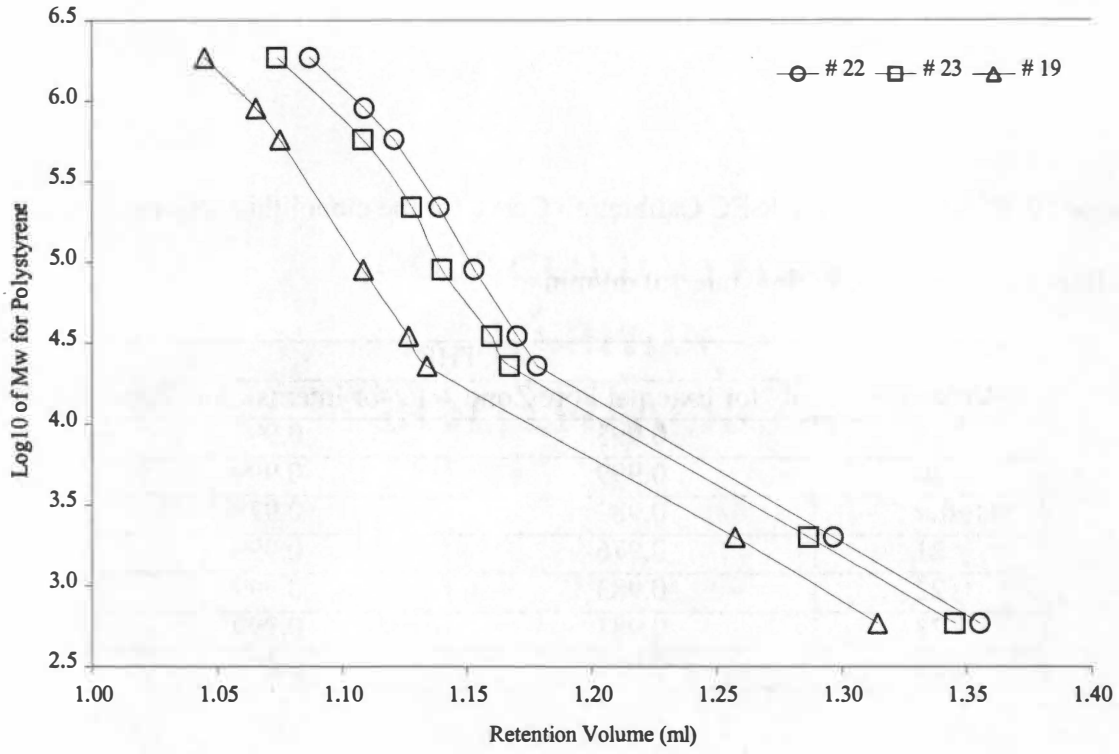


Figure 20. ISEC Calibration Curve for the columns # 19, # 22, and # 23.

THF is a mobile phase at 1.0 ml/min.

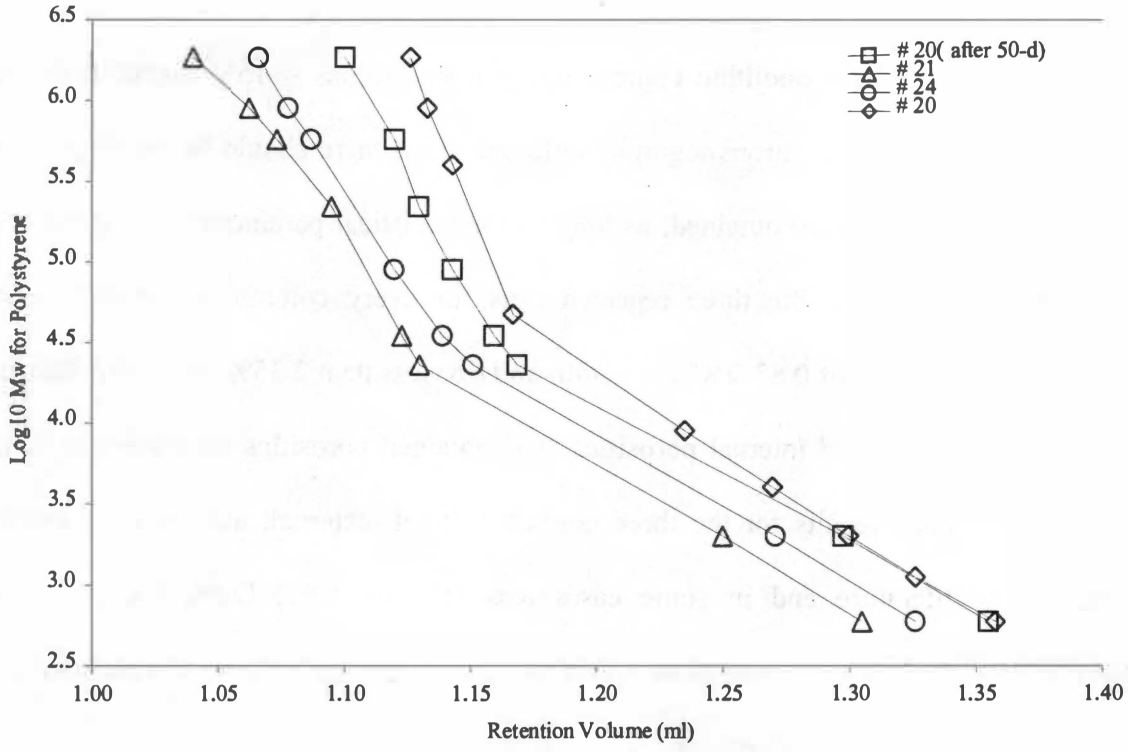


Figure 21. ISEC Calibration Curve for the columns # 20, # 23, and # 24.

THF is a mobile phase at 1.0 ml/min.

However, the average pore-size diameters results with their SD's and RSD's values reflect the high quality of the columns, which give a high level of reducibility. As a conclusion for these figures, inverse size exclusion chromatography is a good method to characterize these kinds of columns.

4.3. Porosities

In general, the monolithic column has porosity values ≈ 15% higher than the normal practical packing chromatography column. Thus, there should be no surprise if slightly high porosities are obtained, as long as the statistical parameters are within the acceptable range. From the three repeated runs for every column, the overall total porosities in the range of 0.83-0.87 are within an error less than 2.35%. The same hold is true for the external and internal porosities. The obtained porosities are shown in table (12). The obtained results for the three porosities (total, external, and internal) match those in the literature and in some cases less than by 10%. Later, the pore-size distributions (PSD) characterize these columns by giving every column three categories of pores: macropores, mesopores, and micropores.

As it will be shown later, the macropores (throughpores) occupy more than 80% of the total pores. This high percentage distinguishes the monolithic from the other chromatographic columns. So, the high value for the porosities is a result of the high percentage of throughpores. These macropores allow the analytes to pass and to be transported through the column under low pressure. Overall, the mesopores occupy ≈ 15% of the total pores and that has been used to cover and generate the activated surface for subsequent chromatographic separation.

Table 11. The Pore- Size Diameters (ϕ) for the monolithic columns # 19-# 24.

THF is a mobile phase at 1.0 ml/min.

Column #	Φ (Å)	RSD %
19	223 ± 6	2.59
20	314 ± 6	1.91
20 (after 50-d)	279 ± 15	5.49
21	204 ± 5	2.34
22	217 ± 6	2.93
23	208 ± 6	2.94
24	255 ± 10	4.00

Table 12. The three porosities, total (ε_t), external (ε_e), and internal (ε_i) porosities, for the monolithic columns # 19-# 24. THF is a mobile phase at 1.0 ml/min.

Column #	ε_T	RSD %	ε_e	RSD %	ε_i	RSD %
19	0.830 ± 0.011	1.33	0.685 ± 0.011	1.61	0.145 ± 0.001	0.69
20	0.845 ± 0.011	1.32	0.704 ± 0.011	1.56	0.141 ±	0.71
20 (after 50-d)	0.855 ± 0.011	1.29	0.678 ± 0.011	1.62	0.176 ± 0.010	5.67
21	0.826 ± 0.010	1.21	0.688 ± 0.010	1.45	0.138 ± 0.001	0.72
22	0.857 ± 0.020	2.33	0.713 ± 0.020	2.80	0.143 ± 0.001	0.70
23	0.850 ± 0.010	1.18	0.708 ± 0.020	2.83	0.142 ± 0.001	0.70
24	0.868 ± 0.021	2.42	0.688 ± 0.011	1.60	0.180 ± 0.002	1.11

Table 13. The Excluded Molecular Weights (Mw) for the monolithic columns # 19-# 24.

THF is a mobile phase at 1.0 ml/min.

Column #	Excluded Mw (g/mol)	RDS %
19	21700 ± 198	0.91
20	38117 ± 190	0.50
20 (after 50-d)	33000 ± 153	0.47
21	18100 ± 193	1.07
22	19900 ± 162	0.83
23	19400 ± 123	0.63
24	29800 ± 195	0.65

4.4. Excluded Molecular Weights

The precision and accuracy of the monolithic columns are very high for the excluded molecular weights, which have been obtained by ISEC, table (13). The obtained results are a good indication that ISEC is a suitable method for studying and characterizing the monolithic column. As a matter of fact, each column can exclude a certain molecular weight, which differs from one to another, for standards polystyrene. The ability of the columns to acquire and achieve the principles of ISEC, which are no absorption for the test material on the stationary phase, no aggregation for the samples, presence of instantaneous equilibrium between the two phase during the whole experiment, no mutation in the nature of the stationary phase, and obtaining the elution peak similar to Gaussian peak, is very high.

4.5. Pore-Size Distributions

The obtained results for the pore-size diameters (ϕ), the porosities (ε), and the excluded molecular weights indicate that ISEC is a suitable technique for characterizing the monolithic column. Depending on those results the pore-size distributions for all of the columns were obtained. The obtained data for the average pore-size diameters (ϕ), in general, indicate that all the columns (except column # 20 and # 24) have values approximately in the range of 228Å with a volume fraction of $\approx$ 8.5% of the total volume. For column # 20, both its values, at low and high pressure, lie in the range of 350Å with a volume fraction of 4.5% of the total volume. The reason behind that is column # 20 has a high pressure of around 70 bar compared with the others that had around 13 bar. Also,

column # 24 experienced a high pressure around 75 bar, the pore diameter (ϕ) is 260Å. It should be mentioned that column # 24 has been under experiment by some one was using unfiltered chemicals. Once again, the pressure here is responsible for this value. The pressure could be used as a block or an obstacle, preventing the sample and the solvent to penetrate some of the pores. Consequently, the obtained pore-size diameter, while the pressure is high, is not the actual one that represents the pores.

Opening the outlet and the inlet of the column and brushing the surface could remove the contamination, very fine brown dust that is generated from the unfiltered chemicals, and return the pressure to normal.

By looking at table 14 and 15, the pores in all of the six columns can be divided as micropores (<15Å), mesopores (15Å-500Å), and macropores (>500Å). Approximately, 5-4% of the pores are micropores except for columns # 20 and # 24, whereas, 3% and 8%, respectively. A value from 14% to 15% of the total pores is mesopores that has been obtained for each column except # 20, which has a value of 13%. Most of the pores lie on the range of the macropores, whereas, they represent 80%, 84%, 81%, 81% 81%, and 77% for # 19, # 20, # 21, # 22, # 23, and # 24, respectively. The high percentage of macropore gives the monolithic column the power to separate the compound and study the analytical issue with rapidity and efficiency.

The pore-size distributions for all the columns # 19, # 22, and # 23, # 21 and # 24, are shown in figures (22) and (23), respectively. Columns # 20 is shown separately in figure (24) because it has a slightly different scale from the others due to the lack of the same molecular weights for the standard polystyrene. These distributions are taking in the

account the pressure influence, which has been built-up by the impurities of the chemicals for columns # 20 and # 24

Table 14. Incremental pore distribution on the monolithic column #20.

THF is a mobile phase at 1.0 ml/min.

Mw (g/mol)	(ϕ) Å	Range of ϕ	% Volume Fraction for column # 20
92	9	9--26	3.08
590	26	26--38	2.03
1110	38	38--54	2.03
2000	54	54--82	2.10
4000	82	82--131	2.29
9000	131	131--350	4.52
47500	350	350--509	0.46
90000	509	509--1225	1.51
400000	1225	1225--1974	0.59
9000000	1974	1974--3025	0.46
18600000	3025	3025--	80.94

Table 15. Incremental pore distribution on the monolithic columns #19, #21, #22, #23, and # 24. THF is a mobile phase at 1.0 ml/min.

Mw (g/mol)	(ϕ) Å	Range of ϕ	% Volume Fraction for columns				
			19	21	22	23	24
92	9	9--26	4.68	2.88	4.78	4.75	7.98
590	26	26--42	2.21	2.04	2.18	2.12	1.94
1300	42	42--54	1.92	1.97	1.90	1.98	1.87
2000	54	54--228	8.92	8.75	8.36	8.50	8.33
23000	228	228--292	0.54	0.51	0.56	0.50	0.83
35000	292	292--509	1.31	1.24	1.19	1.42	1.32
90000	509	509--862	0.83	0.80	0.98	0.85	0.90
220000	862	862--1517	1.60	1.53	1.26	1.35	1.39
575000	1517	1517--1974	0.69	0.80	0.84	0.64	0.62
900000	1974	1974--3025	1.49	1.68	1.55	1.84	0.76
1860000	3025	3025--	75.78	75.80	76.39	76.06	74.05

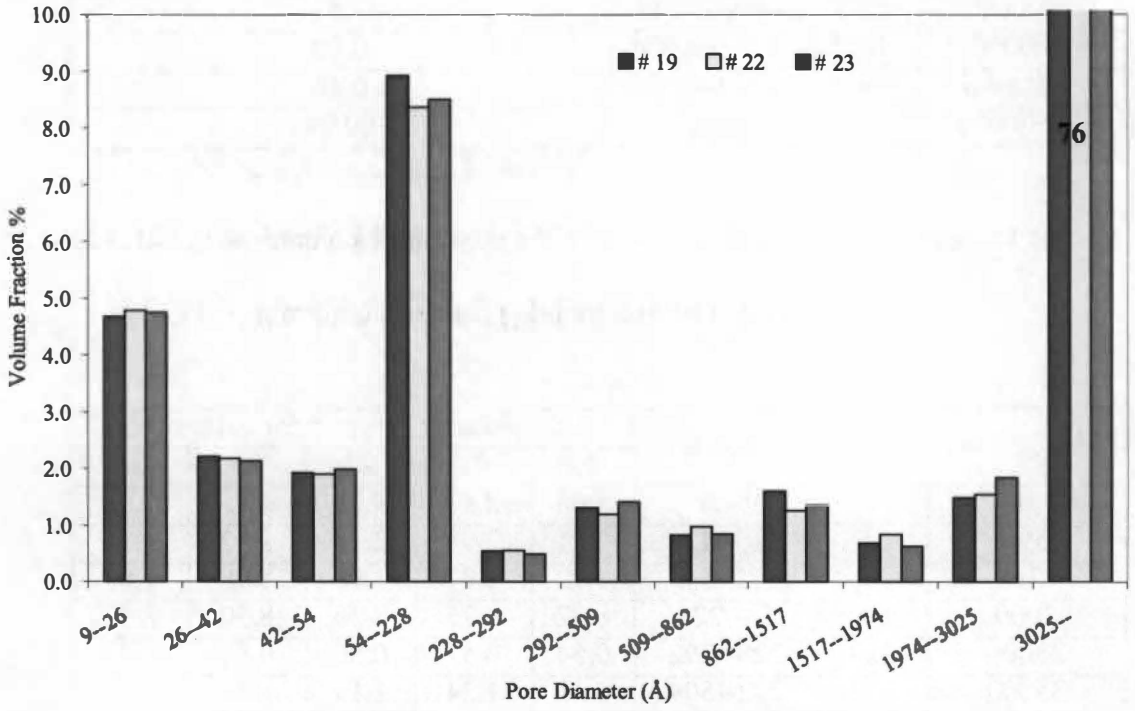


Figure 22. Average Pore-Size Distributions for the monolithic columns # 19, # 22, and # 23. THF is a mobile phase at 1.0 ml/min.

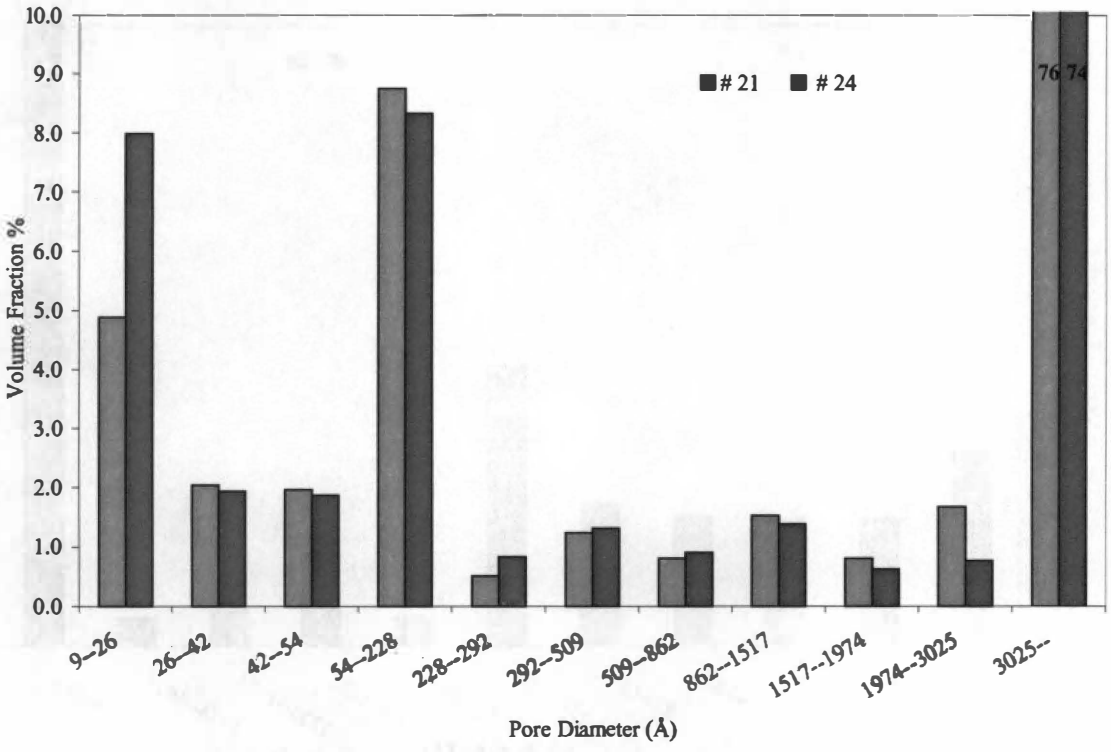


Figure 23. Average Pore-Size Distributions for the monolithic for columns # 21 and # 24. THF is a mobile phase at 1.0 ml/min.

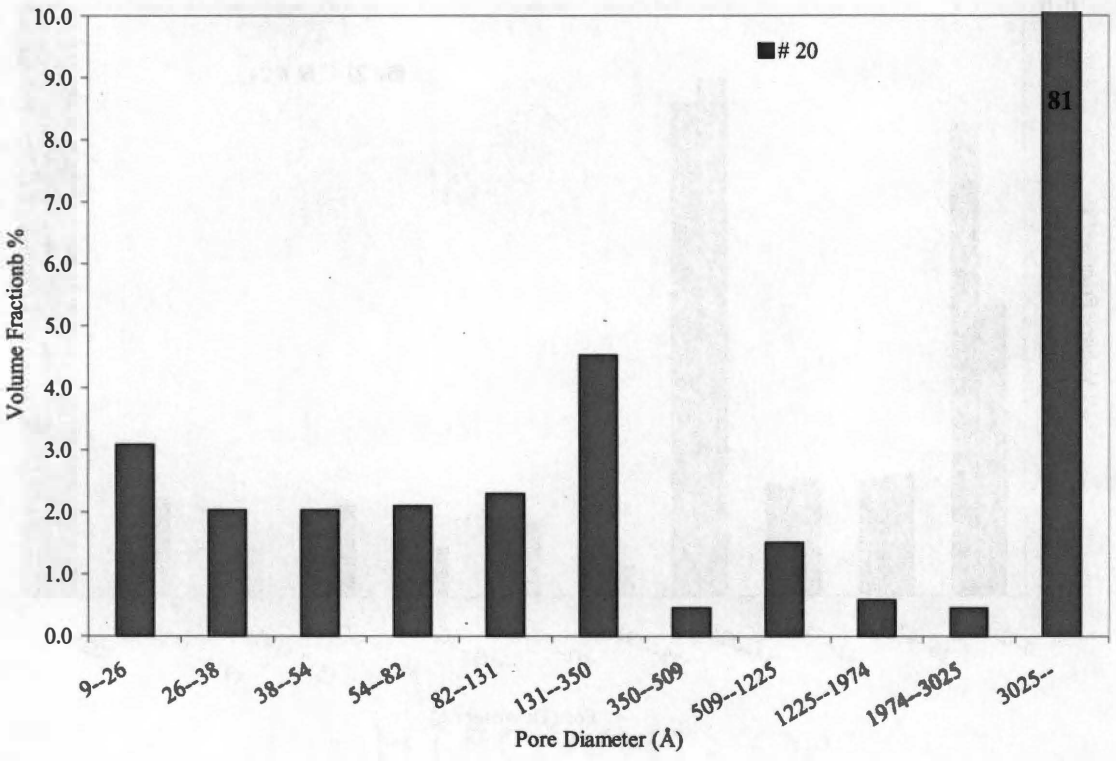


Figure 24. Average Pore-Size Distributions For the monolithic column # 20.

THF is a mobile phase at 1.0 ml/min.

CHAPTER 5

CONCULSION

Inverse Size Exclusion Chromatography has been used for more than 30 years. Each time, the analytical chemist performs this technique it exhibits reliable results. Not only in the analytical chemistry field, but also, in fields such as material science and polymer science, can this technique be utilized to evaluate products. Thereupon, the election to characterize our work by this *modus operandi* arrives at two major points: the accessible and proper facilities that are available at the research laboratory, and the limited time with which we have been controlled by.

In order to apply (ISEC) to the investigated material, five major conditions must be considered. First, the stationary phase must not absorb the injected sample. Second, instantaneous equilibrium must be occurring between the flowing eluent and the holding one within the pores. Third, the ratio of unity should be applied evenly to the injected sample in the flowing eluent and the accessible pore (Bunsen distribution coefficient). Fourth, Gaussian peak for the elution must be obtained, and fifth, the matrix of the stationary phase must be rigid during the whole experiment. According to the results that are obtained, depending on the standard materials that match the certificated data from the manufacturing company, there is a firm belief in the conditions chosen for this methodology. There is a strong believe that THF being is an organic solvent with a high degree of polarity can have two indirectly influences on the principles of the ISEC. THF could be acting like a tansio-active agent as well as swelling agent. In former acting, both the stationary phase and the standard polystyrene will be coated by THF and produce a

kind of partial adsorption. The latter acting, molecule of the standard polystyrene can be swelled by THF and consequently, got prevented from penetrating the pores of the packing material.

The five synthesized prepared packing materials reveal very different behavior ranks between a quasi-ideal ISEC Calibration Curve and quasi-poor one. For the sake of making this investigating and characterizing work consistent and as accurate as possible, six runs have been carried out for the synthesized materials by ISEC: Three times by using THF as mobile phase and the others by CH_2CL_2. Since both of the solvents are considered as good solvents for doing Inverse Size Exclusion Chromatography, they should give almost the same results. That has been observed for the prepared packing material for the column (**A**). The results for that material were very reliable and consistent. Its indication of a sharp calibration curve allowed us to characterize this material. It has a pore size of 354Å, reasonable efficiency for a prepared material ≈ 3250 plate for toluene/THF, porosities of 0.74, 0.40, and 0.34 for total, external, and internal porosity, respectively, and 55% of the total pores are macropores. Indeed, all its results within acceptable errors do not exceed 3 %.

On the other hand, the packing materials for columns B and C did not reflect a proper ISEC Calibration Curve, neither in THF, nor in CH_2CL_2 as mobile phase, which gave an indication that these materials are not preferable to be studied by ISEC due to the failure of achieving the boundaries of ISEC. Furthermore, it demonstrated this material to be unreliable for drawing reliable data by ISEC. The efficiency for these materials proved their vulnerabilities, where they gave 1450 plate and 525 plate for toluene/THF or CH_2CL_2 for columns B and C, respectively. The last two prepared packing materials

(columns D & E) that had been received from ORNL did give and reflect the ISEC Calibration Curve. But the results obtained from both runs, once in THF and the other in CH_2CL_2, did not match one another. This conflict confers the idea that ISEC cannot be applied to characterize such kinds of material. The only rationalization that could be reasonable is failure to achieve one or more from the boundaries and conditions for Inverse Size Exclusion Chromatography. As a final thought for the prepared materials, we can say that the ones for columns B, C, D, and E require many improvements, whether by changing the recipes, or by adding new function groups or modifying the particle's shape if we want them to be studied by ISEC.

The second part of this work is characterizing the six monolithic columns. These columns have been manufactured by MERCK Company. As mentioned before, these columns have been prepared by using a proprietary in-situ surface-modification process; however, each still has its own properties, which differ from one to another. All of them displayed a good efficiency for toluene/THF at flow rate of 1.0 ml/min, where they have 5213, 4252, 5714, 5822, and 5723, and 4301plate for columns # 19, # 20, # 21, # 22, # 23, and # 24, respectively, with an excellent percent of errors that did not exceed 1.64%. The pressure drop for monolithic column is sensitive to any kind of contamination that has resulted from being the solvents or samples unfiltered. Column # 20 exhibited an excellent Van Deemter behavior for (**HETP**) vs. flow rate when the used chemicals and samples are filtered. On the other hand, no longer we can acquire a reasonable Van Deemter behavior by using unfiltered reagents to do an experiment through monolithic column (column # 20 after 50 days). Consequently, that will affect the performance at the high flow rate of $\approx$ 3.0 ml/min. The pore diameters can be divided into two groups: a

group of diameters of 223Å, 204Å, 217Å, 208Å, and 314Å for columns # 19, # 21, # 22, # 23, and # 20, respectively, which have a low pressure drop ≈ 13 bar. And a group of 279Å and 255Å for columns # 20 (after 50-day) and # 24, which experienced a very high pressure drop ≈ 70bar and 75bar, respectively. Thus, as a conclusion here, the pressure has a tremendous influence on the pore diameter. Height Equivalent Theoretical Plate, (**HETP**), at a high flow rate; will have a fluctuation in the value of plate at certain flow rate if the column experiences a high pressure. The total porosity for those columns is higher by 15% than the ones of normal columns. Overall, the total porosities of the monolithic columns are in the range of 0.83-0.87, which match the published ones. The external and internal porosities were in the expected range of the values for the monolithic column. In general, Pore-Size Distributions (**PSD**) of the six monolithic columns show that 80% of the total pores are macroporse, and 20% are mesopores and micropores, where they vary from one column to another.

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VITA

Majed Mohammed-Ali Al-Bokari was born in Riyadh, Kingdom of Saudi Arabia, on October 26th, 1974. He was reared in and attended school in Riyadh where he graduated from one of the country's most prestigious and challenging high schools, Al-Farouk High School, in 1993. In 1993, Majed commenced his education by pursuing a Bachelor of Science degree in Chemistry at King Saud University. Throughout his undergraduate study, Majed maintained a superior GPA, which placed him within the top ones percentile by being the best of three in his department. Upon his graduation in 1997, Majed was among the superlatives when he graduated Summa Cum Laude. 1998 was a significant year in his life: He entered upon his professional field at the eminent King Abdualaziz City for Science and Technology (KACST), in Riyadh, as an analytical chemist. Within this realm, he published two papers and presented one at the 8th **International Symposium on Environmental Radiochemical Analysis,** in Blackpool, United Kingdom. Receiving the honored scientific scholarship from KACST for having achieved the highest ratings in Chemistry concluded the year. In his personal domain, Majed began his life of matrimony with Sarah Al-Momen. In Spring 2000, he entered graduate school at the University of Tennessee, Knoxville. In August of 2002, he completed the requirements for a Master of Science in Chemistry. In Summer 2002, he will resume his position at KACST, as well as embark on his future goal of a Doctorate of Philosophy in Chemistry.