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UNIVERSITY OF ALBERTA

IMMOBILIZATION OF POLY(AMIDOAMINE) (PAMAM) DENDRIMERS ON ZIRCONIA CHROMATOGRAPHIC SUPPORT

BY

MIN HE

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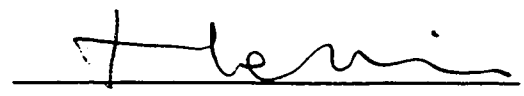

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ABSTRACT

The motivation of this work was to develop a method of immobilizing dendrimers on chromatographic supports as stationary phases and characterize them by studying the interactions between the immobilized dendrimer and small solutes using high-performance liquid chromatography.

Commercially available poly(amidoamine) (PAMAM) dendrimers were immobilized on the surface of zirconia liquid chromatographic support by exploiting Lewis acid-base interactions between multiple carboxylic acid groups on the periphery of the dendrimer and the zirconia surface Lewis acid sites. The modified surface exhibited good stability and the zirconia column can be regenerated by citrate buffer or sodium hydroxide. Small polycyclic aromatic hydrocarbons were employed to investigate the chemical properties of the surface-adsorbed dendrimers.

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Immobilization of poly(amidoamine) (PAMAM) dendrimers on zirconia chromatographic support submitted by Min He in partial fulfillment of the requirements for the degree of Master of Science.

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Dr. X. Le

Date: July 20, 2006

TO

MY GRANDFATHER

AND TO THE MEMORY OF MY GRANDMOTHER

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CHAPTER 1

AN INTRODUCTION TO DENDRIMERS

1.1 Dendrimers

1.1.1 What are dendrimers?

Dendrimers, which possess a branch point at every monomer unit, are probably the most highly branched structures that may exist.[1] Figure 1-1 shows the structure of carboxyl-terminated polyamidoamine (PAMAM) dendrimer (generation 2.5) as an example, which represents one of the first commercially available dendrimers. These new polymers were called "dendrimers" to describe their tree-like branching structure. Dendrimers have spawned a burst of interest in studies of their characteristics and applications since the discovery around 20 years ago [2].

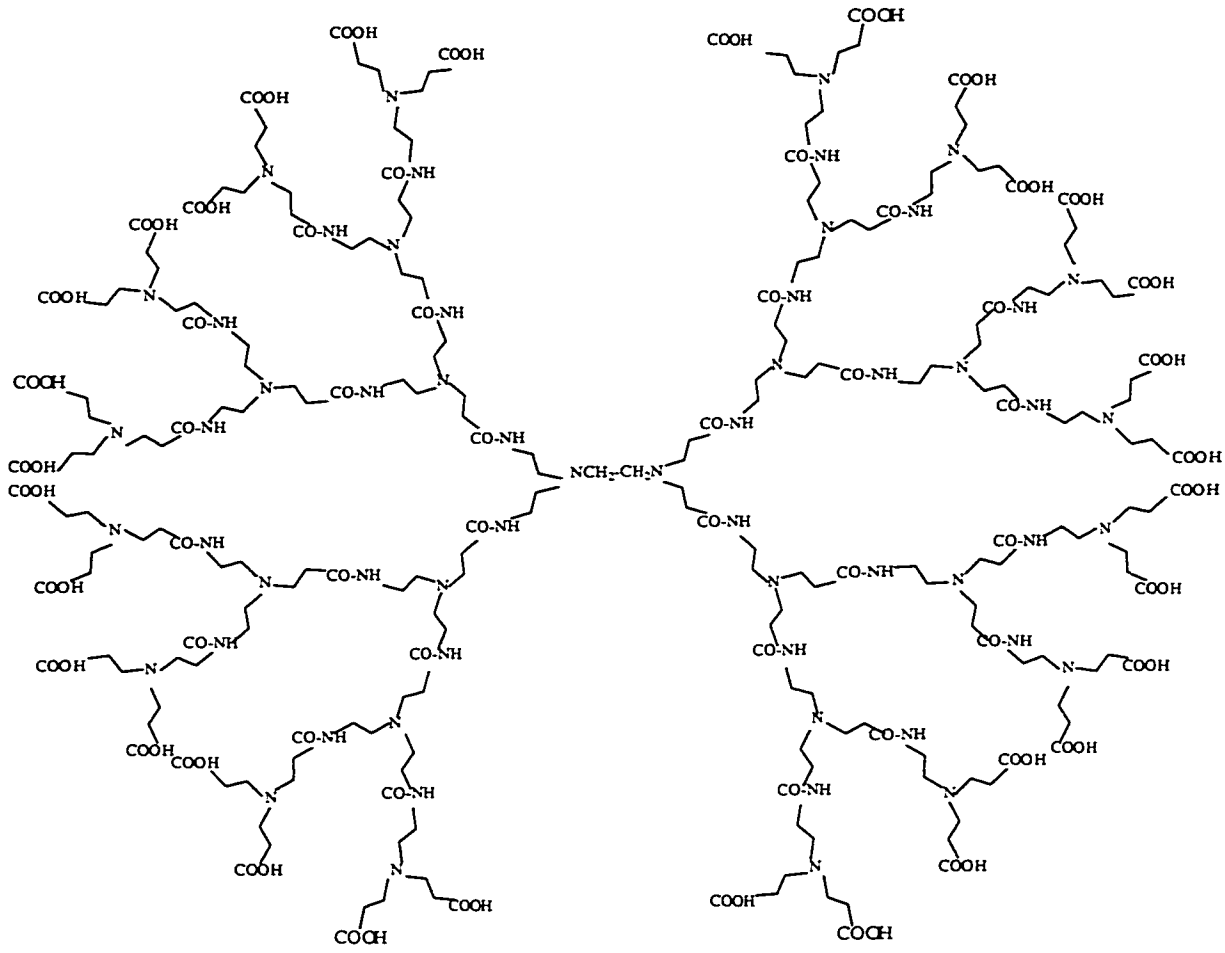

Figure 1-1 Structure of carboxyl-terminated PAMAM dendrimer (generation 2.5).

This class of macromolecules distinguish themselves from linear polymers in two critical ways. First, they contain AB_n segments (n usually 2 or 3) while the linear polymers contain the standard AB segments. Thus, they are hyperbranched structures. Secondly, they are synthesized in an iterative fashion. These two features lead to a nonlinear, stepwise growth where the number of monomer units in each successive iteration roughly doubles for AB_2 type or triples for AB_3 type that in the previous cycle. Usually, each repetition cycle results in the addition of one more layer of branches, called a *generation*, to the dendrimer framework. The generation number of the dendrimer is hence equal to the number of repetition cycles, and may be readily determined by counting the number of branch points from the core to the periphery.

1.1.2 Characterization of dendrimers

One of the motivations for studying these macromolecules has been the belief that these highly branched materials will have fundamentally different properties compared to traditional linear polymers. Unlike those classical polymers, dendrimers have narrow molecular weight distribution, specific size and shape characteristics, and a highly functional terminal surface. As a consequence, the unique chemical and physical properties of dendrimers have instigated intense interests in this field.

Structural characterization and host-guest chemistry

In structural characterizations, Hawker et.al[3] used a solvatochromic probe to explore the microenvironment in dendritic polyethers. They found the transition of the shape of the dendrimer from an extended fan to a globular structure as the generation number increased to 3. Tomalia et al[4] reported similar observation of half-generation PAMAM dendrimers with photoinduced electron-transfer reactions where the transition in dendrimer shape occurred from generation 3.5 to 4.5. Scherrenberg et.al[5] employed small-angle neutron scattering, viscosimetry and molecular dynamics simulations to study the structures and found that the dimension of dendrimers increased linearly with the generation number. Intermolecular interactions of dendrimers[6,7,8,9] were studied both in concentrated and dilute solutions. In dilute solutions, no significant interpenetration occurred between dendrimers while in

concentrated solutions, dendrimers would collapse and interpenetrate. The host-guest chemistry based on dendrimers has received a growing interest. It was found that both the interior and the periphery of a dendrimer could serve as hosts.[10] Jansen et al.[11] demonstrated that it is possible to encapsulate guest molecules in a dendritic polymer by constructing a dense shell in the presence of the guest molecules. Shape-selective liberation was also studied for molecules trapped in the dendrimer. The capability of starburst molecules to act as hosts for small molecules makes them attractive as drug-delivery agents[12]. More recently, colloids prepared by Crooks[13] were encapsulated in dendrimers and characterized by infrared and UV-vis spectroscopy and transmission electron microscopy. They found that the dendrimer generation used in the synthesis controls the size of the resultant colloids.

Theoretical studies

Theoretical simulations have also been conducted with mean-field[14,15], and renormalized field theory models[16], as well as Monte Carlo simulations[17]. However, so far, no perfect model has been developed for simulating dendrimers. For example, in Biswas's[16] calculation, only pair interactions were included while higher body interactions were neglected that are almost certainly important at the higher generations of chain growth. Naylor's[18] model for PAMAM dendrimers did not include hydrogen bonding which plays an important role in determining the structure. At the same time, the studies on dendrimers are still in controversy. For example, as to the density changes of dendrimers, many groups developed different, even opposite

proposals. de Gennes and Hervet[14] found the density to be minimal at the core and to increase monotonically to the outer edge. However, numerical simulation by Lescanec et al.[19] showed a density maximum at the core with a monotonic decrease to the edge. Studies with Monte Carlo simulations[17], molecular dynamics simulation[20], and self-consistent mean field model[15] all agreed with the latter postulation. More recently, Scherrenberg et.a[5] demonstrated that PAMAM dendrimers could be considered as flexible molecules with a relatively homogeneous radial density distribution.

Application of chromatography in dendrimer chemistry

What interests us particularly is the use of chromatography for studying dendrimers. Fréchet et al.[21] used reversed-phase high-performance liquid chromatography (RP-HPLC) to separate functionalized dendrictic macromolecules. RP-HPLC allows the separation of individual components and the estimation of the mass distribution of each component. Wal et al. utilized reversed-phase[22] and size exclusion chromatography[23] in combination with electrospray mass spectrometry to analyze the compositions of nitrile teminated poly(propylene imine) dendrimers. Furthermore, when the surface of an apolar dendrimer has polar terminal groups, it would resemble the structure of a micelle in solution[24]. By dissolving probe molecules in dendrimers, their micellar behavior has been characterized[25,26]. Unlike those low molar mass surfactants, for example sodium dodecyl sulfate (SDS), dendrimers can retain their micellar structure at a low concentration, regardless of ionic strength, or temperature. In contrast, surfactants of low molecular weight can only form micelles

when their concentration exceeds the critical micelle concentration (cmc), which is ionic strength and temperature dependent. Therefore, dendritic unimolecular micelles can be used at a much wider concentration range. Tanaka[27,28,29,30] and Kuzdzal[51] demonstrated the use of starburst dendrimers as a pseudostationary phase for the separation of polycyclic aromatic hydrocarbons (PAHs) in electrokinetic chromatography (EKC). Kuzdzal et al.[31] and Muijselaar et al.[32] employed dendritic macromolecules as pseudostationary phase to separate mixtures of other solutes. Castagnola[33] separated dansylated amino acids with a much higher efficiency by using starburst dendrimers as pseudostationary phase compared to dodecyltrimethylammonium bromide (DTAB). The enhancement in efficiency was attributed to the greater microhomogeneity of dendrimers compared to micelles formed by low molar mass surfactants. Kuzdzal et al.[51] also performed a thermodynamic study of dendrimer-solute interactions via electrokinetic chromatography and concluded there are great differences between dendrimers and SDS in their solvation properties.

Modification of dendrimers on solid substrates

The modification of a solid surface with a dendrimer monolayer or multilayers has been discussed in several studies. Work has been performed on the adsorption of dendrimers on metal substrates[34,35], charged alumina[36,37], and silica particles[38]. Smooth and homogeneous monolayers from both carboxyl terminated (negative) and amine terminated (positive) PAMAMs on opposite charged surfaces have been formed[39]. The adsorption via strong electrostatic interactions on both silica and alumina particles was considerably affected by the generation number: the high generation dendrimers behave like polyelectrolytes while the low generation dendrimers behave like electrolyte. Furthermore, strong deformation of the surface-bound dendrimers has been observed where dendrimers were collapsed and compressed, resulting in a flattened, disklike conformation[40,41]. This was attributed to the interactions between the terminal functional groups and the activated substrate. Actually, Mansfield[42] has already predicted this spreading out and flattening down of dendrimers on the surface with computer simulation. He demonstrated the dependence of adsorption of dendrimers on the generation number. The adsorption strength was found to be relatively high for dendrimers of high generations (G>3) and high interaction strength between terminal groups of dendrimers and substrate surface would facilitate this deformation. In addition, multilayer films of oppositely charged PAMAMs (amine and carboxyl termini) have also been built through *electrostatic layer-by-layer self-assembly* of dendrimer molecules of different generations[43]. Electrochemical methods have been applied on the study of the thermodynamics and kinetics of the adsorption

of PAMAM dendrimers with ruthenium complexes termini on Pt electrode[44]. The kinetics of adsorption appeared to be activation controlled instead of diffusion controlled and dependent on the identity of dendrimer rather than concentration.

1.1.3 Applications of dendrimers

The variety in molecular structure, size, shape, topology, flexibility, and surface chemistry offered a wide range of applications of dendrimers. Carboxylated starburst dendrimers have been employed as calibration standards for aqueous size exclusion chromatography because of their compact, symmetrical structures and narrow mass distribution.[45] Compared to linear macromolecules, dendrimers have an enhanced solubility and reactivity in a vast range of organic solvents[46,47]. With the enhanced solubility, they have been used as catalysts more efficient compared to linear macromolecules[47]. The branches of dendrimers can shield the core from the bulk solvent to some extent, resulting in a micro-solvent environment inside the dendrimers. The formation of internal cavities makes dendrimer a good candidate for biomolecule transfer[48], DNA transport[49], and drug delivery[50]. This type of unimolecular micelle has also been employed as a pseudostationary phase for capillary electrophoresis[51] and a novel recyclable solubilization agents[52] that can solvate hydrophobic molecules over a much wide range of concentration compared to sodium dodecyl sulfate. These dendrictic macromolecules are also used as models for branched polysaccharides[53]. The ability to control their size, shape, and surface chemistry offers options for applications as molecular electronic devices, biosensors, and chemical sensors. For example, Wells and Crooks[54] showed that dendrimer monolayers provide suitable interfaces for chemical sensing applications.

1.1.4 Motivation to this study

The motivation of this work is two-fold: 1) we wish to develop a method of immobilizing dendrimers on HPLC supports. This is for future chiral separation applications as part of the long-term goal of this group. 2) we wish to characterize immobilized dendrimers with HPLC.

Although some research work has been carried out on dendrimers in chromatography, HPLC has only been employed for the separation of dendrimers. Further fundamental studies are very scarce. The applications are limited to their use as pseudostationary phases in electrokinetic chromatography. However, CE has its limitations, for example, relatively poor reproducibility in electroosmotic flow and the inflexibility of running buffer compositions as the solubility of dendrimers is significantly affected by pH and solvent composition. These defects can be compensated if HPLC is employed. In addition, HPLC studies dendrimers immobilized on surface, which is more relevant to applications of dendrimers as stationary phase.

Despite of the fact that a large amount work has been conducted on dendrimers, the conformational behavior of dendrimers is still an issue of debate. We hope to investigate the following problems: Do dendrimers retain micellar properties once immobilized on a chromatographic support? How do the experimental conditions, for example, temperature, solvents, influence the solvation characteristics

of dendrimers? How would the generation number of dendrimers affect the adsorption and solvation towards small solutes?

As part of the long-term objective of research in this group, we hope to develop chiral selector-bound polymeric micelles as stationary phases for chiral separations of pharmaceutical products using polar mobile phases. Furthermore, we hope to develop surface plasmon resonance (SPR) selective sensors based on the immobilization of artificial receptor-bound cascade polymers for direct sensing in aqueous samples. To realize these ideas, we need to investigate the immobilization of dendrimers on the chromatographic support.

Although studies on surface adsorption of dendrimers have been conducted on various substrates including silicon[55,56] and alumina[57] , the application of these modified particles in chromatography is still under way. Furthermore, the interactions between the terminal functional groups of dendrimers and substrates are mostly based on electrostatic interactions, which are strongly dependent upon ionic strength and pH. On the other hand, zirconia[58,59] has been employed as a chromatographic support where Lewis acid sites dominate the surface chemistry[60] and can interact strongly with carboxyl groups[61]. Therefore, we investigated the possibility of immobilizing commercially available carboxylated poly(amidoamine) (PAMAM) dendrimers on zirconia surface through Lewis acid-base interactions. By characterizing this stationary phase with small solutes, we wish to gain a better understanding of PAMAM dendrimers. We also immobilized the carboxyl terminated polyamide

cascade polymers, a gift from Southern Florida University, on a strong anion-exchange chromatographic support via electrostatic interactions. Linear solvation energy relationships studies were conducted on this novel stationary phase to explore the chemical and physical properties of this class of dendrimers.

1.2 High-performance Liquid Chromatography

1.2.1 Advantages and limitations of HPLC for studying dendrimers

The most important advantages relevant to this study are the following: 1) The range of mobile phase that can be used is much larger in HPLC systems than that in CE. We can explore the effect of organic modifier on the retention of solutes with a wide range of properties. 2) The high reproducibility of HPLC has made it a reliable analysis methodology, while CE has the problem for its poor reproducibility in electroosmotic flow. 3) Investigation of surface immobilized dendrimers provides direct information on the application of dendrimers as stationary phases.

However, HPLC has its limitations as well. As is the case in all other chromatographic methods, HPLC is a poor identifier. Usually, other instruments have to be employed for positive identification of each peak. However, in this work, we used pure test solutes to characterize dendrimers and we could identify the peaks by their retention time. Therefore, the peak identification can be readily achieved. Compared with CE, the efficiency of HPLC is much poorer and tailing peaks were observed. But this disadvantage does not directly impact our work because we are more interested in the capacity factors of the solutes rather than their kinetics.

1.2.2. Chromatographic modes used in this work

In this thesis, three chromatographic modes have been utilized: reversed-phase liquid chromatography (RPLC), ion-exchange chromatography (IEC) and size-exclusion chromatography (SEC).

Separations in RPLC mode are based on non-specific hydrophobic interactions between solutes and stationary phases[62]. Most of the stationary phases are on substrates such as silica[55], zirconia[63] and alumina[57], with a bonded phase that is less polar than mobile phase. Typically, the mobile phases contain water and one organic modifier. Separation of analytes can be achieved with the variation of the composition of mobile phases. In this work, we immobilized dendrimers on zirconia surface and anion-exchange surface. Therefore, we wish to investigate the hydrophobic core of dendrimers in this mode by using small solutes that are commonly used for characterizing reversed-phase stationary phases[64]. The investigation of the solvation of the interior of dendrimer can be performed in a wide range of bulk solvents.

In SEC, molecules are separated on the basis of their hydrodynamic volume or size[65]. Usually, SEC employs small, rigid, porous particles with controlled pore size to obtain average molecular weight distribution. The mobile phase used has to be able to dissolve the samples and have a low viscosity. Particularly, it is optimized to eliminate or reduce the adsorption of solutes on stationary phase. In this work, we used size-exclusion chromatography to examine the purity of dendrimers and verify if there is

any size-exclusion effect on these macromolecules. These experiments were performed on porous zirconia surface and citrate was chosen as the mobile phase because of its efficient competition with the dendrimer molecules for the interaction sites on the zirconia surface.

Separation of sample ions in IEC is dependent on their relative affinity for the stationary phase ion centers compared to that of the mobile phase counterions[62]. Usually, polymeric porous particles, bonded-phase pellicular particles and bonded-phase porous particles are chosen as the IEC packings. IEC separations are often carried out with aqueous salt solutions as mobile phase. In this work, a strong anion-exchange chromatographic support was modified with polyamide dendrimers based on the principle of the ion-exchange chromatography, where the deprotonated carboxyl terminal groups on dendrimers interact with the positive charged anion-exchange surface through electrostatic interaction. Acetate buffer was employed as the mobile phase.

1.2.3 Modification of chromatographic supports

Zirconia chromatographic support

The modification of zirconia particles has converted this metal oxide surface to efficient chromatographic supports, including octadecyl-[66] aromatic polymer-[67], and polybutadiene[68]-coated zirconia. We are particularly interested in those stationary phases based on Lewis acid-base interaction mechanism. The Lewis acid sites on the zirconia surface can form coordination complexes with a number of Lewis bases. For example, the polyoxy anions (borate, sulfate, carboxylate and phosphate)[61,69,70], and fluoride[60,71]. Particularly, fluoride[72] and phosphate[73,74] modified zirconia have been applied as biocompatible stationary phases for high performance liquid chromatography. Although carboxylate has also demonstrated high affinity to the Lewis acid sites on zirconia surface, the applications of carboxylate modified zirconia in chromatography are few. Here, we present a method of modifying zirconia with multiple carboxyl terminated PAMAM dendrimers in Chapter 2.

Anion-exchange resins

The modification of anion-exchange resins has been intensively explored. A range of enzymes on anion-exchanger has been immobilized on the anion-exchangers as chromatographic reactors[75,76]. This modified anion-exchange resin can be recycled through the desorption of enzymes from the carriers[77]. The modification of anion-exchange through electrostatic interaction between the positively charged resin and negatively charged functional groups on the solutes studied has also been applied in catalysis[78,79], trace metal analysis[80], and gas sensors[81]. Here, we prepared a new stationary phase by immobilizing multiple carboxyl terminated polyamide dendrimers on a strong anion-exchanger via the electrostatic interactions between the negatively charged carboxyl groups on the periphery of the dendrimers and positively charged ammonium sites. This work has been summarized for potential publication. However, to keep the thesis concise, this part of work was decided not to be included in the thesis.

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CHAPTER 2

IMMOBILIZATION OF PAMAM DENDRIMERS ON ZIRCONIA CHROMATOGRAPHIC SUPPORT

2.1 INTRODUCTION

2.1.1 Motivation

The motivation of this work is as the following: 1) we wish to develop a method of immobilizing dendrimers on HPLC supports. 2) we wish to characterize immobilized dendrimers with HPLC using small probe solutes.

Studies have shown that in bulk water phase, a dendrimer molecule may resemble a micelle[i,ii,iii], with hydrophilic terminal groups at the periphery that allows water solubility, and a relatively hydrophobic interior. The micellar behavior was confirmed with several hydrophobic probes (e.g., diphyenylhexatriene, phenol blue[iii], and Bengal Rose[iv]) and a variety of techniques including fluorescence microscopy[iii,v] and UV-visible spectroscopy[iv].

However, these spectroscopic methods cannot reveal much quantitative information on the solvation properties of the interior of dendrimers. At the same time, these spectroscopic studies are confined to the use of large hydrophobic dye molecules

that are sensitive to solvation environment. Compared to these methodologies, liquid chromatography allows the use of small solutes to probe the interior solvation properties of dendrimers. Furthermore, liquid chromatography can provide quantitative information on the association of small probing solutes with dendrimers both thermodynamically and kinetically.

The main objective of this work was to investigate whether the carboxyl-terminated poly (amidoamine) (PAMAM) dendrimers can be immobilized on zirconia based chromatographic supports through multiple-point interactions. HPLC can assist to elucidate the mechanism of adsorption of dendrimers and the stability of the immobilized layer under a wide range of mobile phase compositions. Once accomplishing this goal, we aim to employ high-performance liquid chromatography to study the physical and chemical properties of various types of dendrimers with the small solutes as probes. Finally, we would be able to demonstrate the application of the modified zirconia surface as chromatographic stationary phase.

The modification of solid surface with dendrimer monolayer or multilayers has been discussed in several studies. Work has been performed on the adsorption of several types of dendrimers on charged alumina[vi,vii], and silica particles[viii]. Electrostatic interaction between the terminal groups and the activated substrate was attributed to the adsorption. However, the modification of zirconia surface with dendrimers has not been investigated, which can be employed as a chromatographic support with superior stability compared to other substrates. Therefore, the

immobilization of PAMAM dendrimers on zirconia surface should have promising applications. An additional advantage is the possibility of removing the adsorbed dendrimers using basic solutions, so that the renewed zirconia surface can be used for reloading dendrimers.

Strong interactions between carboxyl groups and zirconia surface Lewis acid sites are known[ix,x]. The nature of multiple carboxyl terminal groups on the surface should allow the dendrimer to be strongly adsorbed on the zirconia surface. We will apply the method of immobilizing carboxyl-terminated dendrimer developed in this work to future development of sensors and new chromatographic stationary phases, based on artificial receptor-incorporated dendrimers and polymeric micelles.

2.1.2 PAMAM Dendrimers

This class of star-shaped polymers is illustrative of the over 50 families of dendrimers represented in the patent art, and is the one most widely studied[xi,xii,xiii,xiv]. The poly(amidoamine) (PAMAM) dendrimers are typically based on an ethylenediamine (EDA) core. These dendrimers have repeating tertiary amine/amide branching units. The structure of generation 2.5 dendrimer is shown in Figure 2-1. Each complete reaction sequence would lead to a new dendrimer "generation" with a larger molecular diameter, twice the number of reactive surface sites, and approximately double the molecular weight of the preceding generation (Table 2-1). One of the key features of the PAMAM dendrimers is their synthetic adaptability for fitting a variety of applications. For example, structural modifications can result in dendrimers with amine surfaces[xv], hydroxyl surfaces[xvi], alkyl (hydrophobic) surfaces[xvii], or even mixed surfaces[xviii,xix] to meet specific performance requirements. In this study, we used carboxylate terminated PAMAM dendrimers, which are called half generation. If they are terminated in amine groups, they are called a full generation. We chose this carboxyl-terminated dendrimer in this study to test the hypothesis that carboxyl-terminal groups could interact with the Lewis acid sites on the zirconia surface under optimized conditions through the Lewis acid-base interactions.

Most experiments in this work were carried out at pH around the pKa of tertiary amines and therefore, about half of the tertiary amine groups inside the

PAMAM dendrimers were positively charged. In addition, amide groups in PAMAM dendrimers have hydrogen-bond donating and accepting capabilities. The presence of these groups contributes to the high polarity of the interior of PAMAM. In fact, small alkylbenzenes do not have large partition coefficients between aqueous phase and PAMAM dendrimers. At pH 10.6, only large planar polyaromatic hydrocarbons show significant partition in generation 3.5 PAMAM dendrimer[xx].

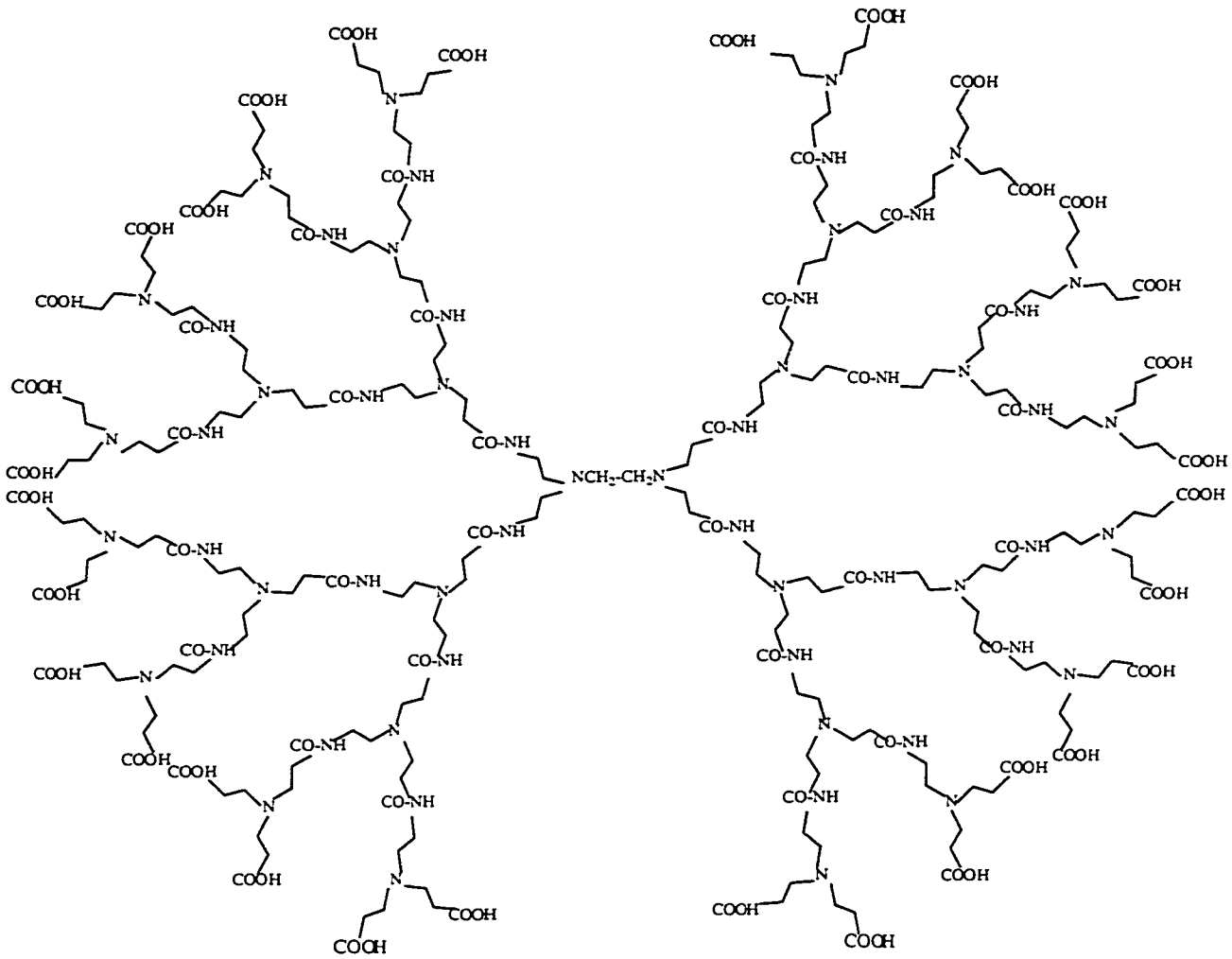


Figure 2-1 Structure of carboxyl-terminated PAMAM dendrimer (generation 2.5).

Table 2-1 Parameters of Starburst™ PAMAM Dendrimers (Carboxyl terminated)

Generation	Molecular Weight	Diameter(Å)[a]	Surface Groups
0.5	1269	15	8
1.5	2935	22	16
2.5	6011	29	32
3.5	12419	36	64
4.5	25234	45	128

[a] Values are from the webpage of Dendritech Inc. which were for the full generations and were determined by intrinsic viscosity measurements. The half-generations studied in this work are slightly larger due to the extra $-CH_2-CH_2-COOH$.

2.1.3 Zirconia Chromatographic Support

2.1.3.1 Chromatographic properties of zirconia

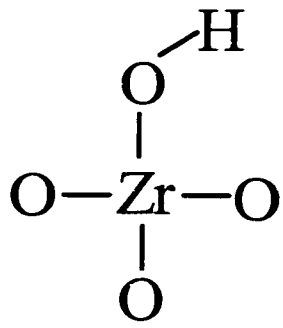
Zirconia, *i.e.* zirconium dioxide, has interesting properties and numerous applications in modern technology[xxi,xxii,xxiii,xxiv]. Particularly, a series of studies of zirconia as a column packing material for high-performance liquid chromatography (HPLC)[xxv,xxvi,xxvii,xxviii,xxix,xxx] have been conducted.

The chief advantage of zirconia relative to silica as chromatographic support is its superior chemical and thermal stability although the physical and mechanical properties of zirconia and silica are quite comparable. Silica-based supports are not stable at extreme pHs (less than 2 and greater than 8)[xxxi], which hinders their application. In contrast, studies have demonstrated the pH stability of zirconia and modified zirconia over a wide range of pHs, from pH 0 to pH 14[xxxii,xxxiii]. After sintering at 900°C, porous zirconia maintains its well-defined pore size and excellent pore connectivity for many hours. Both polybutadiene and carbon-coated zirconia can withstand operating temperature up to 200 °C[xxxiv]. In these respects, zirconia can be an attractive alternative to traditional silica substrates.

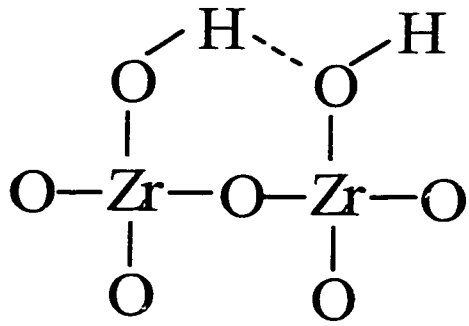
2.1.3.2 Surface chemistry of zirconia support

A second reason for exploring the use of zirconia support is to take advantage of the unique surface chemistry of this particular support.

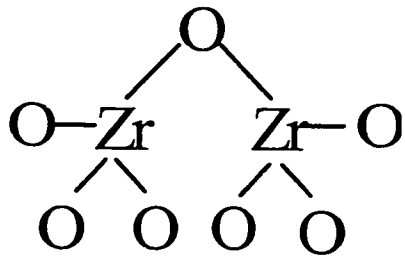
The chemistry of zirconia surface is complex. A number of distinct classes of sites exist which can contribute to the retention of a given solute. These sites can be Brönsted acid sites, Brönsted base sites, and Lewis acid sites[xxxv,xxxvi,xxxvii] as shown schematically in Figure 2-2. These sites will interact differently with different solutes.



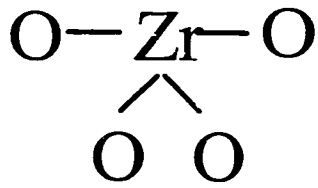
Brönsted Acid Site



Strong Brönsted Acid Site



Brönsted Base Site



Lewis Acid Site

Figure 2-2 Surface sites on Zirconium oxide.

In this study, there are two types of active sites that can potentially interact with terminal carboxyl groups on dendrimer. The first type is the Lewis acid sites that can strongly interact with carboxyl groups. The other is the negatively charged surface hydroxyl groups at pHs higher than 6.7, the isoelectric point of zirconia. The negatively charged surface groups can potentially interact with positively charged tertiary amine groups in PAMAMs.

Various studies have shown that the presence of coordinatively unsaturated zirconium (IV) sites is the dominant feature of the surface chemistry of zirconia. These sites act as rather strong "hard" Lewis acids[xxxviii] and can form coordination complexes with a number of Lewis bases. For example, the polyoxy anions (borate, sulfate, carboxylate and phosphate)[ix,x,xxxix,xl], and fluoride[xxxviii,xli]. It has been reported that the presence of strong Lewis bases such as dicarboxylic acid can generate a negative charge on the zirconia surface resulting in the adsorption of cations[xli]. The strong Lewis acid-base interactions between Lewis bases and zirconia surface have been applied to surface modifications. Particularly, fluoride[xlii] and phosphate[xliii] modified zirconia have been used as biocompatible stationary phases for high performance liquid chromatography.

Infrared studies of zirconia have confirmed the presence of at least two types of surface hydroxyl species[xxxvii,xliv] (Figure 2-3): the single, terminal hydroxyl (A), which is similar to the free silanol of silica, and the bridging hydroxyl (B) where a single oxygen is shared by two zirconium atoms. The negatively charged surface can

interact with the positively-charged tertiary amine groups in dendrimer, which may add complexity to the adsorption of dendrimers.

The surface hydroxyl groups may be protonated or deprotonated depending on the solution pH. Surface hydroxyl groups have a pKa of about 8.1 while protonated surface groups have a pKa of about 4.0.

$$Zr\text{-}OH_2^+ \Leftrightarrow \underset{pKa\ 4.0}{Zr\text{-}OH} + H^+ \Leftrightarrow \underset{pKa\ 8.1}{Zr\text{-}O^-} + 2H^+$$

The modification of zirconia surface with PAMAM dendrimers was conducted at pH 5, at which the net surface charge is close to neutral. Therefore, the electrostatic interactions between the surface hydroxyl groups and the amine groups are minimized.

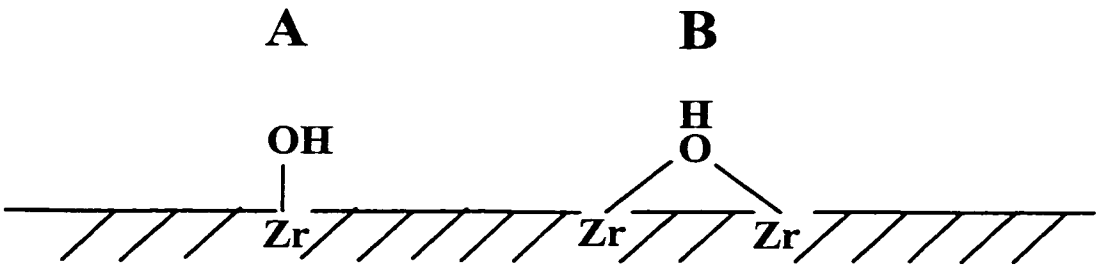


Figure 2-3 Two types of hydroxyl groups on zirconia surface.

2.2 Experimental

2.2.1 Reagents and Materials

Dendrimer:

Starburst™ (PAMAM) Dendrimer Generation 0.5, 20 wt. % solution in water

Starburst™ (PAMAM) Dendrimer Generation 1.5, 20 wt.% solution in methyl alcohol

Starburst™ (PAMAM) Dendrimer Generation 2.5, 10 wt.% solution in methyl alcohol

Starburst™ (PAMAM) Dendrimer Generation 3.5, 10 wt.% solution in methyl alcohol

Starburst™ (PAMAM) Dendrimer Generation 4.5, 20 wt.% solution in methyl alcohol

All of the dendrimers, citric acid monohydrate (99%), sodium nitrate, anthracene and triphenylene were from Aldrich (Milwaukee, WI, USA). Acetic acid glacial (17.4 M) and nitric acid were from Anachemia (Montreal, Canada). Sodium hydroxide (analytical reagent) was from BDH Inc (Toronto, ON, Canada). Trizma base (tris[hydroxymethyl]aminomethane, electrophoresis reagent) was from Sigma (St. Louis, MO, USA). Pyrene was from Fluka AG Chem. Fabrik. Naphthalene was from Fisher Scientific (Fire Lawn, NJ, USA)

Water used in HPLC mobile phases was house-deionized and was further purified by passing it through a Barnstead/Thermolyne (Dubuque, IA, USA) to give 18.3 MΩ· cm resistivity.

All of the buffers were prepared by adjusting pH values with an Accumet AR15 pH Meter (Fisher Scientific, Nepean, ON, Canada) followed by filtration with a 0.45 Micron membrane filter (MSI, Minnetonka, MN, USA). Acetonitrile used for the mobile phase was HPLC grade and was obtained from Fisher Scientific, Nepean, ON, Canada.

The 1% (w/w) dendrimer samples from generation 1.5 to 4.5 were prepared by evaporating the methanol in the original dendrimer solutions through bubbling Nitrogen gas, followed by dissolution in the buffers accordingly. The 1% (w/w) generation 0.5 dendrimer sample was prepared in the same fashion without evaporation.

2.2.2 Column packing:

Stainless steel blank columns of 5 cm x 0.46 cm dimension were packed with porous zirconia particles obtained as a gift from Zirchrom^{TM} (Zirchrom, Anoka, MN). The columns were packed by using a down-slurry column packer from Alltech (Deerfield, IL, USA) using isopropanol as both the slurry and the displacing solvent. The specific surface area of zirconia particles is 30 m^2/g, the average pore size is 300 Å, and the average particle diameter is 3.4 μm.

2.2.3 Chromatographic conditions:

All studies were conducted on a Hewlett-Packard 1100 high-performance liquid chromatograph system. All of the dendrimers were detected at 230 nm and all of the PAH (polycyclic aromatic hydrocarbon) solutes were detected at 254 nm at 25°C or 30°C (column temperature) unless otherwise indicated. In all of the experiments, the flow rate was maintained at 1.000 ml/min unless otherwise indicated.

2.2.4 Surface Modification Procedure

Surface modification of zirconia with PAMAM dendrimers was performed in a home-packed zirconia HPLC column. In this study, generation 3.5 PAMAM dendrimer solutions of 1% (w/w) concentration was repeatedly injected to the column at 100 μL injection volume, which was equivalent to 6.33×10^{-8} moles of generation 3.5 PAMAM dendrimer per injection. Mobile phase used was 10 mM, pH 5.0 acetate buffer at a flow rate of 1 ml/min. After a certain number of injections of a dendrimer solution, naphthalene, anthracene, and pyrene were injected under reversed-phase mobile phase conditions to test the degree of surface coverage by dendrimers.

2.3 RESULTS AND DISCUSSION

2.3.1 Immobilization of PAMAM Dendrimer on Zirconia Surface

We found generation 3.5 PAMAM dendrimer did strongly adsorb on zirconia surface under 10 mM pH 5 acetate mobile phase condition, as the total peak area detected from thirty two injections was about 36% (standard deviation: 5%) of the total area of the same number of injections without the column. Also the peak area for the first injection was about 20% (standard deviation: 5%) of that for the 32nd injection of the dendrimer solution (Figure 2-4). In the calculation of uncertainties, we estimated 10% standard deviation in the peak areas of dendrimer solutions without column and assumed this standard deviation remained constant during the injections for modification of zirconia surface. In fact, the conditions in column are more complicated than that without column. Therefore, we have simplified the calculation and might have underestimated the uncertainties. The area of the 32nd injection was almost the same as the area without column. This indicates that as we repeatedly injected PAMAM dendrimer solution to the zirconia column, the surface coverage increased. The increase in peak area should result from the breakthrough of dendrimer.

Multiple peaks appeared in both chromatograms. This might be due to polydispersity of the PAMAM dendrimer supplied by Aldrich. In an early study, we observed multiple peaks while injecting PAMAM samples to a C_{18} reversed-phase chromatographic column. We can use other techniques, for example, mass

spectroscopy, to confirm this hypothesis. The first sharp peak in the two chromatograms might be system disturbance peak due to mismatch of solvents used for the sample and the mobile phase. As surface coverage increases, the dendrimers injected to the column would be able to interact with the immobilized dendrimers on the zirconia surface. This interaction may be responsible for the multiple peaks in the chromatogram of 32^{nd} injection.

Figure 2-4 Chromatogram of the elution of PAMAM Dendrimers (a) the 1st injection; (b) the 32nd injection. Column with 5.0 x 0.46 cm i.d. was packed with zirconia particles. The injection was 100 μL 1% (w/w) PAMAM dendrimer (generation 3.5), detected at 230 nm. Mobile phase was 10 mM, pH 5 acetate buffer at a flow rate of 1 mL/min. Column temperature was 30°C.

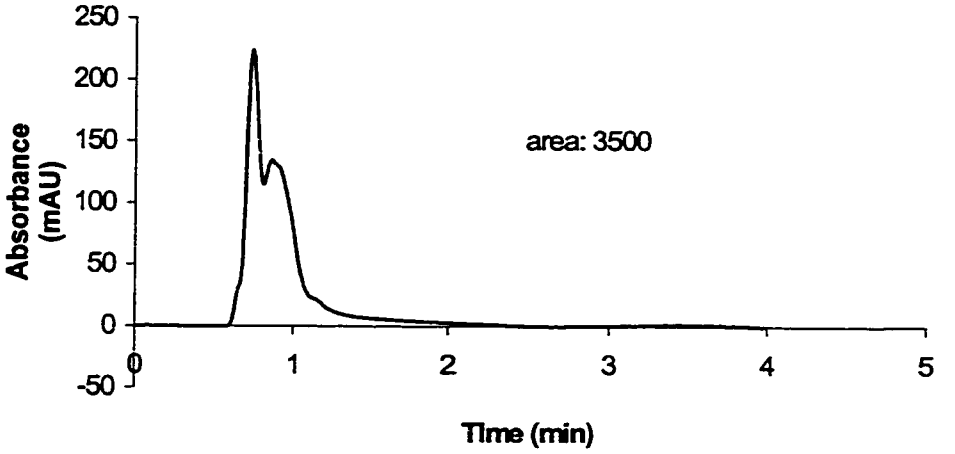


Figure 2-4 (a)

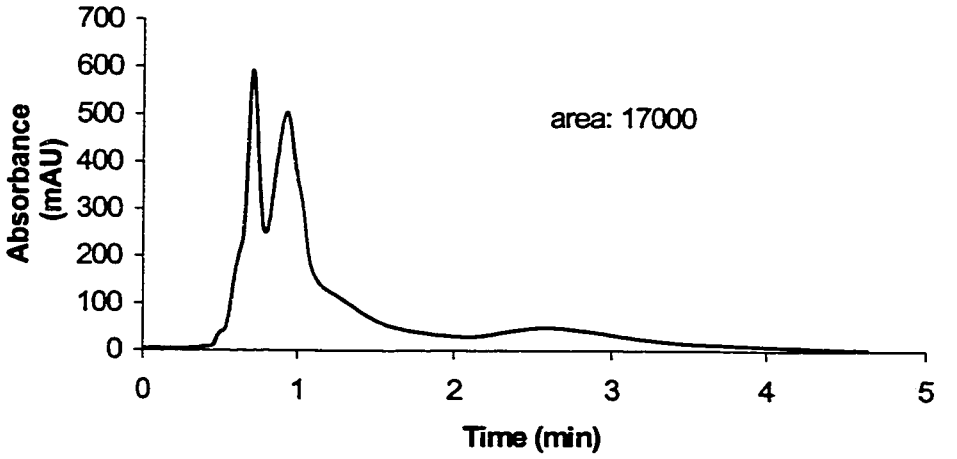


Figure 2-4 (b)

Three polycyclic aromatic hydrocarbons (PAHs) were employed as probing molecules for monitoring the surface coverage. The retention time for naphthalene, anthracene and pyrene increased with the number of injections of dendrimer solutions (Figure 2-5). The maximum points of the chtomatograms were taken as the retention time. The increase in capacity factors of PAHs can also be regarded as a proof of the surface coverage as k' increases with phase ratio Φ according to the following equation, assuming adsorption mechanism did not change during the process of modification:

$$k' = K \times \Phi$$

where K is the partition coefficient. From Figure 2-5, we have three interesting observations: 1) the larger the size of the PAHs, the longer the retention time. This is consistent to the results in reversed phase chromatography and also consistent to the literature observation when using PAMAM dendrimers as pseudostationary phase[xx]. Researchers have shown that the interior of high generation dendrimers is much more hydrophobic than the exterior[xlv]. Therefore, PAHs, one class of very hydrophobic molecules, would prefer to interact with the interior of the dendrimers rather than the surface; 2) after about 30 injections, the retention capacity factors reached a plateau. The plateau points slightly differed from each other for these three molecules. This observation reflected the saturation of surface available adsorption sites and further injections did not have significant effect on the retention time of PAHs; 3) with a few more injections of dendrimers to the column after the first plateau was reached, a second plateau appeared after around 65 injections. This is especially obvious for

anthracene and pyrene. This might be due to the formation of multiple layers. It would be necessary for us to perform more detailed studies to gain a clear understanding.

We found that the chromatograms of the PAHs at large number of injections of dendrimers were tailed. The larger the PAH molecule, the more serious was the tailing. The chromatograms of naphthalene and pyrene after 1^{st} and 80^{th} injections of dendrimers are shown in Figure 2-6. This might be due to formation of multiple layers at high injection numbers, leading to the slow diffusion of the PAHs in the dendrimer-modified stationary phase. Overloading of sample might also contribute to peak tailing. In order to test whether overloading is also responsible for peak tailing, we compared the normalized peak shape of 4 times diluted pyrene sample with the normalized peak shape of sample solute that is 4 times as concentrated (Figure 2-7). The normalization was performed by dividing the absorbance of all the data points under the peak areas by the maximum absorbance. We found that the peak shape was not improved at the low sample concentration. Therefore, we conclude that sample overloading is not the major cause of peak tailing.

In this work, as we assume that the slow mass transfer could be the major reason for the tailing of the peaks, we decided to use an injection number of 32 as our optimal protocol for immobilizing PAMAM dendrimers on zirconia.

Figure 2-5 PAHs as probing molecules for the modification of zirconia surface with PAMAM dendrimers (generation 3.5). (a) naphthalene; (b) anthracene; (c)pyrene. The 5.0 x 0.46 cm column was packed with zirconia particles. Mobile phase composition was acetonitrile/pH 5 10 mM acetate buffer =10/90 (v/v). Flow rate was 1 mL/min with detection at 254 nm. Column temperature was maintained at 30°C.

Figure 2-6 Retention of PAHs during the process of modification. (a) after the 1st injection of dendrimer; (b) after the 80th injection of dendrimer.1: naphthalene; 2: pyrene. 5.0 x 0.46 cm column was packed with zirconia particles that were modified with PAMAM dendrimers (generation 3.5). Mobile phase composition was a 90/10 (v/v) mixture of 10 mM, pH 5 acetate buffer and acetonitrile at a flow rate of 1 mL/min. Column temperature was 30°C. Detection was at 254 nm.

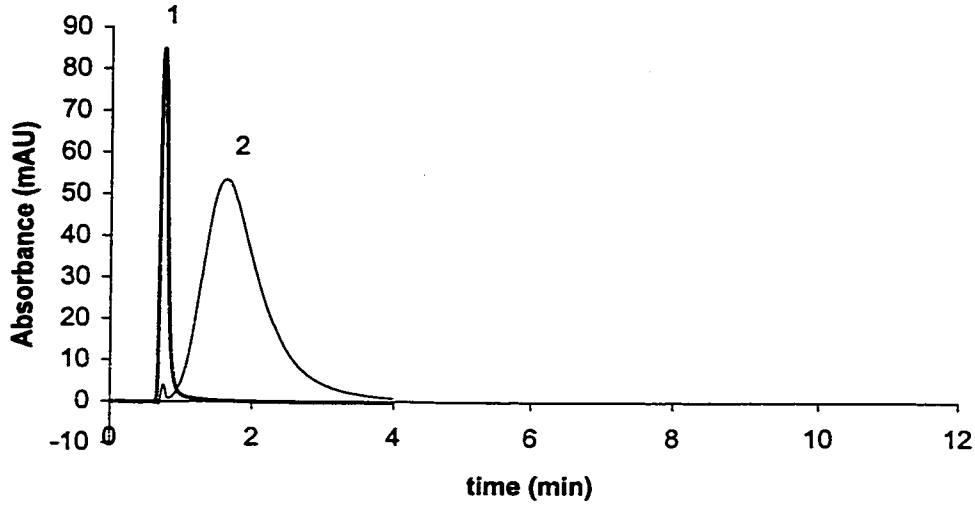


Figure 2-6 (a)

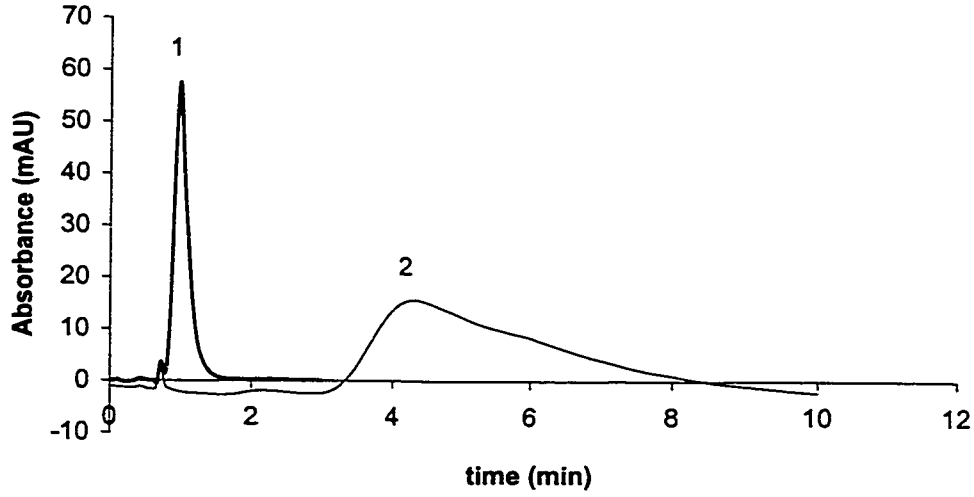


Figure 2-6 (b)

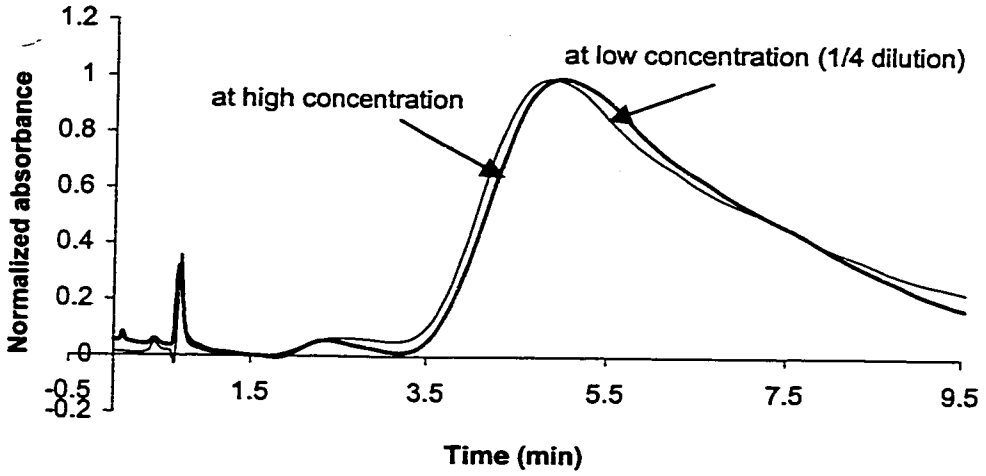


Figure 2-7 Study of effect of sample concentration on peak tailing. The solutes were pyrene. These experiments were done on a PAMAM dendrimer (generation 3.5) modified zirconia surface. The mobile phase was a 90/10 (v/v) mixture of 10 mM pH 5 acetate buffer and ACN. Flow rate was 1.0 mL/min. Detection wavelength was 254 nm. Column temperature was 30 °C. The low concentration solution was obtained by diluting the high concentration solution by 4 folds.

2.3.2 Estimation of surface coverage

2.3.2.1 Calculation scheme of surface coverage

We found that after thirty two injections, the amount of PAMAM dendrimers retained on the zirconia surface is equivalent to a surface coverage of 29 %. In the calculation, we have the following assumptions: 1) dendrimers (generation 3.5) adopted an extended conformation with a radius of 20Å; 2) no interpenetration among dendrimers occurred; 3) immobilized dendrimers adopted compact surface packing as shown in Figure 2-8; 4) all surface area was accessible for dendrimers and no size exclusion effect involved. Therefore, the specific area of zirconia particles, 30 m^2/g, was applied in the calculation. 5) only monolayer formed; 6) zirconia surface was saturated at the 32nd injection. The scheme of calculation is shown in Figure 2-8. As the shaded hexagon area occupied by three circles corresponds to three dendrimers, the effective surface area occupied by a single dendrimer molecule is calculated as $A_d = 2 \times \frac{\sqrt{3}}{4} \times d^2$, which is slightly larger than the area of a circle, πr^2. Hence, the percent of surface coverage was defined as:

$$S\% \equiv \frac{\text{Surface area occupied by retained PAMAM dendrimers}}{\text{Total surface area of the column}} \times 100\%$$

The percentage of retained dendrimers $S_r\%$ was calculated based on the peak areas:

$$S_r\% = \frac{A_p \quad n - A_t}{A_p \quad n} = \frac{17000 \quad 32 - 195000}{17000 \quad 32} = 64\%$$

where "17000" corresponds to the area of one injection detected without a column, A_p; "195000" is the total area detected during the first 32 injections, A_t. Therefore, the percent of surface coverage was calculated as:

$$S\% = \frac{A_d \times N_a \times M \times n \times S_r\%}{m \times s} \times 100\%$$

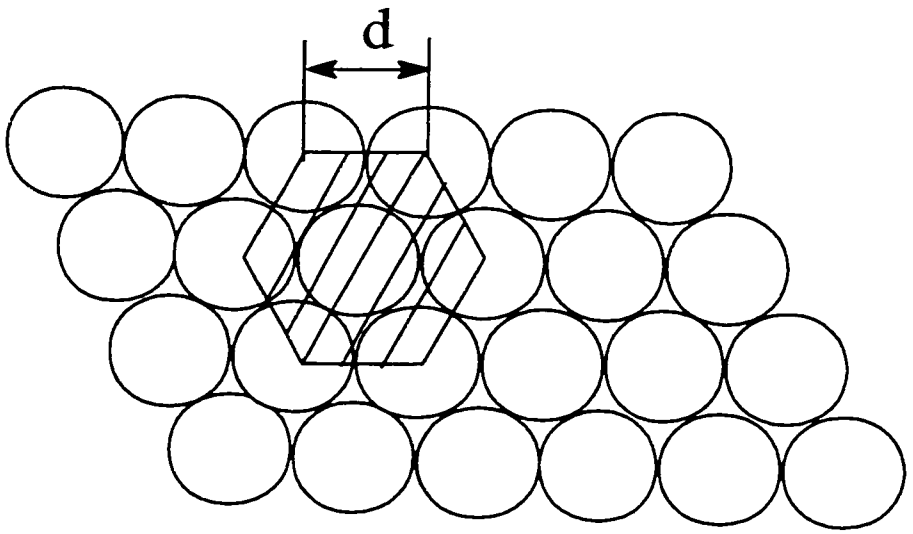
$$= \frac{2 \times \frac{\sqrt{3}}{4} \times (20 \times 2 \times 10^{-10})^2 \times 6.02 \times 10^{23} \times 6.33 \times 10^{-8} (mol/_{inj}) \times 32 inj \times 64\%}{1.2524(g) \times 30(m^2/_g)}$$

$$= 29\%$$

where N_a is the Avogadro constant; M is the amount of dendrimer injected per injection in molar; n is the number of injection to reach the plateau, which is 32 here; m is the mass of zirconia particles when unpacked from the column and s represents the specific area of zirconia particles.

The low coverage is very likely due to the inaccessible surface area in the micropores of the packings. Therefore, a size-exclusion chromatography study was performed to test this assumption.

The surface coverage can also be measured by eluting all the adsorbed dendrimers adsorbed on the zirconia surface and calculating the ratio of the eluted dendrimers to the dendrimers required for the formation of monolayer.



d: diameter of one dendrimer molecule

Figure 2-8 Schematic for the calculation of surface coverage.

2.3.2.2 Size exclusion studies of the PAMAM dendrimers

In order to explain the experimental surface coverage, we studied whether all of the zirconia surface area is accessible to the PAMAM dendrimers. We also hope to know the purity of dendrimers employed. Therefore, we performed size exclusion chromatography of PAMAM dendrimers.

Based on the hydrodynamic molecular volume or size, molecules can be separated by size exclusion chromatography (SEC). The molecular mass distribution and the statistical molecular mass averages can be readily obtained with proper column calibrations[xlvi,xlvii,xlviii] or by the use of molecular-mass-sensitive detectors, such as light scattering[xlix,l], viscometry[li,lii], or mass spectrometry[liii,liv]. Therefore, SEC has been an important method to characterize the molecular mass of polymers[lv,lvi,lvii,lviii]. In this study, we tested whether PAMAM dendrimers were excluded from the pores of the zirconia support by comparing them with low-molecular weight solutes.

The experiments were conducted on a bare zirconia column with a low flow rate, 0.2 mL/min. A pH 7.6, 10 mM citrate buffer was used as the mobile phase. We chose this condition based on our previous findings. Under this condition, dendrimers should have no enthalpic interactions with the zirconia surface and their elution time should be based on the entropic interactions with the stationary phase. Benzene was also assumed to be free of enthalpic interactions with zirconia surface and have a

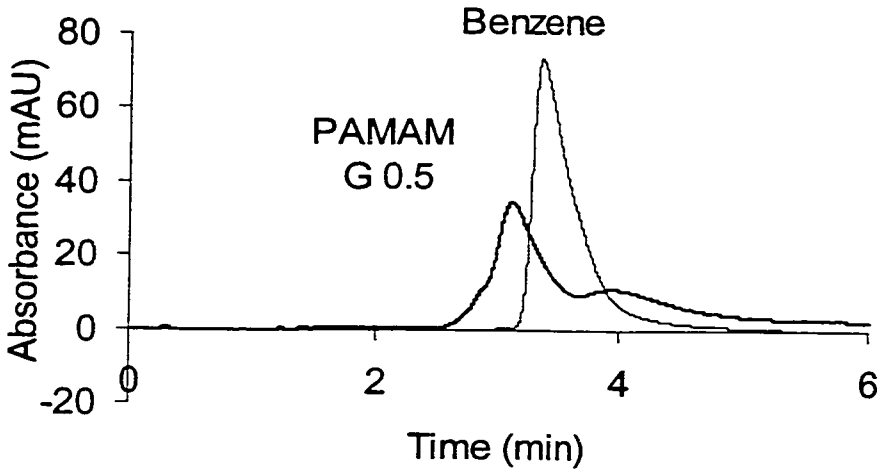
small size. Therefore, benzene was supposed to elute from the column at the void volume without being excluded. For large solutes, exclusion would occur and they would elute before the void volume. The chromatograms of benzene and PAMAM dendrimers of generation 0.5 and 3.5 are shown in Figure 2-9.

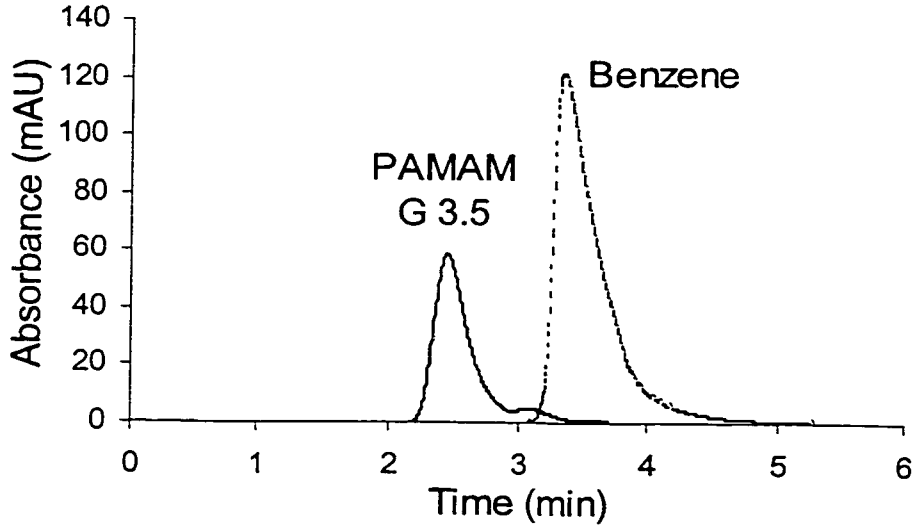
Generation 3.5 PAMAM dendrimer eluted earlier than benzene with a rather sharp peak, confirming that size exclusion effect does exist. As a consequence, some dendrimers injected into the column might have been excluded from micro-pores, which could be one possible explanation for the relative low surface coverage. However, large pores in zirconia support still allow the access of dendrimers, leading to the 29% surface coverage. The sharp peak can be regarded as an indication of narrow molecular mass distribution of the generation 3.5 dendrimer. Dendrimer of generation 0.5 eluted later than generation 3.5, indicating a smaller size. The shoulder-peak is likely due to a low-molecular weight impurity.

It is noteworthy that the plate number computed based on benzene is 221, indicating that the bare zirconia column was reasonably well-packed. We will compare this number to the dendrimer-modified zirconia column in later sections.

Figure 2-9 Size exclusion chromatography of bare zirconia surface. Analytes: PAMAM dendrimers of generation 0.5, 3.5, and benzene.

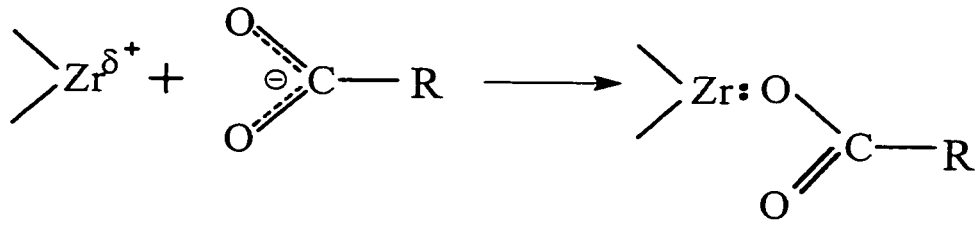
Stationary phase was monoclinic ZrO_2 particles. Mobile phase was 10 mM citrate buffer at pH 7.60. Flow rate: 0.2 mL/min with detection at 230 nm for dendrimers and 254 nm for benzene. Temperature was 30°C


Benzene
PAMAM
G 0.5
Absorbance (mAU)
80
60
40
20
0
-20
0
2
4
6
Time (min)


Benzene
PAMAM
G 3.5
Absorbance (mAU)
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
Time (min)

2.3.2 Mechanism of Adsorption

As we desire to have a dendrimer-modified surface stable under a wide range of experimental conditions, Lewis acid-base interaction is more preferable to electrostatic interaction because it is less dependent upon the pH and the ionic strength of the mobile phase. It has been reported that the dominant feature of the surface chemistry of zirconia is the presence of coordinatively unsaturated zirconium (IV) sites that act as rather strong "hard" Lewis acids[xxxviii]. Therefore, hard Lewis acids tend to form strong complexes with "hard" Lewis bases, for example, carboxylates. The end-on adsorption of carboxylate group on zirconia surface is schematically shown as below:



To test whether the Lewis acid-based interaction played a predominant role in the adsorption of the carboxyl-terminated PAMAM dendrimer on zirconia, we investigated the effect of buffer conditions on the adsorption of dendrimers of various generations. We found that in acetate buffer, PAMAM dendrimers of generation 0.5 to 4.5 were strongly adsorbed on the zirconia surface in a pH range from 3 to 7, regardless of an increase of ionic strength from 10 mM to 2.0 M. However, when the citrate buffer was used, the retention of dendrimers varied with the pH and the ionic

strength of the buffer. In 10 mM citrate buffer, when pHs were below 3.13, the pK_{a1} of the citric acid, dendrimers of generation 0.5 through 4.5 did not elute from the zirconia column. Under this pH condition, the carboxyl groups of citric acids were mostly protonated. Therefore, there were no sufficient number of ionized carboxyl groups on citric acids that could compete with dendrimers for the interaction sites on zirconia. Therefore, the PAMAM dendrimers can interact with the active sites on the zirconia surface. In the pH range between 3.13 and 4.76 (pK_{a2} of citric acid), only the dendrimer of generation 0.5 eluted at the void volume while all the other higher generations did not show noticeable elution. One possible explanation of the difference between generation 0.5 and higher generations is that the number of sites of interaction is fewer in generation 0.5 (Figure 2-10). The high-generation dendrimers have a high density of –COOH groups at the surface so that multiple-point interactions might have occurred. The degree of forming the multiple-point Lewis acid-base interactions would differ for dendrimers with different surface densities of carboxyl groups. The higher generation dendrimers with more available carboxyl groups per molecule would have a better chance to be adsorbed. At pHs higher than 4.76, none of the dendrimers could be retained on the column at an ionic strength of 10 mM. The results are summarized in Table 2-2.

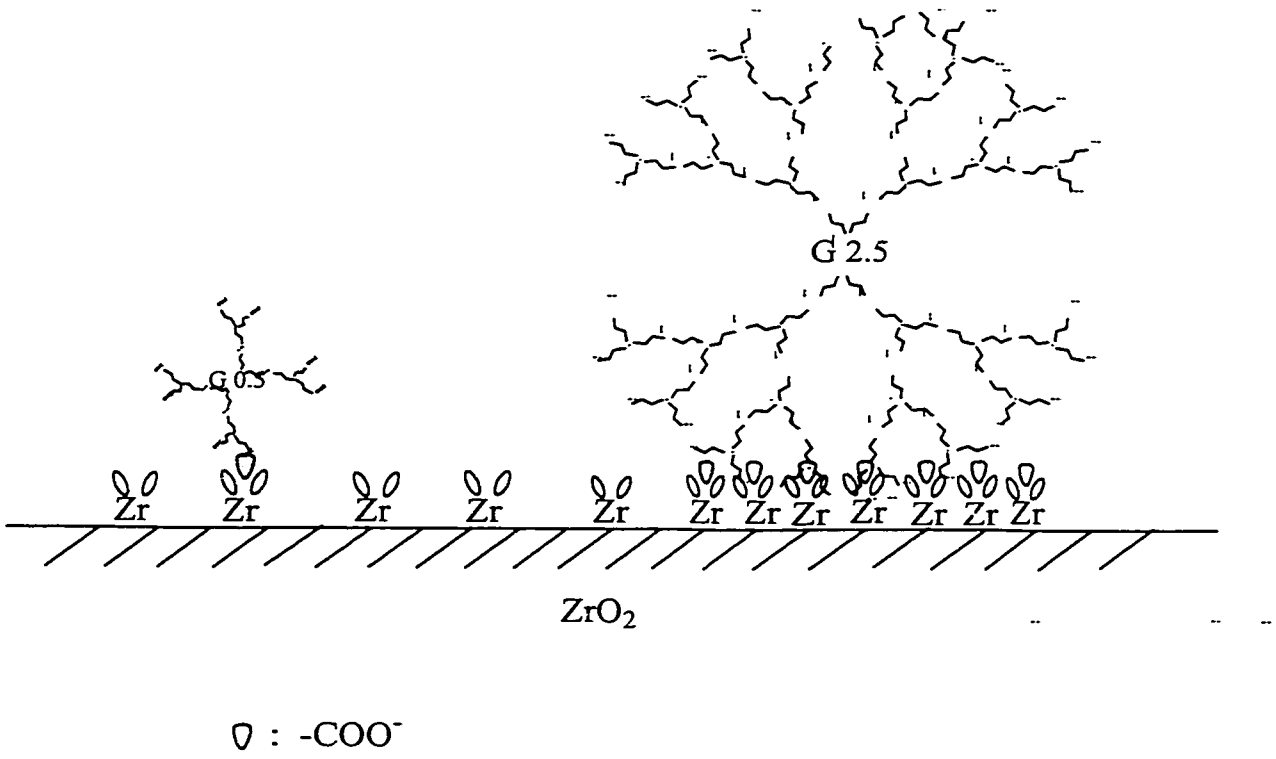


Figure 2-10 Schematic of interactions between dendrimers and zirconia surface.

Table 2-2 Effect of Buffer Conditions on the Adsorption of Dendrimers

Dendrimer[a]	Acetate Buffer pH 3 to 7		Citrate Buffer[c] 10 mM ionic strength			10 mM NaOH
	μ^b = 10 mM	μ =2.0 M[c]	pH < 3.1	3.1 < pH < 4.8	pH > 4.8	pH 12
G 0.5	Ads.	Ads.	Ads.	elute[f]	elute	elute
G 1.5	Ads.	Ads.	Ads.	Ads.	elute	elute
G 2.5	Ads.	Ads.	Ads.	Ads.	elute	elute
G 3.5	Ads.	Ads.	Ads.	Ads.	elute	elute
G 4.5	Ads.	Ads.	Ads.	Ads.	elute	elute

[a] 20 μL of 1 % (w/w) PAMAM dendrimer solutions were injected to a bare-surface zirconia column under different mobile phase compositions. Flow rate was 1.0 mL/min, detector was set at 230 nm, column temperature was 30 °C. [b] Ionic strength. [c] 2.0 M sodium nitrate solution with 10 mM acetate buffer. [d] Ads. = Dendrimer adsorbed. i.e. more than 80% of peak area diminished due to adsorption. [e] Citrate: pK_{a1} = 3.14, pK_{a2} = 4.76. [f] More than 80% of peak area eluted at the void volume.

The fact that citrate buffer was a much more effective displacer of the adsorbed PAMAM dendrimer relative to acetate buffer indicates that the carboxyl terminal groups on the dendrimer contributed to the strong adsorption of the dendrimers on zirconia surface. Because ionic strength played less of a role in displacing dendrimers, we concluded that it was Lewis acid-base interaction rather than electrostatic interaction that was responsible for the adsorption. The structure of citric acid is shown in Figure 2-11. It is likely that citric acid with three carboxyl groups may have a larger association constant with the Lewis acid sites on the zirconia surface compared to acetic acid presumably due to the chelating effect, and hence compete more efficiently with the adsorption of the dendrimers.

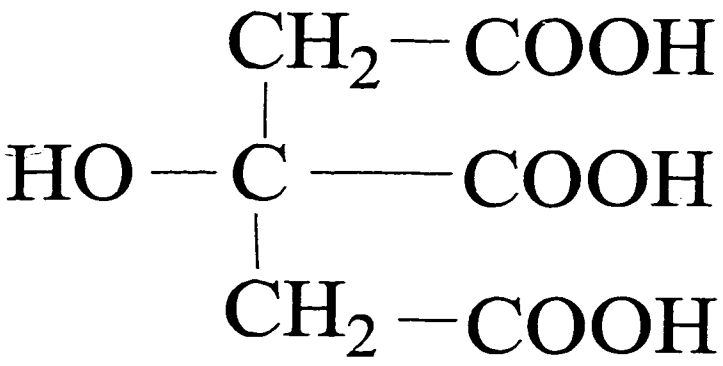


Figure 2-11 Structure of citric acid.

2.3.3 Properties of PAMAM Dendrimer-Modified Surface

2.3.3.1 Stability Studies

To investigate the stability of the immobilized dendrimer monolayer, we monitored the retention of naphthalene, anthracene, and pyrene as a function of the volume of mobile phase pumped through the column. The mobile phase tested was a mixture of acetonitrile and pH 5, 10 mM acetate buffer with a volume ration of 10/90. As shown in Figure 2-12, The capacity factors of these PAHs did not change significantly over 720 column volumes. This suggests a stable dendrimer-modified surface.

However, without being used for 10 days, the surface was checked again and the retention time of the three PAHs decreased to the level of about 24 to 28 injections of PAMAM dendrimers. The capacity factors increased after a few more injections of dendrimer solutions. This change reflected the strength of the interaction. Some dendrimer molecules might have only adsorbed on surface without forming real Lewis acid-base bonds. They may slowly disadsorb from the surface over storage and eluted with the mobile phase, resulting in the decreased retention of PAHs. Similar effect has been reported by Schefer *et al.*[lix] on their porous phosphate-modified zirconia substrate.

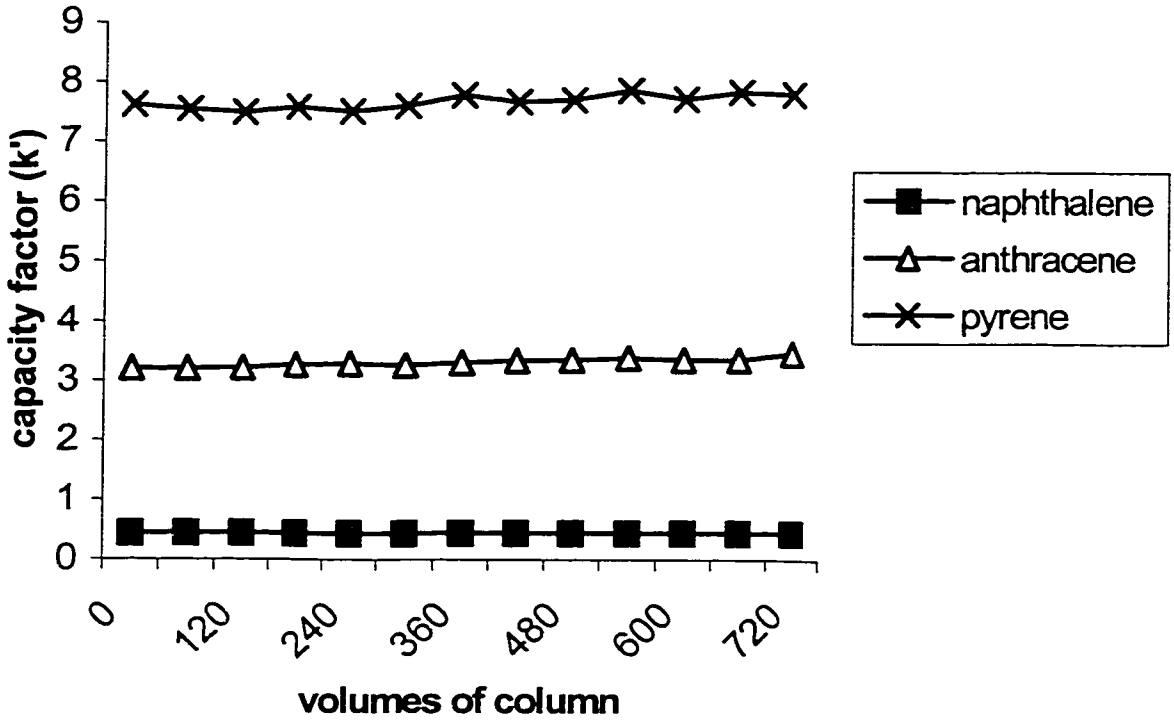
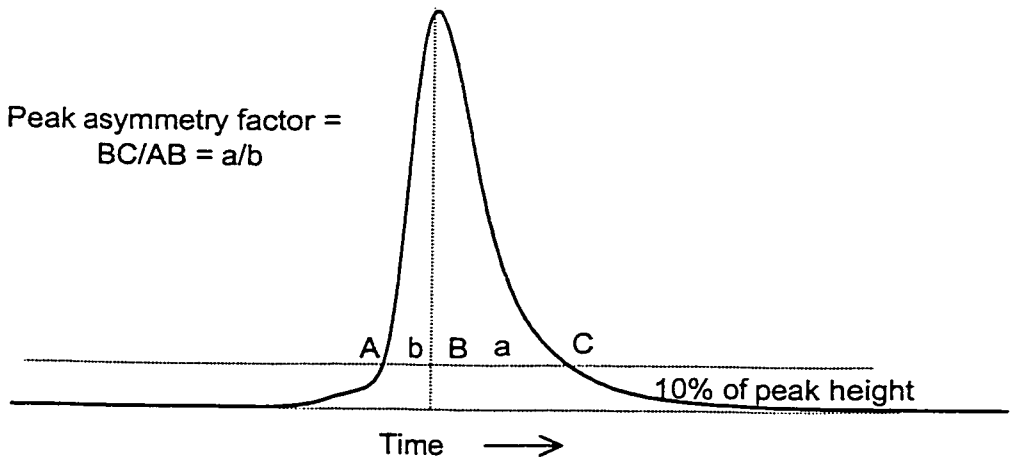


Figure 2-12 Stability of PAMAM dendrimer (generation 3.5)-modified zirconia surface. Tests were done in a 5.0 x 0.46 cm column packed with dendrimer modified zirconia particles. Composition of eluent was a 90/10 (v/v) mixture of 10 mM acetate buffer (pH 5) and acetonitrile. Flow rate was 1 mL/min with detection at 254 nm. Column temperature was 30°C.

Table 2-3 lists the plate numbers and asymmetry factors for benzene on bare zirconia surface and the three PAHs on this dendrimer-modified surface. The plate numbers for these asymmetric bands were calculated based on the following equation[lx]:

$$N = \frac{41.7 \left(\frac{t_r}{w_{0.1}}\right)^2}{\frac{a}{b} + 1.25}$$

where t_r is the retention time for the molecule studied; $w_{0.1}$ is the peak width at 10% of peak height, a/b is the asymmetry factor of this peak, which is defined as following:



Compare the plate number on the bare zirconia surface to those gained from the modified surface, the plate number is much larger in the former case. This is presumably due to the slow mass transfer of molecules in this modified surface.

Table 2-3 Plate Number and Asymmetry Factor of Probing Molecules

	Plate Number (N)	Asymmetry Factor (a/b)
benzene[a]	221	2.54
naphthalene[b]	72	1.75
anthracene	26	9.33
pyrene	15	3.69

[a] On bare zirconia surface

[b] On PAMAM dendrimer generation 3.5 modified zirconia surface

2.3.3.2 Regeneration of zirconia surface

We investigated the possibility of removing the adsorbed PAMAM dendrimer at high pHs because hydroxide is also a hard Lewis base. We found that at pH 12, the adsorbed dendrimers can be completely removed, as indicated by detection of a large peak due to the elution of dendrimers. We also found that PAH molecules were no longer retained on this cleaned zirconia column. Furthermore, addition of citrate may also assist the removal of dendrimers. Therefore, dendrimer-modified surface can be cleaned by flushing the zirconia support with strong alkaline solution and the regenerated surface can be re-modified with other dendrimers. This provides a convenient way to study various dendrimers using one zirconia column.

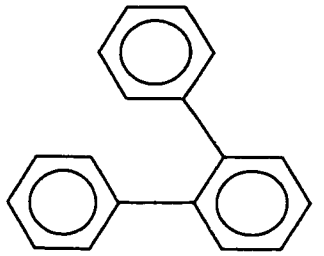
2.3.4 Shape-selectivity of PAMAM–stationary phase

The excellent stability of the PAMAM dendrimer-modified stationary phase allows us to explore the chemical properties of this dendrimer by investigating their interactions with small solute molecules. We studied the shape selectivity of the immobilized dendrimers of three terphenyl isomers. The structure selectivity provided by the starburst dendrimers has been reported as pseudostationary phase in electrokinetic chromatography[xx]. This study will provide information on surface-adsorbed PAMAM dendrimers.

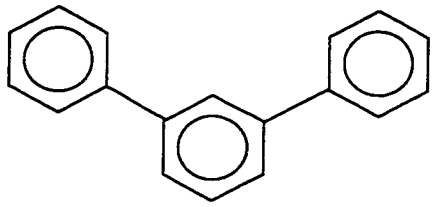
The structure of *o*-terphenyl , *p*-terphenyl and *m*-terphenyl are shown in Figure 2-13. A solvent gradient elution scheme was applied. Figure 2-14 shows the separation of *p,o,m*-terphenyls on this PAMAM dendrimer-modified stationary phase: the *p*-terphenyl was well separated from *o*–terphenyl and *m*-terphenyl while the latter two were barely separated. The selectivity coefficients (*k'* ratio of linear/nonlinear solutes) is 2.3 for *p/m*-terphenyl and 2.5 for *p/o*-terphenyl.

It was reported that for reversed-phase liquid chromatography, the shape of the solute affects the retention and selectivity on C_{18} stationary phases: Linear molecules were preferentially retained over their nonlinear analogs.[lxi] Therefore, *para* isomer of terphenyl were supposed to be retained longer than *ortho* and *meta* isomers. The study reported a selectivity coefficient of 2.1 and 2.6 for *p/m*-terphenyl and *p/o*-terphenyl respectively on a polymeric reversed-phase stationary phase with a mobile phase

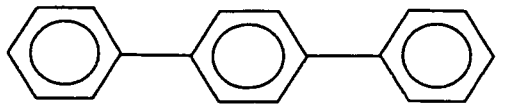
condition of 85/15 acetonitrile/water. The results showed that the PAMAM dendrimer-modified surface behaved similarly as the polymeric alkyl chain surface. The stiff chains of dendrimers protrude to the bulk water-rich solvents and the "dense shell" may have provided a microenvironment for the polycyclic aromatic hydrocarbon isomers, which is less polar than the mobile phase. Therefore, these non-polar compounds would penetrate into the interior of dendrimers. As the chemical nature of the interactions for *p,m,o*-terphenyls with PAMAM dendrimers are the same, the differences in k' must be due to the differences in their shape. The long narrow molecules can fit to the cavities formed by the branches of dendrimers better than square shaped isomers. We will conduct further studies by using other PAH molecules.



o-terphenyl



m-terphenyl



p-terphenyl

Figure 2-13 Structures of isomers of terphenyl.

Figure 2-14 Separations of terphenyl isomers.

Figure (a) chromatogram of *o*-terphenyl and *p*-terphenyl;

Figure (b) chromatogram of *m*-terphenyl and *p*-terphenyl.

Stationary phase was PAMAM dendrimer (generation 3.5) modified zirconia surface.

Composition of mobile phase ranged from 10% ACN/10 mM acetate buffer (pH 5): 90 % ACN to 100% ACN in 10 minutes. Flow rate was 1 mL/min with detection at 254 nm.

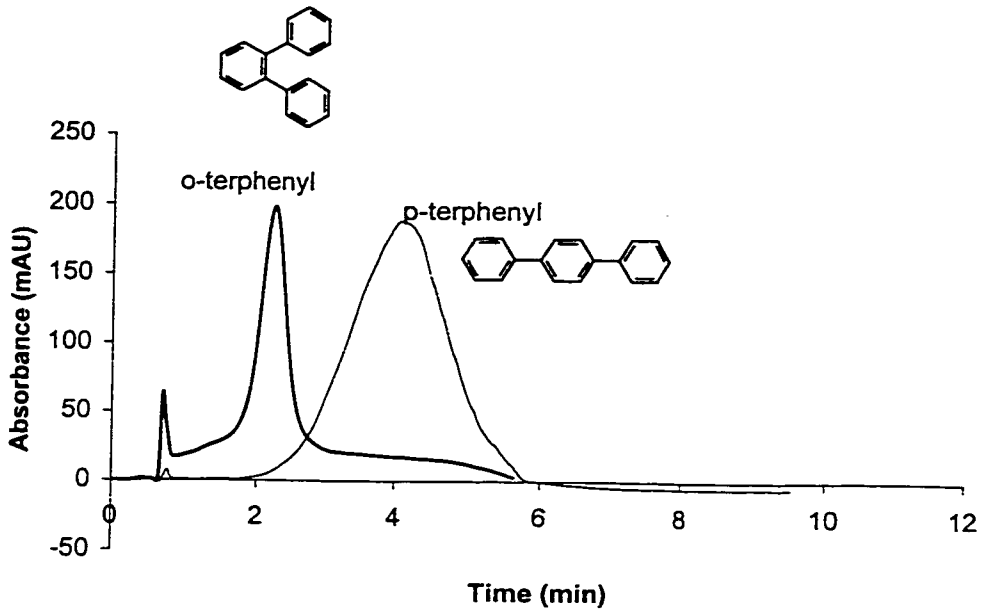


Figure 2-14 (a)

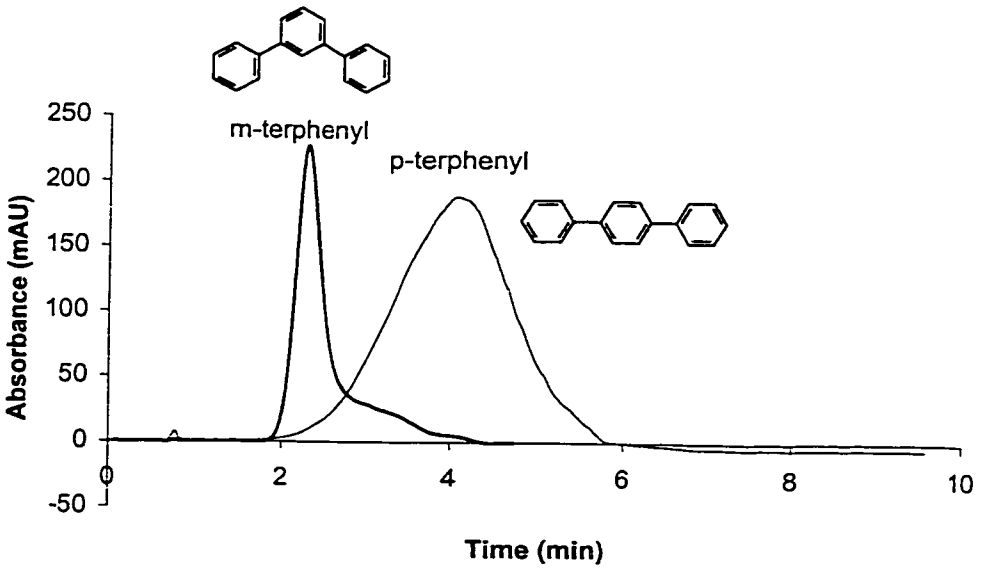


Figure 2-14 (b)

2.3.5 Thermodynamic study

To better understand the nature of the surface-immobilized PAMAM dendrimers, we studied the temperature dependence of the retention of PAH molecules. Numerous studies have been done on reversed-phase stationary phases in order to deduce the enthalpic and entropic contributions to retention[lxii,lxiii,lxiv]. In reversed-phase chromatography, as the temperature increases, retention decreases and chromatographic efficiency increases[lxv]. The increase in efficiency is most often explained as the effect of a decrease in the mobile phase viscosity that facilitates faster mass transfer of the solute in and out of the stationary phase. Of larger scope, the study of temperature effect can provide information about the fundamental properties of mobile phase and stationary phase that dominate retention and selectivity.

Van't Hoff relationship describes the temperature dependence of retention and is expressed as following:

$$\ln k' = \frac{-\Delta H^{o}}{RT} + \frac{\Delta S^{o}}{R} + \ln \Phi$$

where ΔH^{o} and ΔS^{o} represent the standard enthalpy and entropy involved for transferring a solute from the mobile phase to the stationary phase. R is the ideal gas constant, T is the absolute temperature, and Φ is the phase ratio[lxvi]. Typically, retention data is collected over a wide range of temperature and $ln\ k'$ is plotted against $1/T$. The equation predicts a linear relationship between $ln\ k'$ and $1/T$. The slope of the line is $\Delta H^{o}/R$, and the intercept is $\Delta S^{o}/R + ln\ \Phi$. If Φ is known, the thermodynamic constants

ΔH^{o} and ΔS^{o} can be readily determined. As all studies were conducted on the same column, $ln\ \Phi$ is the same for all the three PAHs studied.

Figure 2-15 shows the temperature dependence of the retention of small PAHs on PAMAM dendrimer-modified zirconia surface. This study included the solutes of anthracene, pyrene and triphenylene. The results of this study provide insight into the nature of the modified surface. The related parameters are listed in Table 2-4.

Because the value of phase ratio is difficult to estimate, we cannot obtain the absolute value of the entropy of transfer. However, as the phase ratio was assumed to be constant during the whole study, the results demonstrate that among these three PAHs, the entropy of transfer was the most favorable or least unfavorable for anthracene and the least favorable or most unfavorable for pyrene. On the other hand, the negative values of enthalpy terms indicate that the surface transfer of solutes from mobile phase to stationary phase is exothermal. The enthalpic change is the most favorable for pyrene and the least favorable for anthracene. The most negative free energy for triphenylene indicated that the immobilized PAMAM dendrimers prefer to interact with these PAHs of large hydrophobic area.

It is worthwhile to point out that after the thermodynamic studies, there were no obvious changes in the retention time and peak shape of the PAH molecules on the PAMAM-modified surface, which indicates that the immobilized dendrimer layer is stable during the temperature range studied.

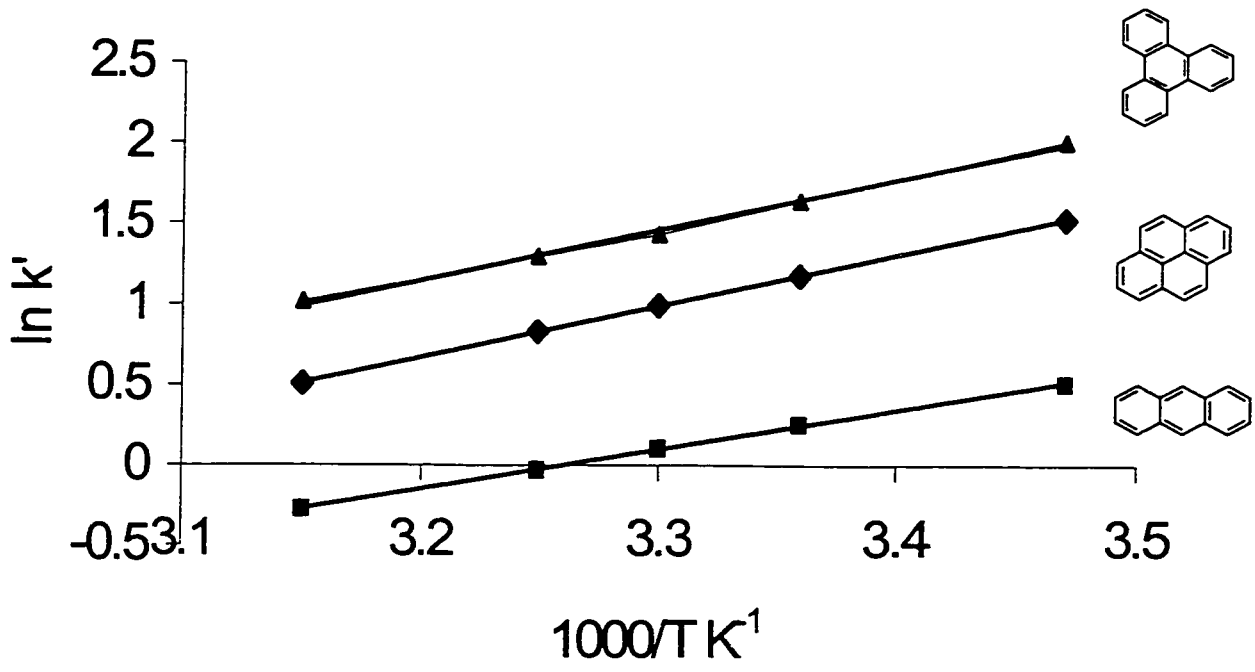


Figure 2-15 Plot of *ln k'* of anthracene, pyrene and triphenylene *vs 1/T* for the PAMAM dendrimer modified zirconia stationary surface. The mobile phase composition was a 90/10 (v/v) mixture of pH 7 10 mM tris buffer and ACN. Flow rate was 1 mL/min with detection at 254 nm.

Table 2-4 Thermodynamic Parameters of PAHs on Dendrimer-Modified Surface

Compounds	triphenylene	pyrene	anthracene
Equation	y=3.1058x-8.7937	y=3.1628x -9.4521	y=2.4525x - 7.9962
R^2	0.9966	0.9999	0.9994
ΔH^o (KJ/mol)	-25.81	-26.29	-20.39
$\Delta S^o/R+ln\Phi$(J/mol/K)	-8.8	-9.5	-8.0

A series of chromatograms were collected on the dendrimer modified zirconia column over a temperature range of 15 °C to 45°C (Figure 2-15). Plate numbers for anthracene, pyrene and triphenylene are listed in Table 2-5. By studying the Table, we have two interesting observations: 1) the plate number is larger for small molecules at a given temperature, with an order of $N_{anthracene} > N_{pyrene} > N_{triphenylene}$. 2) as temperature increased, the plate number increased more significantly for pyrene and triphenylene. This is consistent to the observations made on RPLC stationary phase[lxv]. As stated above, this can be attributed to the enhancement of mass transfer of the solutes into and out of the stationary phase as temperature increased. However, for anthracene, the trend is not obvious and a dramatic drop in efficiency happened at 35 °C.

Figure 2-16 Temperature dependence of PAHs retention. :1) 45 °C; 2) 35 °C; 3) 30 °C; 4) 25 °C.

(a) anthracene

(b) pyrene

(c) triphenylene

Stationary phase was PAMAM dendrimer (generation 3.5) modified zirconia surface. Mobile phase composition was a 90/10 (v/v) mixture of pH 7 10 mM tris buffer and ACN. Flow rate was 1 mL/min with detection at 254 nm.

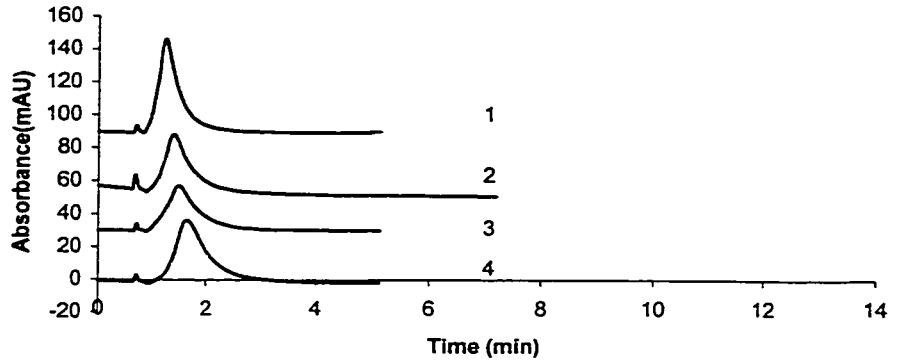


Figure 2-16 (a)

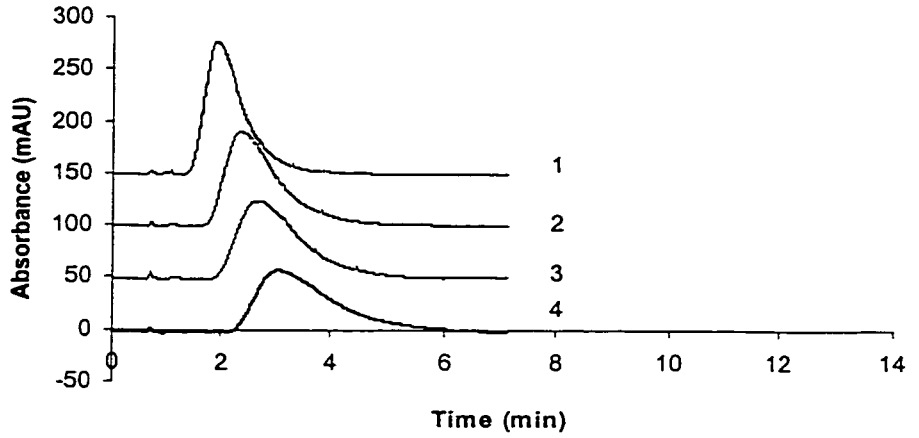


Figure 2-16 (b)

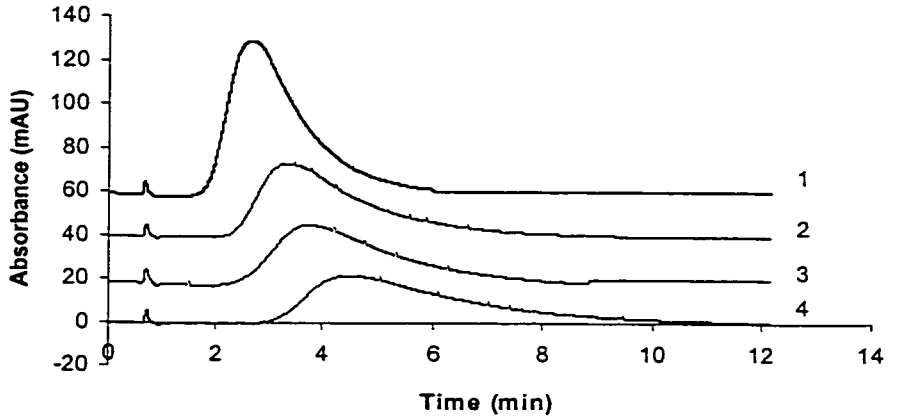


Figure 2-16 (c)

Table 2-5 Plate Numbers for Three PAHs in Temperature Studies

Temperature (°C)	Plate number (N)		
	anthracene	pyrene	triphenylene
25	21.9	11.6	2.8
30	26.1	14.7	5.7
35	11.3	16.1	5.2
45	20.9	16.7	9.6

2.4 Conclusions

In this work, we tested the hypothesis that multiple carboxylic acid groups on the periphery of PAMAM dendrimer molecules can interact with zirconia surface Lewis acid sites, and therefore form a stable surface monolayer. We also demonstrated that by investigating the interactions between dendrimers and small probing molecules, we could study immobilized PAMAM dendrimers through chromatographic techniques.

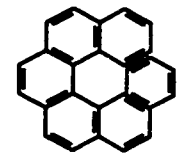
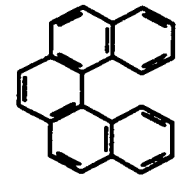
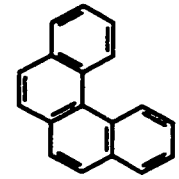
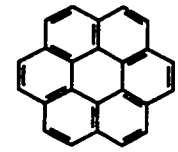
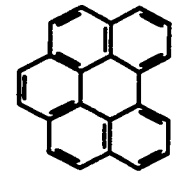
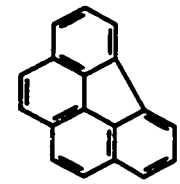
2.5 Future work

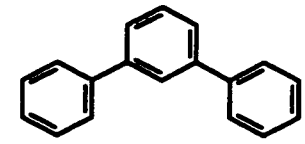
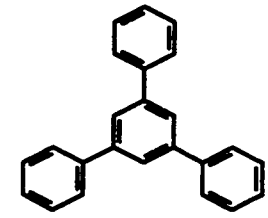
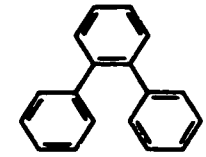
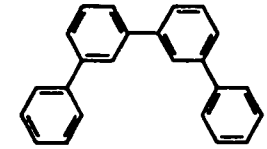
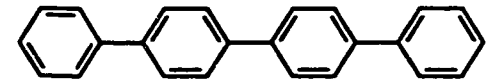
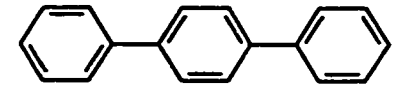
First, we need to optimize the modification conditions. We did this study mostly at pH 5, which was supposed to be higher than the pKa value of carboxyl groups. However, we have to account for the effects of the ionization of tertiary amine groups in the backbone of dendrimer. It was reported[lxvii,lxviii] that pK_a of the tertiary amine groups in PAMAM dendrimers are approximately 4.5~5.5. Therefore, at pH 5, many amine groups will be protonated, which would reduce the hydrophobicity of the interior environment and complicate the study. Actually, MEKC studies have demonstrated a negative electroosmotic flow in Starburst dendrimer- EKC system[Error! Bookmark not defined.]. We might want to process the modification at higher pH.

Second, further work can be done on the shape selectivity of PAMAM dendrimer-modified chromatographic stationary phase. Table 2-6 lists the PAHs that are interesting to us for future studies.

Third, we only studied the PAMAM dendrimer of generation 3.5. Studies with other generations will be of interest. Different characteristics will be expected for different generation of PAMAM dendrimers modified surface.

Table 2-6 List of PAHs for Future Shape-Selective Studies

	Name	Structure
nonplanar	Phenanthro[3,4-c]phenanthrene	
	Dibenzo[c,g] phenanthrene	
	Benzo[c]phenanthrene	
Planar	Coronene	
	Benzo[ghi] perylene	
	Benzo[ghi]fluoranthene	

	Name	Structure
nonlinear	m-tertraphenyl	
	1,3,5- triphenylbenzene	
	o-terphenyl	
	m-terphenyl	
linear	p-quaterphenyl	
	p-terphenyl	

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